PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	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.
AUTHORS	Jørgensen, Stian; Bohn, Marie; Aagaard, Per; Mechlenburg, Inger

VERSION 1 – REVIEW

REVIEWER	Sara R. Piva University of Pittsburgh
REVIEW RETURNED	12-Oct-2019

GENERAL COMMENTS	Thank you for the opportunity to review this research protocol for a multicenter randomized trial examining the preoperative intervention of low-load blood flow restriction exercise (BFRE) on postoperative outcomes of total knee replacement (TKR). The aim of the study is to determine the impact of an 8-week pre-operative course of BFRE versus usual care prior to TKR. Patients will be recruited by the Orthopedics departments at both Horsens and Silkeborg regional hospitals and be randomized (1:1) after baseline. The primary outcome is the 30-second chair stand test with secondary outcomes that include: timed up and go test, 40m fast paced walk test, maximal isometric knee extension and flexion strength (assessed with handheld dynamometer), vastus lateralis myofiber area, fiber type composition, satellite cell content, myonuclei number, KOOS, NRS for pain, and EuroQol. Primary endpoint will be 3 months post TKR, with additional assessments at week of TKR, 6 weeks post TKR, and 12-month TKR. The use of BFRE preoperatively in TKR is a novel and interesting premise. The topic is important to the clinical physical therapist as BFRE increases in popularity. The authors present a nice rationale to support the need for further research on BFRE in this population. The study aims are clear, the study design is correctly justified to answer the research question, and eligibility criteria is clear. The description of the BFRE protocol makes it very easy reproduce in any clinic that has the capability to perform this training. The outcome measures are appropriate and encompass different domains. The methods of measurement and follow-up periods are also detailed and reproducible. However, several weakness decrease the enthusiasm for this protocol paper and warrant clarification or inclusion of new information: TITLE In the title, there is no mention of this being a protocol paper; this needs to be indicated in the title so that the reader is aware of the fact it is a protocol paper from the beginning.

	ABSTRACT The abstract needs better organization. The first paragraph has excessive information about outcome measures. This information is not only repetitive but should be described in the methods section. The methods section leaves the reader wanting further information; the methods should include further details of the parameters of the study` along with statistics measures. INTRODUCTION • There is mention of “mixed results” of pre-rehabilitation of TKR, but the argument could be strengthened; there is more to know from the reader point of view in terms of the successes vs failures of previous studies and why this is an important topic to research. • Readers may question the normal parameters anticipated for a BFRE protocol for this population. For example, is that restriction specific to OA populations? Are there any risks or precautions that should be considered? METHODS • Protocol papers should report dates of the study. There are no dates indicating when recruitment starts, time points to start and complete intervention protocol, and expected date of completion. There should be dates included so the reader has a sense of where the study is in the process, currently recruiting, testing, etc. • The title implies that participants have knee OA. However, TKR surgeries are also done for reasons other than knee OA, such as inflammatory arthritis, tumor, and trauma. Please clarify whether TKR due to knee OA is an inclusion criteria versus not. • There should be further clarity as to who is enrolling the patients in the study; is it the orthopedic surgeon, nurses, physical therapist? I realize you stated on page 7 in line 10 the orthopedic surgeon will perform initial inclusion so if they are also enrolling the patients that should be specifically clarified at this point. • In regards to assessors, how are they being trained for the study protocol? What are the plans to maintain fidelity of testing? This is also true for the interventionists. How are they trained and retrained? What frequency? • How are the assessors being blinded and is there is a protocol to prevent break of blinding? • In regards to the secondary outcomes; specifically, myofiber area, fiber type composition, satellite cell content, and myonuclei number how are theses being determined? As you indicated on page 14 in line 16 the biopsy is being performed by a trained medical professional but how is the procedure being performed, where in the vastus lateralis is the sample being taken, how are the samples being plated and stained? These questions should be clarified or there should be additional sources to indicate these procedures. The paper also indicates explorative outcome variables will be taken on page 10 line 27; what are these variables specifically (information on postoperative rehabilitation, etc) and when will these variables be taken. • Clarify why is the KOOS the only outcome measure assessed at 6 weeks post-op. • Clarify/justify the selection of the endpoint being at 3 months postop as opposed to just prior to the surgery. It seems that choosing a time period just prior to the surgery would have allowed for better control of external variables impacting the study design. This topic should be addressed in the research limitations. • Clarify whether the protocol parameters for the BFRE protocol are supported by evidence. For example, is it common to use 60% restriction? Is that restriction specific to OA populations? • Another area of clarity with the BFRE group is how is the 1RM

	done, one in each machine? Is the 1RM being assessed on the first treatment day for each exercise or is being calculated off of other working sets. What is the frequency of 1RM repeated to adjust dose of exercise? If the 1RM is done frequently, it could contribute to the improvement in the study outcomes. • It is unclear what the usual group receives as treatment (CON group). Is the control group not getting any care or direction? Are patients in these hospitals prescribed any exercise program prior to surgery? There needs to be further clarification as to what the control group is doing for the 8 weeks prior to TKR. • A major weakness of this protocol paper is the lack of information about post-surgical rehabilitation protocol. Will both the control and BFRE group utilize the same rehabilitation protocol post TKR to prevent differences in rehabilitation from impacting the assessments at 6 weeks and 12 months post TKR? There needs to be further clarity on the course of treatment post operatively, including home exercise program and physical therapy regimens. If this is something that is being surveyed/completed, this should be further explained within the paper. This topic should be addressed in the research limitations. • When determining the sample size required for the study, justify why was the HRST data (p. 11 line 11) utilized to determine the power and sample size calculations? • With the multitude of variables being assessed at multiple times, there is no indication as to whether there are plans to account multiplicity or if these are intentional not corrected in an attempt to curtail Type II errors, which would be appropriated. There should be clarification in regards to reasons to adjust versus not for multiplicity. • There are areas of improvement for grammar and typing errors that should be reviewed prior to resubmission.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1

(1) Review comment:
In the title, there is no mention of this being a protocol paper; this needs to be indicated in the title so that the reader is aware of the fact it is a protocol paper from the beginning.

Authors reply
Thank you for your comment.
The title has been rephrased as suggested to include the term 'protocol article' (lines 1-3):
"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"

(2) Review comment:
The abstract needs better organization. The first paragraph has excessive information about outcome measures. This information is not only repetitive but should be described in the methods section. The methods section leaves the reader wanting further information; the methods should include further details of the parameters of the study` along with statistics measures.

Authors reply:
Information about outcomes measures mentioned in the Introduction section has been adjusted and incorporated into the Methods section (lines 41-48). Further, additional information on some outcome parameters have been included in the Methods section . Finally, the statistical analysis methods have been elaborated, as suggested (lines 44-48).

(3) Review comment:
There is mention of “mixed results” of pre-rehabilitation of TKR, but the argument could be strengthened; there is more to know from the reader point of view in terms of the successes vs failures of previous studies and why this is an important topic to research.

Authors reply:
We have now rephrased this paragraph to include more information about previous results related to preoperative training in relation to total knee replacement (lines 82-89))
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 (1, 2). 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 (3, 4). 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 (5-7)"

(4) Review comment:
Readers may question the normal parameters anticipated for a BFRE protocol for this population. For example, is that restriction specific to OA populations? Are there any risks or precautions that should be considered?

Authors reply:
Currently, no recommendations exist for specific BFR parameters for certain patient populations. However, based on previous investigations using BFRE in knee OA-patients and recommendations reported in systematic reviews and meta-analyses, the present parameters used for loading intensity (% 1RM), training frequency, occlusion pressure, etc. were considered safe and affective of promoting adaptations in muscle mass and mechanical muscle function, respectively. We have now elaborated on this (lines 101-106).
" 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 (3, 8, 9). This is in line Hughes et al. (10), who suggested that when BFRE is performed correctly it has been demonstrated to be as safe as free-flow exercise methods (10)."

(5) Review comment:
Protocol papers should report dates of the study. There are no dates indicating when recruitment starts, time points to start and complete intervention protocol, and expected date of completion. There should be dates included so the reader has a sense of where the study is in the process, currently recruiting, testing, etc.

Authors reply:
Thank you for this constructive comment. Information now has been added on the expected time periods of patient recruitment, start and completion of the intervention protocol, and expected date of study completion (lines 144-148):"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."

(6) Review comment:
The title implies that participants have knee OA. However, TKR surgeries are also done for reasons other than knee OA, such as inflammatory arthritis, tumor, and trauma. Please clarify whether TKR due to knee OA is an inclusion criteria versus not.

Authors reply:
Only patients suffering from knee OA were included in the present study. This has been made clearer in the revised manuscript (line 150):

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

(7) Review comment:
There should be further clarity as to who is enrolling the patients in the study; is it the orthopedic surgeon, nurses, physical therapist? I realize you stated on page 7 in line 10 the orthopedic surgeon will perform initial inclusion so if they are also enrolling the patients that should be specifically clarified at this point.

Authors reply:
Our orthopedic surgeons performed the initial screening and invitation to participate in the project. Subsequently, a more comprehensive screening including the formal enrollment was performed by the principal Author (project site 1) or a project manager (physiotherapist in charge of training at project site 2). This information has now been made more clear to the reader in the revised manuscript (lines 161-169):
"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."

(8) Review comment:
In regards to assessors, how are they being trained for the study protocol? What are the plans to maintain fidelity of testing? This is also true for the interventionists. How are they trained and retrained? What frequency?

Authors reply:
All testers and assessors will be trained in their specific experimental procedures prior to study start and subsequently every 3rd months. This information has now been included in the revised manuscript (lines 255-262):
"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."

(9) Review comment:
How are the assessors being blinded and is there is a protocol to prevent break of blinding?

Authors reply:
All assessors will be blinded to intervention allocation (pre surgery BFRE training or usual care). 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. Further, participants will be carefully instructed not to reveal their group allocation to any assessors.

(10) Review comment:
In regards to the secondary outcomes; specifically, myofiber area, fiber type composition, satellite cell content, and myonuclei number how are theses being determined? As you indicated on page 14 in line 16 the biopsy is being performed by a trained medical professional but how is the procedure being performed, where in the vastus lateralis is the sample being taken, how are the samples being plated and stained? These questions should be clarified or there should be additional sources to indicate these procedures. The paper also indicates explorative outcome variables will be taken on page 10 line 27; what are these variabes specifically (information on postoperative rehabilitation, etc) and when will these variables be taken.

Authors reply:
Information now has been included in the revised manuscript about the specific muscle biopsy sampling procedures (location within the VL muscle) as well as on the procedures used to analyze myofiber area, satellite cell content and myonuclei number (lines 356-373). Furthermore, I have included more information on all other outcomes as well (lines 302-354 + 375-431) :
"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 (11-13). 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.) (13). 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 (13-15). All muscle samples will be analyzed as previously described by Nielsen et al. (14) 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 (14). Myogenic stem cells (satellite cells (SC)) will be visualized with an antibody against Pax7 (14). Type I (stained) and Type II (unstained) myofibers will be differentiated, and muscle fiber area will be determined (14): 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 (14)".

"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 (16-18).

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 (19, 20). Good inter-rater reliability has been demonstrated with the TUG test *(18)*.

4x10 meter walk test meter walk test (40m-FWT) **measures the total time taken to walk 4 x 10 m excluding turns (meter/sec) (18). 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 (18). 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 (18).**

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 consisted of 12 repetitions, and the 3rd warm-up set will consist of 8 repetitions. The load of each warm-up set will be increased with 10 kilos. After the 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)] (21).**

1RM knee extension strength **will be estimated from 5-8RM knee extension test as described above for the estimation of 1RM leg press test (21).**

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 (22). 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 (22, 23). 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 (22)

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 (22). The HHD will be positioned posterior aspect of calcaneus (22) 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 (22). 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 (22)"

" **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 (24). 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 (25). The KOOS questionnaire is valid and reliable in patients suffering from knee OA and patients on the waiting-list for TKA for knee OA (24, 26, 27).

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 (28), which will be converted into a summary index ranging from -0.624 (worst) to 1.000 (best), using a Danish value set (29). The second part, EQ-VAS rates the overall current health status from 0 (worst imaginable health) to 100 (best imaginable health) (28). The EQ-5D-5L is reliable and valid in patients with knee osteoarthritis eligible for TKA, (30, 31)

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 (32). 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 (32). 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 (32) 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 (32)."

(11) Review comment:
Clarify why is the KOOS the only outcome measure assessed at 6 weeks post-op.

Authors reply:
In fact, several other outcome measures will be assessed in addition to KOOS (Knee disability and Osteoarthritis Outcome Score) at 6 weeks post-op: EuroQol Group 5-dimensions (EQ-5D-L5) and monitoring of adverse event-
This has now been made clearer in the revised manuscript (lines 245-248):
. 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 completed.

(12) Review comment:
Clarify/justify the selection of the endpoint being at 3 months postop as opposed to just prior to the surgery. It seems that choosing a time period just prior to the surgery would have allowed for better control of external variables impacting the study design. This topic should be addressed in the research limitations.

Authors reply:
Thank you for this comment. The aim of our project almost dictates a postoperative primary end point. We agree that the present study design leaves space for the influence of uncontrolled effects (for instance the individual success of TKR surgery) between surgery and 3 months post-op. Hence, the results obtained/span> at 3 months post-op will reflect the performance level after a 3 months period where patients have returned to their usual activities of daily living. As suggested, we have now included this issue in the Discussion section (lines 519-525):
"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. "

(13) Review comment:
Clarify whether the protocol parameters for the BFRE protocol are supported by evidence. For example, is it common to use 60% restriction? Is that restriction specific to OA populations?

Authors reply:
Both the protocol (30-15-15-to failure), protocol details such as rest periods between sets, occlusion pressure, initial training load, and load progression were based on protocols reported in previous investigations in clinical patients. These intervention parameters have previously been employed with BFRE training in OA patients, but are not specific to this population. A restrictive pressure between 40%-80% of total limb occlusion pressure (LOP) has been suggested by Hughes et al. (10) (please find References listed below).

(14) Review comment:
Another area of clarity with the BFRE group is how is the 1RM done, one in each machine? Is the 1RM being assessed on the first treatment day for each exercise or is being calculated off of other working sets. What is the frequency of 1RM repeated to adjust dose of exercise? If the 1RM is done frequently, it could contribute to the improvement in the study outcomes.

Authors reply:
1RM testing will be performed at all test sessions (i.e. baseline, in the week prior to surgery, 3 months post-op, 12 months post-op). As mentioned in the manuscript exercise load will be progressed in a given exercise when the participant performs more than 15 repetitions in the 4th set. This information has now been included in the revised manuscript (lines 325-330):
"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 consisted of 12 repetitions, and the 3rd warm-up set will consist of 8 repetitions. The load of each warm-up set will be increased with 10 kilos. After the 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)] (21)."

(15) Review comment:
It is unclear what the usual group receives as treatment (CON group). Is the control group not getting any care or direction? Are patients in these hospitals prescribed any exercise program prior to surgery? There needs to be further clarification as to what the control group is doing for the 8 weeks prior to TKR.

Authors reply:
Patients scheduled for total knee replacement surgery typically are invited to an information meeting a few weeks prior to surgery. The information meeting contains information about the surgical procedure, nutrition, physical activity, smoking and an introduction to the unsupervised, home-based, postoperative exercise program. Apart from this information meeting, Danish elektive TKR patients are not offered any preoperative treatment. Both groups (BFRE, CON) will be invited to this preoperative information meeting.
This point has now been made more clear in the revised manuscript (lines 170-175 + 204):
" 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. (33)" + " BFRE group: In addition to receiving ususal 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"

(16) Review comment:
A major weakness of this protocol paper is the lack of information about post-surgical rehabilitation protocol. Will both the control and BFRE group utilize the same rehabilitation protocol post TKR to prevent differences in rehabilitation from impacting the assessments at 6 weeks and 12 months post TKR? There needs to be further clarity on the course of treatment post operatively, including home exercise program and physical therapy regimens. If this is something that is being surveyed/completed, this should be further explained within the paper. This topic should be addressed in the research limitations.

Authors reply:
Thank you for this important comment. We fully agree that it is highly relevant to include information about the postoperative plans for the intervention- and control group. At both hospitals patients are discharged ~1 day after surgery, while receiving a written unsupervised, home-based, postoperative exercise program. However, if the discharge criteria are not met at discharge, patients will be offered supervised rehabilitation at a municipal physical therapy center. To reflect the real-life setting as much as possible, the project does not offer any special postoperative treatment to any of the patient groups. If the surgeon or the physiotherapist decide that the patient needs supervised physiotherapy to profit from the TKR surgery, patients will be offered supervised physical therapy after surgery. Surgeon and physiotherapists will be basing their evaluation and decision on objective

discharge criteria (described in the revised manuscript, c.f. lines 177-183)). A survey 6 weeks-, 3 months, and 12 months post-op will be used to monitor if patients have received any knee-related, supervised physiotherapy postoperatively.
This point has now been made more clear in the revised manuscript (lines 177-191):
" . 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 (33). 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"

(17) Review comment:
When determining the sample size required for the study, justify why was the HRST data (p. 11 line 11) utilized to determine the power and sample size calculations?

Authors reply:
Low-resistance BFRE training and conventional heavy-resistance strength training (HRST) have both demonstrated to increase muscle strength, promote muscle hypertrophy and improve functional performance in young and old adults. Notably, BFRE has been documented to induce similar gains in maximal muscle strength and skeletal muscle mass compared with HRST (34), which may justify the use of HRST-based data to determine the present sample size.

(18) Review comment:
With the multitude of variables being assessed at multiple times, there is no indication as to whether there are plans to account multiplicity or if these are intentional not corrected in an attempt to curtail Type II errors, which would be appropriated. There should be clarification in regards to reasons to adjust versus not for multiplicity.

Authors reply:
According to Guowei et al. (35) "There is no need to adjust for multiplicity when there is a single primary outcome, as findings for secondary [outcome variables] are considered subsidiary and exploratory, rather than confirmatory"(35)

(19) Review comment:
Table citation missing
The in-text citation for ‘table 1’ is missing. Please provide the missing citation and ensure that all citations of tables are in ascending order.

Authors reply:
Thank you for that comment, referencing to Table 1 has now been provided in the text (line 217):
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).

(20) Reviewer comment:
Figure/s should only be uploaded as ‘Image’ and not embedded in the main document

Authors reply:
As requested, Figure 1 has now been removed from the document file and submitted as a separate image.

(21) Review comment:
Required figure/s format
Figures can be supplied in TIFF, JPG or PDF format (figures in document, excel or powerpoint format will not be accepted), we also request that they have a resolution of at least 300 dpi and 90mm x 90mm of width.

Authors reply:
We have now uploaded Figure 1 in PDF format to ensure maximal printing quality.

(22) Review comment:
Please remove table 1 and table 2 uploaded separately as it is already embedded in your main document.

Authors reply:
Tables 1 and 2 are now reported in the main document only.

(23) Review comment:
There are areas of improvement for grammar and typing errors that should be reviewed prior to resubmission.

Authors reply:
The manuscript has been thoroughly checked for grammar- and typing errors before resubmission

VERSION 2 – REVIEW

REVIEWER	Sara Piva University of Pittsburgh
REVIEW RETURNED	08-Apr-2020

GENERAL COMMENTS	Thank you for the thorough revision of the paper. The changes have improved the clarity and quality of this protocol paper. After our second review, we recommend further work on the following areas of your paper. Abstract: Please revise the statement in line 39: "…collected at baseline, in the week of TKR." It is unclear when the baseline takes place and whether the "week of TKR" refers to before or after the operation. Strengths and limitations of this study: It should be made clear that this is a protocol paper. Introduction: Nice new information to support use of BFR, thank you. Please consider these additional suggestions: • The second paragraph is long. It would be beneficial to break the paragraph at line 89 with the sentence starting as "Resistance training..". as the beginning of the new paragraph. • To improve flow, please consider moving sentence in lines 96 to 98 - "The adaptive mechanisms evoked by BFRE….stem cells (satellite cells: SC)" to the beginning of the next paragraph, the one that starts with "Satellite cells are…" It would be helpful to have information about potential mechanisms of BFRE in the same paragraph. Methods: • There remains some question about the time frames noted in lines 144-148. These time frames should be reviewed because this layout currently does not allow for the 8 weeks of BFR treatment, surgery and a 3 month follow up.

	• Additionally, we understand that the patients are screened by an orthopedic surgeon, but it would be beneficial to note how many surgeons at each hospital are participating in this study and their level of experience. Due to the small study sample size, this could be an important variable to consider for adjustment or subgroup analysis. • When describing the HEPs and rehab protocols (lines 174-175), it would be useful for readers to know what exercises are included in your "standard program" to make the findings of this study more reproducible and applicable to clinical settings. Please consider including this information in a table/chart where the exercises are listed with the prescribed reps and sets. • In lines 177-191, the additional information is useful to understand the discharge criteria and basic timeline of a patient undergoing a TKA. There should be further clarification of the "60/90 and 15/5" numbers that explicitly state that these are the different discharge criteria based on the hospital. On first read, these numbers were interpreted as a range that was accepted for the patient to go home. • It became apparent that the discharge criteria and care path after surgery is different in each hospital. Could different pathways of care and protocols between hospitals affect study results? If yes, will hospital be considered for adjustment in the analysis? It is unclear if this is what the authors mean by "normalize" in the limitation paragraph in the discussion section? Please clarity. • The response to comment 9 regarding how blinding of assessors is good, but it should be included in the paper. We were not able to find where this was inserted. • Thank you for adding information about the BFRE protocol. There is still some confusion about the choice of 60% of total limb occlusion pressure for the research protocol. Your rationale for utilizing 60% and not well structured throughout the paper and would be quite confusing for someone who is a novice BFRE user. At different points throughout the manuscript you note 70%, 60%, and 40-80%; you can continue to present in this manner, but there needs to be much more clarity as to why you choose 60% for your study design. Was this percentage arbitrarily selected? Or are you working off of 60% and you are increasing to 80% and/or decreasing to 40% depending on certain criteria? As a protocol paper addressing BFRE the rationale for your occlusion pressure needs to be more concise so that clinicians can replicate your procedure. • Briefly justify the choice of 5 minutes of free flow between exercises. We are familiar with literature which suggests 1 minute free flow minimum between exercises but why have you expanded the time to 5 minutes? • The description of outcome measures needs further organization: o The overview of primary, secondary and other outcome measures at the bottom of page 12 is located inside Data Management section, which does not make sense. o There is no need for the overview of the outcome measures (bottom of page 12 and top of page 13) to be separated from the "Elaborated description of outcome measures. Please consolidate the description of outcome measures in a single location and use appropriate heading for the section. • The reply to the comment on sample size (#17) is hard to follow. Clarify why you used data from HRST rather than BFRE. Could it be because data on primary outcome for BFRE was not available? • We suggest substituting the words "assistive device" in for "crutches" when discussing the progression of patients from the hospital to independent walking in consideration of international variation in the use of walkers, canes and crutches.

	• Overall, we suggest an in depth grammatical and editorial review prior to resubmission: o Inconsistent capitalization of the first letter for the sequence of exclusion criteria. o Repeated expressions such as however in consecutive sentences. o Last sentence before Sample Size section is unfinished.

VERSION 2 – AUTHOR RESPONSE

Reviewer 1

(1) Review comment:
Abstract: Please revise the statement in line 39: "…collected at baseline, in the week of TKR." It is unclear when the baseline takes place and whether the "week of TKR" refers to before or after the operation

Authors reply
The statement has now been further clarified (Lines 39-40).
" Data will be collected before randomization, three-four days prior to TKR, six weeks, three months, and 12 months after TKR.."

(2) Review comment:
Strengths and limitations of this study: It should be made clear that this is a protocol paper.

Authors reply:
A bullet point stating that the current manuscript is a protocol paper has now been added (line 65).
"This is a protocol paper"

(3) Review comment:
Introduction:
Nice new information to support use of BFR, thank you. Please consider these additional suggestions: The second paragraph is long. It would be beneficial to break the paragraph at line 89 with the sentence starting as "Resistance training.". as the beginning of the new paragraph.

Authors reply:
Paragraph has now been adjusted as suggested (lines 86-92).
"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)"

(4) Review comment:
To improve flow, please consider moving sentence in lines 96 to 98 - "The adaptive mechanisms evoked by BFRE….stem cells (satellite cells: SC)" to the beginning of the next paragraph, the one that starts with "Satellite cells are…" It would be helpful to have information about potential mechanisms of BFRE in the same paragraph.

Authors reply:
The sentence has now been moved as suggested (lines 121-125).
" 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)"

(5) Review comment:
Methods:
There remains some question about the time frames noted in lines 144-148. These time frames should be reviewed because this layout currently does not allow for the 8 weeks of BFR treatment, surgery and a 3 month follow up

Authors reply:
Thank you for your thorough review of the time frame. The time layout has now been adjusted to allow for the 8 weeks of BFR treatment, surgery and 3 months follow up. (lines 164-166).
" 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"

(6) Review comment:
Additionally, we understand that the patients are screened by an orthopedic surgeon, but it would be beneficial to note how many surgeons at each hospital are participating in this study and their level of experience. Due to the small study sample size, this could be an important variable to consider for adjustment or subgroup analysis.

Authors reply:
We have now specified the number of surgeons who will be performing the inclusion of participants at each site. All surgeons participating in this study are chief physicians, which indicates their senior level of clinical experience (and expertise) (lines 181-183).
"All patients will be screened for eligibility by four orthopedic chief physicians at Horsens Regional Hospital and by three orthopedic chief physicians Silkeborg Regional Hospital who will perform the initial inclusion of study participants and hand out written project information."

(7) Review comment:
When describing the HEPs and rehab protocols (lines 174-175), it would be useful for readers to know what exercises are included in your “standard program” to make the findings of this study more reproducible and applicable to clinical settings. Please consider including this information in a table/chart where the exercises are listed with the prescribed reps and sets.

Authors reply:
Thank you for this constructive suggestion. This is of course highly relevant due potential between-site differences in the rehabilitation programs applied. We have now included a table where all exercises are listed along with the prescribed repetitions and sets (lines 190-195).
" 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)."

Table 1a. Postoperative rehabilitation program at 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	5 repetitions	3 sets	Slipper minimizes floor friction

	the heel			
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 one time per day.

Table 1b. Postoperative rehabilitation program at 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 once per day. Cycling ergometer exercise is optional.

(8) Review comment:
In lines 177-191, the additional information is useful to understand the discharge criteria and basic timeline of a patient undergoing a TKA. There should be further clarification of the “60/90 and 15/5” numbers that explicitly state that these are the different discharge criteria based on the hospital. On first read, these numbers were interpreted as a range that was accepted for the patient to go home.

Authors reply:
Instead of listing the discharge criterions in the text, we have included all this information in a designated table. Hopefully this should ease the read and explicitly state the difference between sites (lines 197-198).
"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)."

Table 2a. Discharge criterions 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 criterions 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

(9) Review comment:
It became apparent that the discharge criteria and care path after surgery is different in each hospital. Could different pathways of care and protocols between hospitals affect study results? If yes, will hospital be considered for adjustment in the analysis? It is unclear if this is what the authors mean by "normalize" in the limitation paragraph in the discussion section? Please clarity.

Authors reply:
Our clinical experience from both patients undergoing total knee replacement surgery as well as other patients (elective hip- and knee surgery) indicates that the different pathways and protocols between hospitals will not affect the study results to any large extent. The differences in chosen pathways and protocols at each hospital (site) will rely on the specific culture and traditions within each of these hospital departments. Furthermore, no national guidelines exist for discharge criteria or postoperative rehab procedures after TKR. Therefore, the discharge criteria and rehab programs will likely differ slightly between hospitals in Denmark. Furthermore, studies in other knee patient populations do not find any difference in outcomes when comparing home based postoperative rehabilitation to supervised postoperative rehabilitation {Hohmann, 2011 #366;Jokl, 1989 #367}. Thus, we do not think it is relevant to make adjustments in the analysis based on which hospital departments participants will be treated.

(10) Review comment:
The response to comment 9 regarding how blinding of assessors is good, but it should be included in the paper. We were not able to find where this was inserted

Authors reply:
We have now included our response to Comment 9 regarding blinding of assessors (lines 278-281 + lines 283-286)
" 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."
"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."

(11) Review comment:
Thank you for adding information about the BFRE protocol. There is still some confusion about the choice of 60% of total limb occlusion pressure for the research protocol. Your rationale for utilizing 60% and not well structured throughout the paper and would be quite confusing for someone who is a novice BFRE user. At different points throughout the manuscript you note 70%, 60%, and 40-80%; you can continue to present in this manner, but there needs to be much more clarity as to why you choose 60% for your study design. Was this percentage arbitrarily selected? Or are you working off of 60% and you are increasing to 80% and/or decreasing to 40% depending on certain criteria? As a protocol paper addressing BFRE the rationale for your occlusion pressure needs to be more concise so that clinicians can replicate your procedure.

Authors reply:

We agree that it can be confusing to understand which restrictive pressure to use. To date, no strict consensus exists on this topic. Therefore, we have now added more information about our choice of using an exercise pressure corresponding to 60% of total limb occlusion pressure (line 104-120).
"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."

(12) Review comment:
Briefly justify the choice of 5 minutes of free flow between exercises. We are familiar with literature which suggests 1 minute free flow minimum between exercises but why have you expanded the time to 5 minutes?

Authors reply:
The 5 minutes free flow rest period between exercises were actually chosen based on experiences from both our own clinical practice and from a previous pilot project performed at our department. In both cases, we often experience that patients are not ready/wiling to perform any activities in the minutes after the last fatiguing set. Thus, many patients/participants choose to stay seated in the leg press machine to recover in order to regain the confidence to walk on the exercised leg (lines 241-246).
" 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."

(13) Review comment:
The description of outcome measures needs further organization:
o The overview of primary, secondary and other outcome measures at the bottom of page 12 is located inside Data Management section, which does not make sense.
o There is no need for the overview of the outcome measures (bottom of page 12 and top of page 13) to be separated from the “Elaborated description of outcome measures. Please consolidate the description of outcome measures in a single location and use appropriate heading for the section.

Authors reply:
New heading have been added to the outcome section (line 292)
“Outcomes”
Data management has been moved (lines 442-453)

(14) Review comment:
The reply to the comment on sample size (#17) is hard to follow. Clarify why you used data from HRST rather than BFRE. Could it be because data on primary outcome for BFRE was not available?

Authors reply:
Indeed, this was the reason for using data from HRST rather than from BFRE (lines 458-460).
" 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)"

(15) Review comment:
We suggest substituting the words “assistive device” in for “crutches” when discussing the progression of patients from the hospital to independent walking in consideration of international variation in the use of walkers, canes and crutches.

Authors reply:
As suggested, we have now replaced 'crutches' with 'assistive devices' (line 202)
" After discharge, all patients will receive a standard home-based rehabilitation program focusing on improving knee joint mobility (representing standard treatment), to increase the tolerance for standing without assistive devices ~~(i.e. crutches)~~, and lower extremity muscle strength."

(16) Review comment:
Overall, we suggest an in depth grammatical and editorial review prior to resubmission:
- Inconsistent capitalization of the first letter for the sequence of exclusion criteria.
- Repeated expressions such as however in consecutive sentences.
- Last sentence before Sample Size section is unfinished

Authors reply:

Before resubmission the manuscript have now been reviewed by an English expert.
The sentence before Sample Size has now been corrected (line 453).

VERSION 3 – REVIEW

REVIEWER	Sara Piva University of Pittsburgh
REVIEW RETURNED	11-Jun-2020

GENERAL COMMENTS	The authors did a nice job addressing this reviewer’s concerns, particularly providing clarifications related to the rehabilitation protocol in the two hospitals. Although the article reads well, there are some typos and grammatical errors that I believe will be taken care by the editorial team of the journal. Please address these final suggestions: 1. Now lines 90-120 are all one paragraph: this should be broken up. From the start of the new information in line 104 - this can begin a new paragraph. 2. Line 164: The time frames still don't match up. Please consider the following suggestion: “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.” 3. Now that the authors clarify the differences in protocol in both hospitals, it became apparent that these protocols are vastly different between the Horsens Regional Hospital and the Silkeborg Regional Hospital. Please include a strong statement in the limitation section that these differences a likely to affect the results of the study and provide plans of how to deal with this limitation. 4. Table 2a and 2b could be combined by having left hand column remain the same and then have 2 columns to the right (one for Horsens, and one for Silkeborge) - easier to compare side to side.

VERSION 3 – AUTHOR RESPONSE

Reviewer 1

(1) Review comment:
Now lines 90-120 are all one paragraph: this should be broken up. From the start of the new information in line 104 - this can begin a new paragraph.

Authors reply:
The paragraph has now been broken up as suggested by the reviewer (lines 90-120)
" 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 (1-21). 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 (2, 14, 15). 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, 22-24) although not observed in all studies (23). Importantly, BFRE appears to be feasible with a high training adherence in knee OA patients (1, 23, 24). 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, 23, 24). This is in line with Hughes et al. (2), who suggested that when BFRE is performed correctly, it has been demonstrated to be as safe as free-flow exercise methods (2).

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 (25). 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 (3, 6, 7, 26). 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 (26). 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 (26). On the other hand, higher pressures are associated with more discomfort during exercise and in between-set rest pauses (3), 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."

(2) Review comment:
Line 164: The time frames still don't match up. Please consider the following suggestion: “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.”

Authors reply:
We have made the change suggested by the reviewer (lines 164-167):
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.

(3) Review comment:

Now that the authors clarify the differences in protocol in both hospitals, it became apparent that these protocols are vastly different between the Horsens Regional Hospital and the Silkeborg Regional Hospital. Please include a strong statement in the limitation section that these differences a likely to affect the results of the study and provide plans of how to deal with this limitation.

Authors reply:
A recent review and meta-analysis found no superiority of clinic-based or inpatient programs compared with home-based programs in the early subacute period after TKA (27). This observation suggests that home-based rehabilitation as an appropriate first line of therapy after uncomplicated TKA in patients with adequate social supports (27). Furthermore, studies in other knee patient populations do not find any difference in main outcome parameters when comparing home based postoperative rehabilitation to clinic-based supervised postoperative rehabilitation (28, 29). Therefore, despite site-specific differences in the postoperative rehabilitation protocols, we do not think that this will affect the results of the study to any large degree. Thus, we do not think it will be relevant to make adjustments in the analysis based on at which hospital departments participants will be treated. Still, as suggested by the Reviewer this aspect has been addressed in the text section on Study Limitations (lines 474 + 549-557).

"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"

" 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 an separate independent variable in the mixed linear model used for the statistical analysis."

(4) Review comment:
Table 2a and 2b could be combined by having left hand column remain the same and then have 2 columns to the right (one for Horsens, and one for Silkeborg) - easier to compare side to side.

Authors reply:
Thank you for this constructive suggestion. Table 2a and 2b are now combined.

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

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