



Experimental Protocol

Assessing in vivo articular cartilage mechanosensitivity as outcome of high tibial osteotomy in patients with medial compartment osteoarthritis: Experimental protocol



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SUMMARY

Objective: To propose an experimental protocol for using high tibial osteotomy (HTO) as a model for studying in vivo biological effects of large permanent changes in ambulatory load.

Design: This study is a prospective multimodal (clinical, biomechanical, biological) data collection without randomization. The study will examine a cohort of 40 patients with medial compartment knee OA undergoing opening wedge HTO.

Experimental protocol: Before planned HTO, patients will be clinically assessed (including mechanical axis measurement from radiographs) and complete questionnaires on physical function. Patients will complete a walking stress test with blood sampling (30 min walking, 5.5 h sitting), and undergo gait analysis. Six weeks after HTO (at the time of full weight bearing), the mechanical axis will be measured from radiographs. Patients will complete the questionnaires and a walking stress test with blood sampling, and undergo gait analysis 6 months after HTO. The peak external knee adduction moment, knee external knee adduction moment impulse and peak external knee flexion moment will be used as surrogates of ambulatory load. Load-induced changes in cartilage biomarkers will be used as surrogates of metabolic changes in response to ambulatory load. At the 12-month follow-up, subjects will complete the questionnaires.

Conclusion: The results of this study can be considered as proof-of-concept of a potential diagnostic test (walking stress test) for cartilage degeneration and its prognostic value. A direct relationship between ambulatory load and cartilage metabolism assessed as degradation to synthesis ratio would allow developing novel load-modifying interventions and evaluating the efficacy of existing interventions.

1. Introduction

Osteoarthritis (OA) is the most common type of arthritis or degenerative joint disease [1] and occurs in a substantial portion of the population over the age of 50 [2–4]. Ambulatory load plays an important role in the development and progression of knee OA [5–7], and high tibial osteotomy (HTO) is a well-accepted therapy of OA in patients with malaligned knees [8,9]. In patients with medial compartment knee OA, individual variations in the mechanics of walking can influence the rate of progression of knee OA [6] and the outcome of treatment for medial

compartment OA at the knee [5,10–12]. Moreover, the knee adduction moment during walking influences the distribution of bone density in the proximal tibia [13] demonstrating that in vivo biological tissue adapts to functional loads experienced during walking.

HTO involves realigning the tibia to reduce the load on the medial compartment of the knee. Most studies on ambulatory load after HTO have focused on group effects [14–18]. However, patients who adopt a gait pattern with a low knee adduction moment before HTO and patients with less severe disease have a better outcome with an HTO and a slower rate of disease progression [10,12]. Hence, effective load reduction

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during walking may explain regenerative or degenerative pathways after statically successful HTO to mild valgus. At an average 3.2-year follow-up, all patients in the low pre-HTO knee adduction moment group had excellent or good clinical results, while only 50% of the patients in the high pre-HTO knee adduction moment group had an excellent or good result [10]. While these results further emphasize the importance of ambulatory load and that ambulatory load clearly changes with HTO, the relationship between individual changes, clinical outcome and the underlying mechanobiological mechanisms are still poorly understood. Evidence for cartilage regeneration after HTO is scarce [19], and the biological processes in response to load-altering interventions in patients with knee OA are poorly understood. Performing a routine arthroscopy or taking intra-articular biopsies as follow-up methods are not desirable methods to determine cartilage quality after HTO. Hence, easier and less invasive methods for determining cartilage regeneration processes after HTO such as through blood tests would be desirable.

Biomarkers can be considered surrogate measures of tissue health and metabolism and have the potential to become indicators of normal biological processes or pathogenic processes. Cartilage oligomeric matrix protein (COMP) is a prominent constituent of articular cartilage [20]. Serum concentrations of COMP fragments are elevated in patients with knee OA [21–24], and patients with greater serum COMP concentration experience a faster progression of their disease [25]. COMP molecules are presumably important for maintaining the properties and integrity of the collagen network [26], contribute to the material properties of biological tissue [27], and transfer forces from the cartilage matrix to the cell [28]. Thus, COMP may indicate the relationship between biological or pathogenic processes and mechanical loading of articular cartilage. COMP is upregulated following cyclic compression *in situ* [28] and results of *in vivo* studies [29] in competing marathon runners suggested that mechanical variations in the way individuals perform the same activity are related to the differences in the serum COMP levels. Serum COMP levels increased immediately after a 30-min running exercise but not after slow deep knee bends or lymphatic drainage in healthy subjects [30] and did not change after 30-min of mechanical loading of a single osteoarthritic knee in patients with knee OA [31]. A recent well-controlled study [32] has demonstrated that the load-induced changes in COMP depend on the magnitude of ambulatory load. Moreover, *in vitro* experiments using explants of porcine cartilage showed that COMP release increased in proportion to the magnitude of dynamic mechanical stress [33]. The results of these studies suggest that changes in serum COMP levels depend on the type of activity, on load magnitude and on cartilage health. Other potentially mechanosensitive markers are matrix metalloproteinase (MMP)-3, MMP-9 [34], proteoglycan 4 (PRG-4), A disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) –4 and ADAMTS-5 [35–37], type II procollagen (CPII) [38], and fragments such as the COL2–3/4Clong mono epitope (C2C) [39].

The current state of research in the field and of our own research clearly shows that the mechanism of clinical improvement after HTO is poorly understood. Previous reports of regenerated articular cartilage [19] and our results of changes in cartilage biomarker concentrations after a walking stress test [31,40,41] raise the following questions:

1. Does the functional outcome after HTO correlate with the amount of reduction in ambulatory load?
2. Are changes in cartilage biomarkers sensitive to the magnitude of ambulatory load during a walking stress test?
3. Are the changes in physical function related to the amount of ambulatory load reduction by HTO?
4. Can the effectiveness of reducing load by HTO for regenerating cartilage be assessed long before the clinical outcome of HTO can be determined?

In this study, we will use HTO as a model for studying *in vivo* biological effects of permanent changes in ambulatory load.

2. Materials and methods

2.1. Specific aims

Clearly, there are multiple factors ranging from mechanical to biological factors that are important to consider. The questions we are addressing will provide information that help to sort out the processes underlying HTO and the biological response to ambulatory load in knee OA. This project will test the overall hypothesis that a sudden ambulatory load reduction leads to changes in cartilage biology that delay or reverse osteoarthritic processes representing an *in vivo* model for assessing cartilage mechanosensitivity. The following specific aims will be addressed (Fig. 1):

Specific Aim 1: To test the relationship between changes in ambulatory load pre-HTO to 6 months post-HTO, static alignment 6 months post-HTO and changes in clinical scores pre-HTO to 12 months post-HTO.

Hypothesis 1: Reduction in the knee adduction moment will correlate more strongly with improvement in clinical scores than post-operative static alignment.

Previous results [14–18] have shown that the knee adduction moment decreases and clinical scores improve after HTO. It is expected that patients with the greatest improvement in clinical scores will have shown the largest reduction in knee adduction moment during walking and that change in knee adduction moment is a stronger predictor of changes in clinical scores than static alignment.

Specific Aim 2: To test the relationship between magnitude of ambulatory load (modified by HTO) during a walking stress test and the change in cartilage biomarkers.

Hypothesis 2.1: Load-induced changes in biomarker concentrations will be smaller after HTO than before HTO. Previous results have shown that cartilage quality changes after HTO [42,43] and that cartilage biomarkers are sensitive to a 30-min walking test [31,40,41]. It is possible that a specific range of loads affect biomarkers for cartilage regeneration and that smaller or higher loads affect biomarkers for cartilage degeneration.

Hypothesis 2.2: The magnitude of ambulatory load correlates with load-induced changes in some but not all biomarkers.

Previous results have suggested that only some biomarkers respond to changes in load such as during immobilization [44,45]. It is possible that not all of the tested biomarkers are sensitive to changes in ambulatory load.

Specific Aim 3: To test the relationship between changes in biomarkers during a walking stress test 6 months after HTO and functional outcome 12 months post-HTO.

Hypothesis 3.1: Smaller load-induced increases in serum COMP, MMP-3 and MMP-9 but not MMP-1 concentrations 6-months post-HTO correlate with better functional outcome.

Previous results have shown that these COMP, MMP-3 and MMP-9 but not MMP-1 are sensitive to physical activity and exercise and to unloading during bed rest [44–48]. It is possible that the sensitivity of metabolic markers to ambulatory load play a role in cartilage regeneration processes after HTO. It is expected that those with the smallest load-induced changes in COMP, MMP-3 and MMP-9 but not MMP-1 six months post-HTO will have the best functional outcome 12 months post-HTO.

Hypothesis 3.2: A smaller load-induced C2C:CPII ratio 6 months post-HTO correlates with better functional outcome 12 months post-HTO.

Previous results have shown that a greater degradation/synthesis ratio has been associated with an increased odds ratio for OA progression [49] and decreased after load-modifying joint distraction reflecting cartilage regeneration [19]. To date the effect of load on cartilage metabolism after HTO has not been established. It is expected that those with the smallest load-induced C2C:CPII ratio 6 months post-HTO will have the best functional outcome 12-months post-HTO.

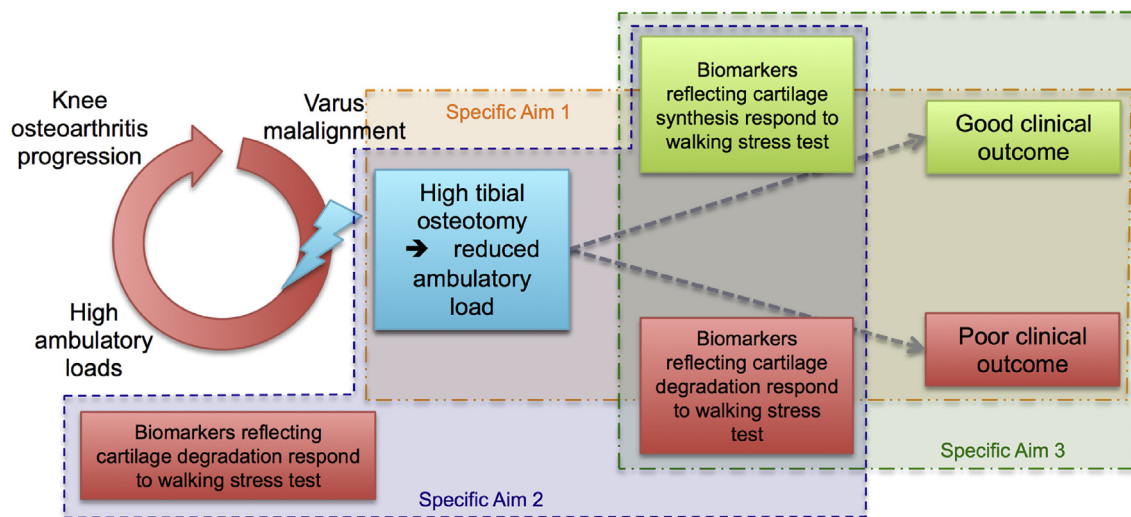


Fig. 1. Illustration of the framework of this study to use HTO as a model for studying in vivo biological effects of sudden large changes in permanent ambulatory load. This framework allows us to address various research questions. In particular we can study the relation between reduction in ambulatory load and clinical and functional outcome after high tibial osteotomy (HTO), also in comparison to static alignment correction (Specific Aim 1), we can relate biomarker response and change in biomarker response to ambulatory load and change in ambulatory load (Specific Aim 2), and finally we can study the relation of biomarker response to good or poor clinical outcome (Specific Aim 3).

These hypotheses will be tested by combining state-of-the-art methods for analyzing human movement to measure ambulatory load, radiographic assessment for measuring static alignment and enzyme-linked immunosorbent assays (ELISAs) for quantifying biomarker concentrations.

2.2. Study design

This study is a prospective multimodal (clinical, biomechanical, biological) data collection without randomization.

2.3. Participants

The study will examine a cohort of 40 patients undergoing opening wedge HTO. The measurement of 40 patients (20 per year) will be realistic and sufficient for the proposed statistical analysis (see below). The participants will be recruited from our orthopaedic clinic by announcing the project during clinic visits before the planned HTO. The study nurse will be contacted and inform interested patients about the study.

2.3.1. Inclusion criteria

Patients suffering from radiographically diagnosed and isolated symptomatic medial compartment knee OA undergoing unilateral opening wedge HTO (Kellgren-Lawrence [50] grade 2 or 3).

2.3.2. Exclusion criteria

Patients will be excluded from the study for the following criteria: use of walking aids; inability to walk for 30 min; age <18 years (before maturation) or age 70 years: due to advanced general sarcopenia (degenerative loss of muscle mass in aging); body mass index (BMI) > 35 kg/m²; excessive skin movement influences the gait analysis (movement between skin mounted marker and underlying bone increases, or locating anatomical landmarks for marker placement not possible); active rheumatic disorder; prior neuromuscular impairment (e.g. stroke); conditions other than knee OA that could cause abnormal patterns of locomotion; prior hip, knee, and ankle prosthesis or osteotomy of the lower extremities; prior spine surgery; ligamentous instability of the knee; other major medical problems; investigators and their immediate families are not permitted to be subjects; persons who have previously completed or withdrawn from this study; patients currently enrolled in another experimental (interventional) protocol.

2.3.3. Ethical considerations

The testing protocol has been approved by the regional ethics board (Ethics Committee Northwest Switzerland EKNZ 2015-224) and registered at clinicaltrials.gov (NCT02622204). Participants in this study will undergo a scheduled HTO that is not subject of this study. Written informed consent will be obtained by all participants prior to participation.

3. Results – a description of the experimental protocol

Within 4 weeks before the planned HTO, patients with medial compartment knee OA will be clinically assessed (including mechanical axis measurement from radiographs) and complete questionnaires on physical function (Fig. 2). Patients will complete a walking stress test with blood sampling (30 min walking, 5.5 h sitting), and undergo gait analysis. Once full load bearing is achieved (6 weeks after HTO), the mechanical axis will be measured from radiographs. Patients will complete the questionnaires and a walking stress test with blood sampling, and undergo gait analysis 6 months after HTO. At the 12-month follow-up, subjects will complete the questionnaires. A 12-month follow-up is considered sufficient for revealing changes in physical function after HTO [8,9].

3.1. Medial wedge opening HTO surgical technique

The surgical procedure for the medial wedge opening HTO is similar to that previously described by Lobenhoffer and Agneskirchner [51]. Two guide pins are placed under fluoroscopic guidance starting 4 cm distal to the medial tibial plateau angling 1 cm distal to the lateral tibial plateau and parallel to the sagittal tibial slope. At the anterior tibia metaphysis, the osteotomy is started 1 cm posterior and parallel to the tibial tuberosity to protect the tibial tuberosity with its patellar tendon insertion. The osteotomy is progressed bi-planar below and parallel to the guide pins. Thin osteotomes are used to complete the osteotomy ending approximately 1 cm short of the lateral femoral cortex to maintain a lateral hinge. Larger osteotomes are then used to slowly open the osteotomy site. A calibrated spreader is then used to open the osteotomy to the desired correction taking care to leave the tibial slope unchanged. A Tomofix (Synthes) osteotomy plate is then placed at the anteromedial tibia spreading the osteotomy site. The osteotomy site is left open in defects below 10-mm opening and otherwise filled with allograft spongy bone. Full range of motion as tolerated and 15 kg weight bearing is

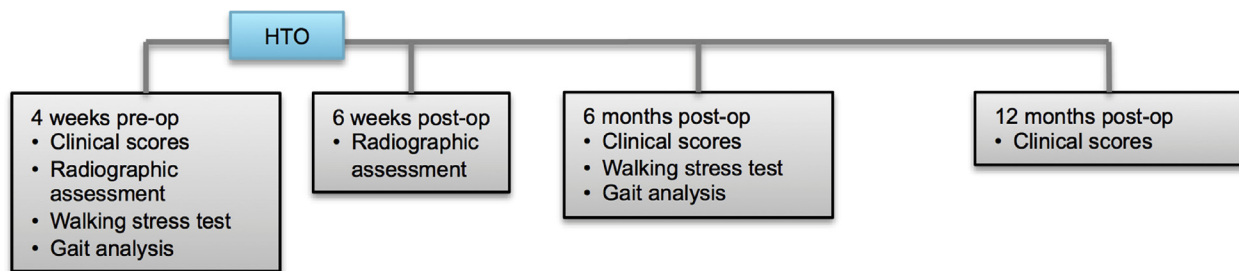


Fig. 2. Illustration of the protocol for testing. The walking stress test comprises repeated blood sampling for biomarker assessment before and after a 30-min walking exercise.

started the next day. Full weight bearing as tolerated is allowed after 4 weeks. Canes are discontinued 6 weeks after the osteotomy, which is the time for radiographic and clinical check-up.

3.2. Measurements

3.2.1. Clinical assessment

Clinical evaluation will be carried out using the Knee Society Score (KSS) and its functional component [52] and the Knee Injury and Osteoarthritis Outcome Score (KOOS) [53]. The active range of movement (ROM) of the knee will be measured pre- and postoperatively with a long arm goniometer.

3.2.2. Radiographic assessment

An anteroposterior weight-bearing radiograph of the knee will be taken pre- and postoperatively, and alignment will be expressed as the femorotibial angle (FTA) defined as the lateral angle between the femoral tibial axes [54].

3.2.3. Walking stress test

Participants will complete a walking stress test pre- and postoperatively. Blood samples will be obtained immediately before (t_0), immediately after (t_1), and 0.5, 1.5, 3.5, and 5.5 h (t_2 - t_5) after a 30-min walking exercise at self-selected walking speed (walking stress test) on a instrumented treadmill (mercury® 3p, h/p/cosmos sports & medical GmbH, Nussdorf-Traunstein, Germany) at least 3 h after waking. For the walking stress test, inertial sensors (RehaGait®, Hasomed GmbH, Magdeburg, Germany) will be attached to the participant's feet to obtain total number of steps and average walking speed, stride length, and cadence of the 30-min walking exercise. For the 5.5 h after the walking exercise, participants will rest while sitting in a chair and their inactivity will be monitored using an activity monitor (ActiGraph wGT3X-BT, Actigraph, 49 E. Chase St., Pensacola, FL, USA) attached to the participant's waist. Blood samples (5 ml each) each will be obtained from the same antecubital vein at each evaluation. A professional nurse will place a thin catheter in a vein of the participant's forearm, which will stay in their vein for 5.5 h. Samples will be collected and allowed to clot for 30 min. Serum will be separated and frozen to -20°C within 1 h of collection and then transferred for storage at -80°C until assayed.

3.2.4. Biological assessment

Serum concentrations of the following biomarkers will be quantified:

3.2.5. Cartilage synthesis

Because type II collagen (CII) is the most abundant protein of cartilage matrix, the assessment of CII synthesis and degradation has been suggested for evaluating OA severity and progression [55]. CII biosynthesis can be evaluated by measuring the C-propeptide of type II procollagen (CPII) [38].

3.2.6. Cartilage degradation

COMP is a structural protein of cartilage and hence specific to cartilage in humans [20]. MMP-3 degrades collagen types II, III, IV, IX, and X,

proteoglycans, fibronectin, laminin, and elastin. MMP-9 digests denatured collagen and other substrates, such as type I collagen and aggrecan, which mainly exist in cartilage [56]. Systemic MMPs may originate from tissues other than cartilage including the synovium but would still act as key mediators of catabolic changes in articular cartilage when in contact with this tissue. Cleavage of type II collagen by collagenases is also excessive in OA cartilage [57]. The C2C epitope is a fragment resulting from cleavage of type II collagen by collagenases reflecting degradation [39].

3.2.7. Assessing serum biomarker concentrations

Serum COMP, MMP-9, MMP-3, C2C and CPII concentrations will be determined using commercial ELISAs. Investigators will be blinded to the samples, which will be analyzed in duplicate and in random order. Differences due to inter-assay variation will be eliminated by comparing concentrations within participants and testing all samples of any participant on the same plate.

3.2.8. Biomechanical assessment

A nine-camera 3-dimensional motion capture system (Vicon Motion Systems Ltd., Oxford, UK; frame rate 120 Hz), and two force plates (Kistler 9260AA6, Kistler Instrumente AG, Winterthur, Switzerland) will be used to capture kinematic and force data. Reflective skin markers will be attached to predefined anatomical landmarks and placed according to the Helen Hayes model for the lower body [58]. Participants will perform a static trial, and the joint coordinate systems will be defined using the Vicon Plug-in-Gait model (PIG) [59]. Knee angles will be calculated from the femur and tibia segments. The ankle angles will be calculated from the tibia and foot segments and hip angles from the pelvis and femur segments [58]. Kinematic data will be filtered using a zero-lag quadratic low-pass Butterworth filter with a cut-off frequency of 12 Hz. Participants will perform three trials at their self-selected preferred walking speed and at each of three walking speeds: slow, normal and fast. In addition, at the follow-up assessment, three trials at the preferred walking speed of the baseline assessment will be captured to facilitate comparison of data between time points.

Net joint moments will be calculated by solving motion equations for the six segments of the lower limbs by incorporating force plate data. Kinetic data will be filtered using a zero-lag quadratic low-pass Butterworth filter with a cut-off frequency of 50 Hz. Joint moments will be calculated using Newton-Euler inverse dynamics [59] and anthropometric mass distributions taken from the literature [60]. The peak external knee adduction moment, external knee adduction moment impulse and peak external knee flexion moment will be used as surrogates of ambulatory load and calculated for each trial.

3.3. Statistical analysis

We will start with an exploration of the individual biomarker response patterns over time in order to define variables reflecting the response to the stress test. We will also explore the biomechanical

measurements in order to define a variable reflecting the ambulatory load. Both initial analyses will be blinded for each other and for all other study results.

We will correlate the response to the load pre- and post-HTO and the change in response to the change in load. Further we will consider regression models to predict functional and clinical outcomes from the post-HTO load, post biomarker response, change in load, change in response and static alignment correction. Model comparisons will be based on R^2 values.

The significance level for all statistical tests will be set a priori to 0.05.

3.3.1. Sample size calculation

Assuming that the biomarker response to the walking stress test will be 20% smaller after compared to before HTO and a standard deviation of differences of 40% and power of 80%, a sample size of 34 is required to detect a significant difference at the $\alpha = 5\%$ level. Previous data from healthy subjects have shown that the biomarker response to no load was 200% smaller than for accumulated load with full weight-bearing [40]. Assuming that KOOS scores will increase by 22 with a standard deviation of 15 [61], and power of 80%, a sample size of 10 is required to detect a significant difference at the $\alpha = 5\%$ level. Calculations were performed in order to detect that if the true correlation has an absolute value 0.50 or greater, we can reject the null hypothesis that the correlation is less than 0.25. This leads to an R^2 of 0.25 or greater. For a power of 80%, a sample size of 30 is required to detect such a correlation at the $\alpha = 5\%$ level (one sided). The correlation value of 0.50 is conservative and likely underestimates the true relationship between accumulated ambulatory load and change in biomarker concentration for several reasons. Pilot data from healthy subjects [62] revealed that the correlation of accumulated ambulatory load, with measures of cartilage metabolism was -0.831 . In the correlation estimate, within these possible options, a conservative value of 0.50 was used. These measurements were obtained from a group of healthy subjects, and the relationships are expected to be stronger in subjects with knee OA. Given the possibility of unanticipated reasons for removing subjects, a recruitment plan targeting for 50 subjects should increase the potential to have sufficient power for this study. If the association based on the proposed data is of the same magnitude as that found in the pilot data, a sample size of 50 will provide at least 80% power to detect a significant relationship.

4. Discussion

In the proposed study, we will use HTO as a model for studying in vivo biological effects of large permanent changes in ambulatory load (Fig. 1). HTO will break the vicious cycle of knee OA progression, varus malalignment and high ambulatory load. On average, the knee adduction moment after HTO is reduced to half of pre-operative levels [10]. Hence, the accumulated load in the affected knee in a post-HTO walking stress test should also only be half of the pre-HTO accumulated load. Depending on the specific ambulatory load reduction with HTO, cartilage may have the capacity to regenerate (sufficient unloading) or further degenerate (insufficient unloading) leading to poor outcome. Changes in cartilage biomarkers in response to a walking stress test are expected to change immediately after the load-altering HTO and depend on the magnitude of load reduction and be related to clinical outcome at 12-months.

Although the mechanosensitivity of articular cartilage has received substantial scientific scrutiny, to date in vivo data on the reaction of articular cartilage to physiological loads in human is limited because of the inability of measuring biological processes directly. Understanding these processes is critical for optimizing care for patients with severe knee OA. Moreover, a model for measuring in vivo mechanosensitivity may also provide unique insights into in vivo cartilage degeneration and regeneration processes in healthy subjects, after injuries or in patients with musculoskeletal disease.

The results of this study can be considered as proof-of-concept of a potential diagnostic test (walking stress test) for cartilage degeneration

and its prognostic value for future outcomes. In this study we will test if the magnitude of ambulatory load affects tissue metabolism assessed as serum biomarker concentrations and if load-induced changes in cartilage biomarkers are related to functional and clinical outcome of HTO. To date, the use of cartilage biomarkers for OA diagnostics and prognostics is limited in part because of the large inter-subject variation in serum markers. A direct relationship between ambulatory load and cartilage metabolism assessed as degradation to synthesis ratio would allow developing novel load-modifying interventions and evaluating the efficacy of existing interventions. Moreover, a predictive relationship between load-induced changes in cartilage biomarkers 6 months post-HTO and functional and clinical outcome 6 months later would suggest that the stress test may be a clinically highly relevant tool for detecting degenerative cartilage processes. Being able to predict the outcome of HTO would allow selecting patients who would likely benefit from this surgical approach.

Author contributions

AM conceived and designed the study and drafted the manuscript; AM, GP, CE and CN contributed methodological details for clinical, biomechanical and biological aspects; CN will collect all data and analyze the biomechanical data; GP and CE will provide patients, perform the surgery and process the clinical data; WV will perform the statistical analyses; all authors will be involved in interpreting the data and critically reviewed and approved the manuscript.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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