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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). A multicenter, randomized controlled trial.

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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 ≥60 years of age [1-5]. 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 [2, 4]. This failure to return to "normal" strength levels has been suggested to be associated with preoperatively lower limb muscle strength and function[2].

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 [6]. Muscle atrophy is known to occur postoperatively, which may explain the marked functional deficits reported to persist for years [6, 7]. Previous research on exercise-based intervention prior to TKR has demonstrated mixed results [6-12], as a likely result of insufficient exercise intensity, training volume, and/or lack of effective progression strategies [9]. Heavy-resistance strength training (HRST) is an often-used method for improving, skeletal muscle strength, hypertrophy and functional capacity in healthy and clinical populations [4, 7, 12-15]. 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[1, 16]. 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 [1, 16-34]. 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 [17,] 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

tissue hypoxia) and activation of myogenic muscle stem cells (satellite cells: SC) [17, 24, 35]. 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, 16, 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].

Satellite cells (SC) are quiescent myogenic stem cells positioned between the sarcolemma and the myofiber basal lamina [24, 36]. SC plays an important role in human skeletal muscle growth due to their ability to donate new myonuclei to the muscle fibers [24, 37-41]. The human skeletal muscle fibers are multinucleated cells with each myonucleus controlling the protein synthesis of a certain cytoplasmatic area in the muscle fiber [37-39, 41, 42]. Myonuclei transcriptional activity can be fully maximized with exercise, hence requiring new myonuclei to support further muscle tissue accretion [38-43]. Furthermore, exercise-induced increases in SC and myonuclei content by means of preconditioning BFRE might represent an effective atrophy-protective mechanism [24, 44]. Previous studies applying short term (10 days) preoperative BFRE before an anterior cruciate ligament rupture-reconstruction demonstrated no atrophy protective effect nor higher postoperative muscle strength compared to performing a low-load exercise without blood flow restriction (placebo) .

Aim and hypothesis of the trial

The primary aim of this trial is to investigate the efficacy of 8 weeks of BFRE compared to receiving usual care prior to TKR on postoperative chair stand performance**.** We hypothesize that 8 weeks of preoperative BFRE will lead to increased perfoemance 30 seconds chair stand performance (30-seconds Chair Stand Test: 30-s CST) when assessed 3 months postoperatively. Secondary aims are to investigate the efficacy of preoperative BFRE on lower limb muscle strength 3 months after TKR and investigate the potential relationship to functional capacity and quality of

life. Furthermore, it will be investigated to which extent 8 weeks of BFRE induces myofiber hypertrophy and gains in satellite cell number and myonuclei content in the knee extensor musculature.

MATERIAL & METHODS

Design

The trial is designed as a multicenter (2 sites), randomized, assessor blinded, controlled trial following the CONSORT guidelines[45]. Primary endpoint will be 3 months after TKR. Additional and secondary endpoints will evaluated during the week of TKR, 6 weeks after TKR (questionnaires only) and 12 months after TKR. Muscle biopsies will be obtained from all patients undergoing surgery at Horsens Regional Hospital at baseline, during surgery and 3 months after TKR.

Participants

Patient will be recruited from the Orthopedic Departments at Horsens and Silkeborg Regional Hospitals.

Inclusion criteria: 1) Patients ≥ 50 years scheduled for TKR at Horsens- or Silkeborg Regional Hospital.

Exclusion criteria: 1) Severe cardiovascular diseases (New York Heart Association (NYHA) class III and IV), previous stroke incident, thrombosis incident; 2) Traumatic nerve injury in affected limb 3) Unregulated hypertension (Systolic ≥180 or diastolic ≥110 mmHg) 4) Spinal cord injury; 5) Planned other lower limb surgery within 12 months; 6) Cancer diagnosis and currently undergoing chemo-, immuno-, or radiotherapy; 7) Inadequacy in written and spoken Danish; 8) an existing

prosthesis in the index limb; 9) living more than 45 minutes from either Horsens Regional Hospital or Silkeborg Regional Hospital; 10) Pregnancy.

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

Randomization

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

CON group: Participants in the CON group will follow usual care before a TKR and be encouraged to continue their usual lifestyle up until TKR.

BFRE group: Will perform supervised BFRE sessions 3 times per week for 8 weeks supervised by a physiotherapist educated in administering BFRE. All BFRE sessions will be performed at either Horsens Regional Hospital or Silkeborg Regional Hospital.

Insert figure 1 here

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. 1st round: 30 repetitions (reps); 2nd round: 15 reps; 3rd round: 15 reps; 4th round: until exhaustion. If patients can perform more than 15 repetitions in the 4th exercise set, the exercise load will be increased with the minimum extra load possible [25]. Participants will be instructed to perform both the eccentric and concentric contraction phases using a steady 2-sec pace duration. The 4th 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

pressure (mmHg) needed for total blood flow occlusion, the cuff will gradually be inflated in 20 mmHg steps until reaching the pressure where the auscultatory pulse is interrupted (LOP). First time the auscultatory pulse is interrupted the examiner releases 10-20 mmHg pressure from the cuff until the auscultatory pulse is present again. When the auscultatory pulse reappears the cuff is inflated with 10 mmHg until LOP is found again. If the second LOP is identical to the first it will be defined as LOP for that specific patient. Otherwise, the procedure will be repeated until determining an identical LOP two consecutive times.

Insert table 1 here

Outcome variables

Outcome assessments will be performed at baseline, in the week of surgery, 6 weeks after TKR, 3 months after TKR, and 12 months after TKR. Six weeks after TKR only questionnaires will be completed. Two testers (the PhD-stipendiate and a trained physiotherapist) blinded to group allocation will perform all baseline and follow-up measurements. Bergstrøm needle muscle biopsies [46] will be taken from vastus lateralis of the quadriceps in both lower limbs from patients included at Regional Hospital Horsens only at baseline, during surgery, and 3 months after TKR by doctors trained in performing the procedure. An overview of the data collection parameters is presented in Table 2.

Primary outcome variable

The primary outcome measure will be the change from baseline to 3 months follow-up in 30s-CST. The 30s-CST measures the number of sit-to-stand repetitions completed within 30 seconds [47, 48] and is a part of the OARSI-recommended minimum outcome core set representing the ability to perform

a sit-to-stand activity [49]. 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 [47-50].

Secondary outcome variables

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

Explorative outcome variables

Type of postoperative rehabilitation received, medication, knee joint range of motion

Demographic data

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

Adherence

Adherence to training will be registered by the physiotherapists in charge of the exercise sessions. High compliance is defined as attendance to the supervised BFRE of ≥80%.

Insert table 2 here

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 [8]. Skoffer et al. [8] 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 [8]. The authors found a between-group difference of 3-4 repetition difference (14.7 ± 4.7 repetitions versus 11.0 ± 4.4 repetitions) 3 months after TKR [8].

To reduce the probability of type I errors and abe able to detect a between-group difference also, α-level is set at 0.05 ($p<0.05$) and β-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[59] and the CONSORT statement[45]. 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 [24]. 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

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

Ethical aspects 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). The trial is registered at Clinicaltrial.gov (NCT04081493). Before inclusion, all patients will provide their written informed consent in accordance with the Helsinki Declaration. All data and information collected in regard to this trial will be treated confidentially (blinded and encrypted) by the researchers and staff connected to the trial.

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

Patient and public involvement

Before developing this clinical trial, a pilot project was performed to determine feasibility and

efficacy og BFRE in patients suffering from lower limb injuries. The experiences with the training modality and also the verbal feedback from patients on training duration, frequency, and intensity resulted in useful knowledge that certainly have improved the development of the present clinical trial.

DISCUSSION

To our best knowledge, this is the first trial to investigate the effect of preoperative BFRE on functional capacity, self-reported outcome, lower limb muscle strength and myofiber morphology/stem cell abundance in patients scheduled for TKR. Only few studies have investigated (short term (10 days)) preoperative BFRE without finding an atrophy protective effect or difference in muscle strength compared to a control group performing a placebo intervention (SHAM group) [61]. However, patients performing short term preoperative BFRE before ACL-R demonstrated higher muscle endurance compared to a SHAM group [62]. Therefore, results of this trial are expected to provide novel information on longer periods of BFRE that will enable to design effective exercise-based preconditioning protocols for elective TKR patients.

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, 16, 17, 27, 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 [17]. 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 [15, 24, 46, 63, 64].

Author contributions

All authors contributed to the design of the trial as well as to the writing of the manuscript and approved the final version of the protocol.

Data statement

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

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Competing interest

None to be declared

Ethics approval

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 (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 1st set	30
Repetitions 2nd & 3rd set	15
Repetitions 4th 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 4th set

Table 2. Outcome measures to be collected.

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

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

*Figure 1.*Patient flow

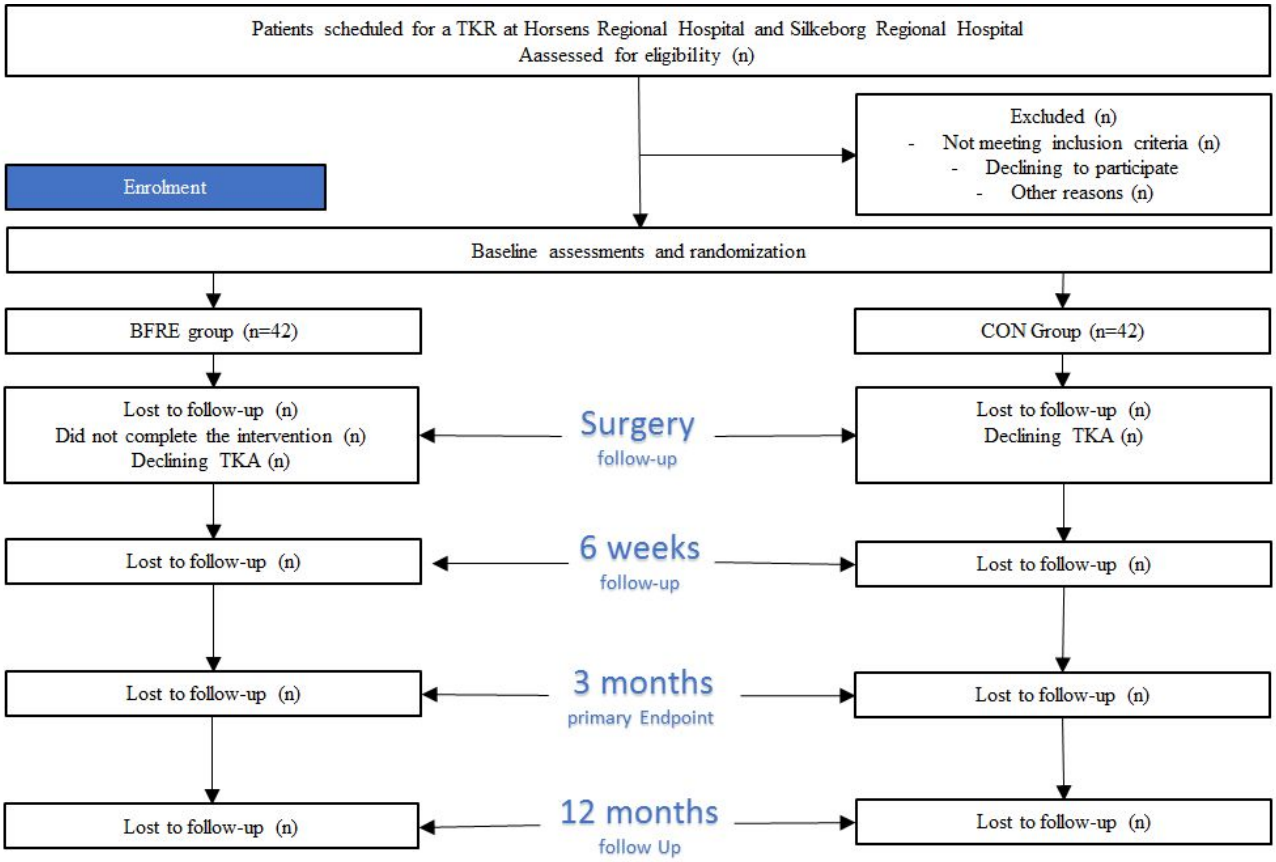


Table and figure legends

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

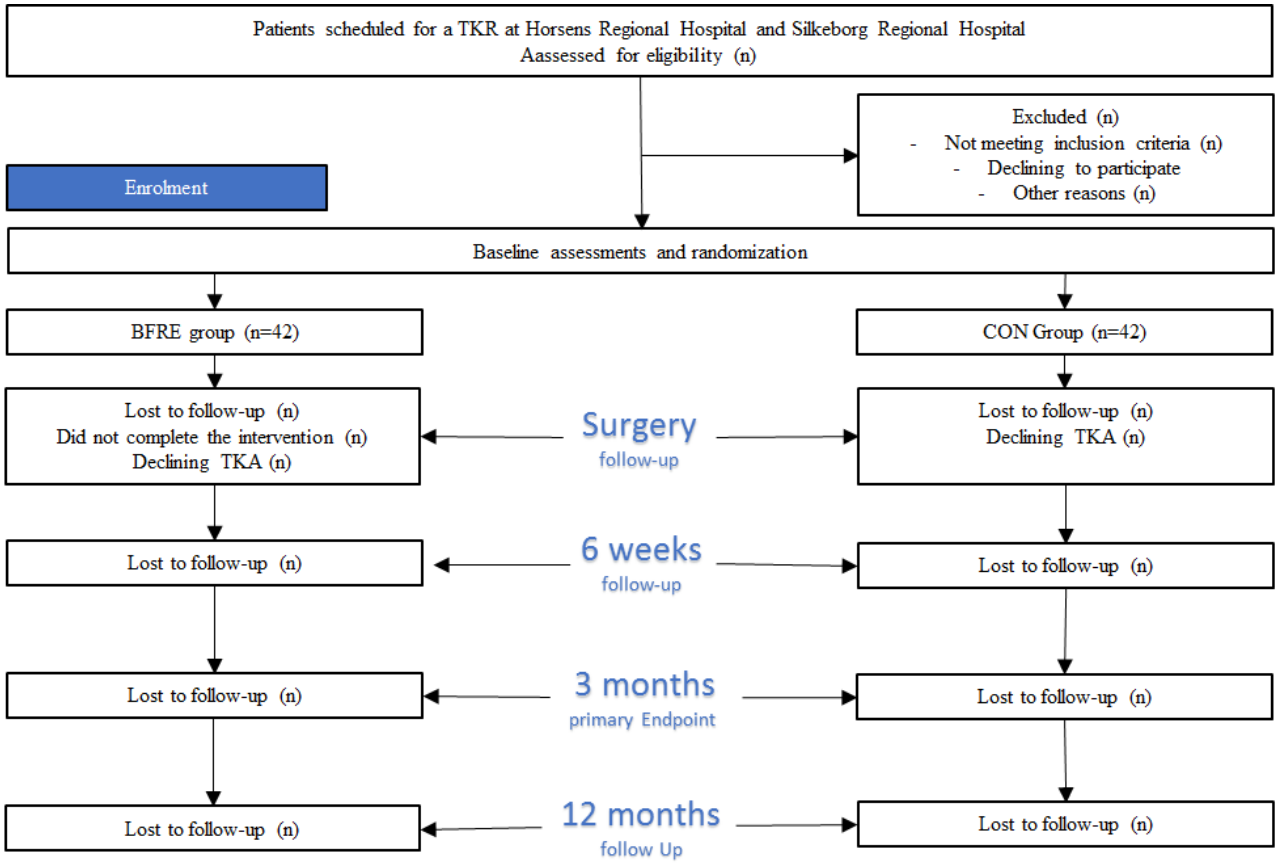
Table 2. KOOS = Knee disability and Osteoarthritis Outcome Score; B = Baseline; S = 0-2 days before surgery; D = during surgery; 3 months = 3 months after TKR; 12 months = 12 after TKR; NRS = Numeric Ranking Scale of pain

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

Figure and figure legend

EXKnee project

Figure 1.Patient flow



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Educational Level	Questionnaire	B
Employment Status	Questionnaire	B
Substance Use (alcohol, smoking)	Questionnaire	B
Duration of knee symptoms	Questionnaire	B
Pain medication during the last week	Questionnaire	B

Com-morbidities	Questionnaire	B
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Primary Subject Heading:	Sports and exercise medicine
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Keywords:	blood flow restriction exercise, knee osteoarthritis, total knee replacement surgery, preconditioning, functional capacity

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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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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, the study aims to investigate to which extent preoperative BFRE will protect against surgery-related atrophy 3 months after TKR.

Methods

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

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

Ethical approval

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

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

Preconditioning exercise designed to prepare the musculoskeletal system to better tolerate stressful events such as the impact of invasive surgery has been suggested to be applicable prior to elective TKR (6). This is supported by the results of two randomized controlled trials indicating that preoperative heavy resistance strength training (HRST) may enhance functional capacity and knee extensor muscle strength 3 months postoperatively (7, 8). However, joint pain resulting from the high mechanical loads associated with HRST may represent a barrier to this type of training in some patients suffering from severe knee OA (1, 9). Therefore, a more tolerable, yet effective, alternative is needed for this population. Also, 3 recent systematic reviews investigating the topic of preoperative physiotherapy-based exercise before TKR have suggested high quality, well-powered evidence to investigate the efficacy of preoperative physiotherapy before TKR (10-12). 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 (1, 13-32). 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, as well as some patient populations (13, 25, 26), 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 tissue hypoxia) and activation of myogenic muscle stem cells (satellite cells: SC) (13, 26, 31). 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). Furthermore, 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) have been applied without any adverse events in mild-degree knee OA (1, 33, 34). This is in line 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).

Satellite cells (SC) are quiescent myogenic stem cells positioned between the sarcolemma and the myofiber basal lamina (31, 35). SC plays an important role in human skeletal muscle growth due to their ability to donate new myonuclei to the muscle fibers (31, 36-40). 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 (36-38, 41). Myonuclei transcriptional activity can be fully maximized with exercise, hence requiring new myonuclei to support further muscle tissue accretion (37, 38, 40). It has been suggested that exercise-related addition of SCs and myonuclei by means of BFRE might reduce the muscle atrophy related to bedrest and/or prolonged inactivity (31, 42). 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 SC 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

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

MATERIAL & METHODS

Design

The trial is designed as a multicenter (2 sites), randomized, assessor blinded, controlled trial following the CONSORT guidelines (43). Primary endpoint will be 3 months after TKR. Additional and secondary endpoints will evaluated during the week of TKR, 6 weeks after TKR (questionnaires only) and 12 months after TKR. Muscle biopsies will be obtained from all patients undergoing surgery at Horsens Regional Hospital at baseline, during surgery and 3 months after TKR.

Participants

Patient will be recruited from the Orthopedic Departments at Horsens and Silkeborg Regional Hospitals in Denmark. Patient enrollment will start September 2nd 2019 at Horsens Regional Hospital and October 1st 2019 at Silkeborg Regional Hospital. Patient recruitment is expected to be completed in June 2021. All patients are expected to have completed baseline testing ultimo June 2021 and have performed 3 months follow-up during September 2021. Thus, at the end of June 2022 all patients are expected to have completed 12 months follow-up testing.

Inclusion criteria: 1) Patients ≥ 50 years scheduled for TKR due to knee OA at Horsens- or Silkeborg Regional Hospital.

Exclusion criteria: 1) Severe cardiovascular diseases (New York Heart Association (NYHA) class III and IV), previous stroke incident, thrombosis incident; 2) Traumatic nerve injury in affected limb 3) Unregulated hypertension (Systolic ≥180 or diastolic ≥110 mmHg) 4) Spinal cord injury; 5) Planned other lower limb surgery within 12 months; 6) Cancer diagnosis and currently undergoing chemo-, immuno-, or radiotherapy; 7) Inadequacy in written and spoken Danish; 8) an existing prosthesis in the index limb; 9) living more than 45 minutes from either Horsens Regional Hospital or Silkeborg Regional Hospital; 10) Pregnancy.

All patients will be screened for eligibility by orthopedic surgeons at Horsens Regional Hospital and Silkeborg Regional Hospital who will perform the initial inclusion of study participants and hand out written project information. All patients accepting to participate will be asked to complete a written informed consent allowing the physiotherapist (at Horsens Regional Hospital and

Silkeborg Regional Hospital) to contact the patients by phone for a final eligibility and exclusion criteria-screening, and book an appointment for baseline testing. In case the patient agrees to participate in the trial, the patient will sign a written informed consent to participate in the project. Subsequently, the patient will be baseline-tested at the hospital by a blinded (to group allocation) assessor. Patients declining to participate in the RCT will be offered the option of participating in a parallel observational cohort trial. All patients included in the project will be scheduled for a TKR and receive a standard multimodal surgical program with standard preoperative care (usual care). Specifically, 2-3 weeks before surgery all patients will be invited to a preoperative information meeting where nurses, surgeons, and physiotherapists will provide detailed information on pain management, nutrition, the surgical procedure, physical activity, postoperative home-based rehabilitation, load management, etc. (44) On the day of surgery, patients will be hospitalized at Horsens Regional Hospital or Silkeborg Regional Hospital where an orthopedic surgeon will perform the TKR procedure. The day after surgery all patients will be trained once or twice per day by a physiotherapist towards fulfilling the following discharge criterions: a minimum knee flexion range of motion (ROM) of 60/90 degree and maximally a knee extension ROM deficit of 15/5 degree knee extension (Horsens Region Hospital/ Silkeborg Regional Hospital), independency in in-and-out of bed and sit-to-stand activities, independency in walking and stair-negotiation with crutches, ADL activities, and sufficient understanding of the home-based exercises during the hospitalization period (44). Patients will generally be discharged within ~1-2 days after fulfilling all the above discharge criteria. After discharge, all patients will as standard receive a standard home-based rehabilitation program focusing on improving knee joint mobility, increasing the tolerance for standing without assistive devices (i.e. crutches), and lower extremity muscle strength. Small variations in the selection of exercises in the standard home-based rehabilitation program exists between hospitals, however, the purpose of the programs is identical. However, if the patients do

not fulfill the discharge criteria the patient will be offered supervised knee-specific exercise therapy at municipal rehabilitation centers, or specialized hospital-based rehabilitation after discharge from the Hospital.

Randomization

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

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

BFRE group: In addition to receiving usual care (cf. above), participants in the BFRE group will perform supervised BFRE sessions 3 times per week for 8 weeks supervised by a physiotherapist educated in administering BFRE. All BFRE training will be performed at Horsens Regional Hospital and Silkeborg Regional Hospital.

Please insert Figure 1 about here

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. 1st round: 30 repetitions (reps); 2nd round: 15 reps; 3rd round: 15 reps; 4th round: until exhaustion (Table 1). If patients can perform more than 15 repetitions in the 4th exercise set, the exercise load will be increased with the minimum extra load possible (30). Participants will be instructed to perform both the eccentric and concentric contraction phases using a steady 2-sec pace duration. The 4th 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 pressure (mmHg) needed for total blood flow occlusion, the cuff will gradually be inflated in 20 mmHg steps until reaching the pressure where the auscultatory pulse is interrupted (LOP). First time the auscultatory pulse is interrupted the examiner releases 10-20 mmHg pressure from the cuff until the auscultatory pulse is present again. When the auscultatory pulse reappears the cuff is

inflated with 10 mmHg until LOP is found again. If the second LOP is identical to the first it will be defined as LOP for that specific patient. Otherwise, the procedure will be repeated until determining an identical LOP two consecutive times.

Please insert Table 1 abot here

Outcome variables

Outcome assessments will be performed at baseline, in the week of surgery, 6 weeks after TKR, 3 months after TKR, and 12 months after TKR. To reduce the number of postoperative visits only questionnaires; The Knee disability and Oteoarthritis Outcome Score (KOOS), EuroQol Group 5-dimensions (EQ-5D-L5), and reporting of adverse event or receiving supervised physiotherapy postoperatively will sent via email 6 weeks after surgery. Two testers (two trained physiotherapists) blinded to group allocation will perform all baseline and follow-up measurements. Bergstrøm needle muscle biopsies (45) will be taken from vastus lateralis of the quadriceps muscle in both lower limbs from patients included at Horsens Regional Hospital only at baseline, during surgery, and 3 months after TKR by doctors trained in performing the procedure. An overview of the data collection parameters is presented in Table 2.

Before starting the baseline testing, all assessors will be thoroughly trained in performing the tests according to the standardized test procedures for each test method. To maintain fidelity of testing during the study period, assessors will be retrained every 3rd month. Also, the physiotherapist in charge of LL-BFRE will be thoroughly trained in performing the exercise on healthy subjects before applying LL-BFRE on study-patients. 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 3rd month.

Data management

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

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

Primary outcome variable

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

Secondary outcome variables

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

Outcome Score (52, 53), EuroQol Group 5-dimensions (54), Numeric Ranking Scale for pain (NRS) (55), and adverse events/postponement of TKR.

Explorative outcome variables

Type of postoperative rehabilitation received, medication and knee joint range of motion.

Demographic data

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

Adherence

Adherence to training will be registered by the physiotherapists in charge of the exercise sessions. High compliance is defined as attendance to the supervised BFRE of ≥80%.

Please insert Table 2 about here

Elaborated description of outcome measures

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 (46, 56, 57).

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 are allowed. The fastest of two trials will be used for further analysis. Up to one minute of rest will be allowed between trials (47, 58). Good inter-rater reliability has been demonstrated with the TUG test *(46)*.

4x10 meter walk test meter walk test (40m-FWT) measures the total time taken to walk 4 x 10 m excluding turns (meter/sec) (46). Patients will be instructed to walk as quickly and as safely as possible without running to a visible mark 10 m away, return and repeat for a total distance of 40 m (46). 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 (46).

1RM leg press strength will be estimated from a 5-8RM leg press test. Patients perform 3 low-load warm-up sets. 1st and 2nd warm-up set consists of 12 repetitions, and the 3rd warm-up set consist of 8 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 3 trials, an 4th all-out trial (as many repetitions as possible) will be performed. The 1RM will be calculated as [1RM = load (kg)/1.0278-0.0278·number of repetitions)] (59).

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 2nd, 3rd, and 4th 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 2nd, 3rd, and 4th measures will be calculated and corrected for bodyweight (50)

Myofiber cross sectional area (CSA), muscle fiber type composition, satellite cell content, and myonuclei number will be assessed by obtaining needle biopsies (100-150 mg) from all patients enrolled at Horsens Regional Hospital. The biopsies will be obtained bilaterally from the middle portion of the vastus lateralis muscle utilizing the percutaneous needle biopsy technique of Bergström (45, 60, 61). Biopsies will be performed by two experienced orthopedic surgeons (chief physicians) trained in performing the needle muscle biopsy technique at Horsens Regional Hospital. Efforts will be made to extract tissue from the same region (2-3 cm apart) and depth (~1-2 cm.) (45). The tissue samples will be dissected of all visible blood, adipose tissue, and connective tissue and mounted in Tissue-Tec (4583, Sakura Finetek, Alphen aan den Rijn, The Netherlands), frozen in isopenate pre-cooled with liquid nitrogen, and stored at -80°C (31, 45, 51). All muscle samples will be analyzed as previously described by Nielsen et al. (31) using immunofluorescence microscopy. Transverse serial sections (8 µm) of the embedded muscle biopsy specimen will be cut at -22°C using a cryostat (HM560; Microm, Walldorf, Germany) and will be mounted on glass slides for subsequent analysis as described in detail elsewhere (31). Myogenic stem cells (satellite cells (SC)) will be visualized with an antibody against Pax7 (31). Type I (stained) and Type II (unstained) myofibers will be differentiated, and muscle fiber area will be determined (31): MSC-derived nuclei will stain positive for Pax7 and be within the basal lamina; nuclei (DAPI stained) with a sublaminar placement will be considered myonuclei (31).

Knee disability and Osteoarthritis Outcome Score (KOOS) is a patient-administered knee specific questionnaire comprising five subscales Pain; Symptoms; Activities of daily living; Sport & Recreation; and Knee-Related Quality of Life. Each item is scored from 0 to 4 (53). The raw score for each of the five subscales is the total sum of the associated item scores. Scores can be transformed to a 0 to 100 scale. The scores of the five subscales can be expressed as a composite

outcome profile, higher scores indicating fewer problems (62). The KOOS questionnaire is valid and reliable in patients suffering from knee OA and patients on the waiting list for TKA for knee OA (52, 53, 63).

EuroQol Group 5-dimension (EQ-5D-5L) is a self-completion questionnaire consisting of two parts; 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 (64), which will be converted into a summary index ranging from -0.624 (worst) to 1.000 (best), using a Danish value set (54). The second part, EQ-VAS rates the overall current health status from 0 (worst imaginable health) to 100 (best imaginable health) (64). The EQ-5D-5L is reliable and valid in patients with knee osteoarthritis eligible for TKA, (65, 66)

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 3-months and 12 months follow-up.

Other Outcome Measures

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

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.

Declining to be operated will 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.

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.

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

maximally (67) The fulcrum of the goniometer will correspond visually to the trans-epicondylar axis of the knee joint. The moveable arms of the goniometer will be pointed towards the greater trochanter and the lateral malleolus while (67).

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 (8). Skoffer et al. (8) 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 in patients receiving a TKR (8). The authors found a between-group difference of 3-4 repetition difference (14.7 ± 4.7 repetitions versus 11.0 ± 4.4 repetitions) 3 months after TKR surgery (8).

To reduce the probability of type I errors and be able to detect a between-group difference also, α-level is set at 0.05 ($p<0.05$) and β-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 78 patients in total). With an anticipated dropout rate of 10%, 84 patients will be recruited for the trial in total.

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 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 (68) and the CONSORT statement (43). 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 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 effect and time and group as fixed effects (31, 69). Also, to gain insights into the potential pre-to-post training differences within the respective training or control groups, paired student t-tests will be performed. Level of statistical significance is $P < 0.05$. *Secondary outcome variables:* Between-intervention comparison from baseline to the week of surgery, 6 weeks after surgery, 3 and 12 months after surgery will be analyzed as described for the primary outcome. Regression analysis will be used to analyze the potential associations between preoperative strength and postoperative lower extremity function and self-reported outcome as well as between preoperative functional capacity and postoperative functional capacity. Additionally, regression analysis will be used to analyze the association between preoperative number of satellite cells and myonuclei on postoperative isometric knee extensor muscle strength, muscle fiber cross sectional area, and functional capacity. All statistical analysis will be performed by the primary investigator using Stata.

Ethical aspects 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). The trial is registered at Clinicaltrial.gov (NCT04081493). Before inclusion, all patients will provide their written informed consent in accordance with the Helsinki Declaration. All data and information collected in regard to this trial will be treated confidentially (blinded and encrypted) by the researchers and staff connected to the trial.

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

Patient and public involvement

Before developing this clinical trial, a pilot project was performed to determine feasibility and efficacy og 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 resulted in useful knowledge that certainly have improved the development of the present clinical trial.

DISCUSSION

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

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

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

Author contributions

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

Data statement

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

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Competing interest

None to be declared

Ethics approval

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 (Reference No 652164).

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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 1st set	30
Repetitions 2nd & 3rd set	15
Repetitions 4th 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 4th set

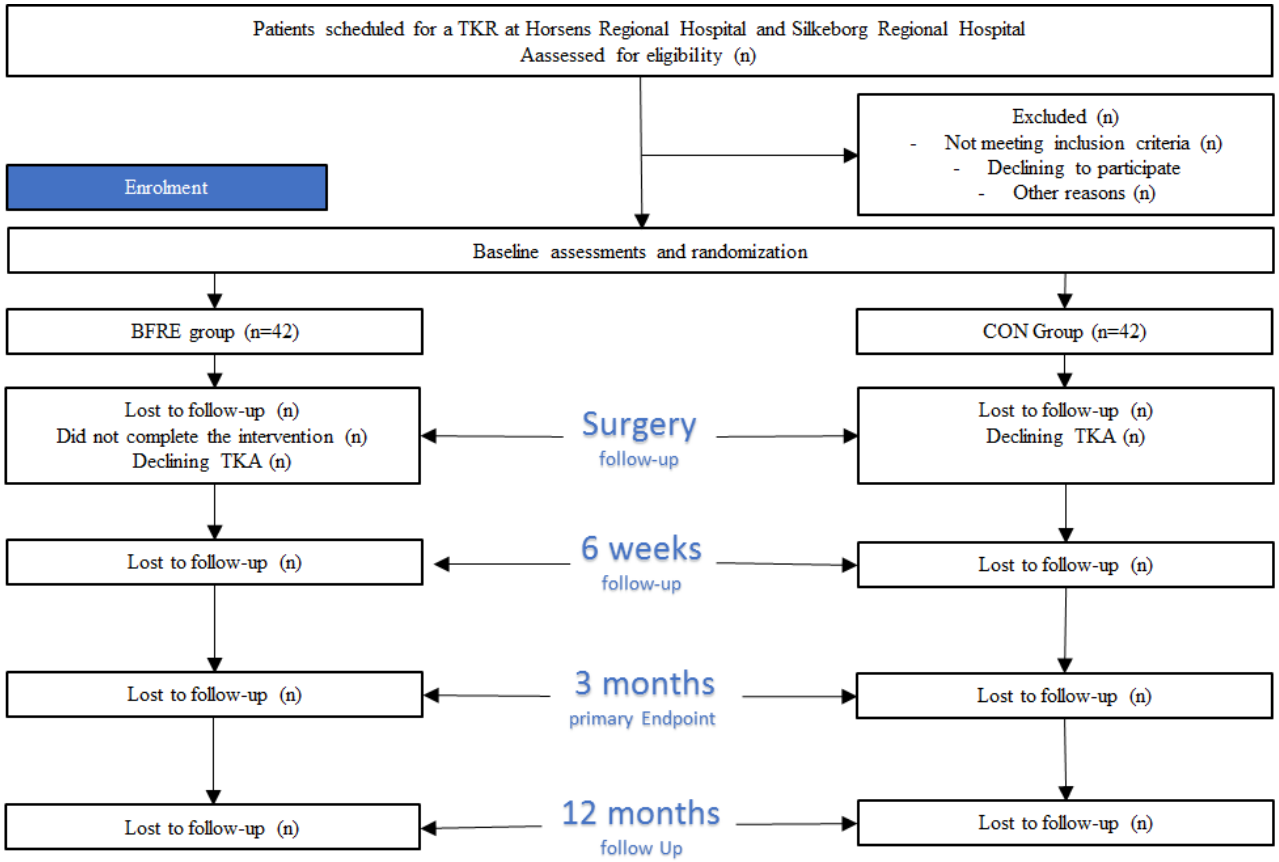
Table 2. Outcome measures to be collected.

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

Postoperative supervised physiotherapy	Questionnaire	6 weeks, 3 and 12 months
Exercise compliance and progression	Physiotherapist records	B, S, at each BBFRE session, 3
NRS Pain	PhD-stipendiate and physiotherapist records	and 12 months

777

Figure 1. Patient flow



SPIRIT
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
Administrative information		
Title (p 1, l 1-3)	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration A: p 2, l 56-57	2a	Trial identifier and registry name. If not yet registered, name of intended registry
B:	2b	All items from the World Health Organization Trial Registration Data Set
Protocol version P 1, l 22	3	Date and version identifier
Funding P 21, l 494-496	4	Sources and types of financial, material, and other support
Roles and responsibilities A: P 1, l 5-11 B: P 1, l 15-20	5a	Names, affiliations, and roles of protocol contributors
	5b	Name and contact information for the trial sponsor
	5c	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
	5d	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)
Introduction		
Background and rationale P 3, l 67-133	6a	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
P 3, l 70-76	6b	Explanation for choice of comparators
Objectives P 5, l 129-136	7	Specific objectives or hypotheses
Trial design P6, l 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, l 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, l 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, l 164-240	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)
C: p12, 283-285	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, l 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, l 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, l 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, l 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

Allocation concealment mechanism P8, l 196-201	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
Implementation P8, l 196-201	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions
Blinding (masking) P8, l 200	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial
Methods: Data collection, management, and analysis		
Data collection methods P 10, l 245-420	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
Statistical methods P 17, l 400-420	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)
P 17, l 400-420	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed

	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, l 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 P7, l 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, l 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, l 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, l 501-502	31b	Authorship eligibility guidelines and any intended use of professional writers

	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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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Primary Subject Heading:	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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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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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, the study aims to investigate to which extent preoperative BFRE will protect against surgery-related atrophy three months after TKR.

Methods

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

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

Ethical approval

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

Trial registration

The trial is registered at Clinical Trials (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)
- This is a protocol paper

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

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

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 (1, 13-32). The application of low

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 arteriel 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),

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

The primary aim of this trial is to investigate the efficacy of eight weeks of BFRE compared to receiving usual care prior to TKR on postoperative chair stand performance**.** We hypothesize that eight weeks of preoperative BFRE will lead to increased 30 second chair stand performance (30-second Chair Stand Test: 30-s CST) when assessed three months postoperatively. Secondary aims are to investigate the efficacy of preoperative BFRE on lower limb muscle strength three months after TKR and investigate the potential relationship to functional capacity and quality of life. Furthermore, it will be investigated to which extent eight weeks of BFRE induce myofiber hypertrophy and gain in satellite cell number and myonuclei content in the knee extensor musculature.

MATERIAL & METHODS

Design

The trial is designed as a multicenter (two sites), randomized, assessor blinded, controlled trial following the CONSORT guidelines (47). Primary endpoint will be three months after TKR. Additional and secondary endpoints will be evaluated during the week of TKR, six weeks after TKR (questionnaires only) and 12 months after TKR. Muscle biopsies will be obtained from all patients undergoing surgery at Horsens Regional Hospital at baseline, during surgery and three months after TKR.

Participants

Patients will be recruited from the Departments of Orthopedic Surgery at Horsens and Silkeborg Regional Hospitals in Denmark. Patient enrollment will start September 2nd 2019 at Horsens Regional Hospital and October 1st 2019 at Silkeborg Regional Hospital. Patient recruitment is expected to be completed in June 2021. All patients are expected to have completed baseline testing

ultimo September 2021 and have performed three-month follow-up ultimo April 2022. Thus, at the end of September 2023 all patients are expected to have completed 12-month follow-up testing.

Inclusion criteria: 1) Patients ≥ 50 years scheduled for TKR due to knee OA at Horsens- or Silkeborg Regional Hospital.

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

Please insert figure 1 around here

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

the hospital by a blinded (to group allocation) assessor. Patients declining to participate in the RCT will be offered the option of participating in a parallel observational cohort trial. All patients included in the project will be scheduled for a TKR. Two-three weeks before surgery all patients will be invited to a, preoperative information meeting where nurses, surgeons, and physiotherapists will provide detailed information on pain management, nutrition, the surgical procedure, physical activity, postoperative home-based rehabilitation (table 1a and 1b), load management, etc. (usual care) (48). On the day of surgery, patients will be hospitalized at Horsens Regional Hospital or Silkeborg Regional Hospital where an orthopedic chief physician will perform the TKR procedure. The day after surgery all patients will receive physiotherapy-supervised training once or twice per day by a physiotherapist in order to fulfill the discharge criteria (table 2a and 2b) (48). Patients will generally be discharged within ~one-two days after fulfilling all the discharge criteria listed above. After discharge, all patients will receive a standard home-based rehabilitation program focusing on improving knee joint mobility, increasing the tolerance for standing without assistive devices, and lower extremity muscle strength. Variations in the selection of exercises and exercise variables exist in the standard home-based rehabilitation programs between the respective hospitals; however, the purpose of the programs is identical. If the patients do not fulfill the discharge criteria, they will be offered supervised knee-specific exercise therapy at a municipal rehabilitation center or specialized hospital-based rehabilitation after discharge from the hospital.

Please insert table 1a and 1 b about here

Please insert table 2a and figure 2b around here

Randomization

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

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

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

Intervention procedures

BFRE

Each BFRE session will consist of a 10-minute warm up (ergometer cycling) followed by two different unilateral lower-limb resistance training exercises: 1) leg press and 2) knee extension performed on standard strength training machines. Each exercise will be performed with the affected lower limb only and consist of four rounds interspaced by 30 seconds of rest (table 3). First round: 30 repetitions (reps); second round: 15 reps; third round: 15 reps; fourth round: until exhaustion (Table 1). If patients can perform more than 15 repetitions in the fourth exercise set, the exercise load will be increased with the minimum extra load possible (30). Participants will be instructed to perform both the eccentric and concentric contraction phases using a steady 2-second

pace duration. The fourth 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 second rest period, patients will rest in a standardized resting position while maintaining the initial cuff-pressure. Between each exercise, patients will have a 5-minute "free-flow" rest period. The 5 minutes rest period applied between exercises was chosen based on experiences from a previous pilot project (Jorgensen & Bohn 2019, unpublished data) and experience with applying BFRE in clinical practice. In both situations, we often experienced that patients stayed seated in the leg press machine for >2 minutes after the last (fatiguing) set to feel sufficiently rested and confident to walk from one exercise machine to another. 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 the starting load intensity will be 30% with 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 pressure (mmHg) needed for total blood flow occlusion, the cuff will gradually be inflated in 20 mmHg steps until reaching the pressure where the auscultatory pulse is interrupted (LOP). The first time the auscultatory pulse is interrupted, the examiner releases 10-20 mmHg pressure from the cuff until the auscultatory pulse is present again. When the auscultatory pulse reappears, the cuff is inflated with 10 mmHg until the LOP is found again. If the second LOP is identical to the first, it

will be defined as the LOP for that specific patient. Otherwise, the procedure will be repeated until determining an identical LOP two consecutive times.

Please insert Table 3 about here

Outcome variables

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

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

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 = load (kg)/1.0278-0.0278·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 HDD will be positioned 5 cm above the medial

malleolus (56). The 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 extensor muscle strength testing with HDD (56, 57). Patients will receive four trials. For analysis, the mean maximal strength of the second, third and fourth measures will be calculated and corrected for bodyweight (56)

MVC of the knee flexors will be measured and performed using HHD at 90° knee flexion with the patients seated identically as during MVC for the knee extensors (56). The HHD will be positioned posterior aspect of calcaneus (56) 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 (56). Patients will receive four trials. For analysis, the mean maximal strength of the second, third and fourth measures will be calculated and corrected for bodyweight (56)

Myofiber cross sectional area (CSA), muscle fiber type composition, satellite cell content, and myonuclei number will be assessed by obtaining needle biopsies (100-150 mg) from all patients enrolled at Horsens Regional Hospital. The biopsies will be obtained bilaterally from the middle portion of the vastus lateralis muscle utilizing the percutaneous needle biopsy technique of Bergström (49, 58, 59). Biopsies will be performed by two experienced orthopedic surgeons (chief physicians) trained in performing the needle muscle biopsy technique at Horsens Regional Hospital. Efforts will be made to extract tissue from the same region (2-3 cm apart) and depth (~1-2 cm.) (49). The tissue samples will be dissected of all visible blood, adipose tissue, and connective tissue and mounted in Tissue-Tec (4583, Sakura Finetek, Alphen aan den Rijn, The Netherlands), frozen

in isopenate pre-cooled with liquid nitrogen, and stored at -80°C (31, 49, 60). All muscle samples will be analyzed as previously described by Nielsen et al. (31) using immunofluorescence microscopy. Transverse serial sections (8 μm) of the embedded muscle biopsy specimen will be cut at -22°C using a cryostat (HM560; Microm, Walldorf, Germany) and will be mounted on glass slides for subsequent analysis as described in detail elsewhere (31). Myogenic stem cells (satellite cells (SC)) will be visualized with an antibody against Pax7 (31). Type I (stained) and Type II (unstained) myofibers will be differentiated, and muscle fiber area will be determined (31): MSC-derived nuclei will stain positive for Pax7 and be within the basal lamina; nuclei (DAPI stained) with a sublaminar placement will be considered myonuclei (31).

Knee disability and Osteoarthritis Outcome Score (KOOS) is a patient-administered knee specific questionnaire comprising five subscales: Pain; Symptoms; Activities of daily living; Sport & Recreation; and Knee-Related Quality of Life. Each item is scored from 0 to 4 (61). The raw score for each of the five subscales is the total sum of the associated item scores. Scores can be transformed to a 0 to 100 scale. The scores of the five subscales can be expressed as a composite outcome profile, higher scores indicating fewer problems (62). The KOOS questionnaire is valid and reliable in patients suffering from knee OA and patients on the waiting list for TKA for knee OA (61, 63, 64).

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.

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

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 maximally (70) The fulcrum of the goniometer will correspond visually to the trans-epicondylar axis of the knee joint. The moveable arms of the goniometer will be pointed towards the greater trochanter and the lateral malleolus (70).

Data management

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

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

Sample size

The power and sample size calculation is based on the expected differences between the two subject groups from baseline to three-month follow-up (8). Due to lack of data on the primary outcome for

investigations applying LL-BFRE before a surgical procedure, we decided to base our sample size calculation on Skoffer 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 ± 4.7 repetitions versus 11.0 ± 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, α-level is set at 0.05 ($p<0.05$) and β-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 and group as fixed effects (31, 72). Also, to gain insight into the

potential pre-to-post training differences within the respective training or control groups, paired student t-tests will be performed. Level of statistical significance is $P < 0.05$. *Secondary outcome variables:* Between-intervention comparison from baseline to the week of surgery, six weeks after surgery, three and 12 months after surgery will be analyzed as described for the primary outcome. Regression analysis will be used to analyze the potential associations between preoperative strength and postoperative lower extremity function and self-reported outcome as well as between preoperative functional capacity and postoperative functional capacity. Additionally, regression analysis will be used to analyze the association between preoperative number of satellite cells and myonuclei on postoperative isometric knee extensor muscle strength, muscle fiber cross sectional area, and functional capacity. All statistical analyses will be performed by the primary investigator using Stata.

Ethical aspects and dissemination

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 (Journal No 652164). The trial is registered at Clinicaltrials.gov (NCT04081493). Before inclusion, all patients will provide their written informed consent in accordance with the Helsinki Declaration. All data and information collected in regard to this trial will be treated confidentially (blinded and encrypted) by the researchers and staff connected to the trial.

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

Patient and public involvement

Before developing this clinical trial, a pilot project was performed to determine the feasibility and

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 resulted in useful knowledge that certainly has improved the development of the present clinical trial.

DISCUSSION

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

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 of 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 has 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, 49, 58, 76, 77).

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

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

Author contributions

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

Data statement

All obtained data will be stored in anonymized form at the Danish National Archives and deleted 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).

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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.

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

-	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 1st set	30
Repetitions 2nd & 3rd set	15
Repetitions 4th 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 4th set

Table 4. Outcome measures to be collected.

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

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.

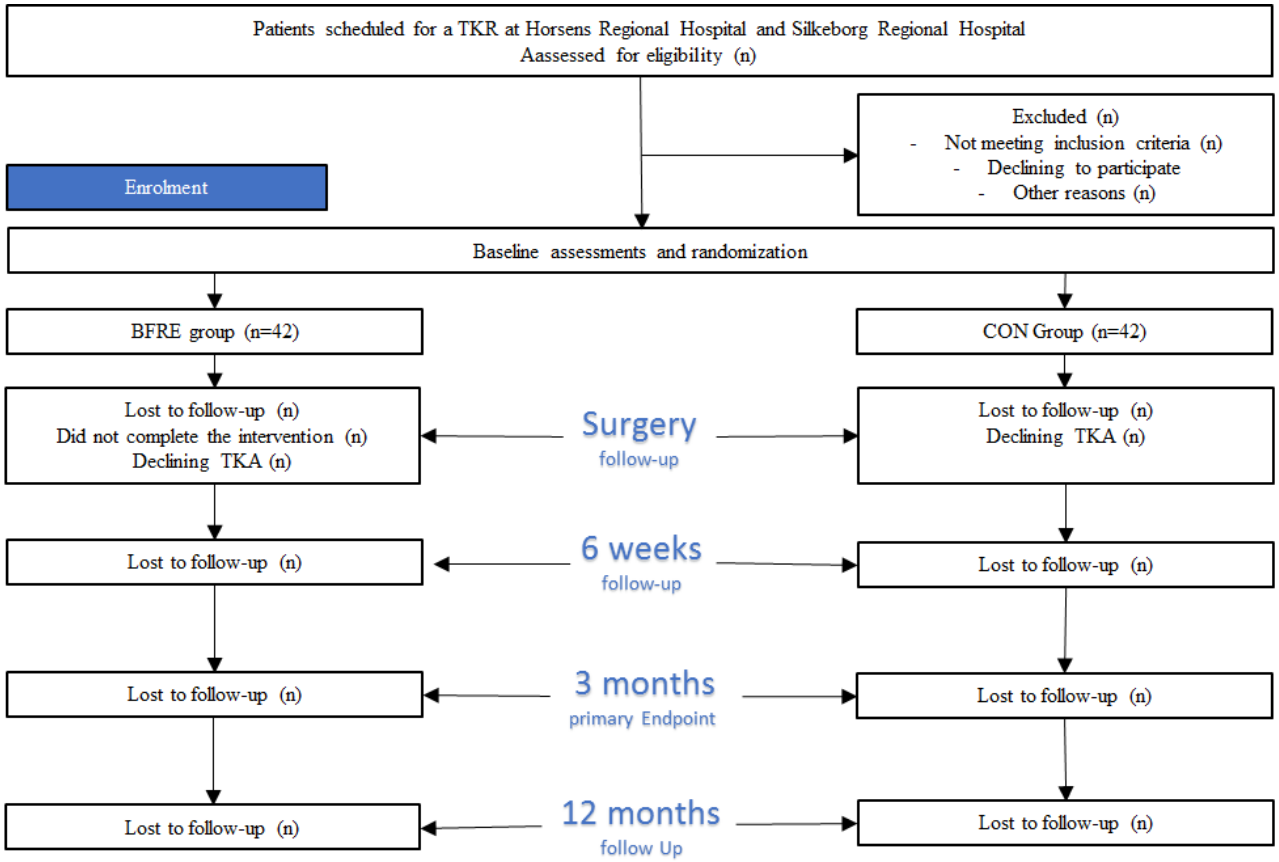
Table 1b. All exercises are performed once per day. Cycling ergometer exercise is optional.

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



SPIRIT
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
Administrative information		
Title (p 1, l 1-3)	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration A: p 2, l 56-57	2a	Trial identifier and registry name. If not yet registered, name of intended registry
B:	2b	All items from the World Health Organization Trial Registration Data Set
Protocol version P 1, l 22	3	Date and version identifier
Funding P 21, l 494-496	4	Sources and types of financial, material, and other support
Roles and responsibilities A: P 1, l 5-11 B: P 1, l 15-20	5a	Names, affiliations, and roles of protocol contributors
	5b	Name and contact information for the trial sponsor
	5c	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
	5d	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)
Introduction		
Background and rationale P 3, l 67-133	6a	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
P 3, l 70-76	6b	Explanation for choice of comparators
Objectives P 5, l 129-136	7	Specific objectives or hypotheses
Trial design P6, l 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, l 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, l 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, l 164-240	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)
C: p12, 283-285	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, l 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, l 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, l 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, l 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

Allocation concealment mechanism P8, l 196-201	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
Implementation P8, l 196-201	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions
Blinding (masking) P8, l 200	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial
Methods: Data collection, management, and analysis		
Data collection methods P 10, l 245-420	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
Statistical methods P 17, l 400-420	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)
P 17, l 400-420	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed

	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, l 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 P7, l 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, l 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, l 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, l 501-502	31b	Authorship eligibility guidelines and any intended use of professional writers

	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated.

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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Primary Subject Heading:	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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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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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, the study aims to investigate to which extent preoperative BFRE will protect against surgery-related atrophy three months after TKR.

Methods

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

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

training or control groups and regression analysis will be used for analyzation of associations between selected outcomes.

Ethical approval

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

Trial registration

The trial is registered at Clinical Trials (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)
- This is a protocol paper

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

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

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 (1, 13-32). The application of low

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 arteriel 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), 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

The primary aim of this trial is to investigate the efficacy of eight weeks of BFRE compared to receiving usual care prior to TKR on postoperative chair stand performance**.** We hypothesize that eight weeks of preoperative BFRE will lead to increased 30 second chair stand performance (30-second Chair Stand Test: 30-s CST) when assessed three months postoperatively. Secondary aims are to investigate the efficacy of preoperative BFRE on lower limb muscle strength three months after TKR and investigate the potential relationship to functional capacity and quality of life. Furthermore, it will be investigated to which extent eight weeks of BFRE induce myofiber hypertrophy and gain in satellite cell number and myonuclei content in the knee extensor musculature.

MATERIAL & METHODS

Design

The trial is designed as a multicenter (two sites), randomized, assessor blinded, controlled trial following the CONSORT guidelines (47). Primary endpoint will be three months after TKR. Additional and secondary endpoints will be evaluated during the week of TKR, six weeks after TKR (questionnaires only) and 12 months after TKR. Muscle biopsies will be obtained from all patients undergoing surgery at Horsens Regional Hospital at baseline, during surgery and three months after TKR.

Participants

Patients will be recruited from the Departments of Orthopedic Surgery at Horsens and Silkeborg Regional Hospitals in Denmark. Patient enrollment will start September 2nd 2019 at Horsens Regional Hospital and October 1st 2019 at Silkeborg Regional Hospital. Patient recruitment is expected to be completed in June 2021. All patients are expected to have completed baseline testing

in September 2021. To account for surgery and intervention, the three-month follow-up will be concluded in April 2022. Thus, at the end of September 2022 all patients are expected to have completed 12-month follow-up testing.

Inclusion criteria: 1) Patients ≥ 50 years scheduled for TKR due to knee OA at Horsens- or Silkeborg Regional Hospital.

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

Please insert figure 1 around here

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

informed consent to participate in the project. Subsequently, the patient will be baseline-tested at the hospital by a blinded (to group allocation) assessor. Patients declining to participate in the RCT will be offered the option of participating in a parallel observational cohort trial. All patients included in the project will be scheduled for a TKR. Two-three weeks before surgery all patients will be invited to a, preoperative information meeting where nurses, surgeons, and physiotherapists will provide detailed information on pain management, nutrition, the surgical procedure, physical activity, postoperative home-based rehabilitation (table 1a and 1b), load management, etc. (usual care) (48). On the day of surgery, patients will be hospitalized at Horsens Regional Hospital or Silkeborg Regional Hospital where an orthopedic chief physician will perform the TKR procedure. The day after surgery all patients will receive physiotherapy-supervised training once or twice per day by a physiotherapist in order to fulfill the discharge criteria (table 2a and 2b) (48). Patients will generally be discharged within ~one-two days after fulfilling all the discharge criteria listed above. After discharge, all patients will receive a standard home-based rehabilitation program focusing on improving knee joint mobility, increasing the tolerance for standing without assistive devices, and lower extremity muscle strength. Variations in the selection of exercises and exercise variables exist in the standard home-based rehabilitation programs between the respective hospitals; however, the purpose of the programs is identical. If the patients do not fulfill the discharge criteria, they will be offered supervised knee-specific exercise therapy at a municipal rehabilitation center or specialized hospital-based rehabilitation after discharge from the hospital.

Please insert table 1a and 1 b about here

Please insert table 2a and table 2b around here

Randomization

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

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

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

Intervention procedures

BFRE

Each BFRE session will consist of a 10-minute warm up (ergometer cycling) followed by two different unilateral lower-limb resistance training exercises: 1) leg press and 2) knee extension performed on standard strength training machines. Each exercise will be performed with the affected lower limb only and consist of four rounds interspaced by 30 seconds of rest (table 3). First round: 30 repetitions (reps); second round: 15 reps; third round: 15 reps; fourth round: until exhaustion (Table 1). If patients can perform more than 15 repetitions in the fourth exercise set, the exercise load will be increased with the minimum extra load possible (30). Participants will be

instructed to perform both the eccentric and concentric contraction phases using a steady 2-second pace duration. The fourth 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 second rest period, patients will rest in a standardized resting position while maintaining the initial cuff-pressure. Between each exercise, patients will have a 5-minute "free-flow" rest period. The 5 minutes rest period applied between exercises was chosen based on experiences from a previous pilot project (Jorgensen & Bohn 2019, unpublished data) and experience with applying BFRE in clinical practice. In both situations, we often experienced that patients stayed seated in the leg press machine for >2 minutes after the last (fatiguing) set to feel sufficiently rested and confident to walk from one exercise machine to another. 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 the starting load intensity will be 30% with 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 pressure (mmHg) needed for total blood flow occlusion, the cuff will gradually be inflated in 20 mmHg steps until reaching the pressure where the auscultatory pulse is interrupted (LOP). The first time the auscultatory pulse is interrupted, the examiner releases 10-20 mmHg pressure from the cuff until the auscultatory pulse is present again. When the auscultatory pulse reappears, the cuff is inflated with 10 mmHg until the LOP is found again. If the second LOP is identical to the first, it

will be defined as the LOP for that specific patient. Otherwise, the procedure will be repeated until determining an identical LOP two consecutive times.

Please insert Table 3 about here

Outcome variables

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

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

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 = load (kg)/1.0278-0.0278·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 HDD will be positioned 5 cm above the medial

malleolus (56). The 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 extensor muscle strength testing with HDD (56, 57). Patients will receive four trials. For analysis, the mean maximal strength of the second, third and fourth measures will be calculated and corrected for bodyweight (56)

MVC of the knee flexors will be measured and performed using HHD at 90° knee flexion with the patients seated identically as during MVC for the knee extensors (56). The HHD will be positioned posterior aspect of calcaneus (56) 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 (56). Patients will receive four trials. For analysis, the mean maximal strength of the second, third and fourth measures will be calculated and corrected for bodyweight (56)

Myofiber cross sectional area (CSA), muscle fiber type composition, satellite cell content, and myonuclei number will be assessed by obtaining needle biopsies (100-150 mg) from all patients enrolled at Horsens Regional Hospital. The biopsies will be obtained bilaterally from the middle portion of the vastus lateralis muscle utilizing the percutaneous needle biopsy technique of Bergström (49, 58, 59). Biopsies will be performed by two experienced orthopedic surgeons (chief physicians) trained in performing the needle muscle biopsy technique at Horsens Regional Hospital. Efforts will be made to extract tissue from the same region (2-3 cm apart) and depth (~1-2 cm.) (49). The tissue samples will be dissected of all visible blood, adipose tissue, and connective tissue and mounted in Tissue-Tec (4583, Sakura Finetek, Alphen aan den Rijn, The Netherlands), frozen

in isopenate pre-cooled with liquid nitrogen, and stored at -80°C (31, 49, 60). All muscle samples will be analyzed as previously described by Nielsen et al. (31) using immunofluorescence microscopy. Transverse serial sections (8 μm) of the embedded muscle biopsy specimen will be cut at -22°C using a cryostat (HM560; Microm, Walldorf, Germany) and will be mounted on glass slides for subsequent analysis as described in detail elsewhere (31). Myogenic stem cells (satellite cells (SC)) will be visualized with an antibody against Pax7 (31). Type I (stained) and Type II (unstained) myofibers will be differentiated, and muscle fiber area will be determined (31): MSC-derived nuclei will stain positive for Pax7 and be within the basal lamina; nuclei (DAPI stained) with a sublaminar placement will be considered myonuclei (31).

Knee disability and Osteoarthritis Outcome Score (KOOS) is a patient-administered knee specific questionnaire comprising five subscales: Pain; Symptoms; Activities of daily living; Sport & Recreation; and Knee-Related Quality of Life. Each item is scored from 0 to 4 (61). The raw score for each of the five subscales is the total sum of the associated item scores. Scores can be transformed to a 0 to 100 scale. The scores of the five subscales can be expressed as a composite outcome profile, higher scores indicating fewer problems (62). The KOOS questionnaire is valid and reliable in patients suffering from knee OA and patients on the waiting list for TKA for knee OA (61, 63, 64).

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.

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

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 maximally (70) The fulcrum of the goniometer will correspond visually to the trans-epicondylar axis of the knee joint. The moveable arms of the goniometer will be pointed towards the greater trochanter and the lateral malleolus (70).

Data management

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

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

Sample size

The power and sample size calculation is based on the expected differences between the two subject groups from baseline to three-month follow-up (8). Due to lack of data on the primary outcome for

investigations applying LL-BFRE before a surgical procedure, we decided to base our sample size calculation on Skoffer 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 ± 4.7 repetitions versus 11.0 ± 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, α-level is set at 0.05 ($p<0.05$) and β-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

the potential pre-to-post training differences within the respective training or control groups, paired student t-tests will be performed. Level of statistical significance is $P < 0.05$. *Secondary outcome variables:* Between-intervention comparison from baseline to the week of surgery, six weeks after surgery, three and 12 months after surgery will be analyzed as described for the primary outcome. Regression analysis will be used to analyze the potential associations between preoperative strength and postoperative lower extremity function and self-reported outcome as well as between preoperative functional capacity and postoperative functional capacity. Additionally, regression analysis will be used to analyze the association between preoperative number of satellite cells and myonuclei on postoperative isometric knee extensor muscle strength, muscle fiber cross sectional area, and functional capacity. All statistical analyses will be performed by the primary investigator using Stata.

Ethical aspects and dissemination

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 (Journal No 652164). The trial is registered at Clinicaltrials.gov (NCT04081493). Before inclusion, all patients will provide their written informed consent in accordance with the Helsinki Declaration. All data and information collected in regard to this trial will be treated confidentially (blinded and encrypted) by the researchers and staff connected to the trial.

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

Patient and public involvement

Before developing this clinical trial, a pilot project was performed to determine the feasibility and

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 resulted in useful knowledge that certainly has improved the development of the present clinical trial.

DISCUSSION

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

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 of 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 has 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, 49, 58, 76, 77).

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

after discharge between sites. However, all patients included in the present project will report whether they have received postoperative supervised physiotherapy at all follow-up assessments. Thus, we will be able to determine (and normalize) a potential between-site difference in patients receiving supervised physiotherapy after TKR. Also, site-specific differences in the postoperative rehabilitation protocols (Tables 1a and 1b) may be considered a limitation. That is, the protocols contain both identical but also different exercises and progression steps. However, a recent review and meta-analysis found no difference in effectiveness between clinic-based or inpatient programs compared with home-based rehabilitation programs in the early subacute period after TKA (27) and studies in other knee patient populations have also been unable to observe differences in main outcome variables when comparing home-based postoperative rehabilitation to supervised postoperative rehabilitation (28, 29). We feel confident therefore that the apparent differences between the postoperative rehabilitation protocols are not highly likely to affect the results of the present study. Nonetheless, to verify this notion we will introduce site allocation (Horsens Hospital vs. Silkeborg Hospital) as a separate independent variable in the mixed linear model used for the statistical analysis.

Author contributions

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

Data statement

All obtained data will be stored in anonymized form at the Danish National Archives and deleted 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).

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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.

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

-	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

All exercises are performed twice per day.

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 1st set	30
Repetitions 2nd & 3rd set	15
Repetitions 4th 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 4th set

Table 4. Outcome measures to be collected.

Outcome measures	Data collection instrument	Time-points of assessment

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

839

840

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.

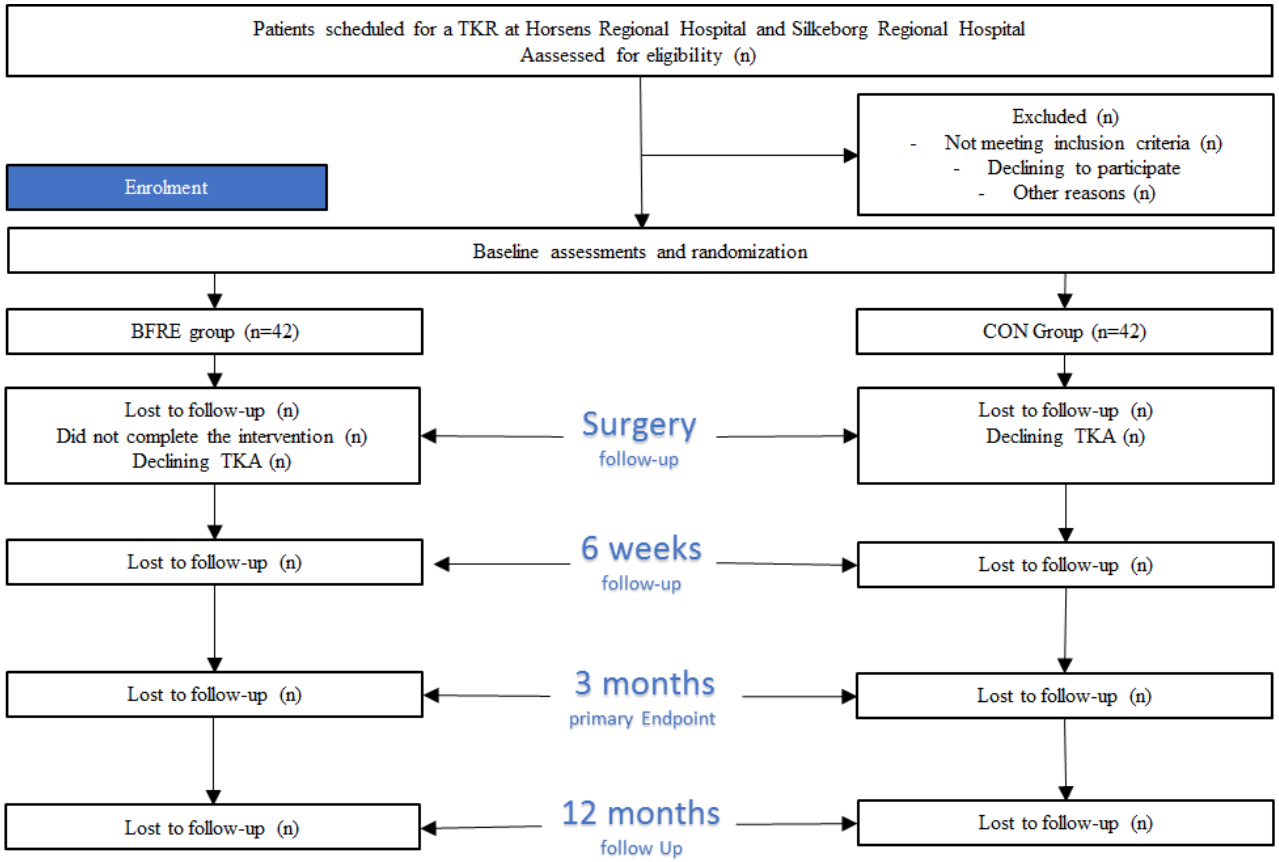
Table 1b. All exercises are performed once per day. Cycling ergometer exercise is optional.

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



SPIRIT
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
Administrative information		
Title (p 1, l 1-3)	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration A: p 2, l 56-57	2a	Trial identifier and registry name. If not yet registered, name of intended registry
B:	2b	All items from the World Health Organization Trial Registration Data Set
Protocol version P 1, l 22	3	Date and version identifier
Funding P 21, l 494-496	4	Sources and types of financial, material, and other support
Roles and responsibilities A: P 1, l 5-11 B: P 1, l 15-20	5a	Names, affiliations, and roles of protocol contributors
	5b	Name and contact information for the trial sponsor
	5c	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
	5d	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)
Introduction		
Background and rationale P 3, l 67-133	6a	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
P 3, l 70-76	6b	Explanation for choice of comparators
Objectives P 5, l 129-136	7	Specific objectives or hypotheses
Trial design P6, l 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, l 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, l 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, l 164-240	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)
C: p12, 283-285	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, l 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, l 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, l 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, l 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

Allocation concealment mechanism P8, l 196-201	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
Implementation P8, l 196-201	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions
Blinding (masking) P8, l 200	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial
Methods: Data collection, management, and analysis		
Data collection methods P 10, l 245-420	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
Statistical methods P 17, l 400-420	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)
P 17, l 400-420	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed

	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, l 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 P7, l 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, l 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, l 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, l 501-502	31b	Authorship eligibility guidelines and any intended use of professional writers

	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated.