MUSCULOSKELETAL FUNCTION IN ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTED INDIVIDUALS WITH AND WITHOUT KNEE PAIN

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Protocol revision history:

Version #	Issue date	Amendment
1.0	14.12.2020	Letter of invitation – added (section 11.6)
1.1	21.01.2021	Health research ethics committee number added (section 1.2). Information about responsibility for study initiation added (section 1.4). Specification regarding compliance of Data Protection Act added (section 9.1). Specification regarding financial issues (section 9.3).
1.2	17.05.2021	Added questionnaire "Intermittent and Constant Osteoarthritis Pain" (ICOAP) (section 7.2.4)
1.2	04.11.2021	Sample size recalculation. Expected group sizes was changed from 1:1 to 1:3 due to low prevalence of symptomatic participants. As per November 2021 we had recruited 17-54 (symptomatic-asymptomatic, respectively) (section 8.1)
1.2	06.12.2021	Per December 2021 no further participant recruitments from Amager-Hvidovre Hospital were possible. A new hospital is added to the protocol to increase the recruitment basis (section 5.3). The orthopedic surgeon at this hospital is added as investigator.

1.0 STUDY IDENTIFIER

1.1 FULL TITLE

Musculoskeletal function in anterior cruciate ligament reconstructed individuals with and without knee pain.

1.2 HEALTH RESEARCH ETHICS COMMITTEE NUMBER

H-20060332

1.3 INTERNAL PROTOCOL NUMBER

APPI2-PT-2020-02

1.4 STUDY INITIATION

Tine Alkjær, MSc, PhD, associate professor, Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark.

2.0 PROTOCOL SYNOPSIS

Study title	Musculoskeletal function in anterior cruciate ligament reconstructed individuals with and without knee pain.
Funder	INNOVATION FUND DENMARK under the frame of ERA PerMed: ERAPERMED2019-331 – DEEPMECHANOKNEE.
Study objectives	The objective is to compare the musculoskeletal function between ACL reconstructed individuals with ("Symptomatic") and without knee pain ("Asymptomatic"). The hypotheses are: 1) ACL reconstructed individuals without knee pain have stronger quadriceps muscles compared to those with knee pain. 2) ACL reconstructed individuals without knee pain develop higher quadriceps muscle forces and knee joint compressive forces during walking and forward lunging compared to those with knee pain.
Study design	Observational cross-sectional study.
Subject populations	Individuals with ACL reconstruction.
Inclusion/exclusion 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. - Pain score of 0 Defines "Asymptomatic" *or* at least 3 Defines "Symptomatic" (verbal rating scale (VRS) 0-10) in the reconstructed knee during activities of daily living (ADL) within the last week. 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. - 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.
Observation	Individuals with an ACL reconstruction in one knee with and without knee pain will be identified and invited to participate in the study where their maximal isometric quadriceps and hamstring muscle strength will be assessed (Biodex System4 Pro, Biodex Medical System, NY, USA). Walking/forward lunge biomechanics will be assessed using standard three dimensional movement analyses (Vicon MX, Vicon Motion Systems, Oxford, UK) and knee joint compression force and quadriceps muscle force production during movement will be estimated from further biomechanical modelling. Knee pain during walking is assessed by a 100 mm visual analogue scale. Pressure pain sensitivity is assessed using computerised cuff pressure algometry. Structural signs of knee OA are determined from standing standardised x-rays of the knees using the Kellgren-Lawrence grading system. Self-reported knee function is obtained by questionnaires

	(IKDC, KOOS) pain and activity level is assessed using the ICOAP and the Tegner score. This study is observational and no interventions will be applied.
Outcomes	Primary outcome: Maximal isometric quadriceps muscle strength. Secondary outcomes: Knee joint biomechanics during walking/forward lunging. Maximal isometric hamstring muscle strength. Knee pain. Pressure pain sensitivity. Self-reported knee function and activity level. Radiographic knee OA level.
Sample size	To detect a group difference of 0.3 Nm/kg in the primary outcome with a common standard deviation of 0.5 Nm/kg, a sample size of 120 with a 1:3 group allocation (n=30 symptomatic; n=90 asymptomatic) will reach a power of 80.6%. Thus, a total sample size of n=120 (30/90 symptomatic/asymptomatic) will be applied.
Study duration	Time for preparation of the study (months): 2 Recruitment period (months): 4 First participant tested to last participant tested (months): 24 Time for data clearance and analysis (months): 9 Duration of the entire study (months): 36
Safety evaluation	No safety issues.
Statistical analysis	All outcomes: Comparison between groups: with and without pain (ANCOVA).
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	To be involved (n): 1, in Denmark.

3.0 INTRODUCTION

3.1 BACKGROUND AND RATIONALE

Knee osteoarthritis (OA) is the most common joint disease and a significant contributor to global disability [1]. The known knee OA risk factors include obesity, surgery, occupational load and injury [2,3]. Anterior cruciate ligament (ACL) rupture is a common knee injury [4,5] and the incidence is increasing, particularly among young people [6]. ACL injury affects the knee joint function and increases the risk of knee OA development [7-10] even at a young age, which prolongs the period of impaired function and pain [11]. Most research has focused on radiographic knee OA while fewer studies have investigated the prevalence of symptomatic knee OA after ACL injury [12]. It is important to discriminate between radiographic and symptomatic knee OA, as knee pain is a decisive criterion to diagnose knee OA [13], whereas radiographic changes more serve as a confirmatory measure. Indeed, the Framingham study showed that the prevalence of radiographic changes (indicative of OA) in the population older than 63 years was 33% whereas the prevalence of symptoms was only 9% [14]. A recent MRI study of 230 asymptomatic knees reported that 97% of these showed abnormalities in at least one knee structure [15]. This emphasizes that image-based signs of knee OA are not always accompanied by pain and OA symptoms.

Conventionally, mechanical joint loading is proposed as a key mechanism contributing to development and progression of OA [16,17]. Thus, the knee joint loading during dynamic tasks in the ACL injured population has been studied extensively due to the supposed link between the knee joint compressive forces and onset of post traumatic knee OA [18-23]. However, the evidence for a causal link between knee joint loading and knee OA development and progression is weak [24,25]. Furthermore, a 15-year follow-up study, showed that ACL reconstructed persons returning to pivoting sport (presumably associated with high loads) had reduced odds of developing knee OA and had better self-reported function in activities of daily living [26]. On the other hand, data suggest that ACL reconstructed individuals develop different adaptive neuromuscular functions [27,28], and it is possible that other mechanical factors than loading magnitude are implicated in the development of knee OA. Such other biomechanical factors may include force dissipation capacity of the musculoskeletal system [29], micro-incoordination [30], muscle strength and other aspects of muscle function. Low quadriceps muscle strength is associated with an increased risk of symptomatic and functional impairment in people with and at risk of radiographic knee OA [31]. The quadriceps muscle strength and function is impaired after ACL injury and strength deficits persist even after ligament reconstruction [32-34]. Altogether, there are indices and a common agreement that poor musculoskeletal function is associated with increased risk of development of both symptomatic and radiographic knee OA, and that an ACL injury and reconstruction may lead to unfavorable changes in the musculoskeletal function accelerating the development of symptoms and/or degenerative OA changes. One study has compared individuals with definitive radiographic OA with and without symptoms and found that the symptomatic group had lower muscle strength and walking biomechanics indicative of a “stiffer” gait, possibly reflecting protective neuromuscular adaptations in the walking pattern [35]. As ACL injuries increase the risk of OA (symptomatic and radiographic) later in life, the musculoskeletal function may be changed alongside with the early onset of symptoms but before definitive radiographic OA is present. Thus, the present study will compare the musculoskeletal function between ACL reconstructed individuals with and without knee pain. By this we can deepen our understanding of the role of the musculoskeletal function in relation to development and progression of knee OA.

4.0 STUDY OBJECTIVE, HYPOTHESES AND OUTCOMES

The objective of the present study is to compare the musculoskeletal function between ACL reconstructed individuals with and without knee pain.

Musculoskeletal function will be assessed by

- Muscle strength of the knee extensor muscle (quadriceps)
- Biomechanics of the knee and quadriceps muscle during level walking and a forward lunge movement

The hypotheses are:

1) ACL reconstructed individuals without knee pain have stronger quadriceps muscles compared to those with knee pain.
2) ACL reconstructed individuals without knee pain develop higher quadriceps muscle forces and knee joint compressive forces during walking and forward lunging compared to those with knee pain.

The rationale for the hypotheses is based on research documenting that quadriceps muscle weakness is associated with an increased risk of symptomatic and functional impairment in people with and at risk for radiographic knee OA[31,35], and that knee joint pain has a negative impact on quadriceps muscle activation and force production[36].

4.1 PRIMARY AND SECONDARY OUTCOMES

Primary outcome:

- Maximal isometric quadriceps muscle strength.

Secondary outcomes:

- Knee joint biomechanics during walking/forward lunging.
- Knee flexor (hamstring) muscle strength.
- Knee pain.
- Pressure pain sensitivity.
- Self-reported knee function and activity level.
- Radiographic knee OA level.

5.0 STUDY DESIGN

5.1 DESCRIPTION OF THE PROTOCOL

This is an observational cross-sectional study investigating the musculoskeletal function in two groups of ACL reconstructed persons discriminated by the presence of knee pain. The participants are invited for one study visit at which all data are collected.

5.2 PARTICIPANTS

In total, 100 (see sample size calculation in section 8.1) ACL reconstructed persons will be identified from the Danish Ligament Reconstruction Register and invited to participate in the study.

As we aim to compare participants with and without knee pain the eligibility criteria are as follows:

Participants with knee pain (“Symptomatic group”):

Inclusion criteria:

- Age between 18 and 40 years at the time of ACL reconstruction.
- Primary ACL reconstruction using the semitendinosus-gracilis tendon graft.
- Post-surgery time of at least 3 years.
- Current body mass index (BMI) of ≤30.
- Pain score of at least 3 (verbal rating scale (VRS) 0-10) in the reconstructed knee during activities of daily living (ADL) within the last week.

Participants without knee pain ("Asymptomatic group"):

Inclusion criteria:

- Age between 18 and 40 years at the time of ACL reconstruction.
- Primary ACL reconstruction using the semitendinosus-gracilis tendon graft.
- Post-surgery time of at least 3 years.
- Current body mass index (BMI) of ≤30.
- Pain score of 0 (VRS 0-10) in the reconstructed knee during activities of daily living (ADL) within the last week.

For both groups, the exclusion criteria are the same:

Exclusion criteria:

- Known neuromuscular diseases.
- Evidence of cartilage lesions ICRS grade 4 (full thickness) from MRI at time of ACL reconstruction or documented peri-surgically.
- ACL reconstruction or other major surgery to the other knee.
- Congenital deformities in the lower extremities preventing full participation in the tests.
- Current musculoskeletal pain in other regions of the lower extremity other than the injured knee.
- 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.

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.

5.3 RECRUITMENT

The participants will be recruited from the Danish Ligament Reconstruction Register, starting with individuals who have had reconstruction surgery at the Department of Orthopedic Surgery, Copenhagen University Hospital, Amager-Hvidovre, Copenhagen, Denmark and Ortopaedic Department, Sealand University Hospital Koege, Koege, Denmark. If recruitment of the scheduled number of participants cannot be reached within a reasonable timeframe, individuals treated at other hospitals in Denmark may be necessary to identify and contact via the Danish Ligament Reconstruction Register.

Also, advertisements in local newspapers, on the participating department's webpages, and on social media may be used if recruitment direct from the Danish Ligament Reconstruction Register is insufficient or too slow (appended).

5.4 PRE-SCREENING AND SCREENING PROCEDURES

Potential participants are contacted and pre-screened and screened as follows:

1) Letter of invitation send via digital mail (e-Boks) 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-Frederiksberg Hospital/The Parker Institute, for the purpose of inclusion (see section 6.0 regarding study procedures).
4) Eligible participants are invited to an X-ray examination of both knees and an examination of musculoskeletal function (see section 7.0 regarding measurements).

6.0 STUDY PROCEDURES

6.1 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.

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.

6.2 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.

6.3 MEASUREMENT VISIT

At the measurement 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)
- Walking and forward lunge biomechanics test (section 7.4)
- Pain sensitivity (section 7.5)

All measurements will be performed at The Parker Institute/Bispebjerg-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 [37].

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) [38] and the Knee Injury and Osteoarthritis Outcome Scale (KOOS) [39], the Intermittent and Constant Osteoarthritis Pain questionnaire (ICOAP)[40], and the Tegner score [41] will be filled out by the participants at the study 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[38]. 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[39]. 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 (http://www.koos.nu), 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[41]. 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[40]. 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.

7.3 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)[42].

7.4 WALKING AND FORWARD LUNGE BIOMECHANICS TEST

All participants will have their walking and forward lunge movement pattern assessed using standard three-dimensional motion capture and software (Vicon MX, Vicon Motion Systems, Oxford, UK). Small reflective markers are attached to the participants' skin over well-defined anatomical landmarks and then the movements (walking/forward lunge) are performed.

Walking and forward lunge kinematics and kinetics of the ankle, knee and hip joints of both legs will be quantified using a standard inverse dynamics calculation model (Vicon Nexus ver 2.10, Vicon Motion Systems, Oxford, UK). Further computational musculoskeletal modeling of the biomechanical data will be used to estimate the knee compression force and the quadriceps muscle force developed during the stance/contact phase of walking/forward lunge.

7.4.1 Walking biomechanics

During walking the participants walk across two ground reaction for plates (AMTI OR 6-5-1000, Watertown, MA, USA) mounted in a 10 m long walkway at a self-selected speed (target speed). The target speed will be determined during the trial habituation procedure. The walking speed are recorded during each trial that are repeated until a sufficient amount of acceptable trials (walking speed within ±0.1 km/h of the target speed) are captured and stored for further processing.

7.4.2 Forward lunge biomechanics

The participants will be instructed to perform forward lunge movements at maximal pace ("as fast as possible"). The forward lunge movements are performed by taking one step forward, placing the foot on the force plate, flexing the knee to 90˚ and subsequently push backwards into the starting position, while having hands on the back of the head, the upper body perpendicular to the ground, and the opposite foot maintaining ground contact. Three forward lunge movements will be recorded with a short resting period (~60 s) in between.

7.4.3 Knee pain during movement

The current knee pain during walking/forward lunging will be assessed by a VRS 0-10 immediately after each walking/lunging trial.

7.5 PAIN SENSITIVITY

The pain sensitivity will be assessed by computerised cuff pressure algometry (CPA)[43]. A double-chambered Tourniquet cuff is wrapped around the calf by the gastrocnemius muscles of the lower extremity of the ACL reconstructed leg. A computer controlled compressor inflates the cuff with air at 1 kPa/s until the person reports the first sensation of pain by pressing a push-button[44]. The recorded pressure defines the pressure pain threshold (PPT) measured in kPa.

8.0 STATISTICAL CONSIDERATIONS

8.1 SAMPLE SIZE

We will compare the quadriceps muscle strength between two groups of ACL reconstructed individuals: 1) with and 2) without knee pain.

The variance in this population is unknown, while our sample size estimation will be pragmatic. To detect a group difference of 0.3 Nm/kg in the primary outcome with a common standard deviation of 0.5 Nm/kg, a sample size of 120 with a 1:3 group allocation (n=30 symptomatic; n=90

asymptomatic) will reach a power of 80.6%. Thus, a total sample size of n=120 (30/90) will be applied.

8.2 GROUP COMPARISONS

All comparisons between groups (with and without knee pain) will be analysed using an analysis of covariance (ANCOVA). The results will be reported as mean ± SD, mean differences with 95% confidence interval (CI) and the level of significance is set to 0.05.

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 with regard to 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.

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

This study is a part of a project entitled "A novel tool for personalised and socio-economically optimal treatment planning for patients with osteoarthritis" that received a grant from ERA PerMed: ERAPERMED2019-331 – DEEPMECHANOKNEE. Tine Alkjær, associate professor, Department of Biomedical Sciences, University of Copenhagen, is the principal investigator of the work package (WP) "Motion analysis and musculoskeletal modeling to characterize the effect of obesity, weight loss and anterior cruciate ligament injury on the onset and progression of osteoarthritis". The national funding agency (Innovation Fund Denmark) has granted the Danish WP DKK: 2.890.757. This grant is transferred to the Department of Biomedical Sciences, University of Copenhagen. This grant will primarily be used to cover salary for a post doc (Lauri Stenroth, study investigator) employed at Department of Biomedical Sciences, University of Copenhagen, Copenhagen,

Denmark and running cost. In case further funding will be granted to the study, the Health research ethics committee and the study participants will be informed.

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 on the basis of 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, biomechanics, pain sensitivity and questionnaires are non-invasive and not associated with any predictable harms or risks to the participants. When measuring pain sensitivity, a mild, short-term pain occurs, which disappears as soon as the participant senses the pain threshold is reached.

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 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: RECRUITMENT MATERIAL

11.5 APPENDIX: GUIDELINES FOR ORAL INFORMATION

11.6 APPENDIX: LETTER OF INVITATION - DIGITAL MAIL (E-BOKS)

12.0 REFERENCES

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