Moderate Exercise Protects Against Joint Disease in a Murine Model of Osteoarthritis

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Abstract

Exercise is recommended as a non-pharmacological therapy for osteoarthritis (OA). Clinical studies investigating the impact of exercise on OA have primarily focussed on the assessment of joint pain and mobilisation, where positive outcomes have been demonstrated. Clinical imaging studies provide limited information on the impact of exercise (positive or negative) on the actual bone and soft tissue pathology. Various exercise regimes, with differing intensities and duration, have been used in a range of OA models, with disparate results. The present study provides definitive insight into the effect of moderate exercise on early joint pathology in the destabilisation of the medial meniscus (DMM) mouse model of OA. Exercise was induced by forced treadmill walking for 3 or 7 weeks. Joints were analysed by microcomputed tomography and histology. Exercise offered protection against cartilage damage and joint inflammation, and a temporary protection against osteosclerosis. Furthermore, exercise modified the metaphyseal trabecular microarchitecture of the osteoarthritic leg. Collectively, our findings provide scientific support for the clinical recommendation of moderate exercise as a physical therapy in OA. In addition to indirect benefit via positive physiological effects of weight loss, our data suggest direct short-term benefits in ameliorating pathology of cartilage, synovitis and bone.

Introduction

Osteoarthritis (OA) affects ~80% of people aged over 50. It is characterised by structural and functional changes in articular joints, with concomitant pain and loss of joint mobility that significantly impairs quality-of-life. There are no disease-modifying treatments available for OA, merely symptomatic pain relief and ultimately joint replacement^{1,2}. To delay rapid progression of OA, international guidelines recommend therapeutic exercise^{1,2}. Numerous studies have shown that exercise regimes, especially land-based, when monitored closely and performed regularly, leads to an improvement in joint movement and pain³. The benefits of exercise are not immediate and many patients do not sustain their exercise programmes, as use of a painful joint seems counterintuitive⁴. Clinical studies employ techniques such as magnetic resonance imaging and radiography as diagnostic tools to assess specific joint lesions⁵, but not to monitor disease progression. Moreover, invasive tissue sampling for mechanistic studies is inappropriate. There exists, therefore, a paucity of data demonstrating the direct effects of exercise (beneficial or detrimental) on joints.

In vivo models of OA mimic many of the features of human OA. Destabilisation of the medial meniscus (DMM)⁶ in rodents results in progressive development of OA with cartilage damage, osteosclerosis, variable synovitis, ligament damage/calcification and osteophyte formation⁶⁻⁹. However, the limited published data regarding the effects of exercise on joint tissues is ambiguous. Forced mobilisation on a rotating cylinder in a rat anterior cruciate ligament model induced increased cartilage degradation, subchondral plate failure and earlier subchondral bone sclerosis, suggesting that repetitive load-bearing exercise is detrimental¹⁰. Restriction of movement in the mouse DMM model revealed that animals housed in restricted cages (15% of normal daily movement) exhibited reduced cartilage damage 8-weeks post-surgery compared to those in standard accommodation¹¹. Moreover, when animals in restricted cages were subjected to gentle treadmill walking, cartilage damage increased¹². In contrast, gentle treadmill walking in the rat DMM model prevented chondrocyte and subchondral osteocyte death, as well as subchondral bone deterioration¹², indicating a reduction in OA pathology. Meanwhile, 4-weeks of voluntary wheel exercise resulted in lower subchondral bone plate thickness but an increased Mankin OA score¹³.

Given these disparate findings, we investigated whether the immediate benefits of exercise were associated with detrimental joint changes. Accordingly, the present study sought to characterise body weight, cartilage damage, inflammation and bone micro-structural changes in response to moderate forced exercise in the DMM murine model of OA.

Results

Moderate exercise decreases weight gain but does not alter overall activity.

Exercise induced a slight ($P=0.006$) reduction in weight gain regardless of the type of surgery (DMM/Sham), which was evident 5 and 6 weeks after initiation of exercise (Figure 1A&B). This was reflected in the loss of white adipose tissue (WAT), measured as percentage tissue weight to total body weight (Figure 1C&D). Subcutaneous and gonadal WAT were significantly lower in the exercise group 8-weeks post-DMM surgery, whilst inter-scapular brown adipose tissue (iBAT)(Figure 1E) was significantly higher in the exercise group, regardless of surgical intervention. No changes in muscle mass were noted (Figure 1F&G). Recognising that forced exercise might induce changes in the overall activity, we measured overnight activity in the DMM group comparing exercised to non-exercised ($n=6$ per

group). There was no significant difference in nocturnal distance travelled (Non-Exercise (NE)=489±34m, Exercise (E)=406±63m; P=0.27) between the groups, and therefore forced exercise did not have a meaningful impact on the total amount of voluntary exercise/activity undertaken (Figure 1H).

Moderate exercise protects against osteoarthritis-related pathology.

While no difference was detected in cartilage damage between exercised and non-exercised groups at 4-weeks (NE=0.6 ± 0.2, E=1.0 ± 0.35; P=0.48) post-surgery, there was lower cartilage damage at 8-weeks in the exercised group (NE=2.3 ± 0.28, E=1.4 ± 0.22; P=0.01) (Figure 2A and B). Low levels of synovitis were noted in DMM, perhaps in part due to surgical intervention, although these were significantly different when compared to the sham (data not shown). As with cartilage damage, synovitis scores were higher in the non-exercised group at 8-weeks (NE=2.5±0.26, E=1.1±0.27; P=0.030) but not 4-weeks (NE=0.5±0.23, E=0.5±0.24; P=0.99) post-surgical intervention when comparing to exercised group (Figure 2C).

DMM led to substantial subchondral osteosclerosis 28 days post induction, measured as the ratio-metric comparison of subchondral bone % BV/TV in the medial tibial compartment of the knee and the contralateral leg (SC % BV/TV, figure 2E and Table 1). This was evident in both DMM groups at 4-weeks post-surgery, but significantly reduced in exercised mice (Table 1, figure 2E). This protection, however, was lost by week 8 (Table 1, figure 2E). The number of osteophytes, measured as protruding bone formation on the medial side of the subchondral bone was not statistically significant at either 4-weeks (figure 2D, NE=1.0±0.38, E=1.7±0.26; P=0.14) or 8-weeks (NE=0.43±0.2, E=0.71±0.29; P=0.43). Despite this, 4 weeks after DMM 70% of exercise samples had 2 or more osteophytes whilst only one sample out of 7 (14%) in the non-exercise group had 2 or more osteophytes. This indicates increased osteophyte formation during the initial phase of the model, when the subchondral bone is adapting to the new loading resulting from the destabilisation of the joint. Unexpectedly, tibial trabecular bone of the operated leg was only significantly different in the exercised DMM group when compared to the contralateral leg, 4 and 8-weeks after surgical intervention. Trabecular bone was more connected (Trabecular pattern factor, Tb.Pf.) and more plate-like (structural model index, SMI), yet less organized (degree of anisotropy, DA). There was also significantly less trabecular bone (%BV/TV) in the operated leg at 4-weeks, which by 8-weeks had normalised (Table 1). Cortical thickness increased significantly at 8-weeks in comparison to 4 weeks in both groups, yet there were no changes induced by exercise (Figure 2F).

Table 1

MicroCT analysis of trabecular and subchondral bone changes in the DMM groups. BV/TV=Bone volume/ Tissue volume. Tb.Th= trabecular thickness. Tb.No= Trabecular number. Tb.Sp.= Trabecular space. Tb.Pf= Trabecular pattern factor (connectivity). SMI = Structural model index (shape). DA= Degree of anisotropy (organisation). Each time point was analysed with a Two-Way ANOVA, comparing relative changes to the contralateral leg and also interrogating the effect of exercise. (*) indicate significant difference between exercise and non-exercise groups. * P < 0.05, ** P < 0.01, *** P < 0.001. (§) indicates significant difference between exercise and non-exercise within the DMM joint normalised against the contralateral leg. § P < 0.05, §§P < 0.01.

4 weeks	Non exercise						Exercise					
	Contralateral			Ipsilateral			Contralateral			Ipsilateral		
BV/TV (%)	12.3	±	0.84	13.2	±	0.71	13.2	±	0.71	12.9	±	0.77*
Tb.Th (µm)	12.0	±	0.38	12.1	±	0.3	12.1	±	0.3	11.9	±	0.35
Tb.Sp (µm)	55.4	±	3.39	52.9	±	2.47	52.9	±	2.47	53.9	±	1.93
Tb.N (µm ⁻¹)	0.010	±	0.0005	0.011	±	0.000542	0.011	±	0.0005	0.011	±	0.000449*
Tb.Pf (µm ⁻¹)	0.097	±	0.0074	0.093	±	0.004942	0.093	±	0.0049	0.093	±	0.007057
SMI	1.97	±	0.0663	1.94	±	0.0548	1.94	±	0.0548	1.88	±	0.0584**
DA	2.35	±	0.141	2.53	±	0.121	2.65	±	0.0243	2.49	±	0.0729
SC bone BV/TV (%)	74.4	±	1.71	83.5	±	1.74***	77.3	±	1.58	81.6	±	1.70**§§
SC Tb.Th (µm)	28.0	±	1.23	33.1	±	1.14	28.7	±	1.24	34.5	±	1.58
8 weeks	Non exercise						Exercise					
	Contralateral			Ipsilateral			Contralateral			Ipsilateral		
BV/TV (%)	17.965	±	0.838	18.14	±	0.724	15.29	±	1.3627	15.69	±	1.450
Tb.Th (µm)	56.67	±	1.434	56.51	±	1.587	54.71	±	1.7477	55.21	±	2.164
Tb.Sp (µm)	199.25	±	7.969	195.97	±	4.177	209.1	±	7.8552	214.7	±	7.54
Tb.N (µm ⁻¹)	0.0032	±	0.00016	0.0032	±	0.00012	0.0028	±	0.0002	0.0028	±	0.00021
Tb.Pf (µm ⁻¹)	0.0164	±	0.0007	0.0158	±	0.00067	0.02	±	0.0021	0.019	±	0.0020*
SMI	1.70	±	0.0639	1.64	±	0.0472	1.89	±	0.1108	1.79	±	0.117*
DA	2.22	±	0.0557	2.30	±	0.0393	2.169	±	0.0939	2.30	±	0.0992*
SC bone BV/TV (%)	84.3	±	1.16	91.7	±	1.04***	78.62	±	2.9674	85.71	±	2.841***
SC Tb.Th (µm)	30.2	±	1.04	38.4	±	1.24	28.6	±	1.65	33.8	±	1.80*** §

Discussion

Recent human studies support a positive benefit of exercise in improving subjective clinical outcomes in OA. These studies however have mainly been focussed on improving joint mobility or reducing pain scores¹⁴, and have not comprehensively interrogated changes in joint tissues and bone microarchitecture to evaluate the consequences of exercise regimes on joint physiology. Animal studies have yielded contradictory results, with some indicating that repetitive load-bearing exercise is detrimental and rest improves cartilage damage scores, whilst others report findings that contradict this view^{12,13,15}. This divergence can be explained partly by differences in the OA models used, but importantly also by the differences in the type and nature of exercise regimes employed in these studies; i.e., high-load bearing^{10,13} to mild voluntary exercise¹². The consensus from these previous reports and the present study is that low load bearing exercise exerts a positive effect on the OA joint. In the present study, we used a moderate form of exercise requiring mice to walk 850m a day, 5 days/week, which, when extrapolating the distance walked by a mouse in a week, the exercise protocol increased said distance by 1.5 fold. It is also important to note that this protocol allowed for recovery from surgical intervention before the start of forced exercise unlike other protocols which start shortly after intervention or later when disease is established. Despite the moderate protocol, exercise resulted in a decrease in weight gain reflected in the loss of WAT mass, indicating that this form of exercise, although moderate, is having a beneficial physiological effect. This is a notable observation, as it is well established that weight loss reduces risk of OA, as well as improving outcomes in established OA^{16,17}. Importantly, our induced moderate form of exercise resulted in protection against cartilage damage and synovitis after 7-weeks of exercise.

This supports the concept that exercise can have indirect benefit on overall physiology that could in the long-term lead to decreased progression of OA pathology. Further studies are needed to fully interrogate the long-term benefits of this form of moderate exercise, and to elucidate molecular and/or biomechanical mechanisms underlying the observed protection.

In addition to the significant changes in cartilage or synovial tissue pathology, evaluation of bone in the exercise DMM group revealed a more plate-like micro-structure with increased connectivity. This type of microarchitecture offers higher bone strength¹⁸. Moreover, there was an early, albeit temporary, improvement in subchondral bone osteosclerosis in the exercise group, expressed by the significantly smaller increase in bone density of the subchondral bone. There was also an initial increase in osteophyte formation, which may indicate an acceleration of the subchondral bone expansion¹⁹ that ensues in the bone adaptation phase of the DMM model to dissipate the increased load. These bone features may indicate an improvement in the way the damaged joint is loaded in the exercised group. Bone adapts to changes in mechanical loading and the DMM model substantially changes the way the joint is loaded. In essence, instead of the meniscus dissipating the load in the joint, this is transmitted primarily through cartilage and subchondral bone²⁰. Notably, the delay in subchondral osteosclerosis we report in the exercise group, together with the positive change in the microarchitecture of the metaphyseal trabecular bone, suggest that exercise changes the way in which the load is dissipated throughout the joint. A possible explanation is that in this scenario, load is shifted to the metaphysis rather than subchondral bone. This delay in osteosclerosis might underpin the 8-week cartilage protection we observed. Indeed, we have observed previously that subchondral bone changes occur rapidly, preceding significant cartilage damage in this OA model⁹, and others (reviewed²¹). Prior studies demonstrated that DMM reduces muscle function at 4 and 8-weeks post-surgery²². It is therefore feasible that exercise-induced improvement in muscle strength results in joint stabilisation and altered load^{23,24}. However, we did not observe any macroscopic changes in muscle mass (data not shown), thus further studies are required to definitively address this question.

It is important to note that the murine exercise protocol used simulates the situation of a human exercising shortly after sustaining a joint injury of a type likely to induce OA onset. This study does not, however, address how this type of exercise regime would affect established OA; this is a key question that future studies should address. Furthermore, longer-term studies would indicate if this form of moderate exercise affords long-term or merely transient benefit to the joint tissues.

In summary, this study demonstrates that early moderate exercise reduced body weight, cartilage degradation, synovitis and temporarily osteosclerosis, with no indication of any detrimental effects to the joint. In addition to inducing weight loss, exercise reduces inflammation¹⁷ and has an important positive psychological impact in humans²⁵. This, taken together with the data presented here, supports the use of moderate low load-bearing exercise as a therapeutic regime following joint injury and/or diagnosis of early OA, based on its collective and overriding physiological benefits.

Methods

Animals and induction of OA and exercise

DMM⁶ was performed on 10-week-old male C57BL/6 mice weighing on average 26.0 ± 1.4 g. A total of 42 mice were purchased (Envigo, UK) and placed in plastic cages with sawdust bedding (4 to 5 animals per cage) in a 12-h light/dark cycle at constant temperature. Animals were monitored daily, allowed to move freely in cages and provided free access to food, water and environmental enrichment. A week before surgery, all mice were tested for a few minutes on regulated rotating wheels (Campden Instruments Ltd., Loughborough) and those capable of using the wheels were selected for the exercise group. Exercise was set for 850m/day at a speed of 3.8m/min, with 18s break every 4min. At surgery animals were given analgesics (Buprenorphine). Exercise commenced 1-week post-surgery and continued 5 days/week for 3 or 7 weeks. Experimental groups are indicated in Table 2. At end point blood and tissues were collected. Legs were harvested for assessment via microcomputed tomography (μ CT) and histology. Subcutaneous, gonadal and brown fat pads, together with quadriceps and soleus muscles, were dissected and weighed (wet weight). The analysis was conducted blind where groups were only revealed at the end of all analysis. All procedures were in accordance with Home Office regulations and experimental design was pre-approved by the Ethical Review Committee at the University of Glasgow. The study is reported in accordance with ARRIVE guidelines (<https://arriveguidelines.org>).

Microcomputed tomography

Knees were fixed (4% paraformaldehyde) for 24h and stored (70% ethanol). Joints were analysed by μ CT using the Skyscan 1272 (Bruker, Belgium; 0.5 aluminium filter, 50kV, 200 μ A, voxel size 4.57 μ m, 0.5° rotation angle). Scans were reconstructed in NRecon software (Bruker, Belgium), with stacks analysed: (1) osteophytes identified in three-dimensional reconstructions as previously described⁹ and (2) subchondral bone within the tibial epiphysis was selected (from the growth plate to subchondral plate) in a volume of interest under the increased loading area²⁰.

Histology and scoring

After μ CT, joints were decalcified (Formical 2000; Decal Chemical, USA) overnight, embedded in paraffin wax and coronal sections (5 μ m cut, and stained with haematoxylin and Safranin-O/Fast-Green. Using a validated scoring system²⁶ ranging from 0 (normal) to 6 (>80% loss of cartilage), the tibial quadrant in 8–10 sections from each mouse was graded by two scorers blinded to the specimens, with scores averaged. There was good agreement between scorers; intraclass correlation coefficient of 0.9 (95% CI 0.82 to 0.95), mean difference in score being 0.12 (95% CI 0.19 to 0.33). Synovitis was assessed using a validated scoring system⁹. This was modified to focus on pannus formation, synovial membrane thickening and subsynovial hyperplasia. There was agreement between scorers; intraclass correlation coefficient of 0.88 (95% CI 0.79 to 0.94), mean difference in score of 0.002 (95% CI -0.07 to 0.35).

Nocturnal activity

Nocturnal activity was measured by placing a mouse in an activity cage (Activmeter, France). Activity monitoring was conducted in the last two weeks of the 8-week protocol. Cage activity measurements were averaged total movements throughout 16h of recording.

Statistics

Data were tested for normality (GraphPad Prism) and presented as mean $\pm$ SEM. Differences were statistically analysed by one-way or two-way analysis of variance (ANOVA). Contralateral and ipsilateral medial bone parameters were compared using a paired t test. The differences between the ipsilateral and contralateral leg (ipsilateral–contralateral) were compared by 2-way-ANOVA. Data is available upon request.

Declarations

The authors have no potential conflicts of interest, including financial and non-financial.

Role of the Funding Source

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Author contributions

Conceptualization	WRF, JCL, CSG, CH
Methodology	CH, WRF, JCL
Formal analysis	CH
Investigation	CH, LD, KM, MF, AO, KM, SM, GJL, AC, vHR, WRF, JCL
Data Curation	CH, LD
Writing - Original Draft	WRF, JCL, CSG, CH
Writing - Review & Editing	GJL, AC,
Visualization	CH, vHR
Supervision	WRF, JCL, CSG, GJL, AC, CH
Project administration	WRF, JCL, CSG, CH
Funding acquisition	WRF, JCL, CSG, CH

Animal studies

All animal experiments were approved by the University of Glasgow Ethical Review Committee and following UK Home Office guidelines for the care and use of laboratory animals.

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Tables

Table 1. MicroCT analysis of trabecular and subchondral bone changes in the DMM groups.

4 weeks	Non exercise						Exercise					
	Contralateral			Ipsilateral			Contralateral			Ipsilateral		
BV/TV (%)	12.3	±	0.84	13.2	±	0.71	13.2	±	0.71	12.9	±	0.77*
Tb.Th (µm)	12.0	±	0.38	12.1	±	0.3	12.1	±	0.3	11.9	±	0.35
Tb.Sp (µm)	55.4	±	3.39	52.9	±	2.47	52.9	±	2.47	53.9	±	1.93
Tb.N (µm ⁻¹)	0.010	±	0.0005	0.011	±	0.000542	0.011	±	0.0005	0.011	±	0.000449*
Tb.Pf (µm ⁻¹)	0.097	±	0.0074	0.093	±	0.004942	0.093	±	0.0049	0.093	±	0.007057
SMI	1.97	±	0.0663	1.94	±	0.0548	1.94	±	0.0548	1.88	±	0.0584**
DA	2.35	±	0.141	2.53	±	0.121	2.65	±	0.0243	2.49	±	0.0729
SC bone BV/TV (%)	74.4	±	1.71	83.5	±	1.74***	77.3	±	1.58	81.6	±	1.70**§§
SC Tb.Th (µm)	28.0	±	1.23	33.1	±	1.14	28.7	±	1.24	34.5	±	1.58

8 weeks	Non exercise						Exercise					
	Contralateral			Ipsilateral			Contralateral			Ipsilateral		
BV/TV (%)	17.965	±	0.838	18.14	±	0.724	15.29	±	1.3627	15.69	±	1.450
Tb.Th (µm)	56.67	±	1.434	56.51	±	1.587	54.71	±	1.7477	55.21	±	2.164
Tb.Sp (µm)	199.25	±	7.969	195.97	±	4.177	209.1	±	7.8552	214.7	±	7.54
Tb.N (µm ⁻¹)	0.0032	±	0.00016	0.0032	±	0.00012	0.0028	±	0.0002	0.0028	±	0.00021
Tb.Pf (µm ⁻¹)	0.0164	±	0.0007	0.0158	±	0.00067	0.02	±	0.0021	0.019	±	0.0020*
SMI	1.70	±	0.0639	1.64	±	0.0472	1.89	±	0.1108	1.79	±	0.117*
DA	2.22	±	0.0557	2.30	±	0.0393	2.169	±	0.0939	2.30	±	0.0992*
SC bone BV/TV (%)	84.3	±	1.16	91.7	±	1.04***	78.62	±	2.9674	85.71	±	2.841***
SC Tb.Th (µm)	30.2	±	1.04	38.4	±	1.24	28.6	±	1.65	33.8	±	1.80*** §

BV/TV=Bone volume/ Tissue volume. Tb.Th= trabecular thickness. Tb.No= Trabecular number. Tb.Sp.= Trabecular space. Tb.Pf= Trabecular pattern factor (connectivity). SMI = Structural model index (shape). DA= Degree of anisotropy (organisation). Each time point was analysed with a Two-Way ANOVA, comparing relative changes to the contralateral leg and also interrogating the effect of exercise. (*) indicate significant difference between exercise and non-exercise groups. * P < 0.05, ** P < 0.01, * P < 0.001. (§) indicates significant difference between exercise and non-exercise within the DMM joint normalised against the contralateral leg. § P < 0.05, §§P < 0.01.**

Table 2. Experimental groups in the study.

Time (weeks)	Non-exercise		Exercise	
	Sham	DMM	Sham	DMM
4	0	7	0	10
8	6	7	5	7

Figures

Figure 1

Effect of the exercise protocol. Overall effect of the exercise protocol on weight expressed as weight gain (A) and area under the curve (B). Subcutaneous (C), gonadal (D) and brown (E) adipose tissue weight expressed as a percentage of total weight. Quad (F) and soleus (G) muscle weight as a percentage of total body weight. H) Overnight distance travelled in DMM mice. Arrow in A indicates start of Exercise protocol. * $P < 0.05$, ** $P < 0.01$

Figure 2

Disease parameters on DMM operated mice. A) Representative images of the joint at 8 weeks, stained with SafraninO for cartilage and Fast Green for bone. B) Cartilage score. C) Synovitis score. D) Osteophyte number. E) Tibial subchondral bone sclerosis normalised to the contralateral leg. F) Tibial cortical thickness. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.