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Purpose: To investigate the association of changes in gait biomechanics, achieved with high tibial osteotomy (HTO) surgery, with subsequent total knee replacement (TKR).

Methods: This prospective cohort study included patients with symptomatic medial compartment knee osteoarthritis (OA) and varus alignment undergoing medial opening wedge HTO between 2002 and 2014. All patients completed a quantitative 3D gait analysis at <3 months prior to their HTO and approximately 12 months following their HTO, using a 12-camera optical motion capture system and a floor-mounted force plate. The present analysis focused on external knee adduction and flexion moments, calculated using inverse dynamics over 100% of stance. Values are expressed in both Nm and %Bw*Ht. For each gait assessment, we used the mean of five walking trials to calculate the 1st and 2nd peak knee adduction moments (KAM), the knee adduction moment impulse (KAI) and the peak knee flexion moment (KFM). To identify patients who underwent TKR on the HTO limb, we used surgery reports and radiographs of the knee from each patient's London Health Sciences Centre hospital electronic database medical record. We followed patients from enrolment to July 31st, 2019. If there was no record of TKR, we censored patients at the time of last contact where we could confirm the absence of TKR using knee radiographs. We censored deceased patients at time of death.

We evaluated the mean change (12 months post-op minus pre-op) in 1st and 2nd peak KAM, KAI and peak KFM using dependent samples t-tests to quantify the direction of changes in gait outcomes following HTO. To estimate the cumulative incidence for time to conversion from HTO to TKR, we used a Kaplan-Meier analysis. We also fitted separate Cox proportional hazards models to estimate the association between 1) the change in KAI (per 10 Nm · s) and 2) the change in KFM (per 20 Nm) with conversion to TKR using hazard ratios (HR) with 95% confidence intervals (CI). We then repeated the analyses while also adjusting for other potential predictors of TKR including change in static alignment (mechanical axis angle - MAA) achieved with surgery, baseline radiographic disease severity (Kellgren & Lawrence [KL] $\leq$ 2 vs. 3 or 4), age (per 10 years), sex and BMI (per 5 kg/m^2). We also controlled for change in gait speed (per 0.2 m/s) for KFM.We adjusted the variance for clustering among different surgeons using robust sandwich estimators to ensure appropriate type 1 error rates. We completed sensitivity analyses substituting KAI for the 1st and 2nd peak KAM, then including only the first HTO in patients who underwent staged bilateral HTOs. Due to substantial multicollinearity between the change in KAI and change in peak KFM, we performed combined and separate models.

Results: The cohort included 610 knees, including 550 patients who underwent HTO, 60 of which underwent staged bilateral procedures. On average, patients were male (n=477; 78%), middle-aged (47.0 ± 9.0 years), overweight (29.7 ± 5.0 kg/m^2) with varus alignment (MAA = -7.9 ± 3.7 degrees). 246 knees (40%) had early-stage radiographic disease (KL $\leq$ 2), while 364 knees (60%) had late-stage radiographic disease (KL 3 or 4). A TKR on the HTO limb was identified on 98 knees (16%) and 6 patients (1%) died during follow-up. The median follow-up time was 7 years (interquartile range = 4 to 10 years). Mean changes from pre-to-post HTO for knee moments are presented in Table 1, showing significant reductions in both knee adduction moment and knee flexion moment after HTO surgery. The cumulative incidence at 5 years was 5% (95%CI, 3% to 7%) and 20% (95%CI, 16% to 25%) at 10 years (Figure 1). The KAI was associated with conversion to TKR (HR = 0.63 [95%CI, 0.40 to 0.98]; per 10 Nm · s) when also adjusting for change in MAA with surgery, KL grade, age, sex, and BMI, all of which were also associated with conversion to TKR (Table 2). Results therefore suggest that at any point in time, individuals who experience larger changes in KAI (ie., larger reductions) after HTO are at a reduced risk of conversion to TKR in the future. KAI was not a significant predictor in the unadjusted model (Table 2). The KFM was also associated with conversion to TKR (HR = 1.18 [95%CI, 1.06 to 1.32]; per 20 Nm) when also adjusting for change in MAA achieved with surgery, KL grade, age, sex, and BMI, all of which were also associated with conversion to TKR, and change in gait speed, which was not associated (Table 3). Results therefore suggest that at any point in time, individuals who experience larger changes in KFM (ie., larger reductions) with HTO are at an increased risk of conversion to TKR in the future. Results were similar in the unadjusted model (Table 2). Sensitivity analyses provided similar results except when substituting KAI for the 1st peak KAM where the confidence interval included 1.

Conclusions: At 5 years, 95% of patients who underwent HTO did not go on to have a TKR, while 79% did not at 10 years. Larger reductions in knee adduction moment achieved with HTO surgery reduced the risk of undergoing future TKR, while larger reductions in knee flexion moment increased the risk. Associations are supported even after controlling for changes in static alignment (MAA) and gait speed and other known predictors of TKR. Results suggest changes in dynamic knee loading may play an important role in contributing to future risk of TKR.

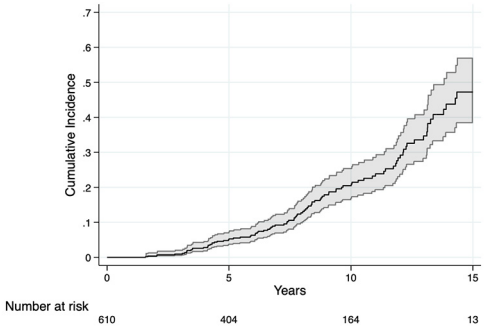

Figure 1. Cumulative incidence curve (with 95% confidence intervals) for knees (n=610) undergoing high tibial osteotomy (HTO). Conversion from HTO to a total knee replacement represents the event of interest. The number of patients at risk are also presented for selected time points.

Table 1. Mean changes in gait biomechanics of the sagittal and frontal plane from pre to 12 months post high tibial osteotomy (n=610).

Outcomes	Pre-HTO	Post-HTO	Mean Difference (95% CI)
External knee adduction moment			
1st Peak, (Nm)	50.4 (18.0)	22.9 (11.6)	-27.4 (-28.9 to -26.2)
(%Bw*Ht)	3.2 (1.0)	1.5 (0.7)	-1.8 (-1.8 to -1.7)
2nd Peak, (Nm)	51.0 (19.2)	24.8 (15.3)	-26.3 (-28.0 to -24.6)
(%Bw*Ht)	3.2 (0.9)	1.5 (0.8)	-1.7 (-1.7 to -1.6)
Impulse, (Nm • s)	25.4 (10.5)	11.6 (6.5)	-13.8 (-14.5 to -13.0)
(%Bw*Ht)	1.6 (0.6)	0.7 (0.7 to 0.8)	-0.9 (-0.9 to -0.8)
External knee flexion moment			
Peak, (Nm)	23.0 (23.6)	19.6 (19.5)	-3.4 (-5.1 to -1.7)
(%Bw*Ht)	1.5 (1.4)	1.2 (1.2)	-0.2 (-0.3 to -0.1)

Values presented as means standard deviation unless otherwise specified.
Abbreviations: BW = body weight, CI = confidence intervals, Ht = height, HTO = high tibial osteotomy, m = meter, N = Newton, s = second, SD = standard deviation

Table 2. Cox regression hazard ratio estimates for change knee adduction impulse as a predictor of conversion from high tibial osteotomy to total knee replacement (n=610).

Predictor	Unadjusted Hazard Ratio (95%CIs)	Adjusted Hazard Ratio (95%CIs)
Change in knee adduction impulse (per 10 Nm • s)*	0.94 (0.83 to 1.07)	0.63 (0.40 to 0.98)
Change in mechanical axis angle (degrees)		0.90 (0.84 to 0.97)
Radiographic severity (Kellgren & Lawrence [KL] grade)		
Mild-to-moderate*		Reference
Moderate-to-severe*		2.51 (1.96 to 3.21)
Sex		
Male		Reference
Female		2.13 (1.65 to 2.74)
Age (per 10 years)		1.61 (1.26 to 2.07)
Body mass index (per 5 kg/m^2)		1.28 (1.09 to 1.50)

The variance was adjusted for clustering at the level of the operating surgeon.
Change scores were calculated by subtracting preoperative values from postoperative values.
*Substituting the knee adduction impulse for the 2nd peak external knee adduction moment (Nm) provided similar results for both outcomes. While, substituting for the 1st peak external knee adduction moment (Nm) also provided similar results, confidence intervals included 0.
* Mild-to-moderate = KL grade 2 or less, moderate-to-severe = KL grade 3 or 4
Abbreviations: CI = confidence intervals kg = kilogram, m = meters, N = Newton, s = second

Table 3. Cox regression hazard ratio estimates for change in peak external knee flexion moment as a predictor of conversion from high tibial osteotomy to total knee replacement (n=610).

Predictor	Unadjusted Hazard Ratio (95%CIs)	Adjusted Hazard Ratio (95%CIs)
Change in knee flexion moment (per 20 Nm)	1.22 (1.05 to 1.42)	1.18 (1.06 to 1.32)
Change in gait speed (per 0.2 m/s)		1.36 (0.89 to 2.07)
Change in mechanical axis angle (degrees)		0.93 (0.88 to 0.98)
Radiographic severity (Kellgren & Lawrence [KL] grade)		
Mild-to-moderate*		Reference
Moderate-to-severe*		2.64 (2.14 to 3.26)
Sex		
Male		Reference
Female		1.95 (1.45 to 2.63)
Age (per 10 years)		1.49 (1.20 to 1.86)
Body mass index (per 5 kg/m^2)		1.27 (1.08 to 1.46)

The variance was adjusted for clustering at the level of the operating surgeon.
Change scores were calculated by subtracting preoperative values from postoperative values.
* Mild-to-moderate = KL grade 2 or less, moderate-to-severe = KL grade 3 or 4
Abbreviations: CI = confidence intervals kg = kilogram, m = meters, N = Newton, s = second

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β2-ADRENOCEPTOR DEFICIENCY LEADS TO INCREASED SUBCHONDRAL BONE REMODELING AND CALCIFIED CARTILAGE THICKNESS IN A MURINE KNEE OSTEOARTHRITIS MODEL

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Purpose: Recent *in vitro* studies demonstrated a contribution of the sympathetic neurotransmitter norepinephrine (NE) to cellular processes involved in osteoarthritis (OA) pathogenesis. These effects were mediated by different adrenoceptor (AR) subtypes that are expressed in all joint tissues. Most *in vitro* data suggest that especially the β2-AR plays an important role during OA progression. However, at present, no study exists that investigated the role of the β2-AR in the knee joint in vivo. Therefore, we examined the contribution of the β2-AR to OA progression using β2-AR-deficient ($Adrb2^{-/-}$) mice.

Methods: We used 12 weeks old male wildtype (WT, C57BL/6J) and $Adrb2^{-/-}$ mice (C57BL/6J background). OA was induced by destabilization of the medial meniscus (DMM) or Sham surgery was performed. 8 weeks after surgery, the severity of cartilage degeneration as well as synovial inflammation were evaluated by histological scoring (OARSI and synovitis grading). Calcified cartilage (CC) thickness and subchondral bone parameters such as subchondral bone plate (SCBP) thickness and bone volume density (BV/TV) were analyzed 8 weeks post-surgery in the medial condyles using micro-computed tomography (μCT). In addition, BV/TV of the subarticular trabecular bone was examined. Activity of osteoclasts and osteoblasts in the subchondral bone was analyzed by histological staining of alkaline phosphatase (ALP) and Cathepsin-K (CatK). Moreover, body weight and serum leptin concentrations (ELISA) were evaluated.

Results: WT and $Adrb2^{-/-}$ mice developed comparable changes in cartilage degeneration and synovial inflammation after DMM surgery (mean OARSI score: WT DMM 2.78 ± 0.32, $Adrb2^{-/-}$ DMM 3.09 ± 0.55; mean synovitis score WT DMM 2.75 ± 0.25, $Adrb2^{-/-}$ 2.75 ± 0.25). The μCT analyses revealed no significant differences in bone parameters of sham animals between WT and $Adrb2^{-/-}$. In contrast, DMM-operated $Adrb2^{-/-}$ mice displayed significant elevated CC thickness (WT DMM 64.54 ± 2.07 μm, $Adrb2^{-/-}$ DMM 74.68 ± 2.79 μm; $p = 0.01$), significantly increased SCBP thickness (WT DMM 107.7 ± 3.1 μm, $Adrb2^{-/-}$ DMM 170.50 ± 11.84 μm; $p < 0.001$), as well as significantly increased BV/TV (WT DMM 0.563 ± 0.029, $Adrb2^{-/-}$ DMM 0.713 ± 0.024; $p = 0.006$) in the medial epiphysis. Further examination of the BV/TV in the subarticular trabecular bone exposed no differences between WT and $Adrb2^{-/-}$ (WT DMM 0.134 ± 0.011, $Adrb2^{-/-}$ DMM 0.123 ± 0.019). Analysis of osteoclast activity by ALP-staining showed no difference in ALP^{+}-area in subchondral bone between WT DMM and $Adrb2^{-/-}$ DMM (WT DMM 0.906 ± 0.148 %, $Adrb2^{-/-}$ DMM 1.212 ± 0.083 %). In contrast, when analyzing osteoclast activity by CatK-staining, a significant decrease was detected in $CatK^{+}$-area in $Adrb2^{-/-}$ DMM compared to WT DMM mice (WT DMM 2.316 ± 0.572 %, $Adrb2^{-/-}$ DMM 0.526 ± 0.068 %; $p = 0.002$). $Adrb2^{-/-}$ mice had a significantly higher body weight compared to WT mice, regardless whether DMM or Sham surgery was performed ($Adrb2^{-/-}$ DMM 33.54 ± 1.18 g, WT DMM 28.56 ± 0.28 g; $p < 0.001$; $Adrb2^{-/-}$ Sham 33.51 ± 1.54 g, WT Sham 28.79 ± 0.25 g; $p < 0.001$). The elevated body weight is due to an increase in body fat mass. Serum leptin concentrations were significantly elevated in $Adrb2^{-/-}$ mice compared to WT after DMM surgery (WT DMM 8.48 ± 2.45 ng/ml, $Adrb2^{-/-}$ DMM 85.39 ± 40.07 ng/ml; $p < 0.001$), but not in Sham animals (WT DMM 4.85 ± 1.8 ng/ml, $Adrb2^{-/-}$ DMM 10.28 ± 1.19 ng/ml).

Conclusions: This study demonstrated that β2-AR deficiency did not affect cartilage degeneration and synovial inflammation but contributed to OA progression by aggravating OA-related calcification at the interface of cartilage and subchondral bone as well as subchondral bone remodeling. The thickening of calcified cartilage and subchondral bone in $Adrb2^{-/-}$ DMM mice was on the one hand directly mediated by β2-AR-deficiency by increasing osteoblast and suppressing osteoclast activities. On the other hand, β2-AR deficiency led to an increased fat mass with elevated serum leptin concentration. Elevated leptin release caused by OA-associated inflammation aggravated the OA-related thickening of subchondral bone in the same way by increasing osteoblast and inhibiting osteoclast activities. Thus, subchondral bone changes in $Adrb2^{-/-}$ DMM mice are the result of a synergistic effect of β2-AR deficiency (direct and indirect) and OA induction. Therefore, targeting the β2-AR represents a novel treatment option, which might help to develop tissue-specific therapeutic drugs for the prevention of pathological subchondral bone remodeling in OA patients.

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CD38 DEFICIENCY PROTECTS MICE FROM AGE-RELATED SPONTANEOUS OA DEVELOPMENT

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Purpose: CD38 is recently shown to be the main NADase in mammalian tissue, which contributes substantially to degradation of cellular nicotinamide adenine dinucleotide (NAD^{+}), a key metabolite involved in cellular energy metabolism and adaptive responses of cells to bioenergetics and oxidative stress. CD38 expression and activity are increased during aging, which contributes to age-related decline of NAD^{+}. In our previous studies, we observed an age-related increase in expression of CD38 in human knee cartilage, and that increased CD38 expression in chondrocytes is associated with reduced NAD/NADH levels and increased catabolic responses to pro-inflammatory cytokine IL-1β. In addition, inhibition of CD38 attenuates chondrocyte catabolic responses to IL-1β and significantly inhibited OA development in mice in a post-traumatic OA model. Maintenance of intracellular NAD^{+} content is implicated to be critical for tissue homeostasis. Since NADase activity is almost absent in CD38 knockout (KO) mice, we determined the effect of CD38 deficiency on age-related spontaneous OA development in mice *in vivo*.

Methods: Both male or female CD38 KO and wild type (WT) mice at 12 and 24 months of age were used. Knee joints of these mice were collected, fixed, decalcified and embedded in coronal plane in paraffin, and sectioned for histological staining with safranin-O and fast green, as well as hematoxylin and eosin (H&E). The histological images were used to assess severity of cartilage damage of all four quadrants of the joint using the OARSI score system. The final OARSI scores were average sum scores of four quadrants. Synovitis scores were determined based on changes in synovial lining thickness and cellular density in the synovial stroma. Osteophyte formation was evaluated based on both size and maturity of osteophytes. Prism 9 GraphPad was used for statistical data analysis.

Results: Both male and female WT and CD38KO mice exhibited very little cartilage damage, synovitis, and osteophyte formation at 12 months of age. However, moderate cartilage damage was seen in both male and female WT mice at 24 months of age. Notably, the extent of cartilage damage was less in WT female with the mean OARSI score 2.6 compared to WT male mice (the mean OARSI score 5.47) at 24 months of age. Compared to WT mice, cartilage damage was significantly reduced with the mean OARSI scores 1.14 and 0.4 for male and female CD38 KO mice, respectively, suggesting that CD38 deficiency was chondroprotective (Table I and Figure 1). Synovitis and osteophyte size and maturity were also observed in both male and female WT mice, but they were milder in female compared to male WT mice with the mean scores of 2.07, 2.5 and 2.44 for male and 0.97, 0.69, 1.12 for female, respectively (Table I and Figure 1). These were significantly inhibited in both male and female CD38 KO male mice at 24 months of age (Table I and Figure 1). These results indicate that both male and female mice deficient in CD38 were protected from age-related spontaneous OA development.

Conclusions: Taken together with our previous finding that CD38 inhibitor apigenin limits post-traumatic OA development and associated pain in mice, inhibition of CD38 could be a novel therapeutic approach for OA prevention and treatment.

Table I		WT mean (95% CI)		CD38KO mean (95% CI)	
		12 M	24 M	12 M	24 M
OARSI	male	0.1 (0.02, 0.2)	5.47 (2.91, 8)	0.14 (0.03, 0.3)	1.14 (0.16, 2.1)
	female	0.46 (0.24, 0.67)	2.61 (1.44, 3.78	0.28 (0.03, .54)	0.4 (0.06, 0.87)
synovitis	male	0.34 (0.03, 0.7)	2.07 (0.8, 3.3)	0.08 (0.05, 0.22)	0.41 (0.23, 1.06)
	female	0.45 (0.22, 0.68)	0.97 (0.6, 1.34)	0.05 (0.03, 0.14)	0.37 (0.011, 0.74)
osteophyte-size	male	0	2.5 (1.45, 3.55)	0.14 (0.03, 0.32)	0.61 (0.04, 1.26)
	female	0.026 (0.08, 0.43)	0.69 (0.22, 1.17)	0.33 (0.13, 0.54)	0.22 (0.03, 0.46)
osteophyte-maturity	male	0	2.44 (1.4, 3.47)	0.14 (0.03, 0.32)	0.58 (0.03, 1.2)
	female	0.46 (0.17, 0.75)	1.12 (0.42, 1.82)	0.26 (0.01, 0.52)	0.31 (0.13, 0.76)