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# BMJ Open

**The efficacy of low-load blood flow restricted resistance EXercise in patients with Knee osteoarthritis scheduled for total knee replacement (EXKnee). A multicenter, randomized controlled trial.**

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Keywords:	blood flow restriction exercise, knee osteoarthritis, total knee replacement surgery, preconditioning, functional capacity

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4 **The efficacy of low-load blood flow restricted resistance EXercise in patients with Knee**  
5 **osteoarthritis scheduled for total knee replacement (EXKnee). A multicenter, randomized**  
6 **controlled trial.**  
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10  
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## ABSTRACT

### Introduction

Up to 20% of patients undergoing total knee replacement (TKR) surgery report no or suboptimal pain relief after TKR. Moreover, despite chances of recovering to preoperative functional levels, patients receiving TKR have demonstrated persistent deficits in quadriceps strength and functional performance compared to healthy aged-matched adults. We intend to examine if low-load blood flow restricted exercise (BFRE) is an effective preoperative method to increase functional capacity, lower limb muscle strength and self-reported outcomes after TKR. In addition we seek to investigate to which extent preoperative BFRE will protect against surgery-related atrophy 3 months after TKR. Specifically, the primary aim of this trial is to examine the efficacy 8 weeks of low-load BFRE prior to a scheduled TKR on changes in 30-seconds Chair stand test from baseline to 3 months after TKR. As a secondary aim, the effect of preoperative BFRE on maximal knee extensor strength (MVC), functional capacity, patient-reported outcome (Knee Injury and Osteoarthritis Outcome Score) and selected myofiber properties (fiber CSA, myogenic stem cell content, myonuclei density) also will be examined also.

### Method and analysis

The trial is a multicenter, randomized controlled and assessor blinded trial, where patients scheduled for TKR will be randomized to either 8 weeks of preoperative BFRE or serve as a control group following usual care before TKR. Data will be collected at baseline, in the week of TKR, 6 weeks (questionnaires only), 3 months, and 12 months after TKR.

### Ethical approval and dissemination

The trial has been accepted by Central Denmark Region Committee on Biomedical Research Ethics (Journal No 10-72-19-19) and by The Danish Data Protection Agency (Journal No 652164). All results from the trial will be published in international peer-reviewed scientific journals regardless of whether the results are positive, negative or inconclusive.

### Trial registration

The trial is registered at Clinical Trial (NCT04081493)

### Article Summary

#### Strengths and limitations of this study

- The trial is a multicenter, randomized controlled assessor blinded trial.
- This is the first clinical trial to investigate the effect of low-load ischemic resistance training as a preconditioning method prior to elective knee replacement surgery.
- Patients will not be blinded to their allocation into intervention groups (BFR vs. control)

### Key words

Blood flow restricted exercise, knee osteoarthritis, total knee replacement surgery, preconditioning

## INTRODUCTION

Knee OA is a degenerative joint disease associated with pain, reduced physical activity, and quality of life affecting almost 40% of all individuals  $\geq 60$  years of age<sup>1-5</sup>. Approaching end-stage knee OA, total knee replacement (TKR) is often the preferred treatment choice to reduce pain and regain functional capacity. However, despite TKR patients typically demonstrate long-lasting deficits in quadriceps strength and functional performance<sup>2, 4</sup>. This failure to return to “normal” strength levels has been suggested to be associated with preoperatively lower limb muscle strength and function<sup>2</sup>.

Preconditioning exercise, designed to prepare the musculoskeletal system to better tolerate the stressful events such as the impact of invasive surgery has been suggested to be applicable prior to elective TKR<sup>6</sup>. Muscle atrophy is known to occur postoperatively, which may explain the marked functional deficits reported to persist for years<sup>6, 7</sup>. Previous research on exercise-based intervention prior to TKR has demonstrated mixed results<sup>6-12</sup>, as a likely result of insufficient exercise intensity, training volume, and/or lack of effective progression strategies<sup>9</sup>. Heavy-resistance strength training (HRST) is an often-used method for improving, skeletal muscle strength, hypertrophy and functional capacity in healthy and clinical populations<sup>4, 7, 12-15</sup>. However joint pain resulting from the high mechanical loads associated with HRST often represents a barrier to this type of training in knee OA patients<sup>1, 16</sup>. Resistance training with low exercise loads (~30% 1 repetition maximum) performed with concurrent partial blood flow restriction to the working limb (Blood flow restricted exercise: BFRE) has received increasing clinical interest during the last decade<sup>1, 16-34</sup>. The application of low muscle/tendon/joint forces in BFRE has been documented to increase human skeletal muscle size and to cause substantial strength gains in healthy young and old individuals<sup>17</sup>, despite the low magnitude of mechanical stress imposed on the trained tissue. The adaptive mechanisms evoked by BFRE seem to involve accumulation of metabolites, ischemia (transient

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4 tissue hypoxia) and activation of myogenic muscle stem cells (satellite cells: SC) <sup>17, 24, 35</sup>. When  
5  
6 applied in the clinical setting, BFRE has demonstrated positive effects on skeletal muscle  
7  
8 hypertrophy, strength, and functional capacity in mild-degree knee OA patients <sup>1, 16, 33, 34</sup>, although  
9  
10 not observed in all studies <sup>33</sup>. Importantly, BFRE appears to be feasible with a high training  
11  
12 adherence in knee OA patients <sup>1, 33, 34</sup>.

13  
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15  
16 Satellite cells (SC) are quiescent myogenic stem cells positioned between the sarcolemma and  
17  
18 the myofiber basal lamina <sup>24, 36</sup>. SC plays an important role in human skeletal muscle growth due to  
19  
20 their ability to donate new myonuclei to the muscle fibers <sup>24, 37-41</sup>. The human skeletal muscle fibers  
21  
22 are multinucleated cells with each myonucleus controlling the protein synthesis of a certain  
23  
24 cytoplasmic area in the muscle fiber <sup>37-39, 41, 42</sup>. Myonuclei transcriptional activity can be fully  
25  
26 maximized with exercise, hence requiring new myonuclei to support further muscle tissue accretion  
27  
28 <sup>38-43</sup>. Furthermore, exercise-induced increases in SC and myonuclei content by means of  
29  
30 preconditioning BFRE might represent an effective atrophy-protective mechanism <sup>24, 44</sup>. Previous  
31  
32 studies applying short term (10 days) preoperative BFRE before an anterior cruciate ligament  
33  
34 rupture-reconstruction demonstrated no atrophy protective effect nor higher postoperative muscle  
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36 strength compared to performing a low-load exercise without blood flow restriction (placebo) .  
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### 45 **Aim and hypothesis of the trial**

46  
47 The primary aim of this trial is to investigate the efficacy of 8 weeks of BFRE compared to  
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49 receiving usual care prior to TKR on postoperative chair stand performance. We hypothesize that 8  
50  
51 weeks of preoperative BFRE will lead to increased performance 30 seconds chair stand  
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53 performance (30-seconds Chair Stand Test: 30-s CST) when assessed 3 months postoperatively.  
54  
55 Secondary aims are to investigate the efficacy of preoperative BFRE on lower limb muscle strength  
56  
57 3 months after TKR and investigate the potential relationship to functional capacity and quality of  
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4 life. Furthermore, it will be investigated to which extent 8 weeks of BFRE induces myofiber  
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6 hypertrophy and gains in satellite cell number and myonuclei content in the knee extensor  
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8 musculature.  
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## 10 11 12 13 14 **MATERIAL & METHODS**

### 15 16 **Design**

17  
18 The trial is designed as a multicenter (2 sites), randomized, assessor blinded, controlled trial  
19  
20 following the CONSORT guidelines<sup>45</sup>. Primary endpoint will be 3 months after TKR. Additional  
21  
22 and secondary endpoints will be evaluated during the week of TKR, 6 weeks after TKR  
23  
24 (questionnaires only) and 12 months after TKR. Muscle biopsies will be obtained from all patients  
25  
26 undergoing surgery at Horsens Regional Hospital at baseline, during surgery and 3 months after  
27  
28 TKR.  
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### 36 **Participants**

37  
38 Patient will be recruited from the Orthopedic Departments at Horsens and Silkeborg Regional  
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40 Hospitals.  
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43 Inclusion criteria: 1) Patients  $\geq 50$  years scheduled for TKR at Horsens- or Silkeborg Regional  
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45 Hospital.  
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48 Exclusion criteria: 1) Severe cardiovascular diseases (New York Heart Association (NYHA) class  
49  
50 III and IV), previous stroke incident, thrombosis incident; 2) Traumatic nerve injury in affected  
51  
52 limb 3) Unregulated hypertension (Systolic  $\geq 180$  or diastolic  $\geq 110$  mmHg) 4) Spinal cord injury; 5)  
53  
54 Planned other lower limb surgery within 12 months; 6) Cancer diagnosis and currently undergoing  
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56 chemo-, immuno-, or radiotherapy; 7) Inadequacy in written and spoken Danish; 8) an existing  
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4 prosthesis in the index limb; 9) living more than 45 minutes from either Horsens Regional Hospital  
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6 or Silkeborg Regional Hospital; 10) Pregnancy.

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9 The orthopedic surgeon will perform the initial inclusion of study participants. In case the  
10  
11 patient agrees to participate in the trial, the patient will be baseline-tested at the hospital by a  
12  
13 blinded (to group allocation) assessor. Patients declining to participate in the RCT will be offered  
14  
15 the option of participating in a parallel observational cohort trial.  
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## 21 **Randomization**

22  
23 After baseline assessment, patients will be randomized (1:1) using Research Electronic Data  
24  
25 Capture (REDCap) randomization system to either the training (BFRE) group or the control (CON)  
26  
27 group. Prior to randomization, all patients will be booked for follow-up test sessions and surgery.  
28  
29 All randomization procedures will be performed by the physiotherapists in charge of the BFRE  
30  
31 training. Assessors performing the tests will be blinded to group allocation until completion of the  
32  
33 trial. A flow chart of the patient allocation procedures is depicted in Figure 1.  
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40 CON group: Participants in the CON group will follow usual care before a TKR and be encouraged  
41  
42 to continue their usual lifestyle up until TKR.  
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48 BFRE group: Will perform supervised BFRE sessions 3 times per week for 8 weeks supervised by a  
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50 physiotherapist educated in administering BFRE. All BFRE sessions will be performed at either  
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52 Horsens Regional Hospital or Silkeborg Regional Hospital.  
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58 *Insert figure 1 here*  
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## Intervention procedures

### BFRE

Each BFRE session will consist of a 10-min warm up (ergometer cycling) followed by two different unilateral lower-limb resistance training exercises: 1) leg press and 2) knee extension performed in standard strength training machines. Each exercise will be performed with the affected lower limb only and consist of 4 rounds interspaced by 30 seconds of rest. 1<sup>st</sup> round: 30 repetitions (reps); 2<sup>nd</sup> round: 15 reps; 3<sup>rd</sup> round: 15 reps; 4<sup>th</sup> round: until exhaustion. If patients can perform more than 15 repetitions in the 4<sup>th</sup> exercise set, the exercise load will be increased with the minimum extra load possible<sup>25</sup>. Participants will be instructed to perform both the eccentric and concentric contraction phases using a steady 2-sec pace duration. The 4<sup>th</sup> and final exercise set will be performed to the point of exhaustion defined as being unable to complete the final concentric contraction phase in 2 seconds. During the 30 sec rest period, patients will rest in a standardized resting position while maintaining the initial cuff-pressure. Between each exercise patients will have a 5-min "free-flow" rest period. The cuff will be released immediately after completion of the final exercise set.

The occlusion pressure during both exercises will be set at 60% of total limb occlusion pressure (LOP) and starting load intensity will be 30% 1 repetition maximum (1RM) in both exercises.

Individual LOP will be determined using a pneumatic, conically shaped, 12 cm wide, rigid cuff (Occlude Aps, Denmark) attached to the patient's most proximal area of the thigh on the affected side. While sitting on an examination table with the ankle and 1/3 of the lower limb off the table, a vascular Doppler probe (EDAN Instruments, inc., China) will be placed posterior to the medial malleolus over the posterior tibial artery to capture the auscultatory pulse. To determine the cuff

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4 pressure (mmHg) needed for total blood flow occlusion, the cuff will gradually be inflated in 20  
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6 mmHg steps until reaching the pressure where the auscultatory pulse is interrupted (LOP). First  
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8 time the auscultatory pulse is interrupted the examiner releases 10-20 mmHg pressure from the cuff  
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10 until the auscultatory pulse is present again. When the auscultatory pulse reappears the cuff is  
11  
12 inflated with 10 mmHg until LOP is found again. If the second LOP is identical to the first it will be  
13  
14 defined as LOP for that specific patient. Otherwise, the procedure will be repeated until determining  
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16 an identical LOP two consecutive times.  
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22 *Insert table 1 here*  
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## 28 **Outcome variables**

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30 Outcome assessments will be performed at baseline, in the week of surgery, 6 weeks after TKR, 3  
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32 months after TKR, and 12 months after TKR. Six weeks after TKR only questionnaires will be  
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34 completed. Two testers (the PhD-stipendiate and a trained physiotherapist) blinded to group  
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36 allocation will perform all baseline and follow-up measurements. Bergström needle muscle biopsies  
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38 <sup>46</sup> will be taken from vastus lateralis of the quadriceps in both lower limbs from patients included at  
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40 Regional Hospital Horsens only at baseline, during surgery, and 3 months after TKR by doctors  
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42 trained in performing the procedure. An overview of the data collection parameters is presented in  
43  
44 Table 2.  
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## 51 Primary outcome variable

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53 The primary outcome measure will be the change from baseline to 3 months follow-up in 30s-CST.  
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55 The 30s-CST measures the number of sit-to-stand repetitions completed within 30 seconds <sup>47, 48</sup> and  
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57 is a part of the OARSI-recommended minimum outcome core set representing the ability to perform  
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4 a sit-to-stand activity<sup>49</sup>. The 30s-CST is considered a valid and sensitive measure of lower-  
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6 extremity sit-to-stand function with good to excellent intra- and inter-observer reliability<sup>47-50</sup>.  
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### 10 11 Secondary outcome variables

12  
13 Secondary outcome measures comprise The Timed Up and Go test<sup>50-52</sup>, 40-m fast-paced walk test<sup>50</sup>,  
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15 maximal isometric knee extensor and knee flexor strength assessed with hand-held dynamometry<sup>53</sup>,  
16  
17 <sup>54</sup>, knee extensor (VL) myofiber area, fibertype composition, satellite cell content, myonuclei  
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19 number<sup>14</sup>, the Knee disability and Osteoarthritis Outcome Score<sup>55, 56</sup>, EuroQol Group 5-  
20  
21 dimensions<sup>57</sup>, Numeric Ranking Scale for pain (NRS)<sup>58</sup>, and adverse events/postponement of TKR.  
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### 25 Explorative outcome variables

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27 Type of postoperative rehabilitation received, medication, knee joint range of motion  
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### 32 Demographic data

33  
34 Gender, age, height, weight, civil status, level of educational, employment status, substance use  
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36 (alcohol and smoking), duration of knee symptoms, pain medication during past week due to knee-  
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38 related pain, and co-morbidities.  
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### 43 Adherence

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45 Adherence to training will be registered by the physiotherapists in charge of the exercise sessions.  
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48 High compliance is defined as attendance to the supervised BFRE of  $\geq 80\%$ .  
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53 *Insert table 2 here*  
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## Sample size

The power and sample size calculation is based on the expected differences between the two subject groups from baseline to 3 months follow up<sup>8</sup>. Skoffer et al.<sup>8</sup> investigated the efficacy of 4 weeks of preoperative and 4 weeks postoperative HRST (intervention group) compared to 4 weeks of postoperative HRST only (control group) on 30-s CST 3 months after TKR<sup>8</sup>. The authors found a between-group difference of 3-4 repetition difference ( $14.7 \pm 4.7$  repetitions versus  $11.0 \pm 4.4$  repetitions) 3 months after TKR<sup>8</sup>.

To reduce the probability of type I errors and be able to detect a between-group difference also,  $\alpha$ -level is set at 0.05 ( $p < 0.05$ ) and  $\beta$ -level is set at 0.20 (80% power). Expecting a 3-repetitions between-group difference 3 months postoperatively and assuming a SD of 4.7 in both groups, 39 patients are required in each group (yielding a total of 78 patients). With an anticipated dropout rate of 10%, a total of 84 patients will be recruited for the trial.

## Statistical considerations

The primary efficacy analysis will be assessment of the between group difference in change in the 30-S CST from baseline to 3 months follow up (primary end point).

All descriptive statistics and tests will be reported in accordance with the recommendations of the “Enhancing the QUALity and Transparency Of health Research” (EQUATOR) network<sup>59</sup> and the CONSORT statement<sup>45</sup>. Intention-to-treat principle (i.e. all patients as randomized independent of departures from allocation treatment, compliance and/or withdrawals) and per protocol analysis will be conducted. A one-way analysis of variance (one-way ANOVA) model will be used to analyze between group mean changes in continuous outcome measures<sup>24</sup>. The model includes changes from baseline to 12 months follow-up. Between-intervention comparison from baseline to 3 months after surgery will be analyzed using a mixed linear model with patient ID as a random

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4 effect and time and group as fixed effects<sup>24,60</sup>. Also, to gain insights into the potential pre-to-post  
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6 training differences within the respective training or control groups, paired student t-tests will be  
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8 performed. Level of statistical significance is  $P < 0.05$ . *Secondary outcome variables*: Between-  
9  
10 intervention comparison from baseline to the week of surgery, 6 weeks after surgery, 3 and 12  
11  
12 months after surgery will be analyzed as described for the primary outcome. Regression analysis  
13  
14 will be used to analyze the potential associations between preoperative strength and postoperative  
15  
16 lower extremity function and self-reported outcome as well as between preoperative functional  
17  
18 capacity and postoperative functional capacity. Additionally, regression analysis will be used to  
19  
20 analyze the association between preoperative number of satellite cells and myonuclei on  
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22 postoperative isometric knee extensor muscle strength, muscle fiber cross sectional area, and  
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24 functional capacity. All statistical analysis will be performed using Stata.  
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### 33 **Ethical aspects and dissemination**

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35 The trial has been accepted by Central Denmark Region Committee on Biomedical Research Ethics  
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37 (Journal No 10-72-19-19) and by The Danish Data Protection Agency (Journal No 652164). The  
38  
39 trial is registered at Clinicaltrial.gov (NCT04081493). Before inclusion, all patients will provide  
40  
41 their written informed consent in accordance with the Helsinki Declaration. All data and  
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43 information collected in regard to this trial will be treated confidentially (blinded and encrypted) by  
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45 the researchers and staff connected to the trial.  
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49 All results from the trial will be published in international peer-reviewed scientific journals  
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51 regardless of the results being considered positive, negative or inconclusive.  
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### 55 **Patient and public involvement**

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57 Before developing this clinical trial, a pilot project was performed to determine feasibility and  
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4 efficacy of BFRE in patients suffering from lower limb injuries. The experiences with the training  
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6 modality and also the verbal feedback from patients on training duration, frequency, and intensity  
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8 resulted in useful knowledge that certainly have improved the development of the present clinical  
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10 trial.  
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## 15 **DISCUSSION**

16  
17 To our best knowledge, this is the first trial to investigate the effect of preoperative BFRE on  
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19 functional capacity, self-reported outcome, lower limb muscle strength and myofiber  
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21 morphology/stem cell abundance in patients scheduled for TKR. Only few studies have investigated  
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23 (short term (10 days)) preoperative BFRE without finding an atrophy protective effect or difference  
24  
25 in muscle strength compared to a control group performing a placebo intervention (SHAM group)  
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27 <sup>61</sup>. However, patients performing short term preoperative BFRE before ACL-R demonstrated higher  
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29 muscle endurance compared to a SHAM group <sup>62</sup>. Therefore, results of this trial are expected to  
30  
31 provide novel information on longer periods of BFRE that will enable to design effective exercise-  
32  
33 based preconditioning protocols for elective TKR patients.  
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40 The trial is designed as an assessor blinded randomized controlled trial, thus representing the  
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42 highest evidence level. However, the nature of the trial does not allow blinding of the participants  
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44 which is an inherent limitation of the trial. The trial is conducted at two hospitals that consistently  
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46 perform a high number TKR procedures annually (225 and 460, respectively), thus securing a  
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48 strong expertise in terms of surgery and infrastructure. Both hospitals have all equipment needed  
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50 available for surgery, post-operative hospitalization, training and testing. All outcome variables are  
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52 considered valid and reliable measures and consist of both objective outcomes and self-reported  
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54 patient outcomes.  
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4 No adverse health-related events have been reported in previous studies applying BFRE in  
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6 patients' suffering from knee OA or in healthy older adults <sup>1, 16, 17, 27, 33, 34</sup>. Further, in a recent  
7  
8 review and meta-analysis it was stated that exercise with concurrent blood-flow restriction is a safe  
9  
10 exercise modality when occlusion procedures are applied correctly <sup>17</sup>. The inherent invasive  
11  
12 procedure of muscle biopsies may cause adverse events in rare occasions. Therefore, all muscle  
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14 biopsy samples will be collected by trained medical doctors and performed following administration  
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16 of local anesthesia and in fully sterile conditions. The needle muscle biopsy protocol have been  
17  
18 applied in a large number of previous investigations including very old frail subjects (97 years of  
19  
20 age) without any reporting of adverse events besides occasional muscle soreness <sup>15, 24, 46, 63, 64</sup>.  
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### 27 **Author contributions**

28  
29 All authors contributed to the design of the trial as well as to the writing of the manuscript and  
30  
31 approved the final version of the protocol.  
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### 37 **Data statement**

38  
39 All obtained data will be stored in anonymized form at the Danish National Archives and deleted  
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41 after 10 years.  
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49  
50  
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52  
53 Foundation for health research of Central Denmark Region (99.658 dkk).  
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4 **Competing interest**

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6 None to be declared  
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12 **Ethics approval**

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14 The trial has been accepted by Central Denmark Region Committee on Biomedical Research Ethics  
15  
16 (Journal No 10-72-19-19) and by The Danish Data Protection Agency (Reference No 652164).  
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## Tables

*Table 1.* Exercise variables for the blood-flow restricted exercise (BFRE) protocol

Exercise variable	Week 1-8
Level of LOP	60% LOP
Sets	4
Load intensity	30% 1RM
Repetitions 1 <sup>st</sup> set	30
Repetitions 2 <sup>nd</sup> & 3 <sup>rd</sup> set	15
Repetitions 4 <sup>th</sup> set	To volitional failure
Contraction modes per repetition	
Concentric	2 seconds
Isometric	0 seconds
Eccentric	2 seconds
Rest between repetitions	0 seconds
Time under tension per repetition	4 seconds
Range of movement	maximum
Rest between sets	30 seconds
Rest between sessions	≥36 hours
Progression	The minimal possible load (5 kilo) is added when patients perform >15 repetitions in 4 <sup>th</sup> set

*Table 2.* Outcome measures to be collected.

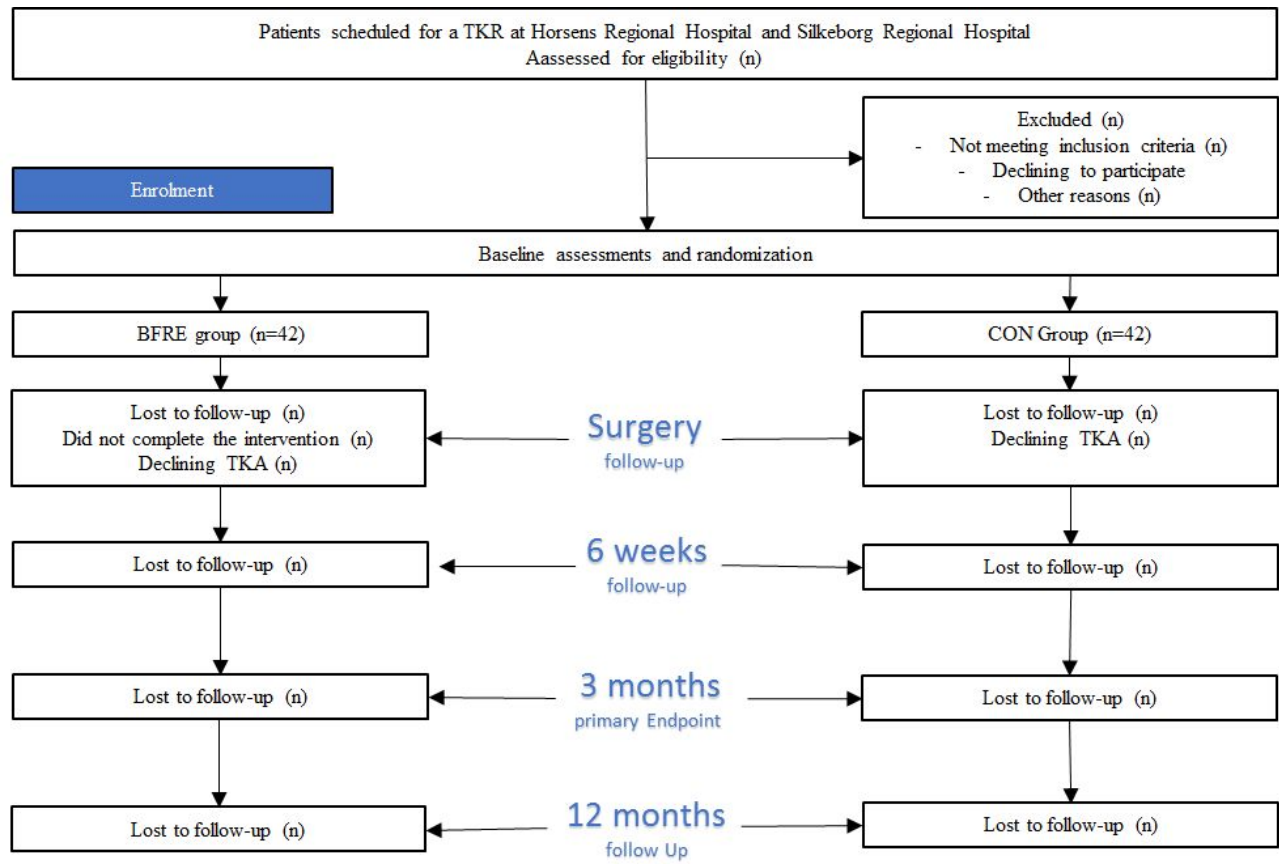
Outcome measures	Data collection instrument	Time-points of assessment
<b>Primary outcome</b>		
<b>Sit-to-stand function</b>	30 seconds chair stand test	B, S, 3 and 12 months
<b>Secondary outcomes</b>		
<b>Isometric Knee extensor muscle strength</b>	Handheld Dynamometer	B, S, 3 and 12 months
<b>Isometric Knee flexion muscle strength</b>	Handheld Dynamometer	B, S, 3 and 12 months
<b>Gait speed</b>	4x10-meter walk test	B, S, 3 and 12 months
<b>Ambulatory capacity</b>	Timed Up & Go	B, S, 3 and 12 months
<b>Muscle morphology and biology</b>	Muscle Biopsies	B, D, 3 months
<b>Pain</b>	KOOS	B, S, 6 weeks, 3 and 12 months
<b>Symptoms</b>	KOOS	B, S, 6 weeks, 3 and 12 months
<b>Activities of daily living</b>	KOOS	B, S, 6 weeks, 3 and 12 months
<b>Sports &amp; Recreation</b>	KOOS	B, S, 6 weeks, 3 and 12 months
<b>Quality of life</b>	KOOS	B, S, 6 weeks, 3 and 12 months
<b>Socioeconomic costs</b>	EQ-5D	B, S, 6 weeks, 3 and 12 months
<b>Adverse Events</b>	Questionnaire and medical records	S, 3 months
<b>Patient characteristics and related measurements</b>		
<b>Gender</b>	Questionnaire	B
<b>Age</b>	Questionnaire	B
<b>Height</b>	Tape measure	B
<b>Body mass</b>	Electronic body mass scale	B
<b>Civil Status</b>	Questionnaire	B
<b>Educational Level</b>	Questionnaire	B
<b>Employment Status</b>	Questionnaire	B
<b>Substance Use (alcohol, smoking)</b>	Questionnaire	B
<b>Duration of knee symptoms</b>	Questionnaire	B
<b>Pain medication during the last week</b>	Questionnaire	B

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<b>Com-morbidities</b>	Questionnaire	B
<b>Blood pressure</b>	Electronic upper limb blood pressure monitor	During the exercise period
<b>Postoperative supervised physiotherapy</b>	Questionnaire	B, S, at each BBFRE session, 3
<b>Exercise compliance and progression</b>	Physiotherapist records	and 12 months
<b>NRS</b>	PhD-stipendiate and physiotherapist records	

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Figure 1. Patient flow



view only



## Table and figure legends

Table 1. LOP = Limb occlusion pressure; RM = Repetition maximum

Table 2. KOOS = Knee disability and Osteoarthritis Outcome Score; B = Baseline; S = 0-2 days before surgery; D = during surgery; 3 months = 3 months after TKR; 12 months = 12 after TKR; NRS = Numeric Ranking Scale of pain

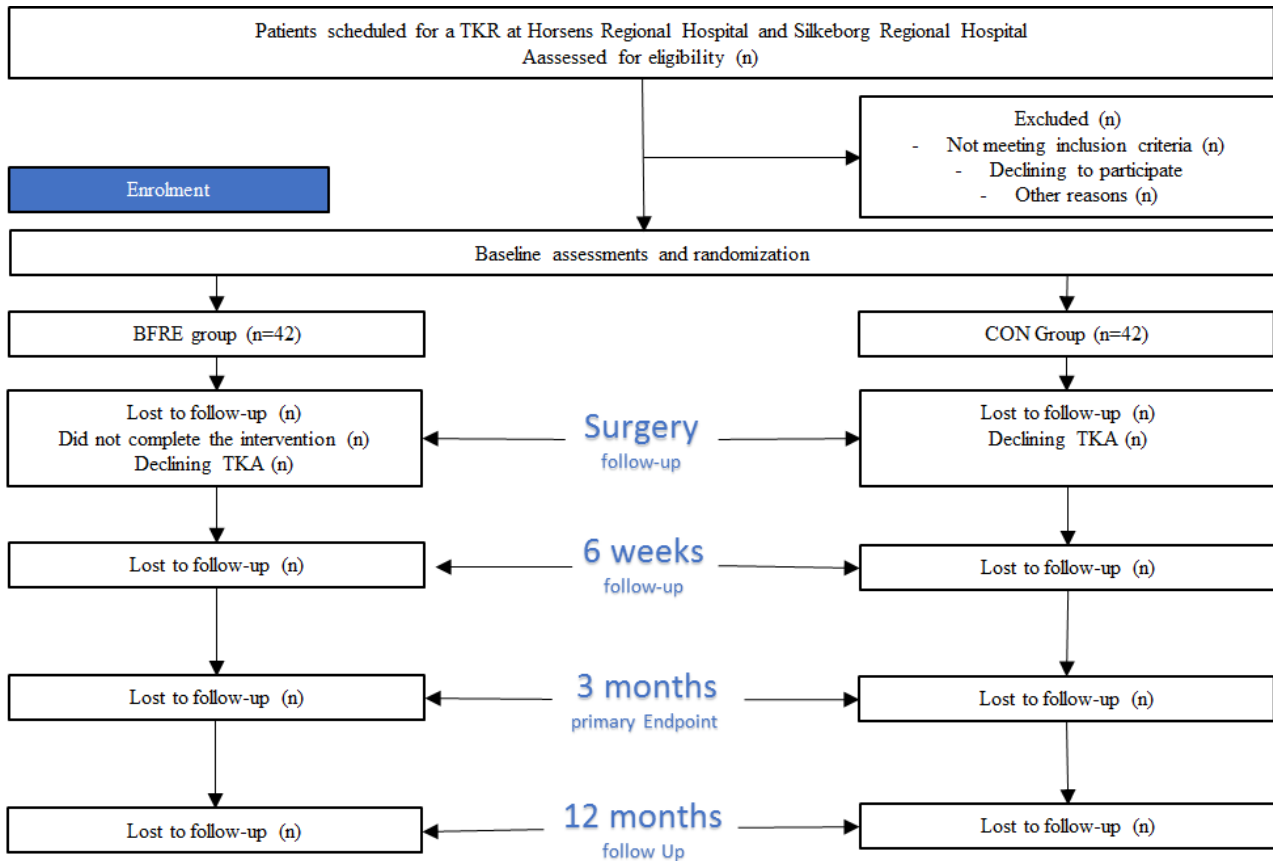
Figure 1. Flow chart of the enrollment, treatment, and follow-up phases. TKR: Total Knee Replacement, BFRE: Low-load blood-flow restricted exercise

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# Figure and figure legend

EXKnee project

Figure 1. Patient flow



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16 Figure 1. Flow chart of the enrollment, treatment, and follow-up phases. TKR: Total Knee Replacement, BFRE: Low-load blood-flow  
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## Tables

*Table 1.* Exercise variables for the blood-flow restricted exercise (BFRE) protocol

Exercise variable	Week 1-8
Level of LOP	60% LOP
Sets	4
Load intensity	30% 1RM
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Repetitions 2 <sup>nd</sup> & 3 <sup>rd</sup> set	15
Repetitions 4 <sup>th</sup> set	To volitional failure
Contraction modes per repetition	
Concentric	2 seconds
Isometric	0 seconds
Eccentric	2 seconds
Rest between repetitions	0 seconds
Time under tension per repetition	4 seconds
Range of movement	maximum
Rest between sets	30 seconds
Rest between sessions	≥36 hours
Progression	The minimal possible load (5 kilo) is added when patients perform >15 repetitions in 4 <sup>th</sup> set

*Table 2.* Outcome measures to be collected.

Outcome measures	Data collection instrument	Time-points of assessment
<b>Primary outcome</b>		
<b>Sit-to-stand function</b>	30 seconds chair stand test	B, S, 3 and 12 months
<b>Secondary outcomes</b>		
<b>Isometric Knee extensor muscle strength</b>	Handheld Dynamometer	B, S, 3 and 12 months
<b>Isometric Knee flexion muscle strength</b>	Handheld Dynamometer	B, S, 3 and 12 months
<b>Gait speed</b>	4x10-meter walk test	B, S, 3 and 12 months
<b>Ambulatory capacity</b>	Timed Up & Go	B, S, 3 and 12 months
<b>Muscle morphology and biology</b>	Muscle Biopsies	B, D, 3 months
<b>Pain</b>	KOOS	B, S, 6 weeks, 3 and 12 months
<b>Symptoms</b>	KOOS	B, S, 6 weeks, 3 and 12 months
<b>Activities of daily living</b>	KOOS	B, S, 6 weeks, 3 and 12 months
<b>Sports &amp; Recreation</b>	KOOS	B, S, 6 weeks, 3 and 12 months
<b>Quality of life</b>	KOOS	B, S, 6 weeks, 3 and 12 months
<b>Socioeconomic costs</b>	EQ-5D	B, S, 6 weeks, 3 and 12 months
<b>Adverse Events</b>	Questionnaire and medical records	S, 3 months
<b>Patient characteristics and related measurements</b>		
<b>Gender</b>	Questionnaire	B
<b>Age</b>	Questionnaire	B
<b>Height</b>	Tape measure	B
<b>Body mass</b>	Electronic body mass scale	B
<b>Civil Status</b>	Questionnaire	B
<b>Educational Level</b>	Questionnaire	B
<b>Employment Status</b>	Questionnaire	B
<b>Substance Use (alcohol, smoking)</b>	Questionnaire	B
<b>Duration of knee symptoms</b>	Questionnaire	B
<b>Pain medication during the last week</b>	Questionnaire	B



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<b>Com-morbidities</b>	Questionnaire	B
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<b>Exercise compliance and progression</b>	Physiotherapist records	and 12 months
<b>NRS</b>	PhD-stipendiate and physiotherapist records	

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## Table legends

Table 1. LOP = Limb occlusion pressure; RM = Repetition maximum

Table 2. KOOS = Knee disability and Osteoarthritis Outcome Score; B = Baseline; S = 0-2 days before surgery; D = during surgery; 3 months = 3 months after TKR; 12 months = 12 after TKR; NRS = Numeric Ranking Scale of pain

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**The efficacy of low-load blood flow restricted resistance EXercise in patients with Knee osteoarthritis scheduled for total knee replacement (EXKnee). Protocol for a multicenter, randomized controlled trial.**

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4 1 **The efficacy of low-load blood flow restricted resistance EXercise in patients with Knee**  
5 **osteoarthritis scheduled for total knee replacement (EXKnee). Protocol for a multicenter,**  
6 **2 randomized controlled trial.**  
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10  
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## 25 **ABSTRACT**

### 26 **Introduction**

27 Up to 20% of patients undergoing total knee replacement (TKR) surgery report no or suboptimal  
28 pain relief after TKR. Moreover, despite chances of recovering to preoperative functional levels,  
29 patients receiving TKR have demonstrated persistent deficits in quadriceps strength and functional  
30 performance compared to healthy aged-matched adults. We intend to examine if low-load blood  
31 flow restricted exercise (BFRE) is an effective preoperative method to increase functional capacity,  
32 lower limb muscle strength and self-reported outcomes after TKR. In addition, the study aims to  
33 investigate to which extent preoperative BFRE will protect against surgery-related atrophy 3  
34 months after TKR.

### 36 **Methods**

37 In this multicenter, randomized controlled and assessor blinded trial, 84 patients scheduled for TKR  
38 will be randomized to receive usual care and 8 weeks of preoperative BFRE or to follow usual care-  
39 only. Data will be collected at baseline, in the week of TKR, 6 weeks, 3 months, and 12 months  
40 after TKR. Primary outcome will be the change in 30-seconds chair stand test from baseline to 3  
41 months follow-up. Key secondary outcomes will be Timed Up & Go, 40-meter fast-paced walk test,  
42 isometric knee extensor and flexor strength, patient-reported outcome, and selected myofiber  
43 properties.

44 Intention-to-treat principle and per protocol analyses will be conducted. A one-way analysis of  
45 variance model will be used to analyze between group mean changes. Between-intervention  
46 comparison will be analyzed using a mixed linear model. Also, paired student t-tests will be  
47 performed and regression analysis will be used for analyzation of associations between selected  
48 outcomes.

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5**6 50 Ethical approval**

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9 51 The trial has been accepted by Central Denmark Region Committee on Biomedical Research Ethics  
10  
11 52 (Journal No 10-72-19-19) and The Danish Data Protection Agency (Journal No 652164). All results  
12  
13 53 will be published in international peer-reviewed scientific journals regardless of positive, negative  
14  
15  
16 54 or inconclusive results.  
17

18 55  
19**20 56 Trial registration**

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23 57 The trial is registered at Clinical Trial (NCT04081493)  
24  
25 58

**27 59 Article Summary****30 60 Strengths and limitations of this study**

- 31  
32 61 • The trial is a multicenter, randomized controlled assessor blinded trial.  
33  
34 62 • This is the first clinical trial to investigate the effect of low-load ischemic resistance training  
35  
36 63 as a preconditioning method prior to elective knee replacement surgery.  
37  
38  
39 64 • Patients will not be blinded to their allocation into intervention groups (BFR vs. control)  
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41 65

**44 66 Key words**

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46 67 Blood flow restricted exercise, knee osteoarthritis, total knee replacement surgery, preconditioning  
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## 68 INTRODUCTION

69 Knee OA is a degenerative joint disease associated with pain, reduced physical activity, and quality  
70 of life and affects almost 40% of all individuals  $\geq 60$  years of age (1-5). Approaching end-stage knee  
71 OA, total knee replacement (TKR) is often the preferred treatment choice to reduce pain and regain  
72 functional capacity. That is, TKR is considered a highly successful treatment to improve quality of  
73 life and long-term function (6). However, despite being considered highly successful approximately  
74 20% of the patients undergoing TKA experience a suboptimal outcome (6), which has been  
75 suggested often to be related to incomplete restoration of physical function (7). In addition, TKR  
76 patients typically demonstrate long-lasting deficits in quadriceps strength and functional  
77 performance (2, 4). This failure to return to “normal” strength levels has been suggested to be  
78 associated with preoperatively lower limb muscle strength and function (2) .

79 Preconditioning exercise designed to prepare the musculoskeletal system to better tolerate  
80 stressful events such as the impact of invasive surgery has been suggested to be applicable prior to  
81 elective TKR (6). This is supported by the results of two randomized controlled trials indicating that  
82 preoperative heavy resistance strength training (HRST) may enhance functional capacity and knee  
83 extensor muscle strength 3 months postoperatively (7, 8). However, joint pain resulting from the  
84 high mechanical loads associated with HRST may represent a barrier to this type of training in some  
85 patients suffering from severe knee OA (1, 9). Therefore, a more tolerable, yet effective, alternative  
86 is needed for this population. Also, 3 recent systematic reviews investigating the topic of  
87 preoperative physiotherapy-based exercise before TKR have suggested high quality, well-powered  
88 evidence to investigate the efficacy of preoperative physiotherapy before TKR (10-12). Resistance  
89 training with low exercise loads ( $\sim 30\%$  1 repetition maximum) performed with concurrent partial  
90 blood flow restriction to the working limb (Blood flow restricted exercise: BFRE) has received  
91 increasing clinical interest during the last decade (1, 13-32). The application of low



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4 92 muscle/tendon/joint forces in BFRE has been documented to increase human skeletal muscle size  
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6 93 and to cause substantial strength gains in healthy young and old individuals, as well as some patient  
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9 94 populations (13, 25, 26), despite the low magnitude of mechanical stress imposed on the trained  
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11 95 tissue. The adaptive mechanisms evoked by BFRE seem to involve accumulation of metabolites,  
12  
13 96 ischemia (transient tissue hypoxia) and activation of myogenic muscle stem cells (satellite cells:  
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15  
16 97 SC) (13, 26, 31). When applied in the clinical setting, BFRE has demonstrated positive effects on  
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18 98 skeletal muscle hypertrophy, strength, and functional capacity in mild-degree knee OA patients(1,  
19  
20 99 9, 33, 34) although not observed in all studies (33). Importantly, BFRE appears to be feasible with a  
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22  
23 100 high training adherence in knee OA patients (1, 33, 34). Furthermore, the use of different restrictive  
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25 101 pressures (absolute restrictive pressures: 160-200 mmHg and individualized pressure of 70% the  
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27 102 pressure needed to provide complete blood flow restriction) have been applied without any adverse  
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30 103 events in mild-degree knee OA (1, 33, 34). This is in line Hughes et al. (13), who suggested that  
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32 104 when BFRE is performed correctly it has been demonstrated to be as safe as free-flow exercise  
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34 105 methods (13).

35  
36 106 Satellite cells (SC) are quiescent myogenic stem cells positioned between the sarcolemma and  
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38  
39 107 the myofiber basal lamina (31, 35). SC plays an important role in human skeletal muscle growth  
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41 108 due to their ability to donate new myonuclei to the muscle fibers (31, 36-40). That is, the human  
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43 109 skeletal muscle fibers are multinucleated cells with each myonucleus controlling the protein  
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45  
46 110 synthesis of a certain cytoplasmic area in the muscle fiber (36-38, 41). Myonuclei transcriptional  
47  
48 111 activity can be fully maximized with exercise, hence requiring new myonuclei to support further  
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50 112 muscle tissue accretion (37, 38, 40). It has been suggested that exercise-related addition of SCs and  
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52  
53 113 myonuclei by means of BFRE might reduce the muscle atrophy related to bedrest and/or prolonged  
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55 114 inactivity (31, 42). Previous studies applying short term (10 days) preoperative BFRE before an  
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57 115 anterior cruciate ligament rupture-reconstruction found no atrophy protective effect or higher  
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4 116 postoperative muscle strength compared to performing a low-load exercise without blood flow  
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6 117 restriction (placebo). However, it might be questionable if the applied training frequency, intensity  
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9 118 and training period have been sufficient to promote SC and myonuclei addition. Thus, longer  
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11 119 periods of intensive training might be necessary to promote the desired muscle morphological  
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13 120 adaptations (addition of myonuclei and increased SC content).  
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### 17 18 122 **Aim and hypothesis of the trial**

19  
20 123 The primary aim of this trial is to investigate the efficacy of 8 weeks of BFRE compared to  
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23 124 receiving usual care prior to TKR on postoperative chair stand performance. We hypothesize that 8  
24  
25 125 weeks of preoperative BFRE will lead to increased 30 seconds chair stand performance (30-seconds  
26  
27 126 Chair Stand Test: 30-s CST) when assessed 3 months postoperatively. Secondary aims are to  
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29  
30 127 investigate the efficacy of preoperative BFRE on lower limb muscle strength 3 months after TKR  
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32 128 and investigate the potential relationship to functional capacity and quality of life. Furthermore, it  
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34 129 will be investigated to which extent 8 weeks of BFRE induces myofiber hypertrophy and gains in  
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36 130 satellite cell number and myonuclei content in the knee extensor musculature.  
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## 40 41 132 **MATERIAL & METHODS**

### 42 43 133 **Design**

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46 134 The trial is designed as a multicenter (2 sites), randomized, assessor blinded, controlled trial  
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48 135 following the CONSORT guidelines (43). Primary endpoint will be 3 months after TKR. Additional  
49  
50 136 and secondary endpoints will be evaluated during the week of TKR, 6 weeks after TKR  
51  
52  
53 137 (questionnaires only) and 12 months after TKR. Muscle biopsies will be obtained from all patients  
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55 138 undergoing surgery at Horsens Regional Hospital at baseline, during surgery and 3 months after  
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57 139 TKR.  
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7 141 **Participants**

8  
9 142 Patient will be recruited from the Orthopedic Departments at Horsens and Silkeborg Regional  
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11 143 Hospitals in Denmark. Patient enrollment will start September 2<sup>nd</sup> 2019 at Horsens Regional  
12  
13 144 Hospital and October 1<sup>st</sup> 2019 at Silkeborg Regional Hospital. Patient recruitment is expected to be  
14  
15  
16 145 completed in June 2021. All patients are expected to have completed baseline testing ultimo June  
17  
18 146 2021 and have performed 3 months follow-up during September 2021. Thus, at the end of June  
19  
20 147 2022 all patients are expected to have completed 12 months follow-up testing.  
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23 148  
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25 149 Inclusion criteria: 1) Patients  $\geq 50$  years scheduled for TKR due to knee OA at Horsens- or  
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27 150 Silkeborg Regional Hospital.  
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30 151  
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32 152 Exclusion criteria: 1) Severe cardiovascular diseases (New York Heart Association (NYHA) class  
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34 153 III and IV), previous stroke incident, thrombosis incident; 2) Traumatic nerve injury in affected  
35  
36 154 limb 3) Unregulated hypertension (Systolic  $\geq 180$  or diastolic  $\geq 110$  mmHg) 4) Spinal cord injury; 5)  
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38  
39 155 Planned other lower limb surgery within 12 months; 6) Cancer diagnosis and currently undergoing  
40  
41 156 chemo-, immuno-, or radiotherapy; 7) Inadequacy in written and spoken Danish; 8) an existing  
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43 157 prosthesis in the index limb; 9) living more than 45 minutes from either Horsens Regional Hospital  
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46 158 or Silkeborg Regional Hospital; 10) Pregnancy.  
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48 159  
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50 160 All patients will be screened for eligibility by orthopedic surgeons at Horsens Regional Hospital  
51  
52 161 and Silkeborg Regional Hospital who will perform the initial inclusion of study participants and  
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54  
55 162 hand out written project information. All patients accepting to participate will be asked to complete  
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57 163 a written informed consent allowing the physiotherapist (at Horsens Regional Hospital and  
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4 164 Silkeborg Regional Hospital) to contact the patients by phone for a final eligibility and exclusion  
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7 165 criteria-screening, and book an appointment for baseline testing. In case the patient agrees to  
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9 166 participate in the trial, the patient will sign a written informed consent to participate in the project.  
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11 167 Subsequently, the patient will be baseline-tested at the hospital by a blinded (to group allocation)  
12  
13  
14 168 assessor. Patients declining to participate in the RCT will be offered the option of participating in a  
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16 169 parallel observational cohort trial. All patients included in the project will be scheduled for a TKR  
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18 170 and receive a standard multimodal surgical program with standard preoperative care (usual care).  
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21 171 Specifically, 2-3 weeks before surgery all patients will be invited to a preoperative information  
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23 172 meeting where nurses, surgeons, and physiotherapists will provide detailed information on pain  
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25 173 management, nutrition, the surgical procedure, physical activity, postoperative home-based  
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27 174 rehabilitation, load management, etc. (44) On the day of surgery, patients will be hospitalized at  
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29  
30 175 Horsens Regional Hospital or Silkeborg Regional Hospital where an orthopedic surgeon will  
31  
32 176 perform the TKR procedure. The day after surgery all patients will be trained once or twice per day  
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34 177 by a physiotherapist towards fulfilling the following discharge criteria: a minimum knee flexion  
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36  
37 178 range of motion (ROM) of 60/90 degree and maximally a knee extension ROM deficit of 15/5  
38  
39 179 degree knee extension (Horsens Region Hospital/ Silkeborg Regional Hospital), independency in  
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41 180 in-and-out of bed and sit-to-stand activities, independency in walking and stair-negotiation with  
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43 181 crutches, ADL activities, and sufficient understanding of the home-based exercises during the  
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46 182 hospitalization period (44). Patients will generally be discharged within ~1-2 days after fulfilling all  
47  
48 183 the above discharge criteria. After discharge, all patients will as standard receive a standard home-  
49  
50 184 based rehabilitation program focusing on improving knee joint mobility, increasing the tolerance for  
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52  
53 185 standing without assistive devices (i.e. crutches), and lower extremity muscle strength. Small  
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55 186 variations in the selection of exercises in the standard home-based rehabilitation program exists  
56  
57 187 between hospitals, however, the purpose of the programs is identical. However, if the patients do  
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4 188 not fulfill the discharge criteria the patient will be offered supervised knee-specific exercise therapy  
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6 189 at municipal rehabilitation centers, or specialized hospital-based rehabilitation after discharge from  
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9 190 the Hospital.  
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## 11 191 12 13 **Randomization** 14

15  
16 193 After baseline assessment, patients will be randomized (1:1) using Research Electronic Data  
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18 194 Capture (REDCap) randomization system to either the training (BFRE) group or the control (CON)  
19  
20 195 group. Prior to randomization, all patients will be booked for follow-up test sessions and surgery.  
21  
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23 196 All randomization procedures will be performed by the physiotherapists in charge of the BFRE  
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25 197 training. Assessors performing the tests will be blinded to group allocation until completion of the  
26  
27 198 trial. A flow chart of the patient allocation procedures is depicted in Figure 1.  
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32 200 CON group: Participants in CON will receive usual care (see above) prior to TKR and be  
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34 201 encouraged to continue their usual lifestyle up until TKR.  
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36 202  
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38 203 BFRE group: In addition to receiving usual care (cf. above), participants in the BFRE group will  
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41 204 perform supervised BFRE sessions 3 times per week for 8 weeks supervised by a physiotherapist  
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43 205 educated in administering BFRE. All BFRE training will be performed at Horsens Regional  
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45 206 Hospital and Silkeborg Regional Hospital.  
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50 208 *Please insert Figure 1 about here*  
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## 52 209 53 54 **Intervention procedures** 55

### 56 57 211 BFRE 58 59 60

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4 212 Each BFRE session will consist of a 10-min warm up (ergometer cycling) followed by two different  
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6 213 unilateral lower-limb resistance training exercises: 1) leg press and 2) knee extension performed in  
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9 214 standard strength training machines. Each exercise will be performed with the affected lower limb  
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11 215 only and consist of 4 rounds interspaced by 30 seconds of rest. 1<sup>st</sup> round: 30 repetitions (reps); 2<sup>nd</sup>  
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13 216 round: 15 reps; 3<sup>rd</sup> round: 15 reps; 4<sup>th</sup> round: until exhaustion (Table 1). If patients can perform  
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15  
16 217 more than 15 repetitions in the 4<sup>th</sup> exercise set, the exercise load will be increased with the  
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18 218 minimum extra load possible (30). Participants will be instructed to perform both the eccentric and  
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20 219 concentric contraction phases using a steady 2-sec pace duration. The 4<sup>th</sup> and final exercise set will  
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23 220 be performed to the point of exhaustion defined as being unable to complete the final concentric  
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25 221 contraction phase in 2 seconds. During the 30 sec rest period, patients will rest in a standardized  
26  
27 222 resting position while maintaining the initial cuff-pressure. Between each exercise, patients will  
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30 223 have a 5-min "free-flow" rest period. The cuff will be released immediately after completion of the  
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32 224 final exercise set.

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34 225 The occlusion pressure during both exercises will be set at 60% of total limb occlusion pressure  
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36 226 (LOP) and starting load intensity will be 30% 1 repetition maximum (1RM) in both exercises.

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39 227 Individual LOP will be determined using a pneumatic, conically shaped, 12 cm wide, rigid cuff  
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41 228 (Occlude Aps, Denmark) attached to the patient's most proximal area of the thigh on the affected  
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43 229 side. While sitting on an examination table with the ankle and 1/3 of the lower limb off the table, a  
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46 230 vascular Doppler probe (EDAN Instruments, inc., China) will be placed posterior to the medial  
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48 231 malleolus over the posterior tibial artery to capture the auscultatory pulse. To determine the cuff  
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50 232 pressure (mmHg) needed for total blood flow occlusion, the cuff will gradually be inflated in 20  
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52 233 mmHg steps until reaching the pressure where the auscultatory pulse is interrupted (LOP). First  
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55 234 time the auscultatory pulse is interrupted the examiner releases 10-20 mmHg pressure from the cuff  
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57 235 until the auscultatory pulse is present again. When the auscultatory pulse reappears the cuff is  
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4 236 inflated with 10 mmHg until LOP is found again. If the second LOP is identical to the first it will be  
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6 237 defined as LOP for that specific patient. Otherwise, the procedure will be repeated until determining  
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9 238 an identical LOP two consecutive times.  
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11 239  
12 240 *Please insert Table 1 abot here*  
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## 15 242 **Outcome variables**

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18 243 Outcome assessments will be performed at baseline, in the week of surgery, 6 weeks after TKR, 3  
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20 244 months after TKR, and 12 months after TKR. To reduce the number of postoperative visits only  
21  
22 245 questionnaires; The Knee disability and Oteoarthritis Outcome Score (KOOS), EuroQol Group 5-  
23  
24 dimensions (EQ-5D-L5), and reporting of adverse event or receiving supervised physiotherapy  
25 246 postoperatively will sent via email 6 weeks after surgery. Two testers (two trained physiotherapists)  
26  
27 247 blinded to group allocation will perform all baseline and follow-up measurements. Bergström  
28  
29 248 needle muscle biopsies (45) will be taken from vastus lateralis of the quadriceps muscle in both  
30  
31  
32 249 lower limbs from patients included at Horsens Regional Hospital only at baseline, during surgery,  
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34 250 and 3 months after TKR by doctors trained in performing the procedure. An overview of the data  
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36 251 collection parameters is presented in Table 2.  
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41 253 Before starting the baseline testing, all assessors will be thoroughly trained in performing  
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43 254 the tests according to the standardized test procedures for each test method. To maintain fidelity of  
44  
45 255 testing during the study period, assessors will be retrained every 3<sup>rd</sup> month. Also, the  
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47  
48 256 physiotherapist in charge of LL-BFRE will be thoroughly trained in performing the exercise on  
49  
50 257 healthy subjects before applying LL-BFRE on study-patients. The primary investigator will be in  
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52 258 weekly contact with the physiotherapists supervising the LL-BFRE at Horsens Regional Hospitalet  
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55 259 and Silkeborg Regional Hospital where day-to-day-retraining and supervision can be arranged.  
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260 Furthermore, physiotherapists supervising the LL-BFRE will receive in-depth retraining every 3<sup>rd</sup>  
261 month.

### 263 **Data management**

264 All data from the physical function tests will be entered into RedCap by the assessors, using double  
265 data entry to ensure data quality. All patient-reported outcome data (KOOS, NRS Pain, EQ-5D-5L)  
266 will be entered directly into RedCap by the patients, and usage of the “required fields” will ensure  
267 no missing items from the completed questionnaires. To reduce missing data, a reminder email will  
268 be sent automatically from the RedCap-system. All patient data will be anonymized by assigning  
269 study numbers to each patient (coding). Personal data about the patient will be located separately  
270 from the main dataset to protect confidentiality during all trial phases.

271 The raw dataset will be maintained for ten years after completion of the trial, with indefinite  
272 restricted access due to sensitive date. After publication of the trial, a fully anonymized patient-level  
273 dataset and corresponding statistical description will be made publicly available if required by the  
274 scientific journal, in which the results are published.

### 276 Primary outcome variable

277 The primary outcome measure will be the change in 30s-CST from baseline to 3 months follow-up.

### 279 Secondary outcome variables

280 Secondary outcome measures comprises The Timed Up and Go test (46-48), 40-m fast-paced walk  
281 test (46), maximal isometric knee extensor and knee flexor strength assessed with hand-held  
282 dynamometry (49, 50), knee extensor (VL) myofiber cross sectional area, muscle fibertype  
283 composition, satellite cell content, myonuclei number (51), the Knee disability and Osteoarthritis



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4 284 Outcome Score (52, 53), EuroQol Group 5-dimensions (54), Numeric Ranking Scale for pain  
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6 285 (NRS) (55), and adverse events/postponement of TKR.  
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9 286  
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11 287 Explorative outcome variables  
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13 288 Type of postoperative rehabilitation received, medication and knee joint range of motion.  
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18 290 Demographic data  
19

20 291 Gender, age, height, weight, civil status, level of educational, employment status, substance use  
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22 (alcohol and smoking), duration of knee symptoms, pain medication during past week due to knee-  
23 292 related pain, and co-morbidities.  
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27 294  
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30 295 Adherence  
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32 296 Adherence to training will be registered by the physiotherapists in charge of the exercise sessions.  
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34 297 High compliance is defined as attendance to the supervised BFRE of  $\geq 80\%$ .  
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37 298

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39 299 *Please insert Table 2 about here*  
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41 300  
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43 301 Elaborated description of outcome measures  
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46 302 *Primary outcome*  
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48 303 The 30s-CST will be assessed using a 44 cm (seat height) chair with armrests. The 30s-CST  
49  
50 304 measures the number of sit-to-stand repetitions completed within 30 seconds. The 30s-CST is  
51  
52 305 considered a valid and sensitive measure of lower-extremity sit-to-stand function with good to  
53  
54 306 excellent intra- and inter-observer reliability (46, 56, 57).  
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4 308 Secondary outcomes

6 309 **The Timed Up & Go test (TUG)** assesses the time required for patients to stand from a 44 cm  
8  
9 310 (seat height) chair walk around a tape mark 3 meters away and sit into the chair at return. The  
10  
11 311 patients will be instructed to walk as fast and safely as possible towards the tape mark (and touch  
12  
13 312 the tape mark (with at least one foot), turn around and return to the chair and sit down. Use of  
14  
15  
16 313 armrests are allowed. The fastest of two trials will be used for further analysis. Up to one minute of  
17  
18 314 rest will be allowed between trials (47, 58). Good inter-rater reliability has been demonstrated with  
19  
20 315 the TUG test (46).

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23 316  
24  
25 317 **4x10 meter walk test meter walk test (40m-FWT)** measures the total time taken to walk 4 x 10 m  
26  
27 318 excluding turns (meter/sec) (46). Patients will be instructed to walk as quickly and as safely as  
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29  
30 319 possible without running to a visible mark 10 m away, return and repeat for a total distance of 40 m  
31  
32 320 (46). Prior to the test one practice trial will be provided to check understanding. The 40m-FWT is a  
33  
34 321 valid and responsive measure for assessing short distance maximum walking speed with excellent  
35  
36 322 inter-rater reliability (46).

38  
39 323  
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41 324 **1RM leg press strength** will be estimated from a 5-8RM leg press test. Patients perform 3 low-load  
42  
43 325 warm-up sets. 1<sup>st</sup> and 2<sup>nd</sup> warm-up set consists of 12 repetitions, and the 3<sup>rd</sup> warm-up set consist of 8  
44  
45 326 repetitions. The load of each warm-up set will be increased with 10 kilos. After warm-up, the load  
46  
47  
48 327 will be increased to determine the 5RM. If the 5RM cannot be determined within 3 trials, an 4<sup>th</sup> all-  
49  
50 328 out trial (as many repetitions as possible) will be performed. The 1RM will be calculated as [1RM =  
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52 329 load (kg)/1.0278-0.0278·number of repetitions)] (59).

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**1RM knee extension strength** will be estimated from 5-8RM knee extension test as described above for the estimation of 1RM leg press test (59).

**Maximal isometric voluntary contraction (MVC) of the knee** will be measured using a hand held dynamometer (HHD). The patients will be seated on an examination table with knees and hips positioned at 90° flexion. The patients will be instructed to remain seated in an upright position and place both hands on the shoulder to avoid compensation. The HHD will be fixed with a rigid belt to the examination table. Adjustable straps will be used to allow MVCs of the knee extensors to be performed at 90° knee flexion in all patients. The HDD will be positioned 5 cm above the medial malleolus (50). The patients will be instructed to produce as much force as possible into the HHD as possible. Good to excellent inter- and intra-rater reliability has previously been demonstrated on group-level in patients suffering from knee OA for maximum knee extensor muscle strength testing with HDD (49, 50). Patients will receive 4 trials. For analysis, the mean maximal strength of the 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> measures will be calculated and corrected for bodyweight (50)

**MVC of the knee flexors** will be measured will be performed using HHD at 90° knee flexion with the patients seated identically as during MVC for the knee extensors (50). The HHD will be positioned posterior aspect of calcaneus (50) and patients will be instructed to produce as much force as possible into the HHD. Good to excellent inter- and intra-rater reliability has previously been demonstrated on group-level in patients suffering from knee OA for maximum knee flexor muscle strength testing with HDD (50). Patients will receive 4 trials. For analysis, the mean maximal strength of the 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> measures will be calculated and corrected for bodyweight (50)

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4 355 **Myofiber cross sectional area (CSA), muscle fiber type composition, satellite cell content, and**  
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6 356 **myonuclei number** will be assessed by obtaining needle biopsies (100-150 mg) from all patients  
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8  
9 357 enrolled at Horsens Regional Hospital. The biopsies will be obtained bilaterally from the middle  
10  
11 358 portion of the vastus lateralis muscle utilizing the percutaneous needle biopsy technique of  
12  
13 359 Bergström (45, 60, 61). Biopsies will be performed by two experienced orthopedic surgeons (chief  
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16 360 physicians) trained in performing the needle muscle biopsy technique at Horsens Regional Hospital.  
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18 361 Efforts will be made to extract tissue from the same region (2-3 cm apart) and depth (~1-2 cm.)  
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20 362 (45). The tissue samples will be dissected of all visible blood, adipose tissue, and connective tissue  
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23 363 and mounted in Tissue-Tec (4583, Sakura Finetek, Alphen aan den Rijn, The Netherlands), frozen  
24  
25 364 in isopentane pre-cooled with liquid nitrogen, and stored at -80°C (31, 45, 51). All muscle samples  
26  
27 365 will be analyzed as previously described by Nielsen et al. (31) using immunofluorescence  
28  
29  
30 366 microscopy. Transverse serial sections (8 µm) of the embedded muscle biopsy specimen will be cut  
31  
32 367 at -22°C using a cryostat (HM560; Microm, Walldorf, Germany) and will be mounted on glass  
33  
34 368 slides for subsequent analysis as described in detail elsewhere (31). Myogenic stem cells (satellite  
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37 369 cells (SC)) will be visualized with an antibody against Pax7 (31). Type I (stained) and Type II  
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39 370 (unstained) myofibers will be differentiated, and muscle fiber area will be determined (31): MSC-  
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41 371 derived nuclei will stain positive for Pax7 and be within the basal lamina; nuclei (DAPI stained)  
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43 372 with a sublaminar placement will be considered myonuclei (31).  
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48 374 **Knee disability and Osteoarthritis Outcome Score (KOOS)** is a patient-administered knee  
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50 375 specific questionnaire comprising five subscales Pain; Symptoms; Activities of daily living; Sport  
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53 376 & Recreation; and Knee-Related Quality of Life. Each item is scored from 0 to 4 (53). The raw  
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55 377 score for each of the five subscales is the total sum of the associated item scores. Scores can be  
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57 378 transformed to a 0 to 100 scale. The scores of the five subscales can be expressed as a composite  
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4 379 outcome profile, higher scores indicating fewer problems (62). The KOOS questionnaire is valid  
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6  
7 380 and reliable in patients suffering from knee OA and patients on the waiting list for TKA for knee  
8  
9 381 OA (52, 53, 63).

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11 382  
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13 383 **EuroQol Group 5-dimension (EQ-5D-5L)** is a self-completion questionnaire consisting of two  
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16 384 parts; first part of the EQ-5D-5L comprises five dimensions involving mobility, self-care, usual  
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18 385 activities, pain/discomfort, and anxiety/depression. All dimensions have five response categories  
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20 386 (no problems, slight problems, moderate problems, severe problems, and extreme problems)  
21  
22  
23 387 resulting in a five digit descriptive health state (64), which will be converted into a summary index  
24  
25 388 ranging from -0.624 (worst) to 1.000 (best), using a Danish value set (54). The second part, EQ-  
26  
27 389 VAS rates the overall current health status from 0 (worst imaginable health) to 100 (best imaginable  
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30 390 health) (64). The EQ-5D-5L is reliable and valid in patients with knee osteoarthritis eligible for  
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32 391 TKA, (65, 66)

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34 392  
35 393 **Adverse events** will be defined as unpredicted or unintended events, signs, or disease occurring  
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37  
38 394 during the period from inclusion until the 3-month follow-up (primary end-point) resulting in  
39  
40 395 contact with the healthcare system (hospital or general practitioner) independent of whether or not  
41  
42 396 the event is related to the intervention or outcome assessments. Adverse events will be recorded and  
43  
44 397 categorized in accordance with the definitions established by the United States Food and Drug  
45  
46  
47 398 Administration [88]. Continuous registration of adverse events will be performed and a short open-  
48  
49 399 ended questionnaire will be administered at 3-months and 12 months follow-up.

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54 401 *Other Outcome Measures*

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56 402 **Blood pressure** will be measured by the orthopedic surgeon when patients are visiting the  
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58 403 outpatient clinic. Blood pressure will be used to determine eligibility to participate in the project.  
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**Exercise compliance and progression** will be obtained by the physiotherapist in charge of the training sessions and entered directly into the REDCap-system. The progression will be monitored as the total load lifted by the patient for exercise session.

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**Declining to be operated** will be measured at 3 months follow up, where patients will be asked whether they decided to be operated or not. Patients who declined to be operated will be invited to participate will be invited to participate in all prescheduled follow-up assessments.

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**Postoperative supervised physiotherapy** will be measured at 6 weeks, 3 months, and 12 months follow-up by answering a questionnaire. If patients have participated in postoperative supervised physiotherapy, the patient must specify whether the treatment was related to the TKR or due to other circumstances.

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418

**Knee joint active range of motion** will be measured with a 360° plastic goniometer (scale 1°) with 16.5 cm moveable arms at baseline, in the week of surgery, 3 months, and 12 months after surgery. Laying supine on an examination table, the knee joint flexion and knee joint extension will be measured separately (67). The tester then identifies the most prominent part of the trochanter, the lateral epicondyle of the femur, the lateral head of fibula, and the lateral malleolus. When identified, the patient is asked to flex the knee as much as possible with the heel maintaining contact to the surface at all time (67). Secondly, the patients will be asked to extend the knee joint as much as possible. To allow the knee to extend as much as possible a firm quadratic box (height: 5 cm, width: 8 cm, length: 15 cm) will be placed under the heel of the patient. The procedure of measuring knee extension will be similar to knee flexion, as the patients increases the degree of knee extension

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4 428 maximally (67) The fulcrum of the goniometer will correspond visually to the trans-epicondylar  
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6 429 axis of the knee joint. The moveable arms of the goniometer will be pointed towards the greater  
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8  
9 430 trochanter and the lateral malleolus while (67).  
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### 11 431 12 13 14 432 **Sample size**

15  
16 433 The power and sample size calculation is based on the expected differences between the two subject  
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18 434 groups from baseline to 3 months follow up (8). Skoffler et al. (8) investigated the efficacy of 4  
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20 435 weeks of preoperative and 4 weeks postoperative HRST (intervention group) compared to 4 weeks  
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23 436 of postoperative HRST only (control group) on 30-s CST 3 months in patients receiving a TKR (8).  
24  
25 437 The authors found a between-group difference of 3-4 repetition difference ( $14.7 \pm 4.7$  repetitions  
26  
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28 438 versus  $11.0 \pm 4.4$  repetitions) 3 months after TKR surgery (8).  
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30 439 To reduce the probability of type I errors and be able to detect a between-group difference also,  
31  
32 440  $\alpha$ -level is set at 0.05 ( $p < 0.05$ ) and  $\beta$ -level is set at 0.20 (80% power). Expecting a 3-repetitions  
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35 441 between-group difference 3 months postoperatively and assuming a SD of 4.7 in both groups, 39  
36  
37 442 patients are required in each group (yielding 78 patients in total). With an anticipated dropout rate  
38  
39 443 of 10%, 84 patients will be recruited for the trial in total.  
40

### 41 42 444 43 44 445 **Statistical considerations**

45  
46 446 The primary efficacy analysis will be assessment of the between group difference in change in the  
47  
48 447 30-S CST from baseline to 3 months follow up (primary endpoint).  
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51 448 All descriptive statistics and tests will be reported in accordance with the recommendations of  
52  
53 449 the “Enhancing the QUALity and Transparency Of health Research” (EQUATOR) network (68) and  
54  
55 450 the CONSORT statement (43). Intention-to-treat principle (i.e. all patients as randomized  
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58 451 independent of departures from allocation treatment, compliance and/or withdrawals) and per  
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4 452 protocol analysis will be conducted. A one-way analysis of variance (one-way ANOVA) model will  
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7 453 be used to analyze between group mean changes in continuous outcome measures (31). The model  
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9 454 includes changes from baseline to 12 months follow-up. Between-intervention comparison from  
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11 455 baseline to 3 months after surgery will be analyzed using a mixed linear model with patient ID as a  
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14 456 random effect and time and group as fixed effects (31, 69). Also, to gain insights into the potential  
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16 457 pre-to-post training differences within the respective training or control groups, paired student t-  
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18 458 tests will be performed. Level of statistical significance is  $P < 0.05$ . *Secondary outcome variables:*  
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21 459 Between-intervention comparison from baseline to the week of surgery, 6 weeks after surgery, 3  
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23 460 and 12 months after surgery will be analyzed as described for the primary outcome. Regression  
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25 461 analysis will be used to analyze the potential associations between preoperative strength and  
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27 462 postoperative lower extremity function and self-reported outcome as well as between preoperative  
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30 463 functional capacity and postoperative functional capacity. Additionally, regression analysis will be  
31  
32 464 used to analyze the association between preoperative number of satellite cells and myonuclei on  
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34 465 postoperative isometric knee extensor muscle strength, muscle fiber cross sectional area, and  
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37 466 functional capacity. All statistical analysis will be performed by the primary investigator using  
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39 467 Stata.

#### 43 469 **Ethical aspects and dissemination**

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46 470 The trial has been accepted by Central Denmark Region Committee on Biomedical Research Ethics  
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48 471 (Journal No 10-72-19-19) and by The Danish Data Protection Agency (Journal No 652164). The  
49  
50 472 trial is registered at Clinicaltrial.gov (NCT04081493). Before inclusion, all patients will provide  
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52  
53 473 their written informed consent in accordance with the Helsinki Declaration. All data and  
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55 474 information collected in regard to this trial will be treated confidentially (blinded and encrypted) by  
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57 475 the researchers and staff connected to the trial.



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4 476 All results from the trial will be published in international peer-reviewed scientific journals  
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7 477 regardless of the results being considered positive, negative or inconclusive.  
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## 9 478 10 11 479 **Patient and public involvement**

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13 480 Before developing this clinical trial, a pilot project was performed to determine feasibility and  
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16 481 efficacy of BFRE in patients suffering from lower limb injuries. The experiences with the training  
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18 482 modality and the verbal feedback from patients on training duration, frequency, and intensity  
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20 483 resulted in useful knowledge that certainly have improved the development of the present clinical  
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23 484 trial.  
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## 25 485 26 27 486 **DISCUSSION**

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30 487 To our best knowledge, this is the first trial to investigate the effect of preoperative BFRE on  
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32 488 functional capacity, self-reported outcome, lower limb muscle strength and myofiber  
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34 489 morphology/stem cell abundance in patients scheduled for TKR. Only few studies have investigated  
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36 490 (short term (10 days)) preoperative BFRE without finding an atrophy protective effect or difference  
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39 491 in muscle strength compared to a control group performing a placebo intervention (SHAM group)  
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41 492 (70). However, patients performing short term preoperative BFRE before ACL-R demonstrated  
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43 493 higher muscle endurance compared to a SHAM group (71). Therefore, results of this trial are  
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46 494 expected to provide novel information on longer periods of BFRE that will enable to design  
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48 495 effective exercise-based preconditioning protocols for elective TKR patients. The LL-BFRE  
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50 496 protocol applied in the present project is widely used and follows the recommendations from a  
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52  
53 497 recent position stand by Patterson et al. (72). The authors suggested that exercising 2-3 times per  
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55 498 week at 20-40% of 1RM in 2-4 sets (e.g. 30-15-15-15 or sets to failure) using pressures between 40  
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to 80% of LOP has demonstrated to be effective when aiming at increasing muscle strength and promoting muscle hypertrophy (72).

The trial is designed as an assessor blinded randomized controlled trial, thus representing the highest evidence level. However, the nature of the trial does not allow blinding of the participants which is an inherent limitation of the trial. The trial is conducted at two hospitals that consistently perform a high number TKR procedures annually (225 and 460, respectively), thus securing a strong expertise in terms of surgery and infrastructure. Both hospitals have all equipment needed available for surgery, post-operative hospitalization, training, and testing. All outcome variables are considered valid and reliable measures and consist of both objective outcomes and self-reported patient outcomes.

No adverse health-related events have been reported in previous studies applying BFRE in patients' suffering from knee OA or in healthy older adults (1, 9, 13, 23, 33, 34). Further, in a recent review and meta-analysis it was stated that exercise with concurrent blood-flow restriction is a safe exercise modality when occlusion procedures are applied correctly (13). The inherent invasive procedure of muscle biopsies may cause adverse events in rare occasions. Therefore, all muscle biopsy samples will be collected by trained medical doctors and performed following administration of local anesthesia and in fully sterile conditions. The needle muscle biopsy protocol have been applied in a large number of previous investigations including very old frail subjects (97 years of age) without any reporting of adverse events besides occasional muscle soreness(31, 45, 60, 73, 74).

There are some limitations of the project that must be taken into account. First, our primary end point is 3 months postoperatively. The (uncontrolled) period discharge to 3 months postoperatively renders the project vulnerable to external variabilities. However, from a pragmatic point of view, this uncontrolled period from discharge to 3 months follow-up reflects the reality that Danish patients faces postoperatively. Thus, the results at 3 months follow-up will, indeed, reflect the

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523 impact of performing preoperative LL-BFRE on the postoperative outcome regardless of the  
524 external variable that can hamper the results. Secondly, the discharge criteria at Horsens Regional  
525 Hospital and Silkeborg Regional Hospital withhold slight differences. That is, the acceptable knee  
526 joint ROM at discharge differs between the sites, thus it can be speculated that more patients from  
527 Silkeborg Regional Hospital will be offered a postoperative, supervised rehabilitation program. This  
528 might affect the number of patient receiving supervised physiotherapy after discharge between sites.  
529 However, all patients included in present project will report whether they have received  
530 postoperative supervised physiotherapy at all follow-up assessment. Thus, we will be able to  
531 determine (and normalize?) a potential between-site difference in patients receiving supervised  
532 physiotherapy after TKR.

#### 534 **Author contributions**

535 SLJ, PAA, MBB, and IM were all part of designing the trial and approved the final version of the  
536 protocol. Also, SLJ, PAA, MBB, and IM wrote and revised the protocol.

#### 538 **Data statement**

539 All obtained data will be stored in anonymized form at the Danish National Archives and deleted  
540 after 10 years.

#### 542 **Funding**

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544 Mindeslegat (163.883 dkk) and The Foundation for health research of Central Denmark Region  
545 (99.658 dkk), Hede-Nielsen Foundation (8.000,00 dkk).

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4 547 **Competing interest**

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7 548 None to be declared

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11 550 **Ethics approval**

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14 551 The trial has been accepted by Central Denmark Region Committee on Biomedical Research Ethics  
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16 552 (Journal No 10-72-19-19) and by The Danish Data Protection Agency (Reference No 652164).

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20 554 **Word count**

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23 555 5.650 words

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For peer review only

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774 *Table 1. Exercise variables for the blood-flow restricted exercise (BFRE) protocol*

Exercise variable	Week 1-8
Level of LOP	60% LOP
Sets	4
Load intensity	30% 1RM
Repetitions 1 <sup>st</sup> set	30
Repetitions 2 <sup>nd</sup> & 3 <sup>rd</sup> set	15
Repetitions 4 <sup>th</sup> set	To volitional failure
Contraction modes per repetition	
Concentric	2 seconds
Isometric	0 seconds
Eccentric	2 seconds
Rest between repetitions	0 seconds
Time under tension per repetition	4 seconds
Range of movement	maximum
Rest between sets	30 seconds
Rest between sessions	≥36 hours
Progression	The minimal possible load (5 kilo) is added when patients perform >15 repetitions in 4 <sup>th</sup> set

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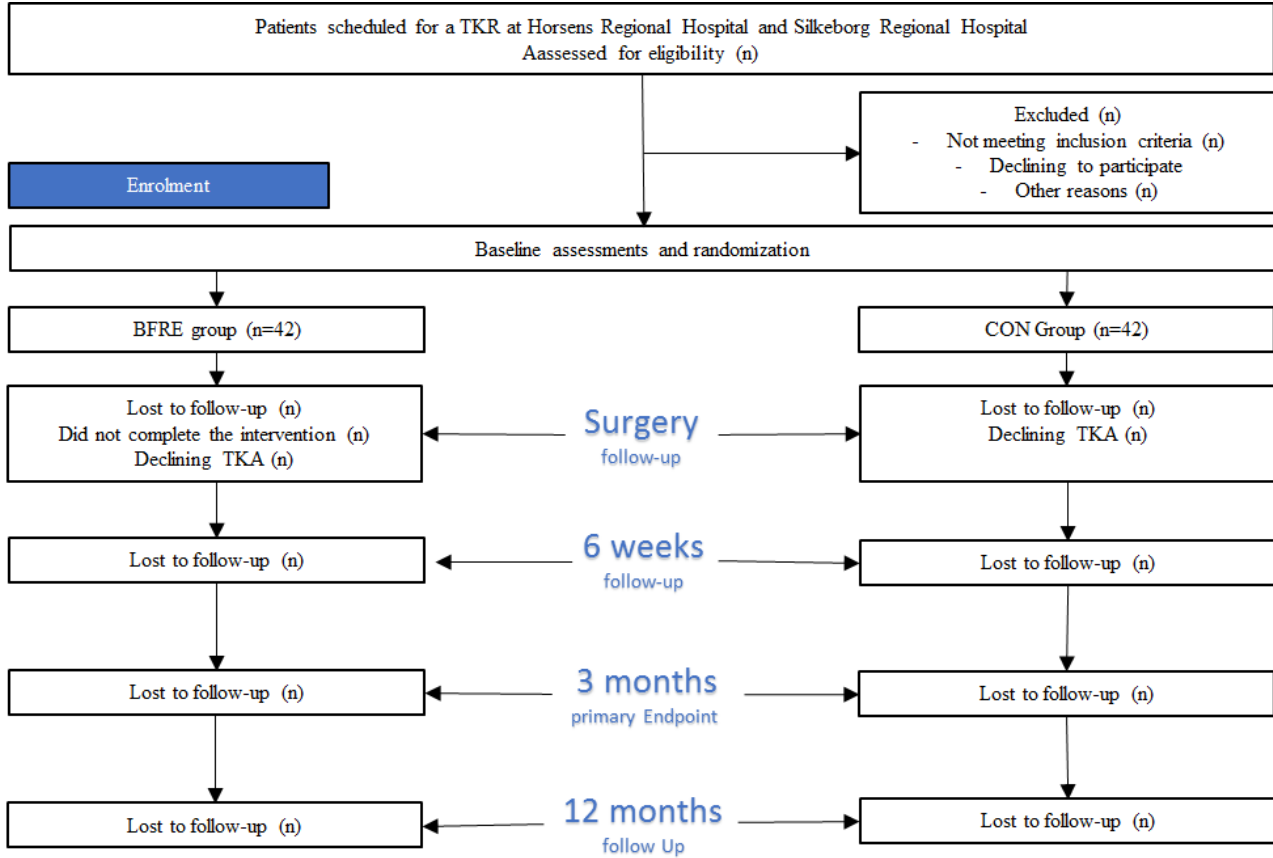
776 *Table 2. Outcome measures to be collected.*

Outcome measures	Data collection instrument	Time-points of assessment
<b>Primary outcome</b>		
<b>Sit-to-stand function</b>	30 seconds chair stand test	B, S, 3 and 12 months
<b>Secondary outcomes</b>		
<b>Isometric Knee extensor muscle strength</b>	Handheld Dynamometer	B, S, 3 and 12 months
<b>Isometric Knee flexion muscle strength</b>	Handheld Dynamometer	B, S, 3 and 12 months
<b>Gait speed</b>	4x10-meter walk test	B, S, 3 and 12 months
<b>Ambulatory capacity</b>	Timed Up & Go	B, S, 3 and 12 months
<b>Muscle morphology and biology</b>	Muscle Biopsies	B, D, 3 months
<b>Pain</b>	KOOS	B, S, 6 weeks, 3 and 12 months
<b>Symptoms</b>	KOOS	B, S, 6 weeks, 3 and 12 months
<b>Activities of daily living</b>	KOOS	B, S, 6 weeks, 3 and 12 months
<b>Sports &amp; Recreation</b>	KOOS	B, S, 6 weeks, 3 and 12 months
<b>Quality of life</b>	KOOS	B, S, 6 weeks, 3 and 12 months
<b>Socioeconomic costs</b>	EQ-5D	B, S, 6 weeks, 3 and 12 months
<b>Adverse Events</b>	Questionnaire and medical records	S, 3 months
<b>Patient characteristics and related measurements</b>		
<b>Gender</b>	Questionnaire	B
<b>Age</b>	Questionnaire	B
<b>Height</b>	Tape measure	B
<b>Body mass</b>	Electronic body mass scale	B
<b>Civil Status</b>	Questionnaire	B
<b>Educational Level</b>	Questionnaire	B
<b>Employment Status</b>	Questionnaire	B
<b>Substance Use (alcohol, smoking)</b>	Questionnaire	B
<b>Duration of knee symptoms</b>	Questionnaire	B
<b>Pain medication during the last week</b>	Questionnaire	B
<b>Co-morbidities</b>	Questionnaire	B
<b>Blood pressure</b>	Electronic upper limb blood pressure monitor	At doctor's visit

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4	<b>Postoperative supervised physiotherapy</b>	Questionnaire	6 weeks, 3 and 12 months
5	<b>Exercise compliance and progression</b>	Physiotherapist records	B, S, at each BBFRE session, 3
6	<b>NRS Pain</b>	PhD-stipendiate and physiotherapist records	and 12 months
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For peer review only

Figure 1. Patient flow





SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents\*

Section/item	Item No	Description
<b>Administrative information</b>		
Title (p 1, I 1-3)	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration A: p 2, I 56-57 B:	2a 2b	Trial identifier and registry name. If not yet registered, name of intended registry All items from the World Health Organization Trial Registration Data Set
Protocol version P 1, I 22	3	Date and version identifier
Funding P 21, I 494-496	4	Sources and types of financial, material, and other support
Roles and responsibilities A: P 1, I 5-11 B: P 1, I 15-20	5a 5b 5c 5d	Names, affiliations, and roles of protocol contributors Name and contact information for the trial sponsor Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
<b>Introduction</b>		
Background and rationale P 3, I 67-133 P 3, I 70-76	6a 6b	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention Explanation for choice of comparators
Objectives P 5, I 129-136	7	Specific objectives or hypotheses
Trial design P6, I 140-145	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)

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**Methods: Participants, interventions, and outcomes**

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Study setting P6, I 148-149	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
Eligibility criteria P6, I 155-163	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)
Interventions A: p7, I 164-240  C: p12, 283-285	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
Outcomes P 10, I 245-384	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
Participant timeline  Table 1	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
Sample size P 17, I 391-401	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
Recruitment P 6, I 148-151	15	Strategies for achieving adequate participant enrolment to reach target sample size

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**Methods: Assignment of interventions (for controlled trials)**

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Allocation:

Sequence generation P8, I 196-201	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions
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2	Allocation	16b	Mechanism of implementing the allocation sequence (eg, central
3	concealment		telephone; sequentially numbered, opaque, sealed envelopes),
4	mechanism		describing any steps to conceal the sequence until interventions are
5	P8, I 196-201		assigned
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7	Implementation	16c	Who will generate the allocation sequence, who will enrol participants,
8	P8, I 196-201		and who will assign participants to interventions
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10	Blinding	17a	Who will be blinded after assignment to interventions (eg, trial
11	(masking)		participants, care providers, outcome assessors, data analysts), and
12	P8, I 200		how
13		17b	If blinded, circumstances under which unblinding is permissible, and
14			procedure for revealing a participant's allocated intervention during
15			the trial
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### Methods: Data collection, management, and analysis

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20	Data collection	18a	Plans for assessment and collection of outcome, baseline, and other
21	methods		trial data, including any related processes to promote data quality (eg,
22	P 10, I 245-420		duplicate measurements, training of assessors) and a description of
23			study instruments (eg, questionnaires, laboratory tests) along with
24			their reliability and validity, if known. Reference to where data
25			collection forms can be found, if not in the protocol
26		18b	Plans to promote participant retention and complete follow-up,
27			including list of any outcome data to be collected for participants who
28			discontinue or deviate from intervention protocols
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30	Data	19	Plans for data entry, coding, security, and storage, including any
31	management		related processes to promote data quality (eg, double data entry;
32			range checks for data values). Reference to where details of data
33			management procedures can be found, if not in the protocol
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35	Statistical	20a	Statistical methods for analysing primary and secondary outcomes.
36	methods		Reference to where other details of the statistical analysis plan can be
37	P 17, I 400-420		found, if not in the protocol
38		20b	Methods for any additional analyses (eg, subgroup and adjusted
39			analyses)
40	P 17, I 400-420	20c	Definition of analysis population relating to protocol non-adherence
41			(eg, as randomised analysis), and any statistical methods to handle
42			missing data (eg, multiple imputation)
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### Methods: Monitoring

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50	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role
51			and reporting structure; statement of whether it is independent from
52			the sponsor and competing interests; and reference to where further
53			details about its charter can be found, if not in the protocol.
54			Alternatively, an explanation of why a DMC is not needed
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2		21b	Description of any interim analyses and stopping guidelines, including
3			who will have access to these interim results and make the final
4			decision to terminate the trial
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6	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and
7			spontaneously reported adverse events and other unintended effects
8			of trial interventions or trial conduct
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10	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and
11			whether the process will be independent from investigators and the
12			sponsor
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### Ethics and dissemination

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17	Research ethics	24	Plans for seeking research ethics committee/institutional review board
18	approval		(REC/IRB) approval
19	P 18, I 423-424		
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22	Protocol	25	Plans for communicating important protocol modifications (eg,
23	amendments		changes to eligibility criteria, outcomes, analyses) to relevant parties
24			(eg, investigators, REC/IRBs, trial participants, trial registries,
25			journals, regulators)
26			
27	Consent or assent	26a	Who will obtain informed consent or assent from potential trial
28	P7, I 164-173		participants or authorised surrogates, and how (see Item 32)
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30		26b	Additional consent provisions for collection and use of participant data
31			and biological specimens in ancillary studies, if applicable
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34	Confidentiality	27	How personal information about potential and enrolled participants will
35	P 11, I 265-275		be collected, shared, and maintained in order to protect confidentiality
36			before, during, and after the trial
37			
38	Declaration of	28	Financial and other competing interests for principal investigators for
39	interests		the overall trial and each study site
40	P 22, I 514		
41			
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43	Access to data	29	Statement of who will have access to the final trial dataset, and
44			disclosure of contractual agreements that limit such access for
45			investigators
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47	Ancillary and	30	Provisions, if any, for ancillary and post-trial care, and for
48	post-trial care		compensation to those who suffer harm from trial participation
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50	Dissemination	31a	Plans for investigators and sponsor to communicate trial results to
51	policy		participants, healthcare professionals, the public, and other relevant
52	P 18, 442-444		groups (eg, via publication, reporting in results databases, or other
53			data sharing arrangements), including any publication restrictions
54			
55			
56	P 21, I 501-502	31b	Authorship eligibility guidelines and any intended use of professional
57			writers
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- 1  
2 31c Plans, if any, for granting public access to the full protocol, participant-  
3 level dataset, and statistical code  
4

## 5 **Appendices**

- 6 Informed consent 32 Model consent form and other related documentation given to  
7 materials participants and authorised surrogates  
8  
9 Biological 33 Plans for collection, laboratory evaluation, and storage of biological  
10 specimens specimens for genetic or molecular analysis in the current trial and for  
11 future use in ancillary studies, if applicable  
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14 \*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013  
15 Explanation & Elaboration for important clarification on the items. Amendments to the  
16 protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT  
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# BMJ Open

**The efficacy of low-load blood flow restricted resistance EXercise in patients with Knee osteoarthritis scheduled for total knee replacement (EXKnee). Protocol for a multicenter, randomized controlled trial.**

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<b>Primary Subject Heading</b>:	Sports and exercise medicine
Secondary Subject Heading:	Rehabilitation medicine
Keywords:	blood flow restriction exercise, knee osteoarthritis, total knee replacement surgery, preconditioning, functional capacity

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4 1 **The efficacy of low-load blood flow restricted resistance EXercise in patients with Knee**  
5 **osteoarthritis scheduled for total knee replacement (EXKnee). Protocol for a multicenter**  
6 **randomized controlled trial.**  
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11 5 *Stian Langgård Jørgensen*<sup>1,2,5</sup>, *Marie Bagger Bohn*<sup>2</sup>, *Per Aagaard*<sup>3</sup>, *Inger Mechlenburg*<sup>4,5</sup>  
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20 Phone: +45 22 71 17 82

23 Word count: 5.650

## 25 **ABSTRACT**

### 26 **Introduction**

27 Up to 20% of patients undergoing total knee replacement (TKR) surgery report no or suboptimal  
28 pain relief after TKR. Moreover, despite chances of recovering to preoperative functional levels,  
29 patients receiving TKR have demonstrated persistent deficits in quadriceps strength and functional  
30 performance compared to healthy aged-matched adults. We intend to examine if low-load blood  
31 flow restricted exercise (BFRE) is an effective preoperative method to increase functional capacity,  
32 lower limb muscle strength and self-reported outcomes after TKR. In addition, the study aims to  
33 investigate to which extent preoperative BFRE will protect against surgery-related atrophy three  
34 months after TKR.

### 36 **Methods**

37 In this multicenter, randomized controlled and assessor blinded trial, 84 patients scheduled for TKR  
38 will be randomized to receive usual care and eight weeks of preoperative BFRE or to follow usual  
39 care-only. Data will be collected before randomization, three-four days prior to TKR, six weeks,  
40 three months, and 12 months after TKR. Primary outcome will be the change in 30-second chair  
41 stand test from baseline to three-month follow-up. Key secondary outcomes will be Timed Up &  
42 Go, 40-meter fast-paced walk test, isometric knee extensor and flexor strength, patient-reported  
43 outcome, and selected myofiber properties.

44 Intention-to-treat principle and per protocol analyses will be conducted. A one-way analysis of  
45 variance model will be used to analyze between group mean changes. Between-intervention  
46 comparison will be analyzed using a mixed linear model. Also, paired student t-tests will be  
47 performed and regression analysis will be used for analyzation of associations between selected  
48 outcomes.

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7 **50 Ethical approval**  
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9 51 The trial has been accepted by the Central Denmark Region Committee on Biomedical Research  
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11 52 Ethics (Journal No 10-72-19-19) and the Danish Data Protection Agency (Journal No 652164). All  
12  
13 53 results will be published in international peer-reviewed scientific journals regardless of positive,  
14  
15 54 negative or inconclusive results.  
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18 55  
1920 **56 Trial registration**  
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23 57 The trial is registered at Clinical Trials (NCT04081493)  
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27 **59 Article Summary**  
2829 **60 Strengths and limitations of this study**  
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- 31  
32 61 • The trial is a multicenter, randomized controlled assessor blinded trial.  
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34 62 • This is the first clinical trial to investigate the effect of low-load ischemic resistance training  
35  
36 63 as a preconditioning method prior to elective knee replacement surgery.  
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38 64 • Patients will not be blinded to their allocation into intervention groups (BFR vs. control)  
39  
40 65 • This is a protocol paper  
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46 **67 Key words**  
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48 68 Blood flow restricted exercise, knee osteoarthritis, total knee replacement surgery, preconditioning  
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## 69 INTRODUCTION

70 Knee OA is a degenerative joint disease associated with pain, reduced physical activity, and quality  
71 of life and affects almost 40% of all individuals  $\geq 60$  years of age (1-5). Approaching end-stage knee  
72 OA, total knee replacement (TKR) is often the preferred treatment choice to reduce pain and regain  
73 functional capacity. That is, TKR is considered a highly successful treatment to improve quality of  
74 life and long-term function (6). However, despite being considered highly successful,  
75 approximately 20% of the patients undergoing TKR experience a suboptimal outcome (6), which  
76 has often been suggested to be related to incomplete restoration of physical function (7). In  
77 addition, TKR patients typically demonstrate long-lasting deficits in quadriceps strength and  
78 functional performance (2, 4). This failure to return to “normal” strength levels has been suggested  
79 to be associated with preoperatively lower limb muscle strength and function (2).

80 Preconditioning exercise designed to prepare the musculoskeletal system to better tolerate  
81 stressful events such as the impact of invasive surgery has been suggested to be applicable prior to  
82 elective TKR (6). This is supported by the results of two randomized controlled trials indicating that  
83 preoperative heavy resistance strength training (HRST) may enhance functional capacity and knee  
84 extensor muscle strength three months postoperatively (7, 8). Joint pain resulting from the high  
85 mechanical loads associated with HRST may represent a barrier to this type of training in some  
86 patients suffering from severe knee OA (1, 9). Therefore, a more tolerable, yet effective, alternative  
87 is needed for this population. Also, three recent systematic reviews investigating the topic of  
88 preoperative physiotherapy-based exercise before TKR all warrant high quality, well-powered  
89 evidence to investigate the efficacy of preoperative physiotherapy before TKR (10-12).

90 Resistance training with low exercise loads ( $\sim 30\%$  1 repetition maximum) performed with  
91 concurrent partial blood flow restriction to the working limb (Blood flow restricted exercise: BFRE)  
92 has received increasing clinical interest during the last decade (1, 13-32). The application of low

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muscle/tendon/joint forces in BFRE has been documented to increase human skeletal muscle size and to cause substantial strength gain in healthy young and old individuals, as well as some patient populations, despite the low magnitude of mechanical stress imposed on the trained tissue (13, 25, 26). When applied in the clinical setting, BFRE has demonstrated positive effects on skeletal muscle hypertrophy, strength, and functional capacity in mild-degree knee OA patients (1, 9, 33, 34) although not observed in all studies (33). Importantly, BFRE appears to be feasible with a high training adherence in knee OA patients (1, 33, 34). The use of different restrictive pressures (absolute restrictive pressures: 160-200 mmHg and individualized pressure of 70%; the pressure needed to provide complete blood flow restriction (total limb occlusion pressure: LOP) has been applied without any adverse events in mild-degree knee OA (1, 33, 34). This is in line with Hughes et al. (13), who suggested that when BFRE is performed correctly, it has been demonstrated to be as safe as free-flow exercise methods (13). Currently, no consensus exists about the appropriate restrictive pressure to induce favorable muscle adaptation in patients suffering from knee OA. This might be due to the fact that the effective occlusion pressure seems to be dictated by the exercise load/intensity (35). Thus, the effective occlusion pressure varies between studies due to use of different exercises or differences in exercise load and intensity. Restrictive pressures ranging from 40%-80% of total arterial leg occlusion pressure (LOP) have been suggested to be sufficient to evoke muscular adaptation in healthy adults (14, 17, 18, 36). If the load is less than 30% 1RM, higher restrictive pressures seems required to evoke muscle hypertrophy, while lower pressures (40% LOP) requires training loads of 30% 1RM or above to be performed (36). Injury or joint pain (i.e. from the knee) might limit the amount of resistance applied during strength testing, and may thus compromise the ability to rely fully on a given 30% 1RM estimation. Therefore, higher pressures than 40% LOP are suggested to be used in clinical settings (36). On the other hand, higher pressures are associated with more discomfort during exercise and in between-set rest pauses (14),



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4 117 which potentially can affect exercise motivation negatively in patients. Thus, an occlusion pressure  
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6 118 sufficiently high to evoke measurable muscle adaptation despite potentially exercising at loads  
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9 119 lower than 30% 1RM; yet tolerable to maintain a high adherence, seems a favorable choice for this  
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11 120 particular patient population.

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13 121 The adaptive mechanisms evoked by BFRE seem to involve accumulation of metabolites,  
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16 122 ischemia (transient tissue hypoxia), which may increase recruitment of higher threshold (Type II)  
17  
18 123 fibers through stimulation of group III and IV afferent nerve fibers (37, 38), and also activation of  
19  
20 124 myogenic muscle stem cells (satellite cells: SC) (13, 26, 31). SC are cells positioned between the  
21  
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23 125 sarcolemma and the myofiber basal lamina (31, 39). SC play an important role in human skeletal  
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25 126 muscle growth due to their ability to donate new myonuclei to the muscle fibers (31, 40-44). That  
26  
27 127 is, the human skeletal muscle fibers are multinucleated cells with each myonucleus controlling the  
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30 128 protein synthesis of a certain cytoplasmatic area in the muscle fiber (40-42, 45). Myonuclei  
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32 129 transcriptional activity can be fully maximized with exercise, hence requiring new myonuclei to  
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34 130 support further muscle tissue accretion (41, 42, 44). It has been suggested that exercise-related  
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36 131 addition of SC and myonuclei by means of BFRE might reduce the muscle atrophy related to  
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39 132 bedrest and/or prolonged inactivity (31, 46). Previous studies applying short term (10 days)  
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41 133 preoperative BFRE before an anterior cruciate ligament rupture-reconstruction found no atrophy  
42  
43 134 protective effect or higher postoperative muscle strength compared to performing a low-load  
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46 135 exercise without blood flow restriction (placebo). However, it might be questionable if the applied  
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48 136 training frequency, intensity and training period have been sufficient to promote SCs and myonuclei  
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50 137 addition. Thus, longer periods of intensive training might be necessary to promote the desired  
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53 138 muscle morphological adaptations (addition of myonuclei and increased SC content).

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## 57 140 **Aim and hypothesis of the trial**

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4 141 The primary aim of this trial is to investigate the efficacy of eight weeks of BFRE compared to  
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7 142 receiving usual care prior to TKR on postoperative chair stand performance. We hypothesize that  
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9 143 eight weeks of preoperative BFRE will lead to increased 30 second chair stand performance (30-  
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11 144 second Chair Stand Test: 30-s CST) when assessed three months postoperatively. Secondary aims  
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14 145 are to investigate the efficacy of preoperative BFRE on lower limb muscle strength three months  
15  
16 146 after TKR and investigate the potential relationship to functional capacity and quality of life.  
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18 147 Furthermore, it will be investigated to which extent eight weeks of BFRE induce myofiber  
19  
20 148 hypertrophy and gain in satellite cell number and myonuclei content in the knee extensor  
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23 149 musculature.  
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## 27 151 **MATERIAL & METHODS**

### 29 152 **Design**

31  
32 153 The trial is designed as a multicenter (two sites), randomized, assessor blinded, controlled trial  
33  
34 154 following the CONSORT guidelines (47). Primary endpoint will be three months after TKR.  
35  
36 155 Additional and secondary endpoints will be evaluated during the week of TKR, six weeks after  
37  
38  
39 156 TKR (questionnaires only) and 12 months after TKR. Muscle biopsies will be obtained from all  
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41 157 patients undergoing surgery at Horsens Regional Hospital at baseline, during surgery and three  
42  
43 158 months after TKR.  
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### 48 160 **Participants**

49  
50 161 Patients will be recruited from the Departments of Orthopedic Surgery at Horsens and Silkeborg  
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52  
53 162 Regional Hospitals in Denmark. Patient enrollment will start September 2<sup>nd</sup> 2019 at Horsens  
54  
55 163 Regional Hospital and October 1<sup>st</sup> 2019 at Silkeborg Regional Hospital. Patient recruitment is  
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57 164 expected to be completed in June 2021. All patients are expected to have completed baseline testing  
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165 ultimo September 2021 and have performed three-month follow-up ultimo April 2022. Thus, at the  
166 end of September 2023 all patients are expected to have completed 12-month follow-up testing.

167  
168 Inclusion criteria: 1) Patients  $\geq 50$  years scheduled for TKR due to knee OA at Horsens- or  
169 Silkeborg Regional Hospital.

170  
171 Exclusion criteria: 1) Severe cardiovascular diseases (New York Heart Association (NYHA) class  
172 III and IV), previous stroke incident, thrombosis incident; 2) traumatic nerve injury in affected limb  
173 3) unregulated hypertension (systolic  $\geq 180$  or diastolic  $\geq 110$  mmHg) 4) spinal cord injury; 5)  
174 planned other lower limb surgery within 12 months; 6) cancer diagnosis and currently undergoing  
175 chemo-, immuno-, or radiotherapy; 7) inadequacy in written and spoken Danish; 8) an existing  
176 prosthesis in the index limb; 9) living more than 45 minutes from either Horsens Regional Hospital  
177 or Silkeborg Regional Hospital; 10) pregnancy.

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179 *Please insert figure 1 around here*

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181 All patients will be screened for eligibility by four orthopedic chief physicians at Horsens Regional  
182 Hospital and by three orthopedic chief physicians at Silkeborg Regional Hospital who will perform  
183 the initial inclusion of study participants and hand out written project information. All patients  
184 accepting to participate will be asked to complete a written informed consent allowing the  
185 physiotherapist (at Horsens Regional Hospital and Silkeborg Regional Hospital) to contact the  
186 patients by phone for a final eligibility and exclusion criteria-screening and book an appointment  
187 for baseline testing. If the patient agrees to participate in the trial, he/she will sign a written  
188 informed consent to participate in the project. Subsequently, the patient will be baseline-tested at

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4 189 the hospital by a blinded (to group allocation) assessor. Patients declining to participate in the RCT  
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6  
7 190 will be offered the option of participating in a parallel observational cohort trial. All patients  
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9 191 included in the project will be scheduled for a TKR. Two-three weeks before surgery all patients  
10  
11 192 will be invited to a, preoperative information meeting where nurses, surgeons, and physiotherapists  
12  
13 193 will provide detailed information on pain management, nutrition, the surgical procedure, physical  
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15  
16 194 activity, postoperative home-based rehabilitation (table 1a and 1b), load management, etc. (usual  
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18 195 care) (48). On the day of surgery, patients will be hospitalized at Horsens Regional Hospital or  
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20 196 Silkeborg Regional Hospital where an orthopedic chief physician will perform the TKR procedure.  
21  
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23 197 The day after surgery all patients will receive physiotherapy-supervised training once or twice per  
24  
25 198 day by a physiotherapist in order to fulfill the discharge criteria (table 2a and 2b) (48). Patients will  
26  
27 199 generally be discharged within ~one-two days after fulfilling all the discharge criteria listed above.  
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30 200 After discharge, all patients will receive a standard home-based rehabilitation program focusing on  
31  
32 201 improving knee joint mobility, increasing the tolerance for standing without assistive devices, and  
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34 202 lower extremity muscle strength. Variations in the selection of exercises and exercise variables exist  
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37 203 in the standard home-based rehabilitation programs between the respective hospitals; however, the  
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39 204 purpose of the programs is identical. If the patients do not fulfill the discharge criteria, they will be  
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41 205 offered supervised knee-specific exercise therapy at a municipal rehabilitation center or specialized  
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43 206 hospital-based rehabilitation after discharge from the hospital.  
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48 208 *Please insert table 1a and 1 b about here*  
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52 210 *Please insert table 2a and figure 2b around here*  
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## 55 211 56 57 212 **Randomization** 58 59 60

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4 213 After baseline assessment, patients will be randomized (1:1) using the Research Electronic Data  
5  
6 214 Capture (REDCap) randomization system to either the training (BFRE) group or the control (CON)  
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8  
9 215 group. Prior to randomization, all patients will be booked for follow-up test sessions and surgery.  
10  
11 216 All randomization procedures will be performed by the physiotherapists in charge of the BFRE  
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13 217 training. Assessors performing the tests will be blinded to group allocation until completion of the  
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16 218 trial. A flow chart of the patient allocation procedures is depicted in Figure 1.

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20 220 CON group: Participants in CON will receive usual care (see above) prior to TKR and be  
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23 221 encouraged to continue their usual lifestyle up until TKR.

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27 223 BFRE group: In addition to receiving usual care (cf. above), participants in the BFRE group will  
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29 224 perform supervised BFRE sessions three times per week for eight weeks supervised by a  
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31 225 physiotherapist educated in administering BFRE. All BFRE training will be performed at Horsens  
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33 226 Regional Hospital and Silkeborg Regional Hospital.

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38 228 **Intervention procedures**

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41 229 BFRE

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43 230 Each BFRE session will consist of a 10-minute warm up (ergometer cycling) followed by two  
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45 231 different unilateral lower-limb resistance training exercises: 1) leg press and 2) knee extension  
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48 232 performed on standard strength training machines. Each exercise will be performed with the  
49  
50 233 affected lower limb only and consist of four rounds interspaced by 30 seconds of rest (table 3). First  
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52 234 round: 30 repetitions (reps); second round: 15 reps; third round: 15 reps; fourth round: until  
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54 235 exhaustion (Table 1). If patients can perform more than 15 repetitions in the fourth exercise set, the  
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56 236 exercise load will be increased with the minimum extra load possible (30). Participants will be  
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59 237 instructed to perform both the eccentric and concentric contraction phases using a steady 2-second  
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4 238 pace duration. The fourth and final exercise set will be performed to the point of exhaustion defined  
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6 239 as being unable to complete the final concentric contraction phase in 2 seconds. During the 30  
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9 240 second rest period, patients will rest in a standardized resting position while maintaining the initial  
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11 241 cuff-pressure. Between each exercise, patients will have a 5-minute "free-flow" rest period. The 5  
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13 242 minutes rest period applied between exercises was chosen based on experiences from a previous  
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16 243 pilot project (Jorgensen & Bohn 2019, unpublished data) and experience with applying BFRE in  
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18 244 clinical practice. In both situations, we often experienced that patients stayed seated in the leg press  
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20 245 machine for >2 minutes after the last (fatiguing) set to feel sufficiently rested and confident to walk  
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23 246 from one exercise machine to another. The cuff will be released immediately after completion of the  
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25 247 final exercise set.

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27 248 The occlusion pressure during both exercises will be set at 60% of total limb occlusion pressure  
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30 249 (LOP) and the starting load intensity will be 30% with 1 repetition maximum (1RM) in both  
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32 250 exercises.

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34 251 Individual LOP will be determined using a pneumatic, conically shaped, 12 cm wide, rigid cuff  
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36 252 (Occlude Aps, Denmark) attached to the patient's most proximal area of the thigh on the affected  
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39 253 side. While sitting on an examination table with the ankle and 1/3 of the lower limb off the table, a  
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41 254 vascular Doppler probe (EDAN Instruments, inc., China) will be placed posterior to the medial  
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43 255 malleolus over the posterior tibial artery to capture the auscultatory pulse. To determine the cuff  
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46 256 pressure (mmHg) needed for total blood flow occlusion, the cuff will gradually be inflated in 20  
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48 257 mmHg steps until reaching the pressure where the auscultatory pulse is interrupted (LOP). The first  
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50 258 time the auscultatory pulse is interrupted, the examiner releases 10-20 mmHg pressure from the cuff  
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53 259 until the auscultatory pulse is present again. When the auscultatory pulse reappears, the cuff is  
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55 260 inflated with 10 mmHg until the LOP is found again. If the second LOP is identical to the first, it  
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261 will be defined as the LOP for that specific patient. Otherwise, the procedure will be repeated until  
262 determining an identical LOP two consecutive times.

*Please insert Table 3 about here*

### **Outcome variables**

267 Outcome assessments will be performed at baseline (before randomization), three-four days before  
268 surgery, six weeks after TKR, three months after TKR, and 12 months after TKR. To reduce the  
269 number of postoperative visits, only questionnaires; The Knee disability and Osteoarthritis Outcome  
270 Score (KOOS), EuroQol Group 5-dimensions (EQ-5D-L5) and reporting of adverse event or  
271 receiving supervised physiotherapy postoperatively will be sent via email six weeks after surgery.  
272 Two testers (two trained physiotherapists) blinded to group allocation will perform all baseline and  
273 follow-up measurements. Bergström needle muscle biopsies (49) will be taken from vastus lateralis  
274 of the quadriceps muscle in both lower limbs from patients included at Horsens Regional Hospital  
275 only at baseline, during surgery, and three months after TKR by doctors trained in performing the  
276 procedure. An overview of the data collection parameters is presented in Table 4.

277 Before starting the baseline testing, all assessors will be thoroughly trained in performing  
278 the tests according to the standardized test procedures for each test method. All assessors will be  
279 blinded to intervention allocation (pre surgery BFRE training or usual care). Further, assessors will  
280 be trained in how to communicate with the participants at follow-up test sessions to avoid break of  
281 blinding due to miscommunication. Also, all cases where blinding is being broken will be  
282 registered. Also, the physiotherapist in charge of LL-BFRE will be thoroughly trained in  
283 performing the exercise on healthy subjects before applying LL-BFRE on study-patients. At the last  
284 scheduled exercise session (i.e. 24th session), the physiotherapists in charge of LL-BFRE will

carefully remind the participants not to reveal their group allocation to any assessors at any time point during post testing.

The primary investigator will be in weekly contact with the physiotherapists supervising the LL-BFRE at Horsens Regional Hospitalet and Silkeborg Regional Hospital where day-to-day-retraining and supervision can be arranged. Furthermore, physiotherapists supervising the LL-BFRE will receive in-depth retraining every three months.

## Outcomes

*Please insert Table 4 about here*

### *Primary outcome*

**The 30s-CST** will be assessed using a 44 cm (seat height) chair with armrests. The 30s-CST measures the number of sit-to-stand repetitions completed within 30 seconds. The 30s-CST is considered a valid and sensitive measure of lower-extremity sit-to-stand function with good to excellent intra- and inter-observer reliability (50-52).

### *Secondary outcomes*

**The Timed Up & Go test (TUG)** assesses the time required for patients to stand from a 44 cm (seat height) chair walk around a tape mark 3 meters away and sit into the chair at return. The patients will be instructed to walk as fast and safely as possible towards the tape mark (and touch the tape mark (with at least one foot), turn around and return to the chair and sit down. Use of armrests is allowed. The fastest of two trials will be used for further analysis. Up to one minute of



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308 rest will be allowed between trials (53, 54). Good inter-rater reliability has been demonstrated with  
309 the TUG test (52).

310  
311 **4x10 meter walk test (40m-FWT)** measures the total time it takes to walk 4 x 10 meters excluding  
312 turns (meter/sec) (52). Patients will be instructed to walk as quickly and as safely as possible  
313 without running to a visible mark 10 meters away, return and repeat for a total distance of 40 meters  
314 (52). Prior to the test, one practice trial will be provided to check understanding. The 40m-FWT is a  
315 valid and responsive measure for assessing short distance maximum walking speed with excellent  
316 inter-rater reliability (52).

317  
318 **1RM leg press strength** will be estimated from a 5-8RM leg press test. Patients perform three low-  
319 load warm-up sets. The first and second warm-up sets consist of 12 repetitions, and the third warm-  
320 up set consists of eight repetitions. The load of each warm-up set will be increased with 10 kilos.  
321 After warm-up, the load will be increased to determine the 5RM. If the 5RM cannot be determined  
322 within three trials, a fourth all-out trial (as many repetitions as possible) will be performed. The  
323 1RM will be calculated as  $[1RM = \text{load (kg)} / 1.0278 - 0.0278 \cdot \text{number of repetitions}]$  (55).

324  
325 **1RM knee extension strength** will be estimated from 5-8RM knee extension test as described  
326 above for the estimation of 1RM leg press test (55).

327  
328 **Maximal isometric voluntary contraction (MVC) of the knee** will be measured using a handheld  
329 dynamometer (HHD). The patients will be seated on an examination table with knees and hips  
330 positioned at 90° flexion. The patients will be instructed to remain seated in an upright position and  
331 place both hands on the shoulder to avoid compensation. The HHD will be fixed with a rigid belt to  
332 the examination table. Adjustable straps will be used to allow MVCs of the knee extensors to be  
333 performed at 90° knee flexion in all patients. The HDD will be positioned 5 cm above the medial

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334 malleolus (56). The patients will be instructed to produce as much force as possible into the HHD.

335 Good to excellent inter- and intra-rater reliability has previously been demonstrated on group-level

336 in patients suffering from knee OA for maximum knee extensor muscle strength testing with HDD

337 (56, 57). Patients will receive four trials. For analysis, the mean maximal strength of the second,

338 third and fourth measures will be calculated and corrected for bodyweight (56)

339

340 **MVC of the knee flexors** will be measured and performed using HHD at 90° knee flexion with the

341 patients seated identically as during MVC for the knee extensors (56). The HHD will be positioned

342 posterior aspect of calcaneus (56) and patients will be instructed to produce as much force as

343 possible into the HHD. Good to excellent inter- and intra-rater reliability has previously been

344 demonstrated on group-level in patients suffering from knee OA for maximum knee flexor muscle

345 strength testing with HDD (56). Patients will receive four trials. For analysis, the mean maximal

346 strength of the second, third and fourth measures will be calculated and corrected for bodyweight

347 (56)

348

349 **Myofiber cross sectional area (CSA), muscle fiber type composition, satellite cell content, and**

350 **myonuclei number** will be assessed by obtaining needle biopsies (100-150 mg) from all patients

351 enrolled at Horsens Regional Hospital. The biopsies will be obtained bilaterally from the middle

352 portion of the vastus lateralis muscle utilizing the percutaneous needle biopsy technique of

353 Bergström (49, 58, 59). Biopsies will be performed by two experienced orthopedic surgeons (chief

354 physicians) trained in performing the needle muscle biopsy technique at Horsens Regional Hospital.

355 Efforts will be made to extract tissue from the same region (2-3 cm apart) and depth (~1-2 cm.)

356 (49). The tissue samples will be dissected of all visible blood, adipose tissue, and connective tissue

357 and mounted in Tissue-Tec (4583, Sakura Finetek, Alphen aan den Rijn, The Netherlands), frozen

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4 358 in isopenate pre-cooled with liquid nitrogen, and stored at -80°C (31, 49, 60). All muscle samples  
5  
6  
7 359 will be analyzed as previously described by Nielsen et al. (31) using immunofluorescence  
8  
9 360 microscopy. Transverse serial sections (8 µm) of the embedded muscle biopsy specimen will be cut  
10  
11 361 at -22°C using a cryostat (HM560; Microm, Walldorf, Germany) and will be mounted on glass  
12  
13  
14 362 slides for subsequent analysis as described in detail elsewhere (31). Myogenic stem cells (satellite  
15  
16 363 cells (SC)) will be visualized with an antibody against Pax7 (31). Type I (stained) and Type II  
17  
18 364 (unstained) myofibers will be differentiated, and muscle fiber area will be determined (31); MSC-  
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21 365 derived nuclei will stain positive for Pax7 and be within the basal lamina; nuclei (DAPI stained)  
22  
23 366 with a sublaminar placement will be considered myonuclei (31).

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27 368 **Knee disability and Osteoarthritis Outcome Score (KOOS)** is a patient-administered knee  
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30 369 specific questionnaire comprising five subscales: Pain; Symptoms; Activities of daily living; Sport  
31  
32 370 & Recreation; and Knee-Related Quality of Life. Each item is scored from 0 to 4 (61). The raw  
33  
34 371 score for each of the five subscales is the total sum of the associated item scores. Scores can be  
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37 372 transformed to a 0 to 100 scale. The scores of the five subscales can be expressed as a composite  
38  
39 373 outcome profile, higher scores indicating fewer problems (62). The KOOS questionnaire is valid  
40  
41 374 and reliable in patients suffering from knee OA and patients on the waiting list for TKA for knee  
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44 375 OA (61, 63, 64).

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7 377 **EuroQol Group 5-dimension (EQ-5D-5L)** is a self-completion questionnaire consisting of two  
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9 378 parts; the first part of the EQ-5D-5L comprises five dimensions involving mobility, self-care, usual  
10  
11 379 activities, pain/discomfort, and anxiety/depression. All dimensions have five response categories  
12  
13 380 (no problems, slight problems, moderate problems, severe problems, and extreme problems)  
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15  
16 381 resulting in a five digit descriptive health state (65), which will be converted into a summary index  
17  
18 382 ranging from -0.624 (worst) to 1.000 (best), using a Danish value set (66). The second part, EQ-  
19  
20 383 VAS rates the overall current health status from 0 (worst imaginable health) to 100 (best imaginable  
21  
22  
23 384 health) (65). The EQ-5D-5L is reliable and valid in patients with knee OA eligible for TKA (67,  
24  
25 385 68).

26  
27 386  
28 387 **Adverse events** will be defined as unpredicted or unintended events, signs, or disease occurring  
29  
30  
31 388 during the period from inclusion until the 3-month follow-up (primary end-point) resulting in  
32  
33 389 contact with the healthcare system (hospital or general practitioner) independent of whether or not  
34  
35 390 the event is related to the intervention or outcome assessments. Adverse events will be recorded and  
36  
37  
38 391 categorized in accordance with the definitions established by the United States Food and Drug  
39  
40 392 Administration [88]. Continuous registration of adverse events will be performed and a short open-  
41  
42 393 ended questionnaire will be administered at three months follow-up.  
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#### 46 395 *Other Outcome Measures*

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49 396 **Blood pressure** will be measured by the orthopedic chief physicians when patients are visiting the  
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51 397 outpatient clinic. Blood pressure will be used to determine eligibility to participate in the project.  
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**Exercise compliance and progression** will be obtained by the physiotherapist in charge of the training sessions and entered directly into the REDCap-system. The progression will be monitored as the total load lifted by the patient for exercise session.

**Numeric rating scale for pain** is a segmented unidimensional 11-item measure of pain intensity in adults (69) that will be used to rate pain intensity during both testing and exercise sessions. (69). 0 represents no pain while 10 represents worst pain imaginable (69).

**Declining to be operated** will be measured at three month follow-up, where patients will be asked whether they decided to be operated or not. Patients who declined to be operated will be invited to participate in all prescheduled follow-up assessments.

**Postoperative supervised physiotherapy** will be measured at six week, three month, and 12 month follow-up by answering a questionnaire. If patients have participated in postoperative supervised physiotherapy, the patient must specify whether the treatment was related to the TKR or due to other circumstances.

**Knee joint active range of motion** will be measured with a 360° plastic goniometer (scale 1°) with 16.5 cm moveable arms at baseline in the week of surgery, three months, and 12 months after surgery. Laying supine on an examination table, the knee joint flexion and knee joint extension will be measured separately (70). The tester then identifies the most prominent part of the trochanter, the lateral epicondyle of the femur, the lateral head of fibula, and the lateral malleolus. When identified, the patient is asked to flex the knee as much as possible with the heel maintaining contact to the surface at all time (70). Secondly, the patients will be asked to extend the knee joint as much as

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4 423 possible. To allow the knee to extend as much as possible, a firm quadratic box (height: 5 cm,  
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7 424 width: 8 cm, length: 15 cm) will be placed under the heel of the patient. The procedure of  
8  
9 425 measuring knee extension will be similar to knee flexion, as the patients increases the degree of  
10  
11 426 knee extension maximally (70) The fulcrum of the goniometer will correspond visually to the trans-  
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14 427 epicondylar axis of the knee joint. The moveable arms of the goniometer will be pointed towards  
15  
16 428 the greater trochanter and the lateral malleolus (70).  
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### 18 429 19 20 430 **Data management**

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23 431 All data from the physical function tests will be entered into RedCap by the assessors using double  
24  
25 432 data entry to ensure data quality. All patient-reported outcome data (KOOS, NRS Pain, EQ-5D-5L)  
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27 433 will be entered directly into RedCap by the patients, and usage of the “required fields” will ensure  
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30 434 no missing items from the completed questionnaires. To reduce missing data, a reminder email will  
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32 435 be sent automatically from the RedCap-system. All patient data will be anonymized by assigning  
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34 436 study numbers to each patient (coding). Personal data about the patient will be located separately  
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37 437 from the main dataset to protect confidentiality during all trial phases.

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39 438 The raw dataset will be maintained for ten years after completion of the trial with indefinite  
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41 439 restricted access due to sensitive data. After publication of the trial, a fully anonymized patient-level  
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43 440 dataset and corresponding statistical description will be made publicly available if required by the  
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46 441 scientific journal, in which the results are published.  
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### 48 442 49 50 443 51 52 444 **Sample size**

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55 445 The power and sample size calculation is based on the expected differences between the two subject  
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57 446 groups from baseline to three-month follow-up (8). Due to lack of data on the primary outcome for  
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4 447 investigations applying LL-BFRE before a surgical procedure, we decided to base our sample size  
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7 448 calculation on Skoffler et al. (8) who investigated the efficacy of four weeks of preoperative and  
8  
9 449 four weeks postoperative HRST (intervention group) compared to four weeks of postoperative  
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11 450 HRST only (control group) on 30-s CST three months in patients receiving a TKR (8). The authors  
12  
13  
14 451 found a between-group difference of 3-4 repetition difference ( $14.7 \pm 4.7$  repetitions versus  $11.0 \pm$   
15  
16 452  $4.4$  repetitions) three months after TKR surgery (8).

17  
18 453 To reduce the probability of type I errors and enable detection of a between-group difference  
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20  
21 454 also,  $\alpha$ -level is set at 0.05 ( $p < 0.05$ ) and  $\beta$ -level is set at 0.20 (80% power). Expecting a 3-repetition  
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23 455 between-group difference three months postoperatively and assuming a SD of 4.7 in both groups,  
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25 456 39 patients are required in each group (yielding 78 patients in total). With an anticipated dropout  
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28 457 rate of 10%, 84 patients will be recruited for the trial.

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### 31 32 459 **Statistical considerations**

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35 460 The primary efficacy analysis will be an assessment of the between group difference in change in  
36  
37 461 the 30-S CST from baseline to three-month follow-up (primary endpoint).

38  
39 462 All descriptive statistics and tests will be reported in accordance with the recommendations of  
40  
41  
42 463 the “Enhancing the QUALity and Transparency Of health Research” (EQUATOR) network (71) and  
43  
44 464 the CONSORT statement (47). Intention-to-treat principle (i.e. all patients as randomized  
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46 465 independent of departures from allocation treatment, compliance and/or withdrawals) and per  
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48 466 protocol analysis will be conducted. A one-way analysis of variance (one-way ANOVA) model will  
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50  
51 467 be used to analyze between group mean changes in continuous outcome measures (31). The model  
52  
53 468 includes changes from baseline to 12-month follow-up. Between-intervention comparison from  
54  
55 469 baseline to three months after surgery will be analyzed using a mixed linear model with patient ID  
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58 470 as a random effect and time and group as fixed effects (31, 72). Also, to gain insight into the  
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4 471 potential pre-to-post training differences within the respective training or control groups, paired  
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6 472 student t-tests will be performed. Level of statistical significance is  $P < 0.05$ . *Secondary outcome*  
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8  
9 473 *variables:* Between-intervention comparison from baseline to the week of surgery, six weeks after  
10  
11 474 surgery, three and 12 months after surgery will be analyzed as described for the primary outcome.  
12  
13 475 Regression analysis will be used to analyze the potential associations between preoperative strength  
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16 476 and postoperative lower extremity function and self-reported outcome as well as between  
17  
18 477 preoperative functional capacity and postoperative functional capacity. Additionally, regression  
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20 478 analysis will be used to analyze the association between preoperative number of satellite cells and  
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22  
23 479 myonuclei on postoperative isometric knee extensor muscle strength, muscle fiber cross sectional  
24  
25 480 area, and functional capacity. All statistical analyses will be performed by the primary investigator  
26  
27 481 using Stata.

### 31 32 483 **Ethical aspects and dissemination**

33  
34 484 The trial has been accepted by the Central Denmark Region Committee on Biomedical Research  
35  
36 485 Ethics (Journal No 10-72-19-19) and by the Danish Data Protection Agency (Journal No 652164).  
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39 486 The trial is registered at Clinicaltrials.gov (NCT04081493). Before inclusion, all patients will  
40  
41 487 provide their written informed consent in accordance with the Helsinki Declaration. All data and  
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43 488 information collected in regard to this trial will be treated confidentially (blinded and encrypted) by  
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46 489 the researchers and staff connected to the trial.

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48 490 All results from the trial will be published in international peer-reviewed scientific journals  
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50 491 regardless of the results being considered positive, negative or inconclusive.

### 52 53 492 54 55 493 **Patient and public involvement**

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57 494 Before developing this clinical trial, a pilot project was performed to determine the feasibility and  
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495 efficacy of BFRE in patients suffering from lower limb injuries. The experiences with the training  
496 modality and the verbal feedback from patients on training duration, frequency, and intensity  
497 resulted in useful knowledge that certainly has improved the development of the present clinical  
498 trial.

## 500 **DISCUSSION**

501 To the best of our knowledge, this is the first trial to investigate the effect of preoperative BFRE on  
502 functional capacity, self-reported outcome, lower limb muscle strength and myofiber  
503 morphology/stem cell abundance in patients scheduled for TKR. Only few studies have investigated  
504 (short term (10 days)) preoperative BFRE without finding an atrophy protective effect or difference  
505 in muscle strength compared to a control group performing a placebo intervention (SHAM group)  
506 (73). However, patients performing short term preoperative BFRE before ACL-R demonstrated  
507 higher muscle endurance compared to a SHAM group (74). Therefore, results of this trial are  
508 expected to provide novel information on longer periods of BFRE that will enable researchers to  
509 design effective exercise-based preconditioning protocols for elective TKR patients. The LL-BFRE  
510 protocol applied in the present project is widely used and follows the recommendations from a  
511 recent position stand by Patterson et al. (75). The authors suggested that exercising 2-3 times per  
512 week at 20-40% of 1RM in 2-4 sets (e.g. 30-15-15-15 or sets to failure) using pressures between 40  
513 to 80% of LOP has demonstrated to be effective when aiming at increasing muscle strength and  
514 promoting muscle hypertrophy (75).

515 The trial is designed as an assessor blinded randomized controlled trial, thus representing the  
516 highest evidence level. However, the nature of the trial does not allow blinding of the participants  
517 which is an inherent limitation of the trial. The trial is conducted at two hospitals that consistently  
518 perform a high number of TKR procedures annually (225 and 460, respectively), thus securing a

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519 strong expertise in terms of surgery and infrastructure. Both hospitals have all equipment needed  
520 available for surgery, post-operative hospitalization, training, and testing. All outcome variables are  
521 considered valid and reliable measures and consist of both objective outcomes and self-reported  
522 patient outcomes.

523 No adverse health-related events have been reported in previous studies applying BFRE in  
524 patients' suffering from knee OA or in healthy older adults (1, 9, 13, 23, 33, 34). Further, in a recent  
525 review and meta-analysis it was stated that exercise with concurrent blood-flow restriction is a safe  
526 exercise modality when occlusion procedures are applied correctly (13). The inherent invasive  
527 procedure of muscle biopsies may cause adverse events in rare occasions. Therefore, all muscle  
528 biopsy samples will be collected by trained medical doctors and performed following administration  
529 of local anesthesia and in fully sterile conditions. The needle muscle biopsy protocol has been  
530 applied in a large number of previous investigations including very old frail subjects (97 years of  
531 age) without any reporting of adverse events besides occasional muscle soreness(31, 49, 58, 76, 77).

532 There are some limitations of the project that must be taken into account. First, our primary end  
533 point is three months postoperatively. The (uncontrolled) period discharge to three months  
534 postoperatively renders the project vulnerable to external variabilities. However, from a pragmatic  
535 point of view, this uncontrolled period from discharge to three-month follow-up reflects the reality  
536 that Danish patients face postoperatively. Thus, the results at three-month follow-up will, indeed,  
537 reflect the impact of performing preoperative LL-BFRE on the postoperative outcome regardless of  
538 the external variable that can hamper the results. Secondly, the discharge criteria at Horsens  
539 Regional Hospital and Silkeborg Regional Hospital withhold slight differences. That is, the  
540 acceptable knee joint ROM at discharge differs between the sites, thus it can be speculated that  
541 more patients from Silkeborg Regional Hospital will be offered a postoperative, supervised  
542 rehabilitation program. This might affect the number of patients receiving supervised physiotherapy

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543 after discharge between sites. However, all patients included in the present project will report  
544 whether they have received postoperative supervised physiotherapy at all follow-up assessments.  
545 Thus, we will be able to determine (and normalize) a potential between-site difference in patients  
546 receiving supervised physiotherapy after TKR.

547

#### 548 **Author contributions**

549 SLJ, PAA, MBB, and IM were all part of designing the trial and approved the final version of the  
550 protocol. Also, SLJ, PAA, MBB, and IM wrote and revised the protocol.

551

#### 552 **Data statement**

553 All obtained data will be stored in anonymized form at the Danish National Archives and deleted  
554 after 10 years.

555

#### 556 **Funding**

557 This work trial is supported by Aase og Ejnar Danielsen's Foundation (100,000 dkk), Nis-Hanssen's  
558 Mindeslegat (163,883 dkk) and the Health Research Foundation of Central Denmark Region  
559 (99,658 dkk), Hede-Nielsen Foundation (8,000 dkk).

560

#### 561 **Competing interest**

562 None to be declared

563

#### 564 **Ethics approval**

565 The trial has been accepted by the Central Denmark Region Committee on Biomedical Research

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807 *Table 1a.* Postoperative rehabilitation program, Horsens Regional Hospital

Week 0-3				
Step	Exercise	Repetitions	Sets	Resistance
Step 1 & 2	Supine peristaltic pump exercise with feet above heart level	20 minutes	3-4/day	-
Step 1	Supine knee extension mobilization	20 seconds	3 sets	-
Step 1	Supine unilateral knee and hip extension and flexion mobilization with slipper under the heel	5 repetitions	3 sets	Slipper minimizes floor friction
Step 2	Seated knee extension and flexion mobilization with slipper under the foot	5 repetitions	3 sets	Slipper minimizes floor friction
Step 2	Standing weight transfer exercise	15 repetitions each side	1 set	Bodyweight
Step 2	Sit to stand from a high chair or the edge of table	5 repetitions	3 sets	Bodyweight
Week 3 and onwards				
Step 1 & 2	Supine peristaltic pump exercise with feet above heart level	20 minutes	3-4/day	-
Step 1	Seated knee extension mobilization	20 seconds	4 rounds	Arms can be used to apply pressure onto the knee to help extend the knee
Step 1	Step up exercise	10-15 repetitions	2-3 sets	Bodyweight
Step 1	Standing knee isometric knee towel press	10-15 repetitions	2-3 sets	Ball/Towel rolled together
Step 1	Sit to stand from a chair	10-15 repetitions	2-3 sets	Bodyweight
Step 1	One leg standing	30 seconds	1 set	Bodyweight
Step 2	Standing hip flexion	Not informed	Not informed	Elastic band
Step 2	Standing hip abduction	Not informed	Not informed	Elastic band
Step 2	Partial frontal plane sliding lunge	10 repetitions	3 sets, 2-3/day	Bodyweight
Step 2	Partial back sliding lunge	10 repetitions	3 sets, 2-3/day	Bodyweight
Optional	Cycling	10-20 minutes	1 set	Light resistance can be added when it is possible to perform a full round with the operated limb.

Step 1 is performed in the morning and step 2 is performed in the afternoon. All exercises are performed once per day.

809 *Table 1b.* Postoperative rehabilitation program, Silkeborg Regional Hospital

Week 0-2				
Step	Exercise	Repetitions	Sets	Resistance
Optional	Cycling	5-10 minutes	2/day	-
-	Supine peristaltic pump exercise	Not informed	Not informed	-
-	Rest with leg above heart level	30 minutes	4/day	-
-	Seated isometric knee extension	3 seconds	10 sets	Lower leg and the foot
-	Seated knee flexion mobilization	3 seconds	10 sets	-
-	Seated knee extension mobilization	30 seconds	3 sets	Apply pressure to the knee joint using the arms
-	Supine isometric knee extension	3 seconds	10 sets	Lower leg and the foot
-	Supine passive knee extension mobilization			Gravity will extend the knee joint
Week 2 and onwards				
-	Supine knee isometric knee towel press	3seconds hold	10sets	Lower leg and the foot

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-	Sit to stand	10 repetitions	1 set	Body weight
-	Standing knee flexion mobilization	3 seconds	10 sets	Body weight
-	Step Up Exercise	10 repetitions	1 set	Body weight

**Table 2a.** Discharge criteria at Horsens Regional Hospital

Minimum knee flexion range of motion	60 degrees
Maximal knee extension deficit	15 degrees
In-and-out of bed	Independent
Sit-to-stand	Independent
Walking with/without assistive devices	Independent
Stair negotiation with/without assistive devices	Independent
Activities of daily living	Independent
Understanding of the home-based postoperative exercise program	Sufficient

**Table 2b.** Discharge criteria at Silkeborg Regional Hospital

Minimum knee flexion range of motion	90 degrees
Maximal knee extension deficit	5 degrees
In-and-out of bed	Independent
Sit-to-stand	Independent
Walking with/without assistive devices	Independent
Stair negotiation with/without assistive devices	Independent
Activities of daily living	Independent
Understanding of the home-based postoperative exercise program	Sufficient

**Table 3.** Exercise variables for the blood-flow restricted exercise (BFRE) protocol

Exercise variable	Week 1-8
Level of LOP	60% LOP
Sets	4
Load intensity	30% 1RM
Repetitions 1 <sup>st</sup> set	30
Repetitions 2 <sup>nd</sup> & 3 <sup>rd</sup> set	15
Repetitions 4 <sup>th</sup> set	To volitional failure
Contraction modes per repetition	
Concentric	2 seconds
Isometric	0 seconds
Eccentric	2 seconds
Rest between repetitions	0 seconds
Time under tension per repetition	4 seconds
Range of movement	maximum
Rest between sets	30 seconds
Rest between sessions	≥36 hours
Progression	The minimal possible load (5 kilo) is added when patients perform >15 repetitions in 4 <sup>th</sup> set

825 *Table 4. Outcome measures to be collected.*

Outcome measures	Data collection instrument	Time-points of assessment
<b>Primary outcome</b>		
<b>Sit-to-stand function</b>	30 seconds chair stand test	B, S, 3 and 12 months
<b>Secondary outcomes</b>		
<b>Ambulatory capacity</b>	Timed Up & Go	B, S, 3 and 12 months
<b>Gait speed</b>	4x10-meter walk test	B, S, 3 and 12 months
<b>Isometric Knee extensor muscle strength</b>	Handheld Dynamometer	B, S, 3 and 12 months
<b>Isometric Knee flexion muscle strength</b>	Handheld Dynamometer	B, S, 3 and 12 months
<b>Myofiber morphology</b>	Muscle Biopsies	B, S, 3 months
<b>Myogenic stem cell content</b>	Muscle Biopsies	B, S, 3 months
<b>Pain</b>	KOOS	B, S, 6 weeks, 3 and 12 months
<b>Symptoms</b>	KOOS	B, S, 6 weeks, 3 and 12 months
<b>Activities of daily living</b>	KOOS	B, S, 6 weeks, 3 and 12 months
<b>Sports &amp; Recreation</b>	KOOS	B, S, 6 weeks, 3 and 12 months
<b>Quality of life</b>	KOOS	B, S, 6 weeks, 3 and 12 months
<b>Socioeconomic costs</b>	EQ-5D	B, S, 6 weeks, 3 and 12 months
<b>Adverse Events</b>	Questionnaire and medical records	3 months
<b>Exercise compliance and progression</b>	Physiotherapist records	BFRE
<b>Pain during visits</b>	NRS for pain	B, BFRE, S, 3 and 12 months
<b>Declining to be operated</b>	Questionnaire	3 months
<b>Postoperative supervised physiotherapy</b>	Questionnaire	6 weeks, 3 and 12 months
<b>Knee joint range of motion</b>	Goniometer	B, S, 3 and 12 months
<b>Patient characteristics and related measurements</b>	Questionnaire	B
<b>Gender</b>	Tape measure	B
<b>Age</b>	Electronic body mass scale	B
<b>Height</b>	Questionnaire	B
<b>Body mass</b>	Questionnaire	B
<b>Civil Status</b>	Questionnaire	B
<b>Educational Level</b>	Questionnaire	B
<b>Employment Status</b>	Questionnaire	B
<b>Substance Use (alcohol, smoking)</b>	Questionnaire	B
<b>Duration of knee symptoms</b>	Questionnaire	B
<b>Pain medication during the last week</b>	Questionnaire	B
<b>Co-morbidities</b>	Questionnaire	B

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## Table and figure legends

Table 1a. Step 1 is performed in the morning and step 2 is performed in the afternoon. All exercises are performed once per day.

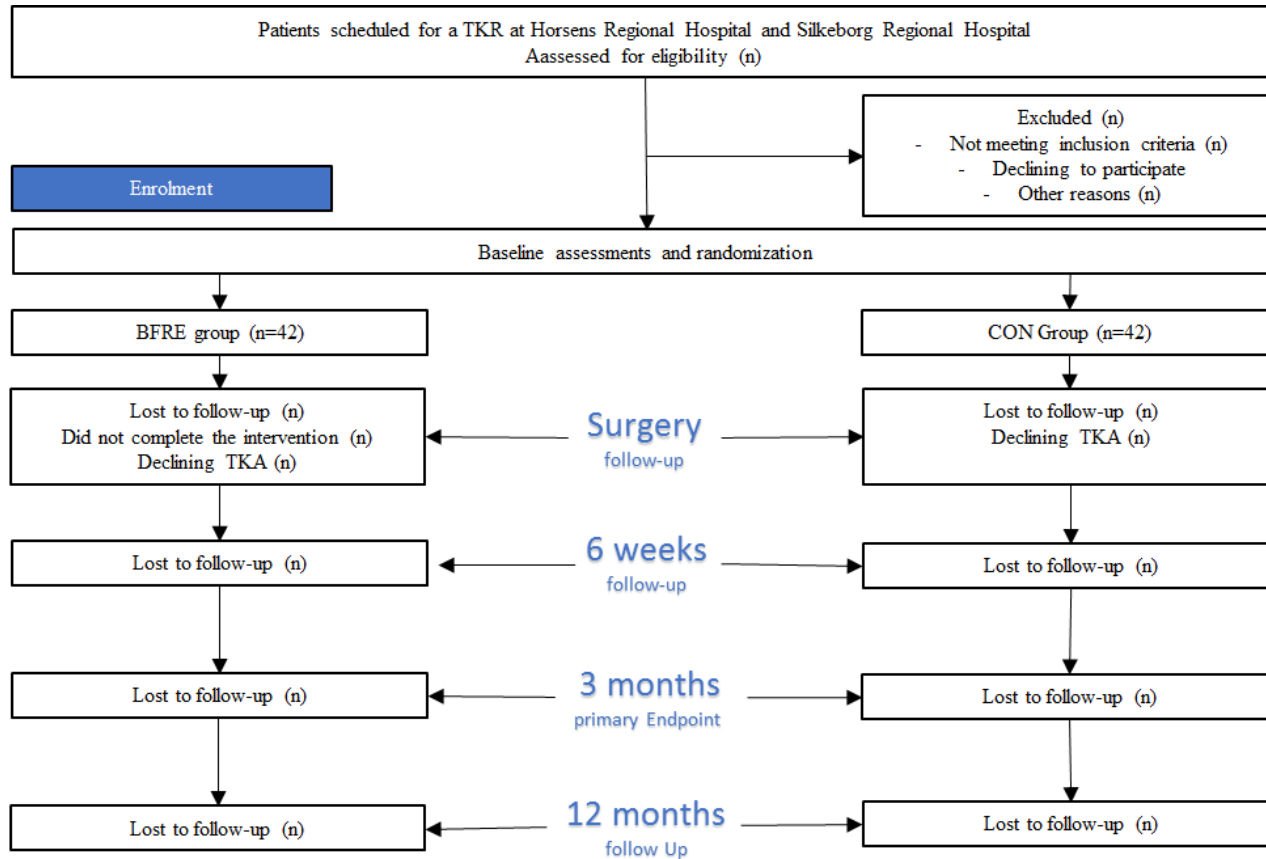
Table 1b. All exercises are performed once per day. Cycling ergometer exercise is optional.

Table 3. LOP: Total limb occlusion pressure; RM: Repetition Maximum

Table 4. KOOS = Knee disability and Osteoarthritis Outcome Score; B = Baseline; S = 0-2 days before surgery; D = during surgery; 3 months = 3 months after TKR; 12 months = 12 after TKR; NRS = Numeric Ranking Scale of pain

Figure 1. Flow chart of the enrollment, treatment, and follow-up phases. TKR: Total Knee Replacement, BFRE: Low-load blood-flow restricted exercise

Figure 1. Patient flow





STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents\*

Section/item	Item No	Description
<b>Administrative information</b>		
Title (p 1, I 1-3)	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration A: p 2, I 56-57 B:	2a 2b	Trial identifier and registry name. If not yet registered, name of intended registry All items from the World Health Organization Trial Registration Data Set
Protocol version P 1, I 22	3	Date and version identifier
Funding P 21, I 494-496	4	Sources and types of financial, material, and other support
Roles and responsibilities A: P 1, I 5-11 B: P 1, I 15-20	5a 5b 5c 5d	Names, affiliations, and roles of protocol contributors Name and contact information for the trial sponsor Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
<b>Introduction</b>		
Background and rationale P 3, I 67-133 P 3, I 70-76	6a 6b	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention Explanation for choice of comparators
Objectives P 5, I 129-136	7	Specific objectives or hypotheses
Trial design P6, I 140-145	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)

## Methods: Participants, interventions, and outcomes

Study setting P6, I 148-149	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
Eligibility criteria P6, I 155-163	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)
Interventions A: p7, I 164-240  C: p12, 283-285	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
Outcomes P 10, I 245-384	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
Table 1		
Sample size P 17, I 391-401	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
Recruitment P 6, I 148-151	15	Strategies for achieving adequate participant enrolment to reach target sample size

## Methods: Assignment of interventions (for controlled trials)

### Allocation:

Sequence generation P8, I 196-201	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions
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2	Allocation	16b	Mechanism of implementing the allocation sequence (eg, central
3	concealment		telephone; sequentially numbered, opaque, sealed envelopes),
4	mechanism		describing any steps to conceal the sequence until interventions are
5	P8, I 196-201		assigned
6			
7	Implementation	16c	Who will generate the allocation sequence, who will enrol participants,
8	P8, I 196-201		and who will assign participants to interventions
9			
10	Blinding	17a	Who will be blinded after assignment to interventions (eg, trial
11	(masking)		participants, care providers, outcome assessors, data analysts), and
12	P8, I 200		how
13		17b	If blinded, circumstances under which unblinding is permissible, and
14			procedure for revealing a participant's allocated intervention during
15			the trial
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### Methods: Data collection, management, and analysis

19			
20	Data collection	18a	Plans for assessment and collection of outcome, baseline, and other
21	methods		trial data, including any related processes to promote data quality (eg,
22	P 10, I 245-420		duplicate measurements, training of assessors) and a description of
23			study instruments (eg, questionnaires, laboratory tests) along with
24			their reliability and validity, if known. Reference to where data
25			collection forms can be found, if not in the protocol
26		18b	Plans to promote participant retention and complete follow-up,
27			including list of any outcome data to be collected for participants who
28			discontinue or deviate from intervention protocols
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30	Data	19	Plans for data entry, coding, security, and storage, including any
31	management		related processes to promote data quality (eg, double data entry;
32			range checks for data values). Reference to where details of data
33			management procedures can be found, if not in the protocol
34			
35	Statistical	20a	Statistical methods for analysing primary and secondary outcomes.
36	methods		Reference to where other details of the statistical analysis plan can be
37	P 17, I 400-420		found, if not in the protocol
38		20b	Methods for any additional analyses (eg, subgroup and adjusted
39			analyses)
40	P 17, I 400-420	20c	Definition of analysis population relating to protocol non-adherence
41			(eg, as randomised analysis), and any statistical methods to handle
42			missing data (eg, multiple imputation)
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### Methods: Monitoring

49			
50	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role
51			and reporting structure; statement of whether it is independent from
52			the sponsor and competing interests; and reference to where further
53			details about its charter can be found, if not in the protocol.
54			Alternatively, an explanation of why a DMC is not needed
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	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor

### Ethics and dissemination

Research ethics approval P 18, I 423-424	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
Consent or assent P 7, I 164-173	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
Confidentiality P 11, I 265-275	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
Declaration of interests P 22, I 514	28	Financial and other competing interests for principal investigators for the overall trial and each study site
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation
Dissemination policy P 18, 442-444	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions
P 21, I 501-502	31b	Authorship eligibility guidelines and any intended use of professional writers

- 1  
2 31c Plans, if any, for granting public access to the full protocol, participant-  
3 level dataset, and statistical code  
4

## 5 **Appendices**

- 6 Informed consent 32 Model consent form and other related documentation given to  
7 materials participants and authorised surrogates  
8  
9 Biological 33 Plans for collection, laboratory evaluation, and storage of biological  
10 specimens specimens for genetic or molecular analysis in the current trial and for  
11 future use in ancillary studies, if applicable  
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14 \*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013  
15 Explanation & Elaboration for important clarification on the items. Amendments to the  
16 protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT  
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# BMJ Open

**The efficacy of low-load blood flow restricted resistance EXercise in patients with Knee osteoarthritis scheduled for total knee replacement (EXKnee). Protocol for a multicenter randomized controlled trial.**

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-034376.R3
Article Type:	Protocol
Date Submitted by the Author:	13-Aug-2020
Complete List of Authors:	Jørgensen, Stian; Regional Hospital Horsens, Department of occupational and physical therapy; Horsens Sygehus, H-HIP Bohn, Marie; Horsens Sygehus, Department of Orthopedic Surgery Aagaard, Per; Institute for Sports Science and Clinical Biomechanics, University of Southern Denmark, Mechlenburg, Inger; Aarhus University Hospital, Department of Orthopedics; Aarhus University, Clinical Medicine
<b>Primary Subject Heading</b>:	Sports and exercise medicine
Secondary Subject Heading:	Rehabilitation medicine
Keywords:	blood flow restriction exercise, knee osteoarthritis, total knee replacement surgery, preconditioning, functional capacity

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Manuscripts



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4 1 **The efficacy of low-load blood flow restricted resistance EXercise in patients with Knee**  
5 **osteoarthritis scheduled for total knee replacement (EXKnee). Protocol for a multicenter**  
6 **randomized controlled trial.**  
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11 5 *Stian Langgård Jørgensen*<sup>1,2,5</sup>, *Marie Bagger Bohn*<sup>2</sup>, *Per Aagaard*<sup>3</sup>, *Inger Mechlenburg*<sup>4,5</sup>  
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## 25 ABSTRACT

### 26 Introduction

27 Up to 20% of patients undergoing total knee replacement (TKR) surgery report no or suboptimal  
28 pain relief after TKR. Moreover, despite chances of recovering to preoperative functional levels,  
29 patients receiving TKR have demonstrated persistent deficits in quadriceps strength and functional  
30 performance compared to healthy aged-matched adults. We intend to examine if low-load blood  
31 flow restricted exercise (BFRE) is an effective preoperative method to increase functional capacity,  
32 lower limb muscle strength and self-reported outcomes after TKR. In addition, the study aims to  
33 investigate to which extent preoperative BFRE will protect against surgery-related atrophy three  
34 months after TKR.

### 36 Methods

37 In this multicenter, randomized controlled and assessor blinded trial, 84 patients scheduled for TKR  
38 will be randomized to receive usual care and eight weeks of preoperative BFRE or to follow usual  
39 care-only. Data will be collected before randomization, three-four days prior to TKR, six weeks,  
40 three months, and 12 months after TKR. Primary outcome will be the change in 30-second chair  
41 stand test from baseline to three-month follow-up. Key secondary outcomes will be Timed Up &  
42 Go, 40-meter fast-paced walk test, isometric knee extensor and flexor strength, patient-reported  
43 outcome, and selected myofiber properties.

44 Intention-to-treat principle and per protocol analyses will be conducted. A one-way analysis of  
45 variance model will be used to analyze between group mean changes. Pre-to-post intervention  
46 comparisons will be analyzed using a mixed linear model. Also, paired student t-tests will be  
47 performed to gain insight into the potential pre-to-post training differences within the respective

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4 48 training or control groups and regression analysis will be used for analyzation of associations  
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6 49 between selected outcomes.  
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### 11 51 **Ethical approval**

12  
13 52 The trial has been accepted by the Central Denmark Region Committee on Biomedical Research  
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15 53 Ethics (Journal No 10-72-19-19) and the Danish Data Protection Agency (Journal No 652164). All  
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17 54 results will be published in international peer-reviewed scientific journals regardless of positive,  
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19 55 negative or inconclusive results.  
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### 25 57 **Trial registration**

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27 58 The trial is registered at Clinical Trials (NCT04081493)  
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### 32 60 **Article Summary**

#### 34 61 **Strengths and limitations of this study**

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36 62 • The trial is a multicenter, randomized controlled assessor blinded trial.  
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38 63 • This is the first clinical trial to investigate the effect of low-load ischemic resistance training  
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40 64 as a preconditioning method prior to elective knee replacement surgery.  
41  
42 65 • Patients will not be blinded to their allocation into intervention groups (BFR vs. control)  
43  
44 66 • This is a protocol paper  
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### 50 68 **Key words**

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52 69 Blood flow restricted exercise, knee osteoarthritis, total knee replacement surgery, preconditioning  
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## 70 INTRODUCTION

71 Knee OA is a degenerative joint disease associated with pain, reduced physical activity, and quality  
72 of life and affects almost 40% of all individuals  $\geq 60$  years of age (1-5). Approaching end-stage knee  
73 OA, total knee replacement (TKR) is often the preferred treatment choice to reduce pain and regain  
74 functional capacity. That is, TKR is considered a highly successful treatment to improve quality of  
75 life and long-term function (6). However, despite being considered highly successful,  
76 approximately 20% of the patients undergoing TKR experience a suboptimal outcome (6), which  
77 has often been suggested to be related to incomplete restoration of physical function (7). In  
78 addition, TKR patients typically demonstrate long-lasting deficits in quadriceps strength and  
79 functional performance (2, 4). This failure to return to “normal” strength levels has been suggested  
80 to be associated with preoperatively lower limb muscle strength and function (2).

81 Preconditioning exercise designed to prepare the musculoskeletal system to better tolerate  
82 stressful events such as the impact of invasive surgery has been suggested to be applicable prior to  
83 elective TKR (6). This is supported by the results of two randomized controlled trials indicating that  
84 preoperative heavy resistance strength training (HRST) may enhance functional capacity and knee  
85 extensor muscle strength three months postoperatively (7, 8). Joint pain resulting from the high  
86 mechanical loads associated with HRST may represent a barrier to this type of training in some  
87 patients suffering from severe knee OA (1, 9). Therefore, a more tolerable, yet effective, alternative  
88 is needed for this population. Also, three recent systematic reviews investigating the topic of  
89 preoperative physiotherapy-based exercise before TKR all warrant high quality, well-powered  
90 evidence to investigate the efficacy of preoperative physiotherapy before TKR (10-12).

91 Resistance training with low exercise loads ( $\sim 30\%$  1 repetition maximum) performed with  
92 concurrent partial blood flow restriction to the working limb (Blood flow restricted exercise: BFRE)  
93 has received increasing clinical interest during the last decade (1, 13-32). The application of low

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4 94 muscle/tendon/joint forces in BFRE has been documented to increase human skeletal muscle size  
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6 95 and to cause substantial strength gain in healthy young and old individuals, as well as some patient  
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9 96 populations, despite the low magnitude of mechanical stress imposed on the trained tissue (13, 25,  
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11 97 26). When applied in the clinical setting, BFRE has demonstrated positive effects on skeletal  
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13 98 muscle hypertrophy, strength, and functional capacity in mild-degree knee OA patients (1, 9, 33,  
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15 99 34) although not observed in all studies (33). Importantly, BFRE appears to be feasible with a high  
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18 100 training adherence in knee OA patients (1, 33, 34). The use of different restrictive pressures  
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20 101 (absolute restrictive pressures: 160-200 mmHg and individualized pressure of 70%; the pressure  
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22 102 needed to provide complete blood flow restriction (total limb occlusion pressure: LOP) has been  
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25 103 applied without any adverse events in mild-degree knee OA (1, 33, 34). This is in line with Hughes  
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27 104 et al. (13), who suggested that when BFRE is performed correctly, it has been demonstrated to be as  
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30 105 safe as free-flow exercise methods (13).

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32 106 Currently, no consensus exists about the appropriate restrictive pressure to induce favorable  
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34 107 muscle adaptation in patients suffering from knee OA. This might be due to the fact that the  
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36 108 effective occlusion pressure seems to be dictated by the exercise load/intensity (35). Thus, the  
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39 109 effective occlusion pressure varies between studies due to use of different exercises or differences  
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41 110 in exercise load and intensity. Restrictive pressures ranging from 40%-80% of total arterial leg  
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43 111 occlusion pressure (LOP) have been suggested to be sufficient to evoke muscular adaptation in  
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45 112 healthy adults (14, 17, 18, 36). If the load is less than 30% 1RM, higher restrictive pressures seems  
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48 113 required to evoke muscle hypertrophy, while lower pressures (40% LOP) requires training loads of  
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50 114 30% 1RM or above to be performed (36). Injury or joint pain (i.e. from the knee) might limit the  
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53 115 amount of resistance applied during strength testing, and may thus compromise the ability to rely  
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55 116 fully on a given 30% 1RM estimation. Therefore, higher pressures than 40% LOP are suggested to  
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57 117 be used in clinical settings (36). On the other hand, higher pressures are associated with more  
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discomfort during exercise and in between-set rest pauses (14), which potentially can affect exercise motivation negatively in patients. Thus, an occlusion pressure sufficiently high to evoke measurable muscle adaptation despite potentially exercising at loads lower than 30% 1RM; yet tolerable to maintain a high adherence, seems a favorable choice for this particular patient population.

The adaptive mechanisms evoked by BFRE seem to involve accumulation of metabolites, ischemia (transient tissue hypoxia), which may increase recruitment of higher threshold (Type II) fibers through stimulation of group III and IV afferent nerve fibers (37, 38), and also activation of myogenic muscle stem cells (satellite cells: SC) (13, 26, 31). SC are cells positioned between the sarcolemma and the myofiber basal lamina (31, 39). SC play an important role in human skeletal muscle growth due to their ability to donate new myonuclei to the muscle fibers (31, 40-44). That is, the human skeletal muscle fibers are multinucleated cells with each myonucleus controlling the protein synthesis of a certain cytoplasmatic area in the muscle fiber (40-42, 45). Myonuclei transcriptional activity can be fully maximized with exercise, hence requiring new myonuclei to support further muscle tissue accretion (41, 42, 44). It has been suggested that exercise-related addition of SC and myonuclei by means of BFRE might reduce the muscle atrophy related to bedrest and/or prolonged inactivity (31, 46). Previous studies applying short term (10 days) preoperative BFRE before an anterior cruciate ligament rupture-reconstruction found no atrophy protective effect or higher postoperative muscle strength compared to performing a low-load exercise without blood flow restriction (placebo). However, it might be questionable if the applied training frequency, intensity and training period have been sufficient to promote SCs and myonuclei addition. Thus, longer periods of intensive training might be necessary to promote the desired muscle morphological adaptations (addition of myonuclei and increased SC content).

### **Aim and hypothesis of the trial**

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4 142 The primary aim of this trial is to investigate the efficacy of eight weeks of BFRE compared to  
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7 143 receiving usual care prior to TKR on postoperative chair stand performance. We hypothesize that  
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9 144 eight weeks of preoperative BFRE will lead to increased 30 second chair stand performance (30-  
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11 145 second Chair Stand Test: 30-s CST) when assessed three months postoperatively. Secondary aims  
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14 146 are to investigate the efficacy of preoperative BFRE on lower limb muscle strength three months  
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16 147 after TKR and investigate the potential relationship to functional capacity and quality of life.  
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18 148 Furthermore, it will be investigated to which extent eight weeks of BFRE induce myofiber  
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20 149 hypertrophy and gain in satellite cell number and myonuclei content in the knee extensor  
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23 150 musculature.  
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## 27 152 **MATERIAL & METHODS**

### 30 153 **Design**

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32 154 The trial is designed as a multicenter (two sites), randomized, assessor blinded, controlled trial  
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34 155 following the CONSORT guidelines (47). Primary endpoint will be three months after TKR.  
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36 156 Additional and secondary endpoints will be evaluated during the week of TKR, six weeks after  
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39 157 TKR (questionnaires only) and 12 months after TKR. Muscle biopsies will be obtained from all  
40  
41 158 patients undergoing surgery at Horsens Regional Hospital at baseline, during surgery and three  
42  
43 159 months after TKR.  
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46 160

### 48 161 **Participants**

49  
50 162 Patients will be recruited from the Departments of Orthopedic Surgery at Horsens and Silkeborg  
51  
52  
53 163 Regional Hospitals in Denmark. Patient enrollment will start September 2<sup>nd</sup> 2019 at Horsens  
54  
55 164 Regional Hospital and October 1<sup>st</sup> 2019 at Silkeborg Regional Hospital. Patient recruitment is  
56  
57 165 expected to be completed in June 2021. All patients are expected to have completed baseline testing  
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166 in September 2021. To account for surgery and intervention, the three-month follow-up will be  
167 concluded in April 2022. Thus, at the end of September 2022 all patients are expected to have  
168 completed 12-month follow-up testing.

169  
170 Inclusion criteria: 1) Patients  $\geq 50$  years scheduled for TKR due to knee OA at Horsens- or  
171 Silkeborg Regional Hospital.

172  
173 Exclusion criteria: 1) Severe cardiovascular diseases (New York Heart Association (NYHA) class  
174 III and IV), previous stroke incident, thrombosis incident; 2) traumatic nerve injury in affected limb  
175 3) unregulated hypertension (systolic  $\geq 180$  or diastolic  $\geq 110$  mmHg) 4) spinal cord injury; 5)  
176 planned other lower limb surgery within 12 months; 6) cancer diagnosis and currently undergoing  
177 chemo-, immuno-, or radiotherapy; 7) inadequacy in written and spoken Danish; 8) an existing  
178 prosthesis in the index limb; 9) living more than 45 minutes from either Horsens Regional Hospital  
179 or Silkeborg Regional Hospital; 10) pregnancy.

180  
181 *Please insert figure 1 around here*

182  
183 All patients will be screened for eligibility by four orthopedic chief physicians at Horsens Regional  
184 Hospital and by three orthopedic chief physicians at Silkeborg Regional Hospital who will perform  
185 the initial inclusion of study participants and hand out written project information. All patients  
186 accepting to participate will be asked to complete a written informed consent allowing the  
187 physiotherapist (at Horsens Regional Hospital and Silkeborg Regional Hospital) to contact the  
188 patients by phone for a final eligibility and exclusion criteria-screening and book an appointment  
189 for baseline testing. If the patient agrees to participate in the trial, he/she will sign a written

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4 190 informed consent to participate in the project. Subsequently, the patient will be baseline-tested at  
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6  
7 191 the hospital by a blinded (to group allocation) assessor. Patients declining to participate in the RCT  
8  
9 192 will be offered the option of participating in a parallel observational cohort trial. All patients  
10  
11 193 included in the project will be scheduled for a TKR. Two-three weeks before surgery all patients  
12  
13  
14 194 will be invited to a, preoperative information meeting where nurses, surgeons, and physiotherapists  
15  
16 195 will provide detailed information on pain management, nutrition, the surgical procedure, physical  
17  
18 196 activity, postoperative home-based rehabilitation (table 1a and 1b), load management, etc. (usual  
19  
20  
21 197 care) (48). On the day of surgery, patients will be hospitalized at Horsens Regional Hospital or  
22  
23 198 Silkeborg Regional Hospital where an orthopedic chief physician will perform the TKR procedure.  
24  
25 199 The day after surgery all patients will receive physiotherapy-supervised training once or twice per  
26  
27 200 day by a physiotherapist in order to fulfill the discharge criteria (table 2a and 2b) (48). Patients will  
28  
29  
30 201 generally be discharged within ~one-two days after fulfilling all the discharge criteria listed above.  
31  
32 202 After discharge, all patients will receive a standard home-based rehabilitation program focusing on  
33  
34 203 improving knee joint mobility, increasing the tolerance for standing without assistive devices, and  
35  
36  
37 204 lower extremity muscle strength. Variations in the selection of exercises and exercise variables exist  
38  
39 205 in the standard home-based rehabilitation programs between the respective hospitals; however, the  
40  
41 206 purpose of the programs is identical. If the patients do not fulfill the discharge criteria, they will be  
42  
43  
44 207 offered supervised knee-specific exercise therapy at a municipal rehabilitation center or specialized  
45  
46 208 hospital-based rehabilitation after discharge from the hospital.  
47  
48 209  
49

50 210 *Please insert table 1a and 1 b about here*  
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53 211  
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55 212 *Please insert table 2a and table 2b around here*  
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4 **214 Randomization**

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6 215 After baseline assessment, patients will be randomized (1:1) using the Research Electronic Data  
7  
8  
9 216 Capture (REDCap) randomization system to either the training (BFRE) group or the control (CON)  
10  
11 217 group. Prior to randomization, all patients will be booked for follow-up test sessions and surgery.  
12  
13 218 All randomization procedures will be performed by the physiotherapists in charge of the BFRE  
14  
15  
16 219 training. Assessors performing the tests will be blinded to group allocation until completion of the  
17  
18 220 trial. A flow chart of the patient allocation procedures is depicted in Figure 1.  
19

20  
21 221  
22  
23 222 CON group: Participants in CON will receive usual care (see above) prior to TKR and be  
24  
25 223 encouraged to continue their usual lifestyle up until TKR.  
26

27 224  
28  
29 225 BFRE group: In addition to receiving usual care (cf. above), participants in the BFRE group will  
30  
31 226 perform supervised BFRE sessions three times per week for eight weeks supervised by a  
32  
33  
34 227 physiotherapist educated in administering BFRE. All BFRE training will be performed at Horsens  
35  
36 228 Regional Hospital and Silkeborg Regional Hospital.  
37

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41 230 **Intervention procedures**

42  
43 231 BFRE

44  
45 232 Each BFRE session will consist of a 10-minute warm up (ergometer cycling) followed by two  
46  
47  
48 233 different unilateral lower-limb resistance training exercises: 1) leg press and 2) knee extension  
49  
50 234 performed on standard strength training machines. Each exercise will be performed with the  
51  
52 235 affected lower limb only and consist of four rounds interspaced by 30 seconds of rest (table 3). First  
53  
54  
55 236 round: 30 repetitions (reps); second round: 15 reps; third round: 15 reps; fourth round: until  
56  
57 237 exhaustion (Table 1). If patients can perform more than 15 repetitions in the fourth exercise set, the  
58  
59 238 exercise load will be increased with the minimum extra load possible (30). Participants will be  
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4 239 instructed to perform both the eccentric and concentric contraction phases using a steady 2-second  
5  
6 240 pace duration. The fourth and final exercise set will be performed to the point of exhaustion defined  
7  
8  
9 241 as being unable to complete the final concentric contraction phase in 2 seconds. During the 30  
10  
11 242 second rest period, patients will rest in a standardized resting position while maintaining the initial  
12  
13 243 cuff-pressure. Between each exercise, patients will have a 5-minute "free-flow" rest period. The 5  
14  
15  
16 244 minutes rest period applied between exercises was chosen based on experiences from a previous  
17  
18 245 pilot project (Jorgensen & Bohn 2019, unpublished data) and experience with applying BFRE in  
19  
20 246 clinical practice. In both situations, we often experienced that patients stayed seated in the leg press  
21  
22  
23 247 machine for >2 minutes after the last (fatiguing) set to feel sufficiently rested and confident to walk  
24  
25 248 from one exercise machine to another. The cuff will be released immediately after completion of the  
26  
27 249 final exercise set.

28  
29  
30 250 The occlusion pressure during both exercises will be set at 60% of total limb occlusion pressure  
31  
32 251 (LOP) and the starting load intensity will be 30% with 1 repetition maximum (1RM) in both  
33  
34 252 exercises.

35  
36 253 Individual LOP will be determined using a pneumatic, conically shaped, 12 cm wide, rigid cuff  
37  
38  
39 254 (Occlude Aps, Denmark) attached to the patient's most proximal area of the thigh on the affected  
40  
41 255 side. While sitting on an examination table with the ankle and 1/3 of the lower limb off the table, a  
42  
43 256 vascular Doppler probe (EDAN Instruments, inc., China) will be placed posterior to the medial  
44  
45  
46 257 malleolus over the posterior tibial artery to capture the auscultatory pulse. To determine the cuff  
47  
48 258 pressure (mmHg) needed for total blood flow occlusion, the cuff will gradually be inflated in 20  
49  
50 259 mmHg steps until reaching the pressure where the auscultatory pulse is interrupted (LOP). The first  
51  
52  
53 260 time the auscultatory pulse is interrupted, the examiner releases 10-20 mmHg pressure from the cuff  
54  
55 261 until the auscultatory pulse is present again. When the auscultatory pulse reappears, the cuff is  
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57 262 inflated with 10 mmHg until the LOP is found again. If the second LOP is identical to the first, it  
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263 will be defined as the LOP for that specific patient. Otherwise, the procedure will be repeated until  
264 determining an identical LOP two consecutive times.

*Please insert Table 3 about here*

### **Outcome variables**

269 Outcome assessments will be performed at baseline (before randomization), three-four days before  
270 surgery, six weeks after TKR, three months after TKR, and 12 months after TKR. To reduce the  
271 number of postoperative visits, only questionnaires; The Knee disability and Osteoarthritis Outcome  
272 Score (KOOS), EuroQol Group 5-dimensions (EQ-5D-L5) and reporting of adverse event or  
273 receiving supervised physiotherapy postoperatively will be sent via email six weeks after surgery.  
274 Two testers (two trained physiotherapists) blinded to group allocation will perform all baseline and  
275 follow-up measurements. Bergström needle muscle biopsies (49) will be taken from vastus lateralis  
276 of the quadriceps muscle in both lower limbs from patients included at Horsens Regional Hospital  
277 only at baseline, during surgery, and three months after TKR by doctors trained in performing the  
278 procedure. An overview of the data collection parameters is presented in Table 4.

279 Before starting the baseline testing, all assessors will be thoroughly trained in performing  
280 the tests according to the standardized test procedures for each test method. All assessors will be  
281 blinded to intervention allocation (pre surgery BFRE training or usual care). Further, assessors will  
282 be trained in how to communicate with the participants at follow-up test sessions to avoid break of  
283 blinding due to miscommunication. Also, all cases where blinding is being broken will be  
284 registered. Also, the physiotherapist in charge of LL-BFRE will be thoroughly trained in  
285 performing the exercise on healthy subjects before applying LL-BFRE on study-patients. At the last  
286 scheduled exercise session (i.e. 24th session), the physiotherapists in charge of LL-BFRE will

carefully remind the participants not to reveal their group allocation to any assessors at any time point during post testing.

The primary investigator will be in weekly contact with the physiotherapists supervising the LL-BFRE at Horsens Regional Hospitalet and Silkeborg Regional Hospital where day-to-day-retraining and supervision can be arranged. Furthermore, physiotherapists supervising the LL-BFRE will receive in-depth retraining every three months.

## Outcomes

*Please insert Table 4 about here*

### *Primary outcome*

**The 30s-CST** will be assessed using a 44 cm (seat height) chair with armrests. The 30s-CST measures the number of sit-to-stand repetitions completed within 30 seconds. The 30s-CST is considered a valid and sensitive measure of lower-extremity sit-to-stand function with good to excellent intra- and inter-observer reliability (50-52).

### *Secondary outcomes*

**The Timed Up & Go test (TUG)** assesses the time required for patients to stand from a 44 cm (seat height) chair walk around a tape mark 3 meters away and sit into the chair at return. The patients will be instructed to walk as fast and safely as possible towards the tape mark (and touch the tape mark (with at least one foot), turn around and return to the chair and sit down. Use of armrests is allowed. The fastest of two trials will be used for further analysis. Up to one minute of

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rest will be allowed between trials (53, 54). Good inter-rater reliability has been demonstrated with the TUG test (52).

**4x10 meter walk test (40m-FWT)** measures the total time it takes to walk 4 x 10 meters excluding turns (meter/sec) (52). Patients will be instructed to walk as quickly and as safely as possible without running to a visible mark 10 meters away, return and repeat for a total distance of 40 meters (52). Prior to the test, one practice trial will be provided to check understanding. The 40m-FWT is a valid and responsive measure for assessing short distance maximum walking speed with excellent inter-rater reliability (52).

**1RM leg press strength** will be estimated from a 5-8RM leg press test. Patients perform three low-load warm-up sets. The first and second warm-up sets consist of 12 repetitions, and the third warm-up set consists of eight repetitions. The load of each warm-up set will be increased with 10 kilos. After warm-up, the load will be increased to determine the 5RM. If the 5RM cannot be determined within three trials, a fourth all-out trial (as many repetitions as possible) will be performed. The 1RM will be calculated as  $[1RM = \text{load (kg)} / 1.0278 - 0.0278 \cdot \text{number of repetitions}]$  (55).

**1RM knee extension strength** will be estimated from 5-8RM knee extension test as described above for the estimation of 1RM leg press test (55).

**Maximal isometric voluntary contraction (MVC) of the knee** will be measured using a handheld dynamometer (HHD). The patients will be seated on an examination table with knees and hips positioned at 90° flexion. The patients will be instructed to remain seated in an upright position and place both hands on the shoulder to avoid compensation. The HHD will be fixed with a rigid belt to the examination table. Adjustable straps will be used to allow MVCs of the knee extensors to be performed at 90° knee flexion in all patients. The HHD will be positioned 5 cm above the medial

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336 malleolus (56). The patients will be instructed to produce as much force as possible into the HHD.

337 Good to excellent inter- and intra-rater reliability has previously been demonstrated on group-level

338 in patients suffering from knee OA for maximum knee extensor muscle strength testing with HDD

339 (56, 57). Patients will receive four trials. For analysis, the mean maximal strength of the second,

340 third and fourth measures will be calculated and corrected for bodyweight (56)

341

342 **MVC of the knee flexors** will be measured and performed using HHD at 90° knee flexion with the

343 patients seated identically as during MVC for the knee extensors (56). The HHD will be positioned

344 posterior aspect of calcaneus (56) and patients will be instructed to produce as much force as

345 possible into the HHD. Good to excellent inter- and intra-rater reliability has previously been

346 demonstrated on group-level in patients suffering from knee OA for maximum knee flexor muscle

347 strength testing with HDD (56). Patients will receive four trials. For analysis, the mean maximal

348 strength of the second, third and fourth measures will be calculated and corrected for bodyweight

349 (56)

350

351 **Myofiber cross sectional area (CSA), muscle fiber type composition, satellite cell content, and**

352 **myonuclei number** will be assessed by obtaining needle biopsies (100-150 mg) from all patients

353 enrolled at Horsens Regional Hospital. The biopsies will be obtained bilaterally from the middle

354 portion of the vastus lateralis muscle utilizing the percutaneous needle biopsy technique of

355 Bergström (49, 58, 59). Biopsies will be performed by two experienced orthopedic surgeons (chief

356 physicians) trained in performing the needle muscle biopsy technique at Horsens Regional Hospital.

357 Efforts will be made to extract tissue from the same region (2-3 cm apart) and depth (~1-2 cm.)

358 (49). The tissue samples will be dissected of all visible blood, adipose tissue, and connective tissue

359 and mounted in Tissue-Tec (4583, Sakura Finetek, Alphen aan den Rijn, The Netherlands), frozen

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4 360 in isopenate pre-cooled with liquid nitrogen, and stored at -80°C (31, 49, 60). All muscle samples  
5  
6  
7 361 will be analyzed as previously described by Nielsen et al. (31) using immunofluorescence  
8  
9 362 microscopy. Transverse serial sections (8 µm) of the embedded muscle biopsy specimen will be cut  
10  
11 363 at -22°C using a cryostat (HM560; Microm, Walldorf, Germany) and will be mounted on glass  
12  
13  
14 364 slides for subsequent analysis as described in detail elsewhere (31). Myogenic stem cells (satellite  
15  
16 365 cells (SC)) will be visualized with an antibody against Pax7 (31). Type I (stained) and Type II  
17  
18 366 (unstained) myofibers will be differentiated, and muscle fiber area will be determined (31); MSC-  
19  
20  
21 367 derived nuclei will stain positive for Pax7 and be within the basal lamina; nuclei (DAPI stained)  
22  
23 368 with a sublaminar placement will be considered myonuclei (31).

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25 369  
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27 370 **Knee disability and Osteoarthritis Outcome Score (KOOS)** is a patient-administered knee  
28  
29  
30 371 specific questionnaire comprising five subscales: Pain; Symptoms; Activities of daily living; Sport  
31  
32 372 & Recreation; and Knee-Related Quality of Life. Each item is scored from 0 to 4 (61). The raw  
33  
34 373 score for each of the five subscales is the total sum of the associated item scores. Scores can be  
35  
36  
37 374 transformed to a 0 to 100 scale. The scores of the five subscales can be expressed as a composite  
38  
39 375 outcome profile, higher scores indicating fewer problems (62). The KOOS questionnaire is valid  
40  
41 376 and reliable in patients suffering from knee OA and patients on the waiting list for TKA for knee  
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44 377 OA (61, 63, 64).

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**EuroQol Group 5-dimension (EQ-5D-5L)** is a self-completion questionnaire consisting of two parts; the first part of the EQ-5D-5L comprises five dimensions involving mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. All dimensions have five response categories (no problems, slight problems, moderate problems, severe problems, and extreme problems) resulting in a five digit descriptive health state (65), which will be converted into a summary index ranging from -0.624 (worst) to 1.000 (best), using a Danish value set (66). The second part, EQ-VAS rates the overall current health status from 0 (worst imaginable health) to 100 (best imaginable health) (65). The EQ-5D-5L is reliable and valid in patients with knee OA eligible for TKA (67, 68).

**Adverse events** will be defined as unpredicted or unintended events, signs, or disease occurring during the period from inclusion until the 3-month follow-up (primary end-point) resulting in contact with the healthcare system (hospital or general practitioner) independent of whether or not the event is related to the intervention or outcome assessments. Adverse events will be recorded and categorized in accordance with the definitions established by the United States Food and Drug Administration [88]. Continuous registration of adverse events will be performed and a short open-ended questionnaire will be administered at three months follow-up.

#### *Other Outcome Measures*

**Blood pressure** will be measured by the orthopedic chief physicians when patients are visiting the outpatient clinic. Blood pressure will be used to determine eligibility to participate in the project.

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401 **Exercise compliance and progression** will be obtained by the physiotherapist in charge of the  
402 training sessions and entered directly into the REDCap-system. The progression will be monitored  
403 as the total load lifted by the patient for exercise session.

404  
405 **Numeric rating scale for pain** is a segmented unidimensional 11-item measure of pain intensity in  
406 adults (69) that will be used to rate pain intensity during both testing and exercise sessions. (69). 0  
407 represents no pain while 10 represents worst pain imaginable (69).

408  
409 **Declining to be operated** will be measured at three month follow-up, where patients will be asked  
410 whether they decided to be operated or not. Patients who declined to be operated will be invited to  
411 participate in all prescheduled follow-up assessments.

412  
413 **Postoperative supervised physiotherapy** will be measured at six week, three month, and 12 month  
414 follow-up by answering a questionnaire. If patients have participated in postoperative supervised  
415 physiotherapy, the patient must specify whether the treatment was related to the TKR or due to  
416 other circumstances.

417  
418 **Knee joint active range of motion** will be measured with a 360° plastic goniometer (scale 1°) with  
419 16.5 cm moveable arms at baseline in the week of surgery, three months, and 12 months after  
420 surgery. Laying supine on an examination table, the knee joint flexion and knee joint extension will  
421 be measured separately (70). The tester then identifies the most prominent part of the trochanter, the  
422 lateral epicondyle of the femur, the lateral head of fibula, and the lateral malleolus. When identified,  
423 the patient is asked to flex the knee as much as possible with the heel maintaining contact to the  
424 surface at all time (70). Secondly, the patients will be asked to extend the knee joint as much as

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4 425 possible. To allow the knee to extend as much as possible, a firm quadratic box (height: 5 cm,  
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6 426 width: 8 cm, length: 15 cm) will be placed under the heel of the patient. The procedure of  
7  
8  
9 427 measuring knee extension will be similar to knee flexion, as the patients increases the degree of  
10  
11 428 knee extension maximally (70) The fulcrum of the goniometer will correspond visually to the trans-  
12  
13 429 epicondylar axis of the knee joint. The moveable arms of the goniometer will be pointed towards  
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15  
16 430 the greater trochanter and the lateral malleolus (70).  
17

### 18 431 19 20 432 **Data management**

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23 433 All data from the physical function tests will be entered into RedCap by the assessors using double  
24  
25 434 data entry to ensure data quality. All patient-reported outcome data (KOOS, NRS Pain, EQ-5D-5L)  
26  
27 435 will be entered directly into RedCap by the patients, and usage of the “required fields” will ensure  
28  
29  
30 436 no missing items from the completed questionnaires. To reduce missing data, a reminder email will  
31  
32 437 be sent automatically from the RedCap-system. All patient data will be anonymized by assigning  
33  
34 438 study numbers to each patient (coding). Personal data about the patient will be located separately  
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36  
37 439 from the main dataset to protect confidentiality during all trial phases.

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39 440 The raw dataset will be maintained for ten years after completion of the trial with indefinite  
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41 441 restricted access due to sensitive data. After publication of the trial, a fully anonymized patient-level  
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43 442 dataset and corresponding statistical description will be made publicly available if required by the  
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45  
46 443 scientific journal, in which the results are published.  
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### 48 444 49 50 445 51 52 446 **Sample size**

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55 447 The power and sample size calculation is based on the expected differences between the two subject  
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57 448 groups from baseline to three-month follow-up (8). Due to lack of data on the primary outcome for  
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investigations applying LL-BFRE before a surgical procedure, we decided to base our sample size calculation on Skoffler et al. (8) who investigated the efficacy of four weeks of preoperative and four weeks postoperative HRST (intervention group) compared to four weeks of postoperative HRST only (control group) on 30-s CST three months in patients receiving a TKR (8). The authors found a between-group difference of 3-4 repetition difference ( $14.7 \pm 4.7$  repetitions versus  $11.0 \pm 4.4$  repetitions) three months after TKR surgery (8).

To reduce the probability of type I errors and enable detection of a between-group difference also,  $\alpha$ -level is set at 0.05 ( $p < 0.05$ ) and  $\beta$ -level is set at 0.20 (80% power). Expecting a 3-repetition between-group difference three months postoperatively and assuming a SD of 4.7 in both groups, 39 patients are required in each group (yielding 78 patients in total). With an anticipated dropout rate of 10%, 84 patients will be recruited for the trial.

### Statistical considerations

The primary efficacy analysis will be an assessment of the between group difference in change in the 30-S CST from baseline to three-month follow-up (primary endpoint).

All descriptive statistics and tests will be reported in accordance with the recommendations of the “Enhancing the QUALity and Transparency Of health Research” (EQUATOR) network (71) and the CONSORT statement (47). Intention-to-treat principle (i.e. all patients as randomized independent of departures from allocation treatment, compliance and/or withdrawals) and per protocol analysis will be conducted. A one-way analysis of variance (one-way ANOVA) model will be used to analyze between group mean changes in continuous outcome measures (31). The model includes changes from baseline to 12-month follow-up. Between-intervention comparison from baseline to three months after surgery will be analyzed using a mixed linear model with patient ID as a random effect and time, group and hospital as fixed effects (31, 72). Also, to gain insight into

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4 473 the potential pre-to-post training differences within the respective training or control groups, paired  
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7 474 student t-tests will be performed. Level of statistical significance is  $P < 0.05$ . *Secondary outcome*  
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9 475 *variables:* Between-intervention comparison from baseline to the week of surgery, six weeks after  
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11 476 surgery, three and 12 months after surgery will be analyzed as described for the primary outcome.  
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14 477 Regression analysis will be used to analyze the potential associations between preoperative strength  
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16 478 and postoperative lower extremity function and self-reported outcome as well as between  
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18 479 preoperative functional capacity and postoperative functional capacity. Additionally, regression  
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21 480 analysis will be used to analyze the association between preoperative number of satellite cells and  
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23 481 myonuclei on postoperative isometric knee extensor muscle strength, muscle fiber cross sectional  
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25 482 area, and functional capacity. All statistical analyses will be performed by the primary investigator  
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27 483 using Stata.  
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### 31 32 485 **Ethical aspects and dissemination**

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34 486 The trial has been accepted by the Central Denmark Region Committee on Biomedical Research  
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37 487 Ethics (Journal No 10-72-19-19) and by the Danish Data Protection Agency (Journal No 652164).  
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39 488 The trial is registered at Clinicaltrials.gov (NCT04081493). Before inclusion, all patients will  
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41 489 provide their written informed consent in accordance with the Helsinki Declaration. All data and  
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44 490 information collected in regard to this trial will be treated confidentially (blinded and encrypted) by  
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46 491 the researchers and staff connected to the trial.  
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48 492 All results from the trial will be published in international peer-reviewed scientific journals  
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50 493 regardless of the results being considered positive, negative or inconclusive.  
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### 54 55 495 **Patient and public involvement**

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57 496 Before developing this clinical trial, a pilot project was performed to determine the feasibility and  
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497 efficacy of BFRE in patients suffering from lower limb injuries. The experiences with the training  
modality and the verbal feedback from patients on training duration, frequency, and intensity  
498 resulted in useful knowledge that certainly has improved the development of the present clinical  
499 trial.

## 502 **DISCUSSION**

503 To the best of our knowledge, this is the first trial to investigate the effect of preoperative BFRE on  
504 functional capacity, self-reported outcome, lower limb muscle strength and myofiber  
505 morphology/stem cell abundance in patients scheduled for TKR. Only few studies have investigated  
506 (short term (10 days)) preoperative BFRE without finding an atrophy protective effect or difference  
507 in muscle strength compared to a control group performing a placebo intervention (SHAM group)  
508 (73). However, patients performing short term preoperative BFRE before ACL-R demonstrated  
509 higher muscle endurance compared to a SHAM group (74). Therefore, results of this trial are  
510 expected to provide novel information on longer periods of BFRE that will enable researchers to  
511 design effective exercise-based preconditioning protocols for elective TKR patients. The LL-BFRE  
512 protocol applied in the present project is widely used and follows the recommendations from a  
513 recent position stand by Patterson et al. (75). The authors suggested that exercising 2-3 times per  
514 week at 20-40% of 1RM in 2-4 sets (e.g. 30-15-15-15 or sets to failure) using pressures between 40  
515 to 80% of LOP has demonstrated to be effective when aiming at increasing muscle strength and  
516 promoting muscle hypertrophy (75).

517 The trial is designed as an assessor blinded randomized controlled trial, thus representing the  
518 highest evidence level. However, the nature of the trial does not allow blinding of the participants  
519 which is an inherent limitation of the trial. The trial is conducted at two hospitals that consistently  
520 perform a high number of TKR procedures annually (225 and 460, respectively), thus securing a

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521 strong expertise in terms of surgery and infrastructure. Both hospitals have all equipment needed  
522 available for surgery, post-operative hospitalization, training, and testing. All outcome variables are  
523 considered valid and reliable measures and consist of both objective outcomes and self-reported  
524 patient outcomes.

525 No adverse health-related events have been reported in previous studies applying BFRE in  
526 patients' suffering from knee OA or in healthy older adults (1, 9, 13, 23, 33, 34). Further, in a recent  
527 review and meta-analysis it was stated that exercise with concurrent blood-flow restriction is a safe  
528 exercise modality when occlusion procedures are applied correctly (13). The inherent invasive  
529 procedure of muscle biopsies may cause adverse events in rare occasions. Therefore, all muscle  
530 biopsy samples will be collected by trained medical doctors and performed following administration  
531 of local anesthesia and in fully sterile conditions. The needle muscle biopsy protocol has been  
532 applied in a large number of previous investigations including very old frail subjects (97 years of  
533 age) without any reporting of adverse events besides occasional muscle soreness(31, 49, 58, 76, 77).

534 There are some limitations of the project that must be taken into account. First, our primary end  
535 point is three months postoperatively. The (uncontrolled) period discharge to three months  
536 postoperatively renders the project vulnerable to external variabilities. However, from a pragmatic  
537 point of view, this uncontrolled period from discharge to three-month follow-up reflects the reality  
538 that Danish patients face postoperatively. Thus, the results at three-month follow-up will, indeed,  
539 reflect the impact of performing preoperative LL-BFRE on the postoperative outcome regardless of  
540 the external variable that can hamper the results. Secondly, the discharge criteria at Horsens  
541 Regional Hospital and Silkeborg Regional Hospital withhold slight differences. That is, the  
542 acceptable knee joint ROM at discharge differs between the sites, thus it can be speculated that  
543 more patients from Silkeborg Regional Hospital will be offered a postoperative, supervised  
544 rehabilitation program. This might affect the number of patients receiving supervised physiotherapy

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4 545 after discharge between sites. However, all patients included in the present project will report  
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6 546 whether they have received postoperative supervised physiotherapy at all follow-up assessments.  
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9 547 Thus, we will be able to determine (and normalize) a potential between-site difference in patients  
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11 548 receiving supervised physiotherapy after TKR. Also, site-specific differences in the postoperative  
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13 549 rehabilitation protocols (Tables 1a and 1b) may be considered a limitation. That is, the protocols  
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16 550 contain both identical but also different exercises and progression steps. However, a recent review  
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18 551 and meta-analysis found no difference in effectiveness between clinic-based or inpatient programs  
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20 552 compared with home-based rehabilitation programs in the early subacute period after TKA (27) and  
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23 553 studies in other knee patient populations have also been unable to observe differences in main  
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25 554 outcome variables when comparing home-based postoperative rehabilitation to supervised  
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27 555 postoperative rehabilitation (28, 29). We feel confident therefore that the apparent differences  
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30 556 between the postoperative rehabilitation protocols are not highly likely to affect the results of the  
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32 557 present study. Nonetheless, to verify this notion we will introduce site allocation (Horsens Hospital  
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34 558 vs. Silkeborg Hospital) as a separate independent variable in the mixed linear model used for the  
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36 559 statistical analysis.  
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### 43 562 **Author contributions**

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45 563 SLJ, PAA, MBB, and IM were all part of designing the trial and approved the final version of the  
46  
47  
48 564 protocol. Also, SLJ, PAA, MBB, and IM wrote and revised the protocol.  
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### 50 565 51 52 566 **Data statement**

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55 567 All obtained data will be stored in anonymized form at the Danish National Archives and deleted  
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57 568 after 10 years.  
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### **Competing interest**

None to be declared

### **Ethics approval**

The trial has been accepted by the Central Denmark Region Committee on Biomedical Research Ethics (Journal No 10-72-19-19) and by the Danish Data Protection Agency (Reference No 652164).

### **Word count**

5.770 words

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peer review only

821 *Table 1a.* Postoperative rehabilitation program, Horsens Regional Hospital

Week 0-3				
Step	Exercise	Repetitions	Sets	Resistance
Step 1 & 2	Supine peristaltic pump exercise with feet above heart level	20 minutes	3-4/day	-
Step 1	Supine knee extension mobilization	20 seconds	3 sets	-
Step 1	Supine unilateral knee and hip extension and flexion mobilization with slipper under the heel	5 repetitions	3 sets	Slipper minimizes floor friction
Step 2	Seated knee extension and flexion mobilization with slipper under the foot	5 repetitions	3 sets	Slipper minimizes floor friction
Step 2	Standing weight transfer exercise	15 repetitions each side	1 set	Bodyweight
Step 2	Sit to stand from a high chair or the edge of table	5 repetitions	3 sets	Bodyweight
Week 3 and onwards				
Step 1 & 2	Supine peristaltic pump exercise with feet above heart level	20 minutes	3-4/day	-
Step 1	Seated knee extension mobilization	20 seconds	4 rounds	Arms can be used to apply pressure onto the knee to help extend the knee
Step 1	Step up exercise	10-15 repetitions	2-3 sets	Bodyweight
Step 1	Standing knee isometric knee towel press	10-15 repetitions	2-3 sets	Ball/Towel rolled together
Step 1	Sit to stand from a chair	10-15 repetitions	2-3 sets	Bodyweight
Step 1	One leg standing	30 seconds	1 set	Bodyweight
Step 2	Standing hip flexion	Not informed	Not informed	Elastic band
Step 2	Standing hip abduction	Not informed	Not informed	Elastic band
Step 2	Partial frontal plane sliding lunge	10 repetitions	3 sets, 2-3/day	Bodyweight
Step 2	Partial back sliding lunge	10 repetitions	3 sets, 2-3/day	Bodyweight
Optional	Cycling	10-20 minutes	1 set	Light resistance can be added when it is possible to perform a full round with the operated limb.

Step 1 is performed in the morning and step 2 is performed in the afternoon. All exercises are performed once per day.

824 *Table 1b.* Postoperative rehabilitation program, Silkeborg Regional Hospital

Week 0-2				
Step	Exercise	Repetitions	Sets	Resistance
Optional	Cycling	5-10 minutes	2/day	-
-	Supine peristaltic pump exercise	Not informed	Not informed	-
-	Rest with leg above heart level	30 minutes	4/day	-
-	Seated isometric knee extension	3 seconds	10 sets	Lower leg and the foot
-	Seated knee flexion mobilization	3 seconds	10 sets	-
-	Seated knee extension mobilization	30 seconds	3 sets	Apply pressure to the knee joint using the arms
-	Supine isometric knee extension	3 seconds	10 sets	Lower leg and the foot
-	Supine passive knee extension mobilization			Gravity will extend the knee joint
Week 2 and onwards				
-	Supine knee isometric knee towel press	3seconds hold	10sets	Lower leg and the foot

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4	-	Sit to stand	10 repetitions	1 set	Body weight
5	-	Standing knee flexion mobilization	3 seconds	10 sets	Body weight
6	-	Step Up Exercise	10 repetitions	1 set	Body weight
7	826	All exercises are performed twice per day.			

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*Table 2. Discharge criteria at Horsens Regional Hospital and Silkeborg Regional Hospital*

Outcome	Horsens Regional Hospital	Silkeborg Regional Hospital
Minimum knee flexion range of motion	60 degrees	90 degrees
Maximal knee extension deficit	15 degrees	5 degrees
In-and-out of bed	Independent	Independent
Sit-to-stand	Independent	Independent
Walking with/without assistive devices	Independent	Independent
Stair negotiation with/without assistive devices	Independent	Independent
Activities of daily living	Independent	Independent
Understanding of the home-based postoperative exercise program	Sufficient	Sufficient

*Table 3. Exercise variables for the blood-flow restricted exercise (BFRE) protocol*

Exercise variable	Week 1-8
Level of LOP	60% LOP
Sets	4
Load intensity	30% 1RM
Repetitions 1 <sup>st</sup> set	30
Repetitions 2 <sup>nd</sup> & 3 <sup>rd</sup> set	15
Repetitions 4 <sup>th</sup> set	To volitional failure
Contraction modes per repetition	
Concentric	2 seconds
Isometric	0 seconds
Eccentric	2 seconds
Rest between repetitions	0 seconds
Time under tension per repetition	4 seconds
Range of movement	maximum
Rest between sets	30 seconds
Rest between sessions	≥36 hours
Progression	The minimal possible load (5 kilo) is added when patients perform >15 repetitions in 4 <sup>th</sup> set

*Table 4. Outcome measures to be collected.*

Outcome measures	Data collection instrument	Time-points of assessment
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<b>Primary outcome</b>		
<b>Sit-to-stand function</b>	30 seconds chair stand test	B, S, 3 and 12 months
<b>Secondary outcomes</b>		
<b>Ambulatory capacity</b>	Timed Up & Go	B, S, 3 and 12 months
<b>Gait speed</b>	4x10-meter walk test	B, S, 3 and 12 months
<b>1RM Leg press strength</b>	Leg press machine	B, S, 3, and 12 months
<b>1RM Knee extension strength</b>	Knee extension machine	B, S, 3, and 12 months
<b>Isometric Knee extensor muscle strength</b>	Handheld Dynamometer	B, S, 3 and 12 months
<b>Isometric Knee flexion muscle strength</b>	Handheld Dynamometer	B, S, 3 and 12 months
<b>Myofiber morphology</b>	Muscle Biopsies	B, S, 3 months
<b>Myogenic stem cell content</b>	Muscle Biopsies	B, S, 3 months
<b>Pain</b>	KOOS	B, S, 6 weeks, 3 and 12 months
<b>Symptoms</b>	KOOS	B, S, 6 weeks, 3 and 12 months
<b>Activities of daily living</b>	KOOS	B, S, 6 weeks, 3 and 12 months
<b>Sports &amp; Recreation</b>	KOOS	B, S, 6 weeks, 3 and 12 months
<b>Quality of life</b>	KOOS	B, S, 6 weeks, 3 and 12 months
<b>Socioeconomic costs</b>	EQ-5D	B, S, 6 weeks, 3 and 12 months
<b>Adverse Events</b>	Questionnaire and medical records	3 months
<b>Exercise compliance and progression</b>	Physiotherapist records	BFRE
<b>Pain during visits</b>	NRS for pain	B, BFRE, S, 3 and 12 months
<b>Declining to be operated</b>	Questionnaire	3 months
<b>Postoperative supervised physiotherapy</b>	Questionnaire	6 weeks, 3 and 12 months
<b>Knee joint range of motion</b>	Goniometer	B, S, 3 and 12 months
<b>Patient characteristics and related measurements</b>	Questionnaire	B
<b>Gender</b>	Tape measure	B
<b>Age</b>	Electronic body mass scale	B
<b>Height</b>	Questionnaire	B
<b>Body mass</b>	Questionnaire	B
<b>Civil Status</b>	Questionnaire	B
<b>Educational Level</b>	Questionnaire	B
<b>Employment Status</b>	Questionnaire	B
<b>Substance Use (alcohol, smoking)</b>	Questionnaire	B
<b>Duration of knee symptoms</b>	Questionnaire	B
<b>Pain medication during the last week</b>	Questionnaire	B
<b>Co-morbidities</b>	Questionnaire	B

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## Table and figure legends

Table 1a. Step 1 is performed in the morning and step 2 is performed in the afternoon. All exercises are performed once per day.

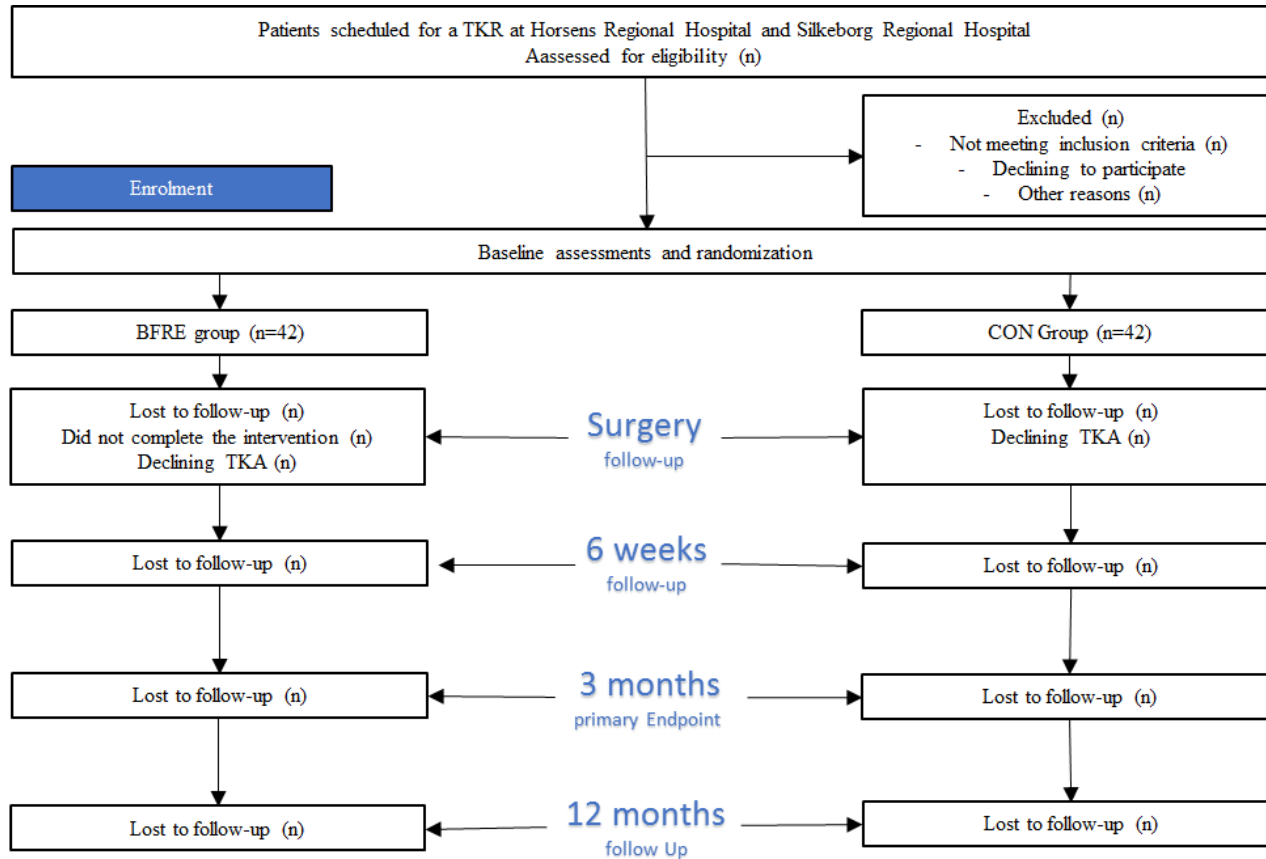
Table 1b. All exercises are performed once per day. Cycling ergometer exercise is optional.

Table 3. LOP: Total limb occlusion pressure; RM: Repetition Maximum

Table 4. KOOS = Knee disability and Osteoarthritis Outcome Score; B = Baseline; S = 0-2 days before surgery; D = during surgery; 3 months = 3 months after TKR; 12 months = 12 after TKR; NRS = Numeric Ranking Scale of pain

Figure 1. Flow chart of the enrollment, treatment, and follow-up phases. TKR: Total Knee Replacement, BFRE: Low-load blood-flow restricted exercise

Figure 1. Patient flow







STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents\*

Section/item	Item No	Description
<b>Administrative information</b>		
Title (p 1, I 1-3)	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration A: p 2, I 56-57 B:	2a 2b	Trial identifier and registry name. If not yet registered, name of intended registry All items from the World Health Organization Trial Registration Data Set
Protocol version P 1, I 22	3	Date and version identifier
Funding P 21, I 494-496	4	Sources and types of financial, material, and other support
Roles and responsibilities A: P 1, I 5-11 B: P 1, I 15-20	5a 5b 5c 5d	Names, affiliations, and roles of protocol contributors Name and contact information for the trial sponsor Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
<b>Introduction</b>		
Background and rationale P 3, I 67-133 P 3, I 70-76	6a 6b	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention Explanation for choice of comparators
Objectives P 5, I 129-136	7	Specific objectives or hypotheses
Trial design P6, I 140-145	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)

## Methods: Participants, interventions, and outcomes

Study setting P6, I 148-149	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
Eligibility criteria P6, I 155-163	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)
Interventions A: p7, I 164-240  C: p12, 283-285	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
Outcomes P 10, I 245-384	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
Table 1		
Sample size P 17, I 391-401	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
Recruitment P 6, I 148-151	15	Strategies for achieving adequate participant enrolment to reach target sample size

## Methods: Assignment of interventions (for controlled trials)

### Allocation:

Sequence generation P8, I 196-201	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions
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2	Allocation	16b	Mechanism of implementing the allocation sequence (eg, central
3	concealment		telephone; sequentially numbered, opaque, sealed envelopes),
4	mechanism		describing any steps to conceal the sequence until interventions are
5	P8, I 196-201		assigned
6			
7	Implementation	16c	Who will generate the allocation sequence, who will enrol participants,
8	P8, I 196-201		and who will assign participants to interventions
9			
10	Blinding	17a	Who will be blinded after assignment to interventions (eg, trial
11	(masking)		participants, care providers, outcome assessors, data analysts), and
12	P8, I 200		how
13		17b	If blinded, circumstances under which unblinding is permissible, and
14			procedure for revealing a participant's allocated intervention during
15			the trial
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### Methods: Data collection, management, and analysis

19			
20	Data collection	18a	Plans for assessment and collection of outcome, baseline, and other
21	methods		trial data, including any related processes to promote data quality (eg,
22	P 10, I 245-420		duplicate measurements, training of assessors) and a description of
23			study instruments (eg, questionnaires, laboratory tests) along with
24			their reliability and validity, if known. Reference to where data
25			collection forms can be found, if not in the protocol
26		18b	Plans to promote participant retention and complete follow-up,
27			including list of any outcome data to be collected for participants who
28			discontinue or deviate from intervention protocols
29			
30	Data	19	Plans for data entry, coding, security, and storage, including any
31	management		related processes to promote data quality (eg, double data entry;
32			range checks for data values). Reference to where details of data
33			management procedures can be found, if not in the protocol
34			
35	Statistical	20a	Statistical methods for analysing primary and secondary outcomes.
36	methods		Reference to where other details of the statistical analysis plan can be
37	P 17, I 400-420		found, if not in the protocol
38		20b	Methods for any additional analyses (eg, subgroup and adjusted
39			analyses)
40	P 17, I 400-420	20c	Definition of analysis population relating to protocol non-adherence
41			(eg, as randomised analysis), and any statistical methods to handle
42			missing data (eg, multiple imputation)
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### Methods: Monitoring

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50	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role
51			and reporting structure; statement of whether it is independent from
52			the sponsor and competing interests; and reference to where further
53			details about its charter can be found, if not in the protocol.
54			Alternatively, an explanation of why a DMC is not needed
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1		21b	Description of any interim analyses and stopping guidelines, including
2			who will have access to these interim results and make the final
3			decision to terminate the trial
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6	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and
7			spontaneously reported adverse events and other unintended effects
8			of trial interventions or trial conduct
9			
10	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and
11			whether the process will be independent from investigators and the
12			sponsor
13			
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### Ethics and dissemination

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17	Research ethics	24	Plans for seeking research ethics committee/institutional review board
18	approval		(REC/IRB) approval
19	P 18, I 423-424		
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21			
22	Protocol	25	Plans for communicating important protocol modifications (eg,
23	amendments		changes to eligibility criteria, outcomes, analyses) to relevant parties
24			(eg, investigators, REC/IRBs, trial participants, trial registries,
25			journals, regulators)
26			
27	Consent or assent	26a	Who will obtain informed consent or assent from potential trial
28	P7, I 164-173		participants or authorised surrogates, and how (see Item 32)
29			
30		26b	Additional consent provisions for collection and use of participant data
31			and biological specimens in ancillary studies, if applicable
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34	Confidentiality	27	How personal information about potential and enrolled participants will
35	P 11, I 265-275		be collected, shared, and maintained in order to protect confidentiality
36			before, during, and after the trial
37			
38	Declaration of	28	Financial and other competing interests for principal investigators for
39	interests		the overall trial and each study site
40	P 22, I 514		
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43	Access to data	29	Statement of who will have access to the final trial dataset, and
44			disclosure of contractual agreements that limit such access for
45			investigators
46			
47	Ancillary and	30	Provisions, if any, for ancillary and post-trial care, and for
48	post-trial care		compensation to those who suffer harm from trial participation
49			
50	Dissemination	31a	Plans for investigators and sponsor to communicate trial results to
51	policy		participants, healthcare professionals, the public, and other relevant
52	P 18, 442-444		groups (eg, via publication, reporting in results databases, or other
53			data sharing arrangements), including any publication restrictions
54			
55			
56	P 21, I 501-502	31b	Authorship eligibility guidelines and any intended use of professional
57			writers
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2 31c Plans, if any, for granting public access to the full protocol, participant-  
3 level dataset, and statistical code  
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## 5 **Appendices**

- 6 Informed consent 32 Model consent form and other related documentation given to  
7 materials participants and authorised surrogates  
8  
9 Biological 33 Plans for collection, laboratory evaluation, and storage of biological  
10 specimens specimens for genetic or molecular analysis in the current trial and for  
11 future use in ancillary studies, if applicable  
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14 \*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013  
15 Explanation & Elaboration for important clarification on the items. Amendments to the  
16 protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT  
17 Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](#)"  
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