THE EFFECT OF BASELINE KNEE PAIN ON KNEE JOINT CARTILAGE LOSS IN ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTED INDIVIDUALS: A TARGET TRIAL EMULATION BASED ON THE MIRAKOS COHORT STUDY

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Protocol revision history:

Version #	Issue date	Amendment
1.0	21.10.2024	1) Health research ethics committee number added (section 1.3). 2) Information regarding utilization of existing data from previous study (MIRAKOS) has been added (section 9.1). 3) Information about written agreement regarding delegation of information to potential participants has been added (section 6.2). 4) Information regarding new financial support to the health research ethics committee (section 9.3). 5) Information on clinical responsible medical doctor of the study (section 1.7). 6) Explanation for no sample size estimation is added (section 8.0).
1.1	07.01.2025	Added Health research ethics committee number (section 1.3). Added Study registration and date (section 1.4)

1.0 STUDY IDENTIFIER

1.1 FULL TITLE

The effect of baseline knee pain on knee joint cartilage loss in anterior cruciate ligament reconstructed individuals: A target trial emulation based on the MIRAKOS cohort study.

1.2 ACRONYM

CIAO-MIRAKOS (The effect of baseline knee pain on knee joint **C**artilage loss **I**n **A**nterior cruciate ligament rec**O**nstructed individuals: A target trial emulation based on the **MIRAKOS** cohort study).

1.3 HEALTH RESEARCH ETHICS COMMITTEE NUMBER

H-24058817

1.4 STUDY REGISTRATION AND DATE

Protocol uploaded to the Parker Institute's web page: https://www.parkerinst.dk/research January 7, 2025.

1.5 INTERNAL PROTOCOL NUMBER

PT-2024

1.6 STUDY INITIATION

Tine Alkjær, MSc, PhD, associate professor, Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark and The Parker Institute, Bispebjerg and Frederiksberg Hospital, Copenhagen, Denmark.

1.7 CLINICAL RESPONSIBLE MEDICAL DOCTOR

Henning Bliddal, professor, MD, DMSc, The Parker Institute, Bispebjerg and Frederiksberg Hospital, Copenhagen, Denmark.

2.0 PROTOCOL SYNOPSIS

Study title	The effect of baseline knee pain on knee joint cartilage loss in anterior cruciate ligament reconstructed individuals: A target trial emulation based on the MIRAKOS cohort study.
Funder	The MIRAKOS study was funded by Innovation Fund Denmark (9088-00006B under the frame of ERA PerMed). The present study will partly be funded by the Oak Foundation Grant Number: OFIL-24-074. Further external funding will be applied for the present study.
Study objective and hypothesis	The objective is to determine the effect of baseline knee pain versus no knee pain on knee joint space narrowing over a period of at least four years in ACL reconstructed individuals. We hypothesize that ACL reconstructed individuals with baseline knee pain will exhibit a significantly greater knee joint space narrowing compared to individuals without knee pain over a period of at least 4 years.
Study design	Target trial emulation study, based on a prospective cohort in Copenhagen, Denmark.
Subject populations	Individuals with ACL reconstruction.
Protocol components	Summarized in Table A (section 5.3.1)
Study duration	Baseline data collection ran from June 2021 to July 2022. Start of follow-up study: January 2025. Follow-up data collection runs from June 2025 to July 2026. End of follow-up study: January 2027.
Safety evaluation	No safety issues.
Data and safety monitoring plan	No safety issues. The data management plan will comply with the common rules regarding data protection (General Data Protection Regulation (GDPR)). The study will be conducted in accordance with Danish law, the Helsinki declaration, and local research ethics committee requirements.
Participating centres	Single-center study. To be involved (n): 1, in Denmark.

3.0 INTRODUCTION

3.1 BACKGROUND AND RATIONALE

Anterior cruciate ligament (ACL) injuries are frequent[1,2] and with increasing incidence, especially among yourth[3,4]. These injuries often require surgical intervention and extended rehabilitation[5]. Furthermore, ACL injury increases the risk of knee osteoarthritis (OA)[6,7]. Despite surgical treatment, the risk of knee OA remains high among the ACL reconstructed population[8]. Knee OA is the most common form of arthritis and a leading cause of chronic pain and long-term disability in adults[9]. The burden of knee OA is rising due to population growth and aging, with no effective cure available. Preventing or delaying the onset of knee OA is crucial, necessitating the detection of early signs of symptoms and targeting modifiable risk factors[10,11]. Knee OA is characterized by two main features: 1) *structural disease* marked by degeneration of articular cartilage, menisci, bone and other joint soft tissues, resulting in joint space narrowing and 2) *illness* where the cardinal symptom is knee joint pain. Identifying factors that contribute to the onset of structural disease in ACL reconstructed individuals is crucial for developing targeted interventions to mitigate this risk.

Knee pain is among the initial symptoms recalled by people with early-stage knee OA[12,13]. As such, the presence of knee pain in ACL reconstructed individuals, is likely an early sign of structural disease onset. Based on this we hypothesize is that ACL reconstructed individuals with knee pain will have a greater loss of knee joint cartilage over a period of at least four years, measured by joint space narrowing on radiographs, compared to those without knee pain. This implies a question about a cause-and-effect relationship between baseline knee pain and the joint space narrowing in ACL reconstructed individuals over a period of at least four years. Ideally, this causal question would be addressed through a randomized trial – the target trial. However, it is impossible to allocate participants to non-experimental pain or pain-free conditions. Instead, we will emulate the components of a target trial using observational data to investigate our hypothesis[14].

4.0 STUDY OBJECTIVE, HYPOTHESES AND OUTCOMES

The objective is to determine the effect of baseline knee pain versus no knee pain on knee joint space narrowing over a period of at least four years in ACL reconstructed individuals.

We hypothesize that ACL reconstructed individuals with baseline knee pain will exhibit a significantly greater knee joint space narrowing compared to individuals without knee pain over a period of at least 4 years.

4.1 PRIMARY AND SECONDARY OUTCOMES

Primary outcome is knee joint space narrowing measured as:

- Change in minimal medial tibiofemoral joint space width from baseline to follow-up (at least 4 years) in the ACL reconstructed knee assessed from clinical standardized weight-bearing anteroposterior knee radiographs without the use of a positioning device.

Secondary/exploratory outcomes:

- Changes in knee extensor/flexor (quadriceps/hamstring) muscle strength.
- Changes in self-reported knee pain, function and activity level.
- Radiographic knee OA level at follow-up (Kellgren-Lawrence (KL)[15] and Osteoarthritis Research Society International (OARSI) [16] grades.

- Changes in varus/valgus angle.

5.0 STUDY DESIGN

5.1 DESCRIPTION OF THE PROTOCOL

This is a target trial emulation study investigating the causal effect of having knee pain after ACL surgery on loss of knee joint space over a period of at least 4 years. We apply the target trial emulation framework for reporting causal inference using observational data because it allows for a structured and rigorous approach to mimic randomized controlled trials (i.e., an average causal design). This includes two steps where the first describes the target trial, and the second describes the emulated trial using observational data adhering to the target trial principles as much as possible[17]. In the following, we describe the data source and the details of the protocol components for the target trial and the target trial emulation.

5.2 DATA SOURCE

We have a cohort of 122 ACL reconstructed individuals from a previous cross-sectional study "MIRAKOS"[18,19], approved by the Capital Region of Denmark's health research ethics committee (H-20060332). The MIRAKOS data were collected from June 2021 to July 2022, with all participants consenting to future follow-up studies. These data include baseline recordings of knee pain, demographics, standard clinical weight-bearing knee radiographs, questionnaires, muscle strength tests, and will serve as baseline data in this study.

For this study, all MIRAKOS participants will be invited for a follow-up measurement visit at least four years after baseline. Participants will be contacted and invited to the follow-up measurement visit via digital mail. Information about the follow-up measurements will comply with Danish Research Ethics Committee guidelines. Thus, all participants will receive written (appended) and oral information about the purpose of the study, the study protocol, the duration and the expectations. They will be offered time to consider participation and asked to sign an informed consent form (appended) before any study related procedures are done. Further details are provided in section 6.0.

5.3 THE TARGET TRIAL AND EMULATION OF THE TARGET TRIAL

The protocol components of both the target trial and the emulated target trial are summarized in Table A.

5.3.1 Table A Summary of the target trial protocol and emulated protocol

Protocol components	**Target trial (ideal randomized controlled trial)**	**Emulation using observational data**
Eligibility criteria	Inclusion criteria: - Age between 18 and 40 years at the time of ACL reconstruction. - Primary ACL reconstruction (semitendinosus-gracilis tendon graft). - Post-surgery time of at least 3 years. - A body mass index (BMI) of ≤30. Exclusion criteria: - Known neuromuscular diseases. - Cartilage lesions ICRS grade 4 (full thickness). - ACL reconstruction or other major surgery to the other knee. - Congenital deformities in the lower extremities preventing full participation in the tests. - Musculoskeletal pain in the lower extremity other than the injured knee. - Knee surgery and/or arthroscopy of the ACL reconstructed knee since baseline including knee replacements. - Any other condition that in the opinion of the investigator makes a potential participant unfit for participation or conditions that puts a potential participant at risk by participation.	Inclusion criteria: Same as target trial. Exclusion criteria: Same as target trial.
Allocation procedure	Participants randomly assigned* to one of the following groups: (A) Knee pain. (B) No knee pain. **In theory, this implies the ability to induce knee pain at baseline in the intervention group using However, this is not possible.*	Participants allocated to two groups (symptomatic/asymptomatic) based on their knee pain rating (ACL reconstructed knee) at baseline: (A) Knee pain score of ≥ 3 on a 0-10 verbal rating scale (VRS) in the reconstructed knee during activities of daily living (ADL) within the last week (*symptomatic*). (B) Knee pain score of 0 on a 0-10 VRS in the reconstructed knee during ADL within the last week (*asymptomatic*).
Follow-up period	Follow-up starts at baseline (time zero, t0). All participants are followed for at least 4 years (t4).	Same as target trial.
Outcome	Primary outcome: Change in minimal medial tibiofemoral joint space width from baseline to follow-up (at least 4 years) assessed from standardized weight bearing anteroposterior knee radiographs. Secondary outcomes: see section 4.1	Same as target trial.
Causal contrast of interest	Intention-to-treat population. Group mean difference in change in minimal medial tibiofemoral joint space width from baseline to follow-up with 95% confidence interval (95%CI)	Same as target trial; while baseline time (t=0) will be defined as in the original MIRAKOS study.
Analysis *(full description see section 8.0)*	Effectiveness is estimated as group mean differences using analysis of covariance with adjustment for baseline values.	Group comparison will be done using an inverse-probability-weighted regression adjustment model to deal with potential selection bias, such that observed groups can be considered balanced and comparable. Effectiveness is estimated as group mean differences using analysis of covariance with inverse-probability weighting and adjustment for pre-exposure values and other pre-exposure covariates.

6.0 STUDY PROCEDURES

6.1 PRE-SCREENING AND SCREENING PROCEDURES

The MIRAKOS participants are invited to participate in the follow-up assessment as follows:

1) Letter of invitation sent via digital mail stating the main criteria for participation.
2) Potential participants contact the research team in case they are interested.
3) Potential participants are invited for a clinical screening examination at Bispebjerg and Frederiksberg Hospital/The Parker Institute, for the purpose of inclusion.
4) Eligible participants are invited to an X-ray examination of both knees and knee muscle strength testing (see section 7.0 regarding measurements).

6.2 ORAL INFORMATION

The oral information visit will be organised as an individual session with an investigator (or his/her delegate) at the OA outpatient clinic at The Parker Institute. Potential participants have the right to bring next of kin or another person of the participant's choice with him/her to the oral information visit. A written agreement will be established to specify the delegation of responsibility when the information is provided by someone other than the principal investigator, detailing who delivers the information, obtains consent, and confirms that verbal information has been given and written information has been provided.

The information will include that

- Participation in the study is voluntary.
- Participants have the right to minimum 24 hours reflection time before deciding to either sign the informed consent or decline.
- Participants can, at any time and without giving any reason, withdraw from the study without affecting the potential participant's right to current or future treatment.

Further, the oral information will include: aim, procedures, potential benefits and risks when participating in the study, procedures for random findings during the project, procedures for securing the participants privacy and data protection, information on the study organisation, funding, as well as contact information on the primary investigator and other key investigators.

The investigator will make sure that participants have received and understood the information given to them. Furthermore, the investigator will make sure they are aware that they have the right to minimum 24 hours reflection time before signing the informed consent.

The written information material will be provided.

6.3 SCREENING VISIT

At the screening visit, the participants provide written informed consent and undergo the screening procedures. The screening procedures will only be done upon signed informed consent.

At the screening visit, the following procedures will be done in this order:

1. Provision of signed informed consent
2. Assessment of in- and exclusion criteria, including
    a. Measurement of height and body mass
    b. Clinical examination by an investigator
    c. Interview about medical history

Participants who meet all inclusion criteria and who do not have exclusions will be scheduled for a measurement visit.

6.4 BASELINE VISIT

The baseline visit took place in June 2021 to July 2022. At this visit, the following procedures were completed in accordance with the study protocol approved by the Capital Region of Denmark's health research ethics committee (H-20060332):

- Knee radiographs
- Questionnaires
- Muscle strength test

6.5 FOLLOW-UP VISIT

At the follow-up measurement visit (scheduled at least four years after the baseline visit), the following procedures will be completed (see section 7.0 for detailed descriptions):

- Knee radiographs (section 7.1)
- Questionnaires (section 7.2)
- Muscle strength test (section 7.3)

All measurements will be performed at The Parker Institute/Bispebjerg and Frederiksberg Hospital, Copenhagen, Denmark.

7.0 OUTCOME MEASUREMENTS

7.1 KNEE RADIOGRAPHS

To assess the radiographic level of knee OA bilateral standing knee radiographs will be acquired. The radiographic recordings will be done at Frederiksberg Hospital. The evaluation of radiographic signs of knee OA are done according to Kellgren-Lawrence grading[15] and OARSI grading using the artificial intelligence (AI) tool RBknee, version 2.1 from Radiobotics, Copenhagen, Denmark. This will also be used to assess minimal medial tibiofemoral joint space width at baseline and follow-up. The results of all radiographic analysis will be approved by an experienced knee OA imaging expert before database lock.

7.2 QUESTIONNAIRES

Information about the participants' perceived knee function and level of activity will be assessed by questionnaires developed for evaluation of ACL injury and knee OA: The International Knee Documentation Committee (IKDC) [20] and the Knee Injury and Osteoarthritis Outcome Scale (KOOS) [21], the Intermittent and Constant Osteoarthritis Pain questionnaire (ICOAP)[22], the Tegner score [23] and the short-form McGill Pain Questionnaire (SF-MPQ)[24] will be filled out by the participants at the follow-up measurement visit. All questionnaires are attached to this protocol.

7.2.1 IKDC

The IKDC questionnaire is an instrument to assess patients with a variety of knee disorders including ligamentous and meniscal injuries as well as patellofemoral pain and osteoarthritis[20]. The questionnaire consists of three subscales: symptoms (7 items), sports activity (2 items), and knee function (2 items) and provides an overall function score. The scores are obtained by summing the individual items and then convert the crude total to a scaled number that ranges from 0 to 100. This

final number represents a measure of function with higher scores representing higher levels of function. Thus, a score of 100 reflects no functional limitations.

7.2.2 KOOS

The Knee injury and Osteoarthritis Outcome Score (KOOS), a disease-specific instrument, is an extension of the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and designed to assess health related quality of life (QoL) in patients with knee injuries and knee OA[21]. The KOOS consists of 42 items covering five domains, namely, Pain (9 items), Symptoms (7 items), Activities of Daily Living (ADL) (17 items), Sports and Recreation (5 items), and knee-related QoL (4 items). The KOOS adopts a five-point Likert scale scoring system (ranging from 0 (least severe) to 4 (most severe)).

A normalized score is calculated for each domain with 100 indicating no symptoms and functional impairment and 0 indicating extreme symptoms and functional impairment. In accordance with the user guide, if the number of missing items is less than or equal to 2 in a subscale they will be substituted by the average item value for that subscale. If more than two items of the subscale are omitted the response will be considered invalid and no subscale score calculated.

7.2.3 Tegner score

The Tegner activity scale is an instrument to measure activity following knee injuries[23]. It grades activity based on work and sports activities on a scale of 0 to 10 one-item score. Zero represents disability due to knee problems and 10 represents competitive sports (soccer - national and international elite level). The subjects report the level of participation that best describes their current level of activity and that before injury.

7.2.4 ICOAP

The Intermittent and Constant Osteoarthritis Pain questionnaire (ICOAP) is a diagnosis-specific 11-item questionnaire designed to assess the pain experience within the last week among people suffering from knee and hip OA[22]. The questionnaire is divided into two domains, a 5-item scale for constant pain and a 6-item scale for intermittent pain (so-called ‘‘pain that comes and goes’’). Each domain captures pain intensity as well as related distress and the impact of OA pain on quality of life. For each of these pain types, single items assess pain intensity, effect on sleep, impact on quality of life, extent to which the pain ‘frustrates or annoys’, and the extent to which it ‘worries or upsets’. For pain that comes and goes, two additional items ask respondents to report the frequency of pain and the degree to which the pain could be predicted. All items are scored on anchored rating scales with five levels of response (0–4) – for questions asking about intensity, response options are ‘not at all’ (0), to ‘extremely’ (4), while those that asked about frequency has the following response options: ‘never’ (0), to ‘very often’ (4). A score is separately produced for the constant pain subscale (0–20) and the intermittent pain subscale (0–24), and for total pain (0–44). Normalized scores for the two subscales and for the total pain score, from 0 (no pain) to 100 (extreme pain), are calculated.

7.2.5 SF-MPQ

The short-form McGill Pain Questionnaire (SF-MPQ) consists of 15 descriptors – 11 sensory (quality descriptor) and 4 affective (quality descriptors)[24]. The patient is asked to choose/mark/ the words describing their knee pain. In the original SF-MPQ the patient ranks each of the pain descriptors as either *none, mild, moderate,* or *severe*. In the version used in this study the patient marks the descriptor as either present (yes) or not present (no).

7.3 MUSCLE STRENGTH TEST

Isometric quadriceps and hamstring muscle strength will be assessed using an isokinetic dynamometer (Biodex System4 Pro, Biodex Medical System, NY, USA). The dynamometer records the torque (Nm) produced by isometric muscle contractions. The participants are seated in a rigid chair firmly strapped to the seat at the hip and distal thigh. The rotation axis of the dynamometer is visually aligned to the lateral femoral epicondyle and the lower leg attached to the lever arm of the dynamometer. The lever arm is placed just above the lateral malleolus and fixed with a cuff. Prior to testing, 15 min. of warm-up will be applied to familiarize the subjects to the dynamometer and the test procedures. Maximal voluntary isometric contractions (MVICs) of the quadriceps and hamstrings, respectively, will be done at 60° knee flexion. The participants are asked to perform the MVICs with maximal effort and verbal feedback and encouragement will be provided during testing that comprises three repetitions of which the highest peak torque value will be defined as the maximal quadriceps/hamstring muscle strength and reported as body mass normalized values (Nm/kg)[25]. Muscle strength will be assessed for both the ACL reconstructed and contralateral leg.

8.0 STATISTICAL CONSIDERATIONS

This section elaborates on the analysis briefly summarized in Table A.

The first step in the analysis emulating a target trial involves formulating the causal question as the protocol of a hypothetical randomized trial designed to provide the answer. This protocol defines the causal estimands by specifying key elements such as eligibility criteria, treatment strategies, treatment assignment, start and end of follow-up, outcomes, causal contrasts, and the data analysis plan. The randomized trial described in this protocol serves as the "target study" for causal inference.

In the target trial, outcomes would be assessed using intention-to-treat analysis (i.e., the full set of all randomly assigned participants). Statistical analysis of endpoints will include two-sided 95% confidence intervals (CIs) and corresponding p-values, with superiority defined as $p<0.05$. Continuous endpoints will be analyzed using an analysis of covariance (ANCOVA) model, with pain phenotype group as a factor and baseline endpoint value as a covariate. Missing data will be imputed at least five times from participants assigned to the same randomized treatment, with results combined using Rubin’s rules. Categorical endpoints will be analyzed using logistic regression, with pain phenotype treatment as a factor and the baseline endpoint value as a covariate.

The second step involves replicating the target trial protocol using observational data: identifying eligible individuals, assigning them to a treatment strategy consistent with their data, following them from the point of assignment (time zero) until the outcome or end of follow-up, and conducting the same analysis as in the target trial. This process includes adjusting for baseline confounders to emulate random treatment assignment, which will be handled using the propensity score method. The propensity score method involves estimating the probability of treatment assignment based on observed baseline characteristics, allowing for the creation of comparable groups for analysis. This adjustment helps to reduce confounding and improve the validity of causal inference in the emulated trial.

This study is explorative in nature and an estimation of the sample size is not applicable. The data source consists of 122 potential participants that will be invited to participate in the present follow-up study.

9.0 REGULATORY STANDARDS AND DATA MANAGEMENT

9.1 NOTIFICATION TO THE DANISH DATA PROTECTION AGENCY

This study will follow the common rules regarding data protection i.e. the General Data Protection Regulation (GDPR) and be conducted in accordance with Danish law, the Helsinki declaration, and local research ethics committee requirements. Thus, the processing of personal data is carried out in compliance with Regulation No 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons regarding the processing of personal data and on the free movement of such data, the Data Protection Act (in Danish: "databeskyttelsesloven") and the Danish Health Care Act (in Danish: "sundhedsloven"). This process will ensure that the data management of the study comply with the data protection regulation.

This project will include participants and data from a previous study (MIRAKOS), in which all participants consented to be contacted for potential follow-up studies. Permissions to utilize the existing data will be obtained through informed consent from participants included in the follow-up study, as well as from the relevant data authorities.

Participant medical information obtained by this study is confidential, and disclosure to third parties other than those noted below is prohibited.

With the participant's permission, information may be shared with his or her personal physician or with other medical personnel responsible for the participant's welfare.

Publication of data from this study will not include names, recognizable photos, personal information or other data that compromises the anonymity of participating participants.

9.2 QUALITY ASSURANCE

All data will be entered into a study database for analysis and reporting. Any data captured electronically will be stored electronically in a separate database according to standard procedures at secured servers. Upon completion of data entry, the databases will be checked to ensure acceptable accuracy and completeness.

Individuals involved in study evaluations will be trained to perform the efficacy evaluations and activity measurements described in the protocol.

9.3 FINANCING AND INSURANCE INFORMATION

The MIRAKOS study that serves as data source for this study was funded by the Innovation Fund Denmark (9088-00006B under the frame of ERA PerMed, grant amount: DKK: 2.890.757, awarded to Tine Alkjær who also initiated the study). The present study will partly be funded by the Oak Foundation Grant Number: OFIL-24-074, grant amount: DKK: 5.125.000. We plan to apply for additional external funding for this study. All current and future financial support will be disclosed to the participants in the written information material. Additionally, if further financial support is received, the health research ethics committee will be informed, including details of the funding amount and any conflicts of interest involving the principal investigator.

The participants are insured by the Danish Patient Insurance Association. Financing and insurance issues are addressed in the written information material.

The research partners involved in the study has no conflicts of interest to declare.

9.4 PUBLICATION

All positive, negative and nonconclusive results will be published in relevant international scientific peer-reviewed journal and presented at national and international conferences. The study findings will be conveyed in a transparent way.

10.0 ETHICS

10.1 GENERAL CONSIDERATIONS

All potential participants are informed, both orally and in writing, about the study purpose, its process and potential risks, as well as costs and benefits of participation. All participants are informed of their rights to withdraw from the study at any time without this influencing any future investigations and/or treatments at any site or by some of the members of the study group. After the information is delivered, read and understood, the participant gives voluntary informed consent by signing a consent form before study participation can take place. The potential participants have at least 24 hours to consider participating in the study.

It is the investigators' opinion that the knowledge and potential individual benefit gained by participation in this study is commensurate with the efforts and difficulties associated with participation. Below are specific research ethics considerations related to information, consent, interventions, and outcome assessments.

10.2 STANDARD TREATMENT

There are no restrictions about medical treatment/other treatments.

10.3 ORAL INFORMATION

The oral information is based on the written information and will be given in an easily understandable language without technical or value-laden terms. The information will be given in a considerate way that is tailored to each potential study participants. The aim is that the conversation takes place without interference. It is the responsibility of the interviewer to ensure that the potential participant has understood the information. The information interview is performed by the investigator or in her absence by a designated delegate.

10.4 WRITTEN INFORMATION

A written information material has been prepared and is attached to this protocol.

10.5 INFORMED CONSENT

Consent to participation in the study is given based on the written and oral information.

An informed consent form (ICF) has been prepared. The form must be signed and dated by the participants prior to participation in the study. A copy of the form is provided to the participants. The investigator or her designated delegates can receive the signed consent form.

The source documentation and case report forms (CRFs) will document for each participant that informed consent was obtained prior to participation in the study. The signed ICF must remain in each participant's study file and must be available for verification by study monitors at any time.

10.6 RESEARCH ETHICS – THE MEASUREMENTS

The measurements regarding muscle strength and questionnaires are non-invasive and not associated with any predictable harms or risks to the participants.

The radiographical examination of the of the participants' knee joints will give the participants a minimal extra dosis of radiation. The effective dose for a single x-ray image of both knees is approximately 3 μSv. The annual background radiation in Denmark is approximately 3000 μSv (≈ 8 μSv / day). When exposed to a dose of 1 Sv (1,000000 μSv), the risk of causing a cancerous disease increases by 5% over the average risk in the population. The risk increments following exposure in this study is 3 μSv (x-ray both knees) can be calculated as 0.000003 Sv x 5% per Sv = 0.00000015% that should be added to the lifetime risk of dying from cancer of 25% in Denmark, that theoretically will change to 25.00000015%.

All measurements are obtained according to well-known methods and are considered justifiable from a health research ethics perspective.

10.7 RESEARCH ETHICS APPROVAL

The study protocol and all attached documents will be submitted to the health research ethics committee to apply for approval.

Furthermore, we will conduct the study in accordance with Danish law, the Helsinki declaration, and local health research ethics committee requirements.

11.0 APPENDICES

11.1 APPENDIX: QUESTIONNAIRES

11.2 APPENDIX: WRITTEN INFORMATION MATERIAL

11.3 APPENDIX: INFORMED CONSENT FORM

11.4 APPENDIX: GUIDELINES FOR ORAL INFORMATION

11.5 APPENDIX: LETTER OF INVITATION - DIGITAL MAIL

12.0 REFERENCES

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