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# Physical inactivity and knee osteoarthritis in guinea pigs

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SUMMARY

*Objective:* To investigate whether and how a sedentary lifestyle contributes to knee osteoarthritis (OA) incidence and severity.
*Design:* An experiment was conducted using Hartley guinea pigs, an established idiopathic knee OA model. To simulate a sedentary lifestyle, growing animals ($n = 18$) were housed for 22 weeks in small cages that restricted their mobility, while another group of animals ($n = 17$) received daily treadmill exercise to simulate moderate physical activity. After the experiment, histological assessments, biochemical assays, and mechanical testing were conducted to compare tibial articular cartilage structure, strength, and degree of OA degeneration between sedentary and physically active animals. Groups were also compared based on body weight and composition, as well as gut microbial community composition assessed using fecal 16S rRNA gene sequencing.
*Results:* Prevalence of knee OA was similar between sedentary and physically active animals, but severity of the disease (cartilage lesion depth) was substantially greater in the sedentary group ($P = 0.02$). In addition, during the experiment, sedentary animals developed cartilage with lower aggrecan quantity ($P = 0.03$) and accumulated more body weight ($P = 0.005$) and visceral adiposity ($P = 0.007$). Groups did not differ greatly, however, in terms of cartilage thickness, collagen quantity, or stiffness, nor in terms of muscle weight, subcutaneous adiposity, or gut microbial community composition.
*Conclusions:* Our findings indicate that a sedentary lifestyle promotes the development of knee OA, particularly by enhancing disease severity rather than risk of onset, and this potentially occurs through multiple pathways including by engendering growth of functionally deficient joint tissues and the accumulation of excess body weight and adiposity.


## Introduction

Knee osteoarthritis (OA) is a painful and disabling degenerative disease whose prevalence has increased in recent decades, especially in developed nations[1]. Although factors such as age, sex, and genes are known to influence knee OA susceptibility, evidence suggests that rising knee OA levels are in large part attributable to environmental exposures that were once rare but now increasingly common[2]. Among the environmental shifts hypothesized to be most detrimental are recent major declines in physical activity.[1,2]

The importance of physical activity for attaining and maintaining optimal knee tissue structure and strength is well documented, particularly by experiments with animal models[3]. For example, prolonged periods of limb immobilization have been shown to lead to thinner articular cartilage with lower aggrecan content, as well as diminished cartilage stiffness[4–6]. In contrast, daily exercise has been demonstrated to augment development of knee cartilage architecture and mechanical properties[7–10]. Moreover, transarticular

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muscles, which stabilize and protect joints, are generally strengthened by physical activity but reduced in size under conditions of disuse[11]. Thus, one way declining physical activity levels might be contributing to the rising prevalence of knee OA is by engendering weaker and more unstable joints that are less resistant to OA degeneration.

Another way that physical inactivity may be increasing knee OA prevalence is by promoting the accumulation of excess body weight and adipose tissue. Routine physical activity is critical for maintaining energy balance and normal metabolism, whereas a persistent surplus of energy from chronic physical inactivity can lead to obesity, a well-known risk factor for knee OA[12,13]. The exact mechanisms by which obesity affects knee OA degeneration are not fully understood but likely include interactions between adiposity-induced metaflammation and joint overloading[2,14]. The importance of avoiding obesity for inhibiting knee OA is highlighted by animal experiments showing that control of caloric intake can limit the deposition of adipose tissue and thereby attenuate knee OA degeneration[15,16]. Presumably, physical activity could have a similar effect as dietary regulation in curbing adiposity and hence knee OA.

A further intriguing but more conjectural possibility is that rising knee OA levels are partly due to the effects of physical inactivity on the gut microbiome. Evidence indicates that gut microbial composition plays a critical role in host innate immune responses and is sensitive to environmental exposures[17], including physical activity[18]. Moreover, animal experiments have shown that deviations from gut microbial homeostasis due to environmental stimuli can trigger host metaflammation and thereby increase risk of knee OA and other diseases whose pathogenesis involves an inflammatory component[19,20]. It is thus conceivable that gut dysbiosis could be an important mediator between physical inactivity and knee OA degeneration[21].

Notwithstanding multiple reasons to hypothesize that declining physical activity levels could play a part in the rising prevalence of knee OA, there have been few prospective investigations of whether and how habitually low levels of physical activity contribute to knee OA incidence or severity. We therefore conducted an experiment to test potential links between physical inactivity and knee OA using guinea pigs as a model system. Guinea pigs are a clinically relevant model because—like the majority of human knee OA patients—they develop knee OA idiopathically, which makes them suitable for prophylactic testing of potential inhibitors of knee OA degeneration such as physical activity[22]. Moreover, previous studies have established that knee OA in guinea pigs is histopathologically comparable to that of humans[23,24], and incidence and severity of the disease in the two species often depend on similar environmental factors such as obesity[15] and abnormal joint loading[25]. In our experiment, to simulate a physically inactive lifestyle (which has been referred to as "sedentary" to distinguish it from immobilization[26]), growing animals were housed for 22 weeks in small cages that restricted (but did not eliminate) their mobility, while another group of animals was housed in similar cages but received daily treadmill exercise to simulate moderate levels of physical activity. Our predictions were that compared to physically active animals, sedentary animals would develop inferior knee cartilage structure and strength, reduced limb muscles, greater body weight and adiposity, and distinct gut microbial communities, all which would ultimately be associated with a higher prevalence and severity of knee OA degeneration.

## Materials and methods

### *Experimental design*

Male, non-sibling Hartley guinea pigs were acquired from Charles River Laboratories (Wilmington, MA, USA) at 7 weeks of age ($n = 36$). Animals were housed individually in small cages (27 × 48 × 20 cm; width × length × height) and maintained on a 12:12-hr light/dark cycle with free access to water and food (LabDiet 5025, PMI Nutrition, St. Louis, MO, USA). At 8 weeks of age, animals were randomly divided into a sedentary group and a physically active group ($n = 18$/group). Physically active animals ran on a flatbed treadmill (Columbus Instruments, Columbus, OH, USA) at a rate of 20–25 m/min, 5 days/week for 22 weeks. Running duration was gradually increased during the first 4 weeks from 10 min/day to 30–45 min/day to acclimate the animals to the treadmill environment and activity. The peak dosage of running (30–45 min/day, 5 days/week) was selected based on the World Health Organization's recommendation for humans of ≥150 min/week of moderate-intensity aerobic exercise[27], as well as prior experiments showing that similar running regimens augment the rodent musculoskeletal system[28,29]. During the initial 4-week training period, one animal was unable to run without excessive motivation and thus removed from the study. At 28 weeks of age, fresh fecal pellets were harvested from the remaining 35 animals and immediately frozen at −80°C for analysis of gut microbial community composition. All animals were euthanized at 30 weeks of age by exsanguination under isoflurane anesthesia; limb muscles (quadriceps, triceps surae) and gonadal fat pads were extracted, weighed, and left and right weights were averaged; right articulated knees were extracted and placed in 10% NBF to be used for histological analyses; and left tibiae were dissected, wrapped in gauze soaked with 1 × PBS, and frozen at −20°C for later mechanical testing and biochemical analyses. Just prior to euthanasia, abdominal subcutaneous fat thickness was measured using ultrasound (L12-4 B-Mode Transducer, Philips, Bothell, WA, USA). Sample sizes were determined based on prior work showing that groups of $n \geq 15$ guinea pigs are sufficient for elucidating statistically significant differences in knee cartilage structure between sedentary and physically active animals.[30]

The use of Hartley guinea pigs between ages 8 and 30 weeks is appropriate for examining the potential detrimental effects of a sedentary lifestyle on knee OA incidence and severity, as it is during this ontogenetic period that idiopathic disease onset typically occurs, with the earliest histological signs present on the medial tibial plateau at approximately 12–16 weeks of age[24,31,32]. Previous work has also shown that when using guinea pigs to investigate potential inhibitors of knee OA, an experiment spanning this ontogenetic interval is adequate for achieving an incidence and severity of OA degeneration in the medial tibiae of untreated animals sufficient for evaluating treatment effects[22]. Importantly, however, OA lesions in the medial femur and lateral compartment of the knee appear later in ontogeny (and are less severe) than those in the medial tibia[23]. Moreover, OA-related changes in the subchondral bone, menisci, and synovium are not yet pronounced in 30-week-old animals[22]. Thus, our experiment was designed specifically to investigate the effects of sedentism on knee OA onset rather than more advanced stages of the disease, and our analyses focused on cartilage structure, strength, and degree of OA degeneration in just the medial tibia.

All procedures were approved by the IACUC of Harvard University.

### *Tibial histology*

Following 10 days of decalcification in 10% formic acid, right knee joints were coronally sectioned and embedded in paraffin in a slightly flexed position[24]. Two 8-μm sections of the medial compartment were prepared, one anterior and one posterior, and stained with toluidine blue (Supplementary Fig. 1). Tibial histological assessments were performed blinded on the sections at a

magnification of 25× using an ocular micrometer. Five histomorphometric parameters were measured: (1) medial tibial cartilage width, defined as the total mediolateral span of cartilage across the load-bearing surface of the plateau; (2) cartilage thickness, defined as the total depth to tidemark of uncalcified cartilage at the mediolateral midpoint of cartilage width; (3 and 4) cartilage degeneration width and depth, defined as the maximum mediolateral and proximodistal spans, respectively, of cartilage affected by any degeneration (matrix fibrillation/loss, aggrecan loss with or without chondrocyte death); and (5) osteophyte thickness, measured from the tidemark to the furthest point extending toward the synovium. In addition, cartilage degeneration was scored using an established semiquantitative method[33] that involves evaluation of chondrocyte death/loss, matrix fibrillation/loss, and aggrecan loss with chondrocyte loss being the main determinant of the score. Following this method, medial tibial cartilage width was divided into three equal-diameter zones (medial, central, and lateral) to assess regional variation in pathology. Cartilage degeneration in each zone was scored as either "none" (no degeneration), "minimal" (5–10% of the total projected cartilage area affected by degeneration), "mild" (11–25% affected), "moderate" (26–50% affected), or "marked" (51–75% affected). None of the animals exhibited cartilage degeneration classifiable by this scoring method as "severe" (>75% affected). Values from anterior and posterior sections were averaged. Cartilage degeneration width and depth are expressed as a percentage of cartilage width and thickness, respectively. Subchondral bone sclerosis, meniscal changes, and synovitis were absent or very minimal in all animals and thus not quantified.

*Cartilage mechanical testing and biochemistry*

Left tibiae were thawed at room temperature in 1 × PBS, then compression was applied to the cartilage of the central region of the medial plateau (with intact tibial bone) using a Dynastat mechanical spectrometer (IMASS, Hingham, MA, USA) fitted with a 1-mm-diameter cylindrical, plane-ended stainless-steel indenter[34]. An initial tare load of 0.02 N was applied to ensure full contact between the indenter and cartilage. Then, consecutive displacements of 12.5, 25, 37.5, and 50 μm were exerted and the resulting loads measured. After the 37.5-μm displacement, sinusoidal displacements with amplitudes of 5 μm and eleven different frequencies ranging from 1.0 to 0.005 Hz were applied and resulting loads measured. Stress–strain curves were constructed using cartilage thickness values derived from the needle-ramp method[34]. Cartilage static stiffness was calculated based on the linear portions of the stress–strain curves. Cartilage dynamic stiffness was calculated as dynamic stress divided by dynamic strain amplitude.

After mechanical testing, cartilage was detached from underlying bone, weighed wet, and digested in Proteinase K (Roche, Indianapolis, IN, USA). Cartilage composition was assessed by measuring aggrecan content using the dimethyl methylene blue dye-binding assay for glycosaminoglycan[35] and collagen content using the hydroxyproline assay[36]. Aggrecan and collagen content are expressed as μg per mg of tissue wet weight.

*Fecal 16S rRNA gene sequencing*

Microbial DNA was isolated from fecal samples using the DNeasy PowerSoil Kit (Qiagen, Venlo, Netherlands) and PCR-amplified using barcoded primers targeting the V4 region of the bacterial 16S rRNA gene (515F and 806R). The following thermocycler protocol was used: 94°C for 3 min, followed by 35 cycles of 94°C for 45 s, 50°C for 30 s, and 72°C for 90 s, with a final extension at 72°C for 10 min[37,38]. Triplicate reactions for each sample were pooled and amplification was confirmed by 1.5% gel electrophoresis. 16S amplicons were cleaned with AmpureXP beads (Beckman Coulter, Brea, CA, USA), quantified using the Quant-iT Picogreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA), and pooled evenly by DNA content (Supplementary Table 1). The pool was run on one lane of an Illumina HiSeq (San Diego, CA, USA) rapid flow cell (1 × 150 bp), generating an average of 26,881 (s.d. = 9,067) high-quality reads per sample. Sequences were analyzed using QIIME software[39]. Operational taxonomic units (OTUs) were picked at 97% similarity against the Greengenes database[40], which we trimmed to span only the 16S V4 region flanked by our sequencing primers (positions 521–773). Bacterial relative abundances at taxonomic levels spanning phylum through OTU were generated using summarize_taxa.py. To ensure unbiased generation of diversity metrics sensitive to sampling depth, sequences were subsampled to a common depth of 12,500 reads (single_rarefaction.py) prior to $\alpha$-diversity (alpha_diversity.py) and $\beta$-diversity (beta_diversity_through_plots.py) computations.

*Statistical analyses*

Microbial community distances were compared between groups using ANOSIM (compare_categories.py) with 999 permutations. Microbial biomarker discovery was performed with LEfSe[41], filtering out species-level OTUs with <100 sequences or those present in only one sample, and treating an LDA score of ≥2 as the threshold for significance. Cartilage degeneration score prevalence was compared using Fisher's exact tests. For all other data, Shapiro–Wilk tests were used to determine whether a normal distribution was followed, and Levene's tests were used to examine the equality of group variances. Depending on the results of these tests, group differences were analyzed with Student's *t*-tests, Mann–Whitney *U* tests, or Welch's *t*-tests (Supplementary Table 2). These tests were performed using JMP Pro software (SAS Institute, Cary, NC, USA) with significance set at $P < 0.05$ (all tests were two-tailed). No allowance was made for multiple comparisons, as the purpose of this study was exploratory rather than confirmatory.

## Results

At the start of the experiment, body weight was similar between animals assigned to the sedentary and physically active groups [$P = 0.35$; Fig. 1(A)]. In both groups, body weight increased throughout the experiment. By week 12 of the experiment, body weights of sedentary animals were, on average, 4% higher compared to physically active animals ($P = 0.041$), and from then on, body weight remained significantly higher among sedentary animals. At the end of the experiment, average body weight among sedentary animals was 7% higher than among physically active animals ($P = 0.005$). At that time, snout-to-rump length did not differ significantly between sedentary and physically active animals [$P = 0.99$; Fig. 1(B)], nor did limb muscle weight [quadriceps and triceps surae: $P = 0.65$ and $P = 0.25$, respectively; Fig. 1(C)] or subcutaneous fat thickness [$P = 0.85$; Fig. 1(D)]. However, gonadal fat pad weight was, on average, 21% greater in sedentary vs physically active animals [$P = 0.007$; Fig. 1(E)].

Gut microbial community composition did not differ significantly between sedentary and physically active animals regardless of the distance metric used [Bray–Curtis distances: $P = 0.49$; weighted Unifrac distances: $P = 0.61$; unweighted Unifrac distances: $P = 0.10$; Fig. 2(A) and (B)]. The number of unique microbial taxa observed per sample was significantly higher among physically active than sedentary animals [observed species: $P = 0.031$; Fig. 2(C)], but a more robust measure of $\alpha$-diversity that accounts for both abundance and evenness was comparable between experimental groups [Shannon Diversity Index: $P = 0.35$; Fig. 2(D)].

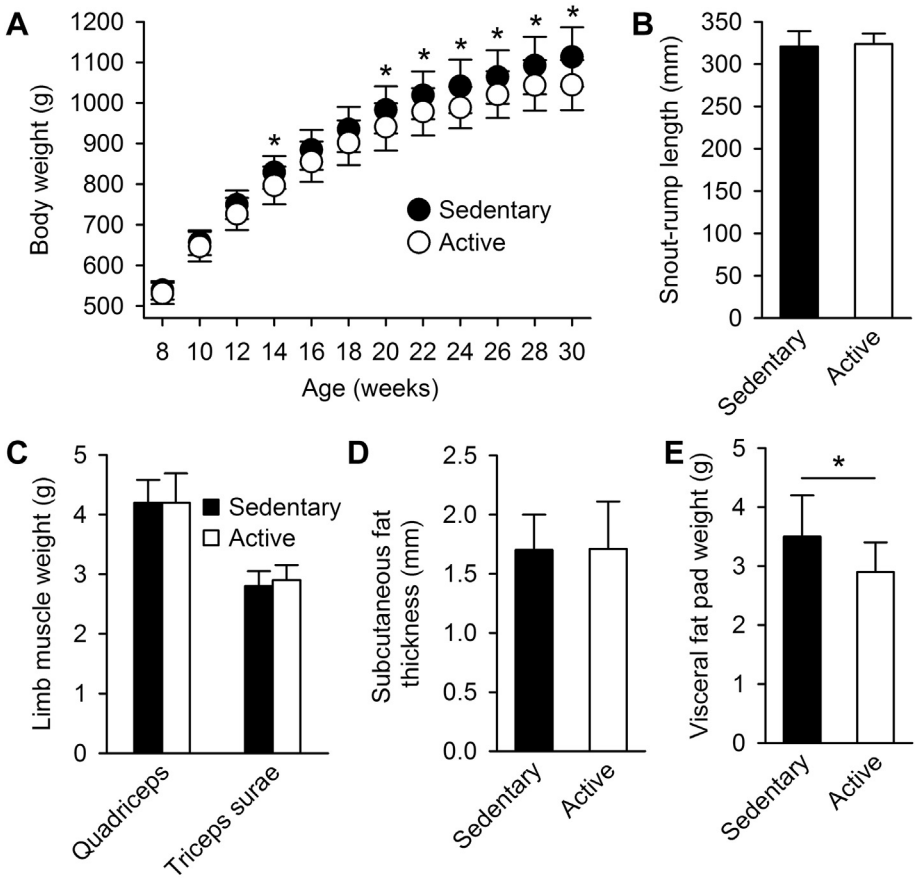


**Fig. 1.** Effects of chronic sedentism on body size and composition. (A) Changes in body weight (±s.d.) among sedentary and physically active animals during the experiment. Average (+s.d.) snout-rump length (B), limb muscle weight (C), abdominal subcutaneous fat thickness (D), and gonadal fat pad weight among sedentary and physically active animals at the end of the treatment period. Asterisks indicate significant ($P < 0.05$) differences between experimental groups.

Additionally, microbial biomarker discovery analyses did not detect any taxa that distinguished the microbial composition of samples collected from sedentary and physically active animals (LEfSe, all LDA <2).

In the medial tibia, sedentary and physically active animals developed cartilage of similar thickness [$P = 0.96$; Fig. 3(A)], but the composition of cartilage was significantly diminished by physical inactivity [Fig. 3(B)]. Specifically, at the end of the experiment, compared to physically active animals, sedentary animals had cartilage with, on average, 16% lower aggrecan content ($P = 0.032$). Cartilage collagen content also tended to be lower among sedentary than physically active animals [Fig. 3(C)], but not significantly so ($P = 0.062$). No significant group differences were detected in cartilage stiffness [static and dynamic: $P = 0.47$ and $P = 0.64$, respectively; Fig. 3(D)]

Prevalence of cartilage degeneration scores did not differ significantly between sedentary and physically active animals [medial, central, and lateral: $P = 0.58$, $P = 0.66$, and $P = 0.66$, respectively; Fig. 3(E)]. There was, however, evidence that cartilage degeneration was more severe in the sedentary group [Fig. 3(F)]. Specifically, while the width of cartilage affected by degeneration was comparable between groups [$P = 0.39$; Fig. 3(G)], the depth of degeneration was, on average, 41% greater among sedentary than physically active animals ($P = 0.024$). In all but three animals, maximum lesion depth occurred in the medial third of the plateau in the area not covered by the meniscus [Fig. 3(E)]. This pattern of localization in cartilage degeneration was evident in both the anterior and posterior histological sections from sedentary and physically active animals, indicating that lesion severity but not location differed between experimental groups (Supplementary Fig. 2). Osteophytes were present in 44% (8/18) of sedentary animals and 71% (12/17) of active animals. Sedentary animals tended to have larger osteophytes than physically active animals [Fig. 3(G)], but not significantly so ($P = 0.078$).

## Discussion

To gain insight into how declining engagement in physical activity might be contributing to the growing burden of knee OA in developed nations[1], this study experimentally investigated the effects of a simulated sedentary lifestyle on the incidence and severity of knee OA degeneration in guinea pigs, an established idiopathic knee OA model[22]. We found that compared to animals who exercised daily for 22 weeks beginning at a young age, habitually sedentary animals who did not exercise and whose movement was restricted by small housing cages developed a similar prevalence of knee OA but the severity of their cartilage degeneration was significantly greater. The more severe knee OA degeneration of sedentary animals was potentially attributable to two general factors. First, compared to physically active animals, sedentary animals developed significantly lower quantities of cartilage aggrecan, suggesting that physical inactivity may have contributed to knee OA development by engendering growth of functionally deficient joint tissues[42]. Second, throughout the experiment, physically active animals accumulated significantly less body weight and visceral adipose tissue, suggesting that

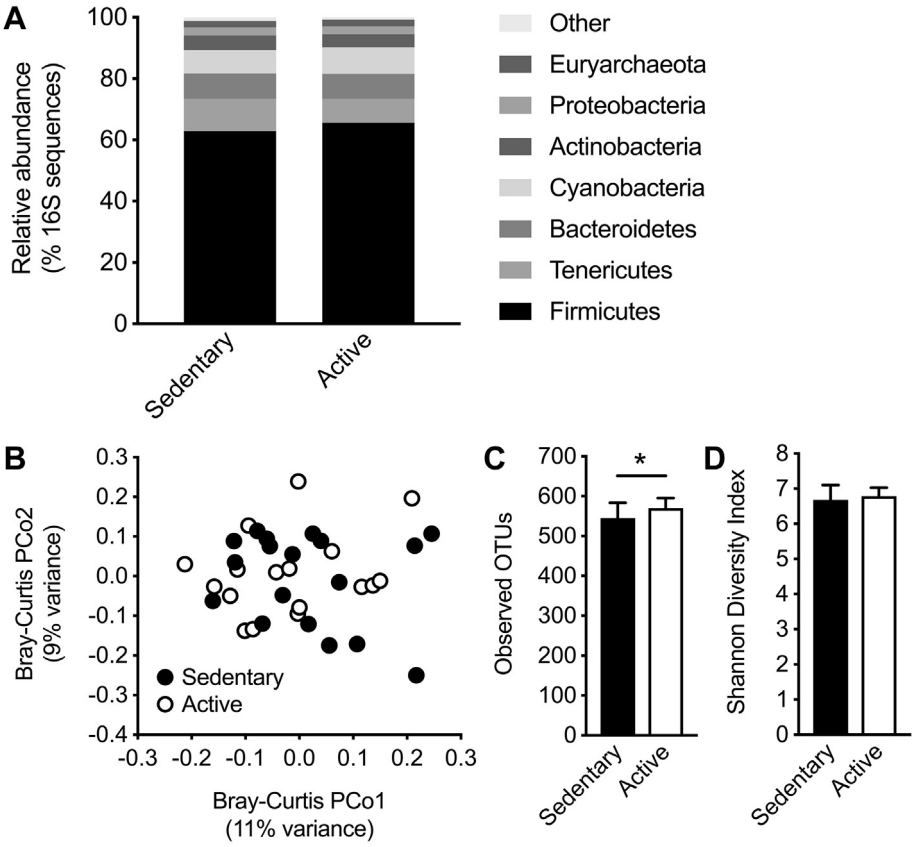


**Fig. 2.** Effects of chronic sedentism on the gut microbiota. (A) Relative abundance of bacterial phyla in the distal guts of sedentary and physically active animals at 28 weeks of age, as indexed by the percentage of 16S sequences. Phyla representing more than 1% of sequences are listed independently. (B) Bray–Curtis principal coordinate analysis (PCoA) showing that sedentary and physically active animals harbored similar gut microbial communities. This result was robust to the choice of distance metric, with ANOSIM tests based on the Bray–Curtis, unweighted Unifrac, and weighted Unifrac distance metrics all returning $P > 0.05$. (C and D) Average (+s.d.) gut microbial $\alpha$-diversity in the sedentary and physically active groups, as indexed by (C) the number of observed OTUs and (D) the Shannon Diversity Index, which considers both community abundance and evenness. The asterisk indicates a significant ($P < 0.05$) difference between experimental groups.

physical inactivity might have exacerbated knee OA degeneration by promoting obesity[15,16]. We also investigated the degree to which the gut microbiota may have mediated the effects of physical inactivity on knee OA degeneration, but gut microbial communities of sedentary and physically active animals were largely indistinguishable, at least in terms of composition. Nevertheless, overall, our results provide support for the hypothesis that a sedentary lifestyle can directly affect knee OA degeneration, particularly the severity of degeneration, and potentially through multiple pathways.[2].

It is interesting yet unclear why physical activity attenuated knee OA degeneration in our experiment but did not prevent the disease outright. One possibility, however, is that the dosage of exercise to which physically active animals were exposed was too modest, amounting to only $\leq$3% of total time per day. This explanation accords with the conclusion of a recent meta-analysis[3] of animal exercise experiments which showed that the potential of physical activity to augment knee cartilage growth generally follows a sigmoid dose–response relationship in which greater amounts of exercise are typically more beneficial than small amounts, but at some point additional exercise has marginal or even negative effects. That sedentary and physically active animals did not differ much in terms of cartilage thickness, muscle size, subcutaneous fat quantity, or gut microbial community composition may also relate to the moderate dose of exercise administered. Ultimately, future studies are needed to test the prediction that higher levels of physical activity would totally prevent knee OA degeneration in guinea pigs. It is also possible that susceptibility to knee OA is so great among Hartley guinea pigs that no amount of physical activity (or other potentially prophylactic measure) could completely inhibit the disease from occurring, as some prior experiments have seemed to suggest[15,16,30]. Indeed, compared to other laboratory guinea pig stocks, Hartley guinea pigs are known to be considerably more vulnerable to knee OA even when maintained under identical environmental conditions[43]. If it is actually the case that knee OA degeneration is essentially inevitable in Hartley guinea pigs, then a different model would be more appropriate for future investigations of the prophylactic potential of physical activity. Nevertheless, regardless of why physical activity failed to completely prevent knee OA in our experiment, the results provide good prospective evidence that habitual engagement in exercise can at least attenuate knee OA degeneration, including when doses of exercise are relatively modest.

It is important to emphasize that at the beginning of the experiment, animals were young and thus undergoing rapid growth. Because musculoskeletal tissues mostly develop prior to maturity, it is possible that physical activity while young provides the greatest benefits for attaining optimal knee tissue structure and strength to resist OA degeneration. It is well established, for example, that the potential for physical activity to augment bone formation is greatest when young and declines with age[44]. Whether cartilage exhibits a similar age-dependency in responding to physical activity has yet to be determined, but it is noteworthy that in the aforementioned meta-analysis of animal exercise

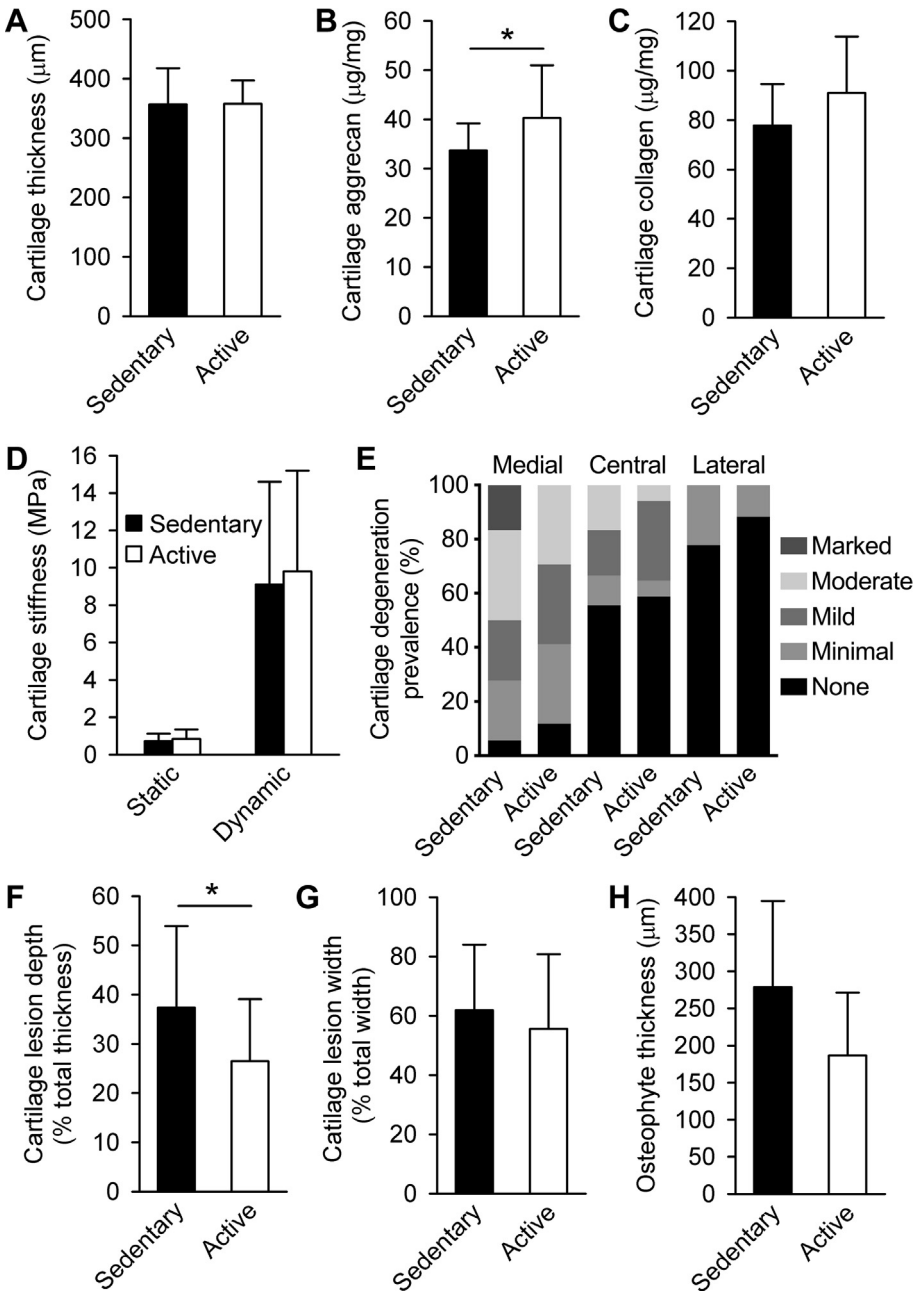


**Fig. 3.** Effects of chronic sedentism on medial tibial articular cartilage structure, composition, and strength and OA severity. Average (+s.d.) articular cartilage thickness (A), cartilage aggrecan content (B), cartilage collagen content (C), and cartilage static and dynamic stiffness (D) among sedentary and physically active animals at the end of the treatment period. (E) Prevalence and histological grading of cartilage degeneration in the medial, central, and lateral thirds of the medial tibial plateaus of sedentary and physically active animals at the end of the treatment period. Average (+s.d.) articular cartilage degeneration width (F), cartilage degeneration depth (G), and osteophyte thickness (H) among sedentary and physically active animals at the end of the treatment period. Asterisks indicate significant ($P < 0.05$) differences between experimental groups.

experiments[3], all studies that found, as we did, that cartilage aggrecan quantity was augmented by physical activity were conducted using young animals, whereas all studies that employed older animals reported nil or negative effects of exercise. In terms of cartilage collagen, consistent with our results, all experiments in the meta-analysis found collagen quantity was not significantly increased by physical activity regardless of the animals' age[3], though there is some evidence that collagen network structure can be improved by exercise during growth but not in older age[30]. Therefore, in considering the results of this study, it should be kept in mind that similar findings would not necessarily be expected if the animals employed were older. Indeed, even at the end of our experiment, animals were still fairly young, which was reflected by the fact that none of them exhibited cartilage degeneration classifiable as severe, or pronounced changes in the subchondral bone, menisci, or synovium. Future studies are thus required to determine how long the benefits of exercise during growth for knee tissues persist with aging.

The precise mechanisms linking the greater body weight and adiposity of sedentary vs physically active animals to knee OA degeneration are uncertain but probably included some combination of metaflammation and joint overloading[2,14]. Although a limitation of this study is that inflammation was not measured, prior research has shown that body weight in Hartley guinea pigs is a

strong predictor of serum concentrations of multiple pro-inflammatory adipokines including IL-6 and TNF-$\alpha$[45], implying that body weight differences between experimental groups likely reflect some degree of variation in metaflammation levels. Moreover, it is notable that differences in body weight between groups were associated with greater visceral adiposity but not greater subcutaneous adiposity among the sedentary animals. Visceral fat is a more potent source of adipokine-induced metaflammation[46] and hence likely to have a greater impact on knee OA risk than subcutaneous fat[47]. Joint overloading is perhaps a less obvious explanation for the more severe knee OA degeneration of sedentary animals given that they loaded their knees much less than did physically active animals. However, it is plausible that the knees of sedentary animals were compromised enough by the development of structurally inferior cartilage, coupled with the tissue weakening effects of metaflammation, that even relatively small and infrequent loads produced by cage activity could have exceeded the cartilage's mechanical capacity. Ultimately, additional research is required to better understand the influence and interaction of the inflammatory and mechanical factors that drive the negative consequences of chronic positive energy balance for joint health.

Although the growing burden of knee OA in developed nations is undoubtedly due to multiple factors[2], the most important conclusion of this study is that declining levels of physical activity are likely making the situation worse and deserve further study. Contrary to the traditional perception of physical activity as primarily a cause of knee tissue wear and tear[48], our findings suggest that regular engagement in physical activity may represent a powerful strategy for attenuating knee OA degeneration[1,42], and that sedentism, by contrast, may be a greater threat to knee health than sometimes assumed[2,26]. This does not mean, however, that all forms of physical activity are likely to benefit knee health. Indeed, evidence indicates that joint overloading produced by specific types of very physically active lifestyles through occupation[49] or athletics[50] can precipitate and exacerbate knee OA degeneration. Nevertheless, that knee OA prevalence has risen in developed countries at the same time that average occupational physical demands and routine exercise have declined implies that too much extreme physical activity is currently not the greatest threat to knee health[1]. Instead, we suggest that the increasing normality of extreme levels of physical inactivity is a more urgent concern for countering the expanding knee OA epidemic and therefore warrants increased research and clinical attention.

## Contributions

IJW, AMB, RNC, AJG, and DEL conceived and designed the study. IJW, GR, NBH, and HLD performed the experiment. IJW, GR, EHF, H-HH, NBH, ASB, EMV, AKY, and HLD collected and analyzed the data. IJW and RNC performed the statistical tests. IJW, AMB, RNC, AJG, and DEL interpreted the results. IJW, RNC, and DEL drafted the article. All authors gave final approval of the article.

## Competing interests

None declared.

## Role of funding sources

The funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the manuscript.

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## Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.joca.2019.07.005.

## References

1. Wallace IJ, Worthington S, Felson DT, Jurmain RD, Wren KT, Maijanen H, *et al*. Knee osteoarthritis has doubled in prevalence since the mid-20th century. Proc Natl Acad Sci USA 2017;114:9332–6.
2. Berenbaum F, Wallace IJ, Lieberman DE, Felson DT. Modern-day environmental factors in the pathogenesis of osteoarthritis. Nat Rev Rheumatol 2018;14:674–81.
3. Bricca A, Juhl CB, Grodzinsky AJ, Roos EM. Impact of a daily exercise dose on knee joint cartilage—a systematic review and meta-analysis of randomized controlled trials in healthy animals. Osteoarthr Cartil 2017;25:1223–37.
4. Jurvelin J, Kiviranta I, Tammi M, Helminen HJ. Softening of canine articular cartilage after immobilization of the knee joint. Clin Orthop 1986;201:246–52.
5. Kiviranta I, Jurvelin J, Tammi M, Säämänen A-M, Helminen HJ. Weight bearing controls glycosaminoglycan concentration and articular cartilage thickness in the knee joint of young beagle dogs. Arthritis Rheum 1987;30:801–9.
6. Nomura M, Sakitani N, Iwasawa H, Kohara Y, Takano S, Wakimoto Y, *et al*. Thinning of articular cartilage after joint unloading or immobilization. An experimental investigation of the pathogenesis in mice. Osteoarthr Cartil 2017;25:727–36.
7. Jurvelin J, Kiviranta I, Tammi M, Helminen HJ. Effect of physical exercise on indentation stiffness of articular cartilage in the canine knee. Int J Sports Med 1986;7:106–10.
8. Kiviranta I, Tammi M, Jurvelin J, Säämänen A-M, Helminen HJ. Moderate running exercise augments glycosaminoglycans and thickness of articular cartilage in the knee joint of young beagle dogs. J Orthop Res 1988;6:188–95.
9. Säämänen A-M, Tammi M, Kiviranta I, Helminen HJ. Running exercise as a modulatory of proteoglycan matrix in the articular cartilage of young rabbits. Int J Sports Med 1988;9:127–33.
10. Säämänen A-M, Tammi M, Kiviranta I, Jurvelin J, Helminen HJ. Levels of chondroitin-6-sulfate and nonaggregating proteoglycans at articular cartilage contact sites in the knees of young dogs subjected to moderate running exercise. Arthritis Rheum 1989;32:1282–92.
11. Gao Y, Arfat Y, Wang H, Goswami N. Muscle atrophy induced by mechanical unloading: mechanisms and potential countermeasures. Front Physiol 2018;9:235.
12. Felson DT, Anderson JJ, Naimark A, Walker AM, Meenan RF. Obesity and knee osteoarthritis. The Framingham Study. Ann Intern Med 1988;109:18–24.
13. Wluka AE, Lombard CB, Cicuttini FM. Tackling obesity in knee osteoarthritis. Nat Rev Rheumatol 2013;9:225–35.
14. Issa RI, Griffin TM. Pathobiology of obesity and osteoarthritis: integrating biomechanics and inflammation. Pathobiol Aging & Age-related Dis 2012;2:17470.

15. Bendele AM, Hulman JF. Effects of body weight restriction on the development and progression of spontaneous osteoarthritis in guinea pigs. Arthritis Rheum 1991;34:1180–4.
16. Brismar BH, Lei W, Hjerpe A, Svensson O. The effect of body mass and physical activity on the development of guinea pig osteoarthritis. Acta Orthop Scand 2003;74:442–8.
17. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. Cell 2014;157:121–41.
18. Allen JM, Mailing LJ, Niemiro GM, Moore R, Cook MD, White BA, *et al*. Exercise alters gut microbiota composition and function in lean and obese humans. Med Sci Sport Exerc 2018;50:747–57.
19. Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, *et al*. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 2009;58:1091–103.
20. Schott EM, Farnsworth CW, Grier A, Lillis JA, Soniwala S, Dadourian GH, *et al*. Targeting the gut microbiome to treat the osteoarthritis of obesity. JCI Insights 2018;3:95997.
21. Huang Z, Kraus VB. Does lipopolysaccharide-mediated inflammation have a role in OA? Nat Rev Rheumatol 2016;12:123–9.
22. Bendele AM. Animal models of osteoarthritis. J Musculoskelet Neuronal Interact 2001;1:363–76.
23. Bendele AM, Hulman JF. Spontaneous cartilage degeneration in guinea pigs. Arthritis Rheum 1988;31:561–5.
24. Kraus VB, Huebner JL, DeGroot J, Bendele A. The OARSI histopathology initiative—recommendations for histological assessments of osteoarthritis in the guinea pig. Osteoarthr Cartil 2010;18:S35–52.
25. Wei L, de Bri E, Lundberg A, Svensson O. Mechanical load and primary guinea pig osteoarthritis. Acta Orthop Scand 1998;69:351–7.
26. Otterness IG, Eskra JD, Bliven ML, Shay AK, Pelletier JP, Milici AJ. Exercise protects against articular cartilage degeneration in the hamster. Arthritis Rheum 1998;41:2068–76.
27. World Health Organization. Global Recommendations on Physical Activity for Health. Geneva: WHO Press; 2010.
28. Hamrick MW, Skedros JG, Pennington C, McNeil PL. Increased osteogenic response to exercise in metaphyseal versus diaphyseal cortical bone. J Musculoskelet Neuronal Interact 2006;6:258–63.
29. Wallace IJ, Pagnotti GM, Rubin-Sigler J, Naeher M, Copes LE, Judex S, *et al*. Focal enhancement of the skeleton to exercise correlates with responsivity of bone marrow mesenchymal stem cells rather than peak external forces. J Exp Biol 2015;218:3002–9.
30. Hyttinen MM, Arokoski JP, Parkkinen JJ, Lammi MJ, Lapveteläinen T, Mauranen K, *et al*. Age matters: collagen birefringence of superficial articular cartilage is increased in young guinea-pigs but decreased in older animals after identical physiological type of joint loading. Osteoarthr Cartil 2001;9:694–701.
31. Bendele AM, White SL, Hulman JF. Osteoarthritis in guinea pigs: histopathologic and scanning electron microscopic features. Lab Anim Sci 1989;39:115–21.
32. Bendele AM, White SL, Hulman JF, Bean JS. Osteoarthritis, model no. 379. Registry of Comparative Pathology. In: Capen CC, Jones TC, Migaki G, Eds. Handbook: Animal Models of Human Disease. Washington, DC: Armed Forces Institute of Pathology; 1991. facsimile 18.
33. Gerwin N, Bendele AM, Glasson S, Carlson CS. The OARSI histopathology initiative—recommendations for histological assessments of osteoarthritis in the rat. Osteoarthr Cartil 2010;18:S24–34.
34. Hoch DH, Grodzinsky AJ, Koob TJ, Albert ML, Eyre DR. Early changes in material properties of rabbit articular cartilage after meniscectomy. J Orthop Res 1983;1:4–12.
35. Farndale RW, Buttle DJ, Barrett AJ. Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue. Biochim Biophys Acta 1986;883:173–7.
36. Woessner JF. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. Arch Biochem Biophys 1961;93:440–7.
37. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, *et al*. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci USA 2011;108:4516–22.
38. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, *et al*. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 2012;6:1621–4.
39. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, *et al*. QIIME allows analysis of high-throughput community sequencing data. Nat Methods 2010;7:335–6.
40. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, *et al*. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 2006;72:5069–72.
41. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, *et al*. Metagenomic biomarker discovery and explanation. Genome Biol 2011;12:1–18.
42. Helminen HJ, Hyttinen MM, Lammi MJ, Arokoski JP, Lapveteläinen T, Jurvelin J, *et al*. Regular joint loading in youth assists in the establishment and strengthening of the collagen network of articular cartilage and contributes to the prevention of osteoarthrosis later in life: a hypothesis. J Bone Miner Metab 2000;18:245–57.
43. Huebner JL, Hanes MA, Beekman B, TeKoppele JM, Kraus VB. A comparative analysis of bone and cartilage metabolism in two strains of guinea-pig with varying degrees of naturally occurring osteoarthritis. Osteoarthr Cartil 2002;10:758–67.
44. Wallace IJ, Demes B, Judex S. Ontogenetic and genetic influences on bone's responsiveness to mechanical signals. In: Percival CJ, Richtsmeier RT, Eds. Building Bones: Bone Formation and Development in Anthropology. Cambridge: Cambridge University Press; 2017:233–53.
45. Huebner JL, Kraus VB. Assessment of the utility of biomarkers of osteoarthritis in the guinea pig. Osteoarthr Cartil 2006;14:923–30.
46. Despres JP, Lemieux I. Abdominal obesity and metabolic syndrome. Nature 2006;444:881–7.
47. Duclos M. Osteoarthritis, obesity and type 2 diabetes: the weight of waist circumference. Ann Phys Rehabil Med 2016;59:157–60.
48. Radin EL, Paul IL, Rose RM. Role of mechanical factors in pathogenesis of primary osteoarthritis. Lancet 1972;299:519–22.
49. Felson DT, Hannan MT, Naimark A, Berkeley J, Gordon G, Wilson PWF, *et al*. Occupational physical demands, knee bending, and knee osteoarthritis: results from The Framingham Study. J Rheumatol 1991;18:1587–92.
50. Tran G, Smith TO, Grice A, Kingsbury SR, McCrory P, Conaghan PG. Does sports participation (including level of performance and previous injury) increase risk of osteoarthritis? A systematic review and meta-analysis. Br J Sports Med 2016;50:1459–66.