(V). Such splicing variants provides cells capacity to generate protein isoforms with different binding properties hence functions. Among the *FN1* splice variants is the intact 70 kDa N-terminus of the full length protein, also known as migration-stimulating factor (MSF), which has been associated with tumor progression in multiple cancers. Despite being prominent in articular cartilage, it is unknown whether *FN1* transcripts are involved in the OA pathophysiology. We, therefore, aimed to identify *FN1* transcripts associated with OA pathophysiology and investigated downstream effects upon modulation of transcripts relative to full length *FN1* expression in our 3D *in vitro* organoid cartilage model with human primary chondrocytes.

Methods: *FN1* transcriptomic data was obtained from our previously assessed RNA-seq dataset of lesioned and preserved OA cartilage samples (N=35, RAAK study). Differential transcript expression analysis was performed on all 27 *FN1* transcripts annotated in Ensembl database. Pearson's correlations were calculated to generate a co-expression network between *FN1* and our previously reported differentially expressed genes in OA cartilage. Human primary chondrocytes obtained from the RAAK study were transduced with lentiviral particles containing shRNA targeting full length *FN1* transcripts or non-targeting shRNA. Subsequently, matrix deposition was induced in our 3D in vitro neo-cartilage model. Effects of changes in *FN1* transcript ratio on sulphated glycosaminoglycan deposition were investigated by Alcian blue staining and dimethylmethylene blue assay. Moreover, gene expression levels of eight cartilage relevant markers and three highly correlating genes with *FN1* were determined by RT-qPCR.

Results: We identified 22 *FN1* transcripts to be robustly expressed in OA cartilage. In line with previous findings, the highest expressed protein-coding transcripts were EDA$^-$ variants, while EDA$^+$ variants were less abundantly expressed. To identify specific *FN1* transcripts associates with the OA process, differential expression analysis was performed on these 22 transcripts between lesioned and preserved OA cartilage, resulting in 16 FDR significantly upregulated transcripts. Of these 16 transcripts, 5 were protein-coding and 11 non-protein coding, suggesting these non-protein coding transcripts may play a role in OA pathophysiology. Notably, *FN1-208*, encoding Migrating Stimulating Factor (MSF), was the most significantly upregulated protein-coding transcript. Upon downregulation of full length *FN1* in our 3D in vitro neo-cartilage model, we generated an increased ratio of *FN1-208* relative to full length *FN1* transcript, as such mimicking cartilage in OA affected state. This resulted in decreased cartilage sulfated glycosaminoglycan deposition (Figure 1), as well as significantly decreased *ACAN* and *COL2A1* and increased *ADAMTS-5*, *ITGB1*, and *ITGB5* gene expression levels, implying a detrimental effect on neo-cartilage deposition. Moreover, *NT5E* expression was downregulated as a result of *FN1* downregulation, indicating *NT5E* expression is regulated downstream of *FN1* signaling.

Conclusions: We identified *FN1-208* as the most significantly upregulated protein-coding transcripts, which has not been previously associated with OA. We show that full length *FN1* downregulation and concomitant relative *FN1-208* upregulation was unbeneficial for deposition of neo-cartilage matrix, likely due to decreased availability of the classical RGD integrin-binding site of fibronectin. We identified *NT5E* as previously unknown *FN1* signaling downstream gene in cartilage. Together, our data highlight the importance of proper balance of *FN1* transcripts for healthy cartilage homeostasis.

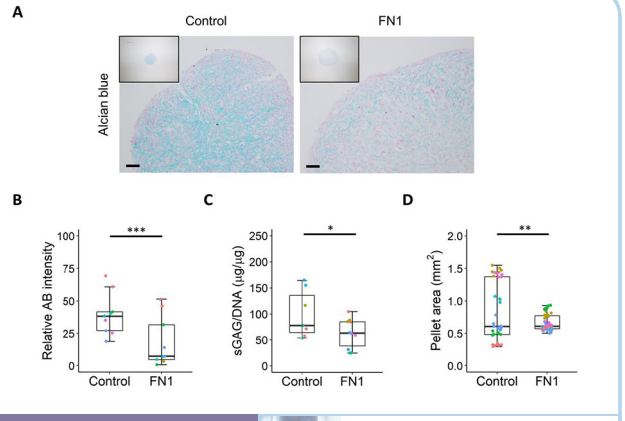

Figure 1 Osteoarthritis and Cartilage

Decreased overall *FN1* expression and change of *FN1* transcript ratios results in decreased matrix deposition in neo-cartilage pellets after three days of chondrogenesis. (A) Representative images of Alcian blue staining of neo-cartilage pellets of primary chondrocytes transduced with non-targeting shRNA (control) and FN1 targeting shRNA (FN1). (B) Quantification of Alcian blue (AB) pixel intensity staining of control and FN1 targeting shRNA transduced pellets (N=9). Colors of dots represent the different donors. (C) Sulphated glycosaminoglycan (sGAG) content normalized to DNA content in pellets of the control (N=7) and FN1 group (N=11) analyzed by dimethylmethylene blue assay. (D) Pellet area of pellets in control (N=40) and FN1 group (N=48). P values were determined by generalized estimation equations, with experimental read-out as dependent variable, and donor and group as covariate. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$.

12
RELIABILITY OF WEARABLE SENSORS FOR ASSESSING GAIT AND CHAIR STAND FUNCTION AT HOME IN PEOPLE WITH KNEE OSTEOARTHRITIS

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Purpose: The COVID-19 pandemic has accelerated the adoption of digital health technologies for remote monitoring of participants in clinical trials, including measuring physical function. For trials in people with knee osteoarthritis (OA), standardized measures of function such as gait and chair stand are considered important outcomes. Wearable sensors have the potential to monitor these outcomes remotely. However, the reliability of wearable sensor metrics of gait and chair stand in participants' homes and agreement between these metrics collected in laboratory and at-home have not been reported to date. Hence, our objective was to assess the reliability of wearable sensors for remote monitoring of gait and chair stand in people with knee OA.

Methods: We used data from a substudy (n=20) embedded within an ongoing, single-arm clinical trial of an exercise intervention in people with knee OA (clinicaltrials.gov NCT04243096). Key inclusion criteria were age $\geq$ 50, BMI $\leq$ 40 kg/m^2, physician diagnosed knee OA, score $\geq$ 3 on weight-bearing questions from the Knee Injury and Osteoarthritis Outcome Score (KOOS) Pain Subscale in the index knee, and ability to walk for 20 minutes without assistance. Key exclusion criteria included other major health conditions; prior, current, or planned major knee OA treatment; prior surgeries for knee OA; and contraindications to exercise. All assessments took place prior to initiation of the intervention. Participants completed two visits, an in-person lab visit and a remote at-home visit. The order of these visits was randomized across participants, with participants completing both visits between 1 and 20 days of each other. For the remote visit, participants were provided a wearable system consisting of three inertial sensors (Opal, APDM, Portland, OR, USA), two cones connected by a 7-meter rope, and an armless chair. Identical equipment was used during the in-person lab visit. During the remote visit, researchers guided the participants via video conference. Participants self-applied the sensors on each foot and on the lower back. They performed two trials each of a standardized gait task (self-selected walk for two laps of a 7-meter path defined by the cones and rope totaling 28 meters of walking) and chair stand task (five chair stands as quickly as possible with arms across the chest) in their

home. Then, the participants removed the sensors, waited 15-minutes, re-applied the sensors, and performed two more trials of each task. At the end of the remote visit, participants completed a survey on their experience. During the in-person lab visit, participants performed two trials of the same tasks after a researcher placed the sensors on the participants. Spatiotemporal metrics of gait function and duration of chair stand were extracted from the sensor data using software (MoveoExplorer) provided by the sensor manufacturer. The mean of sensor metrics across each set of two trials were used in the analyses. We used Pearson's correlation R^2 and the intra-class correlation coefficients (ICC) to determine the correlation and the test-retest reliability of sensor metrics from the two repetitions of the tasks during the remote visit. We used ICCs and Bland-Altman plots and their 95% limits of agreement to examine agreement between sensor metrics from the remote (first two trials) and lab visits.

Results: Participant characteristics are shown in Table 1. All ICCs were good to excellent (between 0.85 and 0.96) for the test-retest reliability during the remote visit and R^2 ranged between 0.81 and 0.95 (Table 2, Figure 1). ICCs were moderate to excellent (between 0.63 and 0.91) for agreement between remote and lab visits (Table 2). Bland-Altman plots showed small bias in all metrics due to participants walking slightly faster during the lab visit compared to the remote visit (Figure 2). Participants were highly accepting of the remote visit (Table 3).

Conclusions: In this cohort of people with knee OA who had moderate pain and disability, our method of estimating gait and chair stand function remotely was found to be reliable, feasible, and acceptable. Wearable sensors could be used to remotely monitor gait and chair stand function in participant's natural environments at a lower cost, reduced participant and researcher burden, and greater ecological validity overcoming many limitations of lab visits. Hence, our approach could be used in future clinical trials of people with knee OA.

Table 1: Participant characteristics.

	Participants (n=20)
Mean Age (SD), years	70.5 (4.7)
Mean BMI (SD), kg/m²	30.6 (4.7)
Mean KOOS Pain (SD)	60.2 (10.6)
Mean KOOS ADL (SD)	68.8 (14.2)
Female, n (%)	17 (85%)
White, n (%)	19 (95%)
Without a college degree, n (%)	2 (10%)
Annual Income <$50,000, n (%)	4 (20%)
Currently Employed, n (%)	11 (55%)

SD = Standard Deviation
KOOS = Knee injury and Osteoarthritis Outcome Score

Table 2: Gait metrics from the left leg* and chair stand duration from home and lab visits. Mean (Standard Deviation) are reported for all metrics along with ICC (lower bound, upper bound). ICC estimates and their 95% confidence intervals were calculated using R based on absolute-agreement, 2-way mixed-effects model.

	Home (1st)	Home (2nd)	Lab	Home vs Home ICC (2,1)	Home vs Lab ICC (2,1)
Gait Speed (m/s)	1.02 (0.11)	1.07 (0.14)	1.07 (0.15)	0.85 (0.62, 0.93)	0.66 (0.35, 0.83)
Cadence (steps/min)	111 (11)	114 (10)	111 (8)	0.94 (0.80, 0.97)	0.73 (0.50, 0.87)
Stride Length (m)	1.10 (0.10)	1.13 (0.12)	1.15 (0.12)	0.92 (0.81, 0.96)	0.63 (0.33, 0.81)
Step Duration (s)	1.09 (0.10)	1.07 (0.10)	1.08 (0.10)	0.93 (0.83, 0.97)	0.76 (0.55, 0.88)
Stride Duration (s)	0.54 (0.04)	0.53 (0.05)	0.54 (0.04)	0.94 (0.78, 0.97)	0.74 (0.52, 0.87)
Stance (%GCT)	61.45 (1.98)	61.16 (2.15)	61.17 (2.29)	0.96 (0.91, 0.98)	0.89 (0.77, 0.95)
Swing (%GCT)	38.55 (1.98)	38.84 (2.15)	38.83 (2.29)	0.96 (0.91, 0.98)	0.89 (0.77, 0.95)
Double Support (%GCT)	23.4 (3.2)	22.9 (3.6)	22.5 (4.1)	0.96 (0.89, 0.98)	0.88 (0.70, 0.95)
Terminal Double Support (%GCT)	11.41 (1.93)	11.28 (2.31)	11.34 (2.49)	0.94 (0.88, 0.97)	0.91 (0.82, 0.96)
Chair Stand Duration (s)	1.05 (0.30)	1.11 (0.32)	0.98 (0.21)	0.89 (0.76, 0.95)	0.66 (0.40, 0.83)

** For gait metrics, only left leg data are reported as results were similar for data from the right leg*

Table 3: Feedback from participants about the remote at-home visit.

Question	Median	Q1	Q3
Overall experience during at-home visit?[a]	4	3.5	5
Difficulty using Zoom?[b]	5	4	5
Difficulty placing sensors on feet and back?[b]	5	5	5
Level of time commitment for the visit?[c]	5	4	5
Comfort performing walking task while wearing sensors?[d]	5	5	5
Comfort performing chair stand task while wearing sensors?[d]	5	5	5
Likelihood of participating in another similar at-home visit?[e]	4	4	4

[a] *1-5 scale from 1 = "definitely not enjoyable" to 5 = "definitely enjoyable"*
[b] *1-5 scale from 1 = "very difficult" to 5 = "very easy"*
[c] *1-5 scale from 1 = "too burdensome" to 5 = "completely manageable"*
[d] *1-5 scale from 1 = "not at all comfortable" to 5 = "completely comfortable"*
[e] *1-4 scale from 1 = "very unlikely" to 4 = "very likely"*

Figure 1: Scatter plots for selected gait metrics from the left leg (A-C) and chair stand duration (D) derived from the wearable sensors during the remote visit.

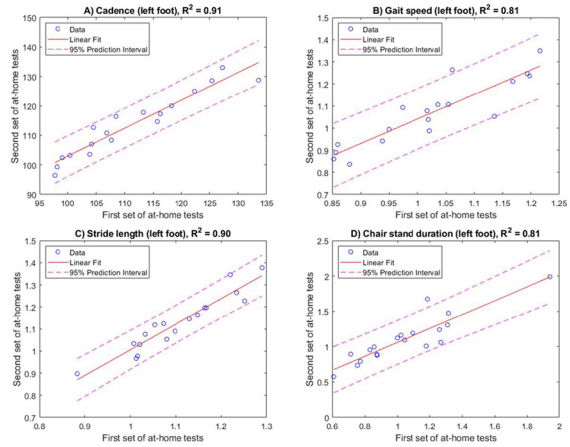
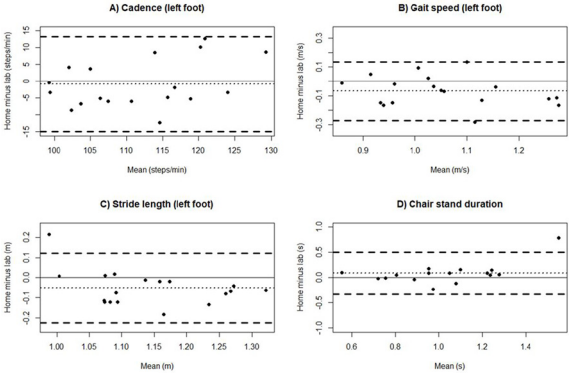


Figure 2: Bland-Altman plots for selected gait metrics (A-C) and chair stand duration (D) derived from the wearable sensors during the remote and lab visits. Lines show mean difference (dotted) and 95% limits of agreement (dashed).



V-13
UNDERLOADING, NOT OVERLOADING, OF THE PATELLOFEMORAL JOINT INCREASES THE RISK OF EARLY OSTEOARTHRITIS AFTER ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

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Purpose: Approximately 50% of adolescents and young adults who rupture their anterior cruciate ligament (ACL) develop knee osteoarthritis (OA) within 10 years. While most reports of OA after ACL injury focus on the tibiofemoral joint, patellofemoral OA is common and more strongly associated with knee pain and impaired function. The mechanisms underpinning the rapid development and progression of patellofemoral OA after ACL injury are unknown but may relate to the way the knee moves (biomechanics). Therefore, this study aimed to determine if altered patellofemoral loading was associated with the presence and/or worsening of patellofemoral OA following ACL reconstruction (ACLR).

Methods: Forty-six participants (30 men, age 27±5 years, BMI 24.5±3.1 kg.m-2) were randomly selected from a cohort of 111 consecutively recruited ACLR patients (primary hamstring-tendon autograft) and completed 3D isotropic MRI and biomechanics testing of their index knee approximately 1-year post-ACLR. Biomechanics testing involved recording trunk and lower-limb movement with a 12-camera 3D motion analysis system (120 Hz) and ground reaction force data (2400 Hz) during the landing phase of a standardised forward hop (equivalent to leg length). These data were input into a musculoskeletal model using OpenSim 4.0 and Matlab to calculate patellofemoral joint contact forces