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A novel *Osteoarthritis* scoring system to separate typical OA joint degeneration from non-typical lesions in male *Sprague Dawley* rats

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ABSTRACT

Objective: To develop a novel scoring system to characterize osteoarthritis-related degeneration distinct from spontaneous subchondral bone lesions observed in the tibia and femur of male Sprague Dawley rats.
Method: Knee joints from male rats following 12 weeks of a diet-induced obesity model of osteoarthritis (OA) were assessed. OA histopathological changes (OAHC) were assessed in the knee joints. All scores were evaluated using a modified Mankin score and a modified Osteoarthritis Research Society International histological score. OAHC were divided into 3 categories: (I) Typical OA score evaluating the changes in cartilage structure, cellularity, proteoglycan depletion, and tidemark integrity, (II) A novel Non-typical OA score evaluating cartilage integrity, and the size of local thickening, fragmentation and degeneration along the tidemark and the size and severity of the subchondral bone lesion, and (III) Total OA score comprised of both, the Typical and the Non-typical scores.
Results: Rats exposed to a high fat/high sucrose diet had higher Typical OA score compared to a control group (Chow). Non-typical and Total OA scores revealed no differences in the severity of the lesions between the HFS and the Chow group animals. All scoring systems had excellent intra- and inter-examiner reliability.
Conclusion: The spontaneous bone lesions observed in male Sprague Dawley rats can obscure the effect of the diet-induced obesity if the classical scoring system is used to assess joint degeneration. The newly proposed scoring method provides a reliable method to distinguish classical OA joint degeneration from spontaneous Non-typical lesions occurring in these rats.

1. Introduction

Osteoarthritis (OA) is a chronic joint disorder that affects 15% of the global population over the age of 30 [1], most frequently affecting the knee joint, followed by the hip and joints in the hand [2]. The pathophysiology of OA is not well understood; however, many risk factors are thought to contribute to the development of OA including joint injury, age, musculoskeletal abnormalities, and obesity [3,4]. Many studies have been conducted to understand the pathophysiology of OA and to identify potential interventions to prevent or slow down the progression of the disease [5–8]. However, human clinical studies are associated with limitations and challenges. Patients with OA generally seek medical help when symptoms of OA such as pain, stiffness, and reduced mobility and function, are well developed, and thus they are typically at an advanced stage of the disease, thereby preventing the study of the onset and early changes [9–11]. The variability of disease progression in patients, the slow onset, and the difficulty in controlling the confounding variables and risk factors affecting OA progression make effective human studies extremely difficult [10]. Animal models of OA have been used for more than 50 years to overcome some of the limitations associated with human studies [12]. Primary OA models that occur naturally due to degenerative changes in the joint, and Secondary OA models associated with the

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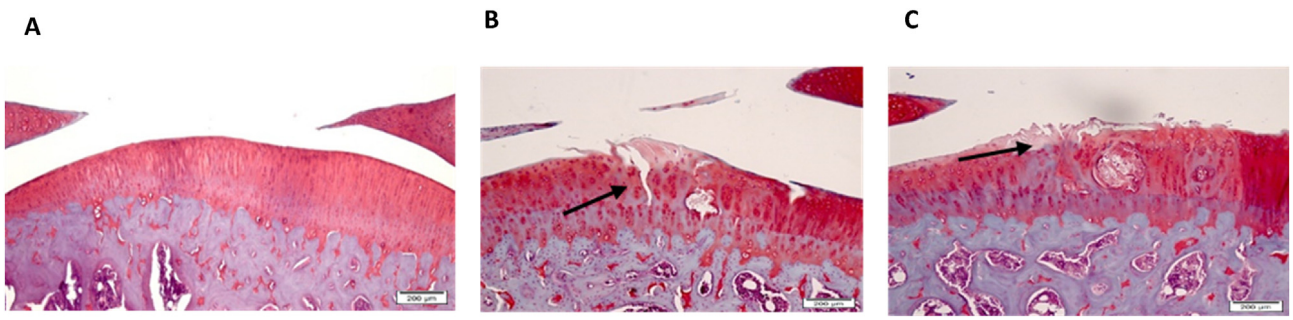


Fig. 1. (A): healthy tibia (score = 0). B–C shows cartilage cysts and fibrillation at varying stages of degeneration commonly seen in typical OA. (B): arrow indicates fibrillation with a crack extending to the tidemark (score = 12). (C): arrow indicates loss of superficial layer and loss of stain (score = 10).

Table 1
Modified Mankin scoring criteria. The criteria were modified from the original Mankin scoring system based on the lesions observed in this model.

Modified Mankin	
I Structure	**Score**
Normal Surface	0
Irregular surface, no panus	1
Surface discontinuity/Delamination	1
Cyst present, single/small	1
Cyst present, multiple/affects surface	2
Superficial layer absent	2
Collagen matrix thinning	2
Defect to the transitional zone	3
Defect to the radial zone	4
Cyst extends surface to tidemark TM	4
Defect extends to calcified zone/TM	5
Fibrillation/fragment loss to TM	5
Plate failure: collapse T/loosebody F	6
II Cells	
Normal distribution	0
Hyper-cellularity	1
Clustring/Cloning	2
Hypo-cellularity	3
III Sfaranin O staining	
Normal/Intense/Even distribution	0
Slight reduction/Uneven distribution	1
Moderate reduction	2
Sever loss/No stain present	3
IV Tidemark integrity	
Intact	0
Non-Intact/Not stained/Non-visible	1
Multiple Tidemarks	2
Total Score	
Minimal	0
Maximal	14

introduction of a cause or risk factor, have been used extensively to study the pathogenesis and pathophysiology of OA in small and large animals [10,13]. There is no gold standard on what constitutes the best animal model, therefore, choosing an animal model depends primarily on the research question and the etiology under investigation [14]. Furthermore, humans are highly heterogeneous and studies with inbred small animal models may not translate to the human condition effectively.

Rats have been widely used as a preclinical model to study OA onset and progression [13,15]. As spontaneous OA is rare in rats [16], they have been used as post-traumatic models of OA [17] inflammation-based models [18] and models of diet-induced obesity and associated metabolic syndrome [19,20].

1.1. Diet-induced obesity model

Obesity, and associated metabolic syndrome, are associated with an increased risk of development and progression of a specific phenotype of OA called metabolic OA [4,20,21]. Historically, the link between obesity and OA was often explained by the increased mechanical loading and associated increase in wear and tear of the weight-bearing joints. However, increased mechanical loading does not explain OA onset in non-weight-bearing joints, such as the hand and wrist joints, these observations suggest a complex etiology of metabolic OA, including low-level of systemic and local inflammation [5,22,23]. Hypertrophic adipocytes that accompany obesity can contribute to systemic inflammation by secreting inflammatory adipokines such as leptin, interleukin-6 (IL-6), tumor necrosis factor-α, monocyte chemoattractant protein-1 (MCP-1), and resistin [24]. Another potential contributing source of systemic inflammation associated with obesity is dysbiosis in the gut microbiota [25,26]. Recent studies provide evidence that feeding rats and mice a high fat/high sucrose diet compromises the intestinal barrier, thereby allowing translocation of lipopolysaccharides (LPS) from the gut lumen into systemic circulation and in turn activating Toll-like receptors on immune cells [25–29]. A high-fat diet, a high-sucrose diet or a combination of the two, has been used to induce obesity and associated metabolic OA in rats and mice [19,20,30–32].

Sprague-Dawley rats have been widely used as a preclinical model of Metabolic OA. Rats are an easy model to implement and study, they develop OA quickly, they are relatively inexpensive and provide sufficient tissues to study [10]. For instance, Collins et al. [22] used Sprague Dawley rats to evaluate the association between systemic and local inflammation and joint degeneration. Obesity and metabolic syndrome were induced by feeding the rats high fat/high sucrose diet for 28 weeks, at which point OA-like damage at the knee joint was observed [20]. In another study, it was found that 12 weeks of feeding Sprague Dawley rats a high fat/high sucrose diet resulted in increased degeneration of the shoulder and knee joints [19]. Rios et al. used the diet-induced obesity model in male Sprague Dawley rats to demonstrate that prebiotic fiber supplementation or progressive moderate treadmill training can protect or slow the progression of knee OA [31]. Also, Warmink et al. reported that Sprague Dawley rats had more pronounced knee joint damage compared to Wistar rats when fed a high-fat diet for 24 weeks [33].

1.2. Spontaneous bone lesions

Although feeding Sprague Dawley rats a high fat/high sucrose diet induced the metabolic changes associated with obesity, metabolic syndrome, and dysbiosis in the gut microbiota [31,32,34–37] a specific phenotype of OA like-degeneration, characterized by changes in the subchondral bone architecture, osteoporosis-like degeneration, and a marked decrease in trabecular bone volume were reported in studies using Sprague Dawley rats [31,38,39]. Most studies accounted for these "bone lesions" as part of the typical joint degeneration resulting from the systemic and local inflammation associated with the high fat, and high sucrose diet. However, Kato and Onodera [42] reported bone lesions in Sprague Dawley rats fed a standard control diet across ages ranging from 19 to 63 weeks that were similar to those observed in rats fed a high fat/high sucrose diet. In their study, the changes to the femur and tibia

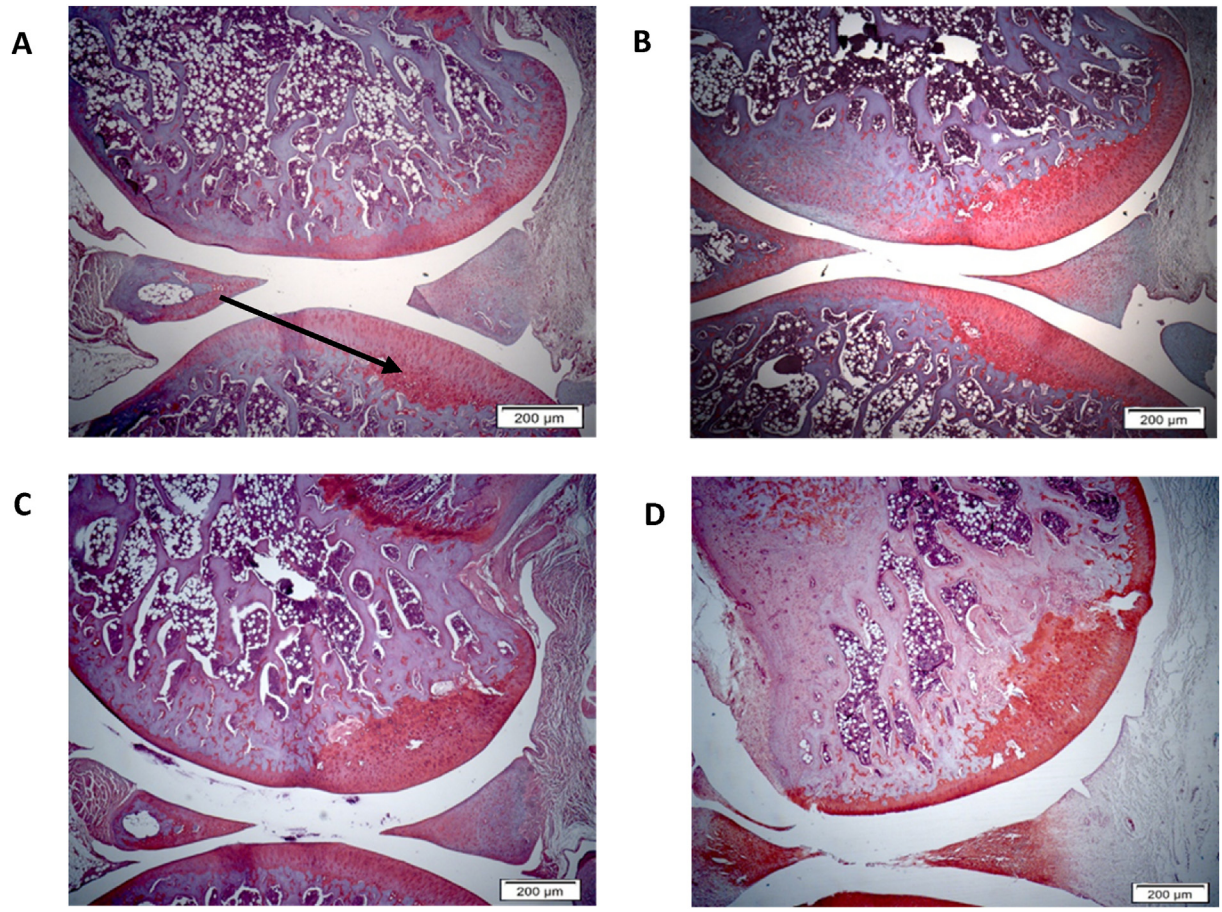


Fig. 2. Non-typical lesions. Local thickening of the articular cartilage on the femur and tibia protruding into the subchondral bone. (A): mild local thickening on the tibia with a total area of less than 0.63 μm^2 (black arrow), (B): medium local thickening on the femur with a total area ranging between 0.64 and 0.94 μm^2. (C) Moderate local thickening on the femur with a total area ranging between 0.95 and 1.25 μm^2, and (D) large local thickening on the femur with an area larger than 1.25 μm^2.

were defined as Osteochondrosis, which is an impaired endochondral ossification of the cartilage in young animals [40]. The same group reported these bone lesions in the medial femoral condyle of chow-fed male and female Sprague Dawley rats as early as 10–12 weeks [41]. The histological changes of osteochondrosis are characterized by local thickening of the articular cartilage protruding into the subchondral bone, accumulation of necrotic chondrocytes with an enlarged lacuna often containing cell debris, and different-sized fissures along the tidemark and in the basal layer of the thickened cartilage. In advanced stages, the fissures formed a large flap parallel to the subchondral bone, a fibrotic lesion subjacent to the thickened cartilage containing many capillaries, and cysts in the subchondral bone, along with the appearance of activated osteoblasts at the surface of sponge bone [40–43]. We observed similar lesions in male Sprague Dawley rats fed a Chow diet and a high fat/high sucrose diet observed in a previous study performed in our lab, these lesions were scored using the traditional scoring system [31]. However, these rats were used to develop a new scoring system by identifying the "Non-typical" lesions and establishing the criterion to assess them. In this study, a new group of rats were used to validate the scoring system and to distinguish classical OA degeneration from bone lesions observed in rats.

1.3. Scoring systems

Degeneration of the articular cartilage and bones in rats is typically assessed using Mankin and/or Osteoarthritis Research Society International (OARSI) scoring systems [44]. Some modifications of the traditional Mankin scoring system have been adapted in the past to include and evaluate OA-related changes in bones and the synovium [19]. These modified Mankin and OARSI scoring systems, that also evaluate changes in cartilage and bone, do not distinguish between features of OA-related degeneration and spontaneous bone lesions that occur in Sprague Dawley rats. Therefore, the traditional scoring systems may lead to an inaccurate assessment and interpretation of the consequences of diet-induced obesity on metabolic OA.

This study aimed to develop a novel, comprehensive scoring system that allows for the characterization of features of osteoarthritis-related degeneration that are distinct from the spontaneous and naturally occurring subchondral bone lesions occurring in the tibia and femur of male Sprague Dawley rats. This new scoring system can be used to determine joint degeneration in all models of knee joint OA using Sprague Dawley rats. Our scoring system includes a Typical histological score assessing the classic changes in cartilage reported in OA joints, a Non-typical score of the spontaneous subchondral bone lesions and the overlying cartilage, and a Total OA score comprising of the sum of the typical and non-typical scores.

2. Methods

A cohort of 15, twelve-week-old male Sprague-Dawley rats were randomized using a block randomization method into two groups; a group fed a high fat/high sucrose diet (n = 8) (20% of total weight as fat, 50% sucrose, 20% protein, and 10% fiber and micronutrients; custom Diet #102412, Dyets, inc., Bethlehem, Pennsylvania, United States) to

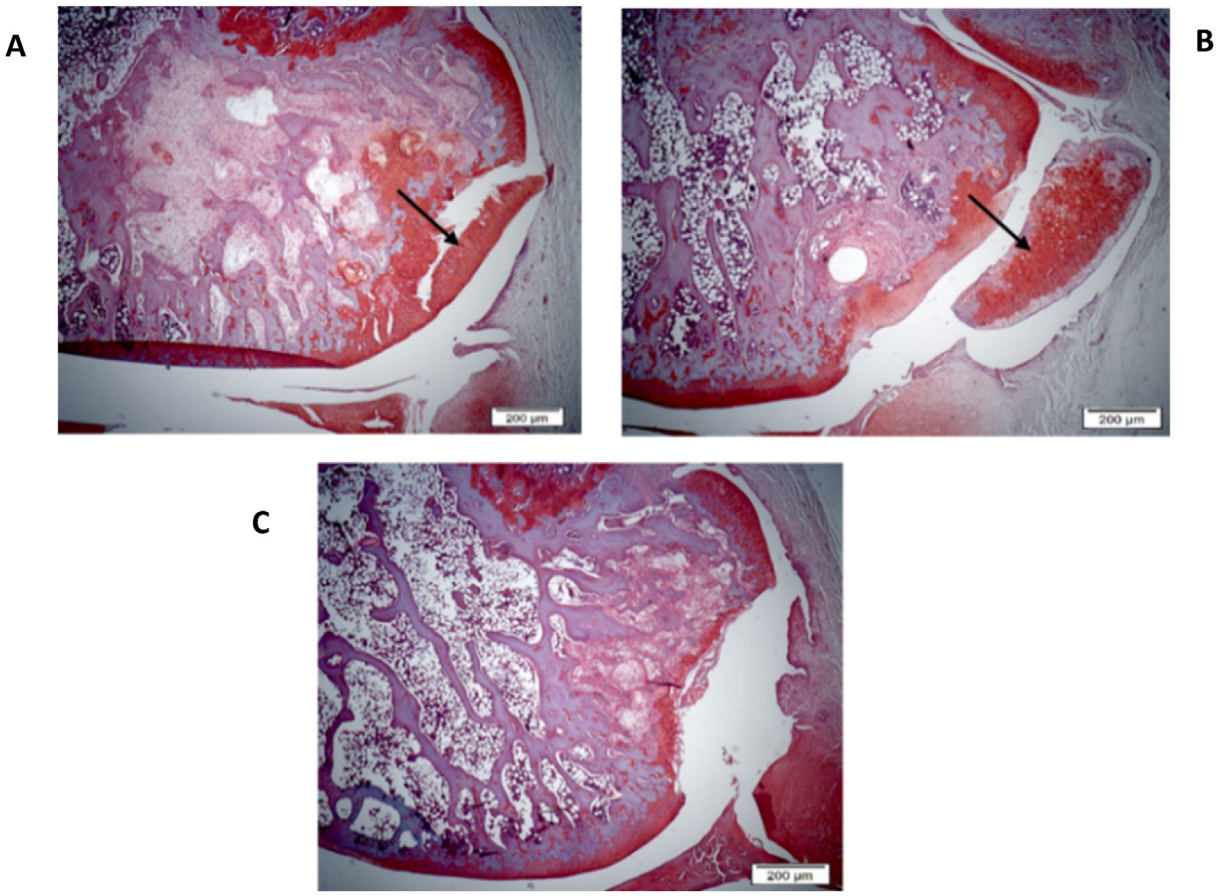


Fig. 3. Non-typical lesions in the femur. The separation of cartilage from the femur forms a loose body (black arrow). (A) Partial separation (score = 20). (B) complete separation (score = 22). (C) Loss of loose body (score = 23).

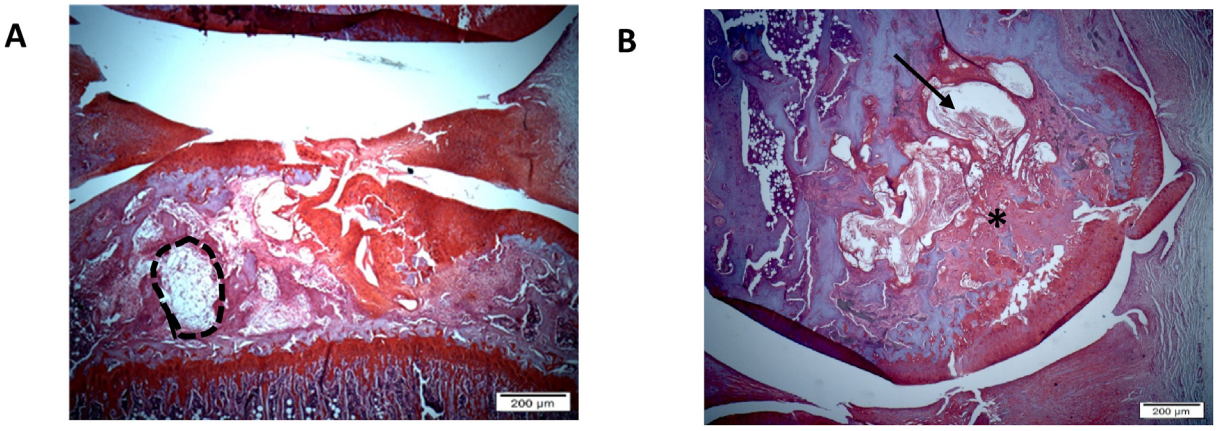


Fig. 4. Non-typical subchondral bone lesions in the tibia, and femur. A: shows a Complete collapse of the cartilage into the epiphysis of the tibia (score = 22). B: Bone cysts and cavities in the epiphysis of the femur (score = 23). The dashed area shows a bone cyst filled with remnants. The black arrow indicates a partially full cyst. * Indicates fibrotic tissue in the subchondral bone.

induce metabolic OA, and a group fed a chow diet (5% of total weight as fat, 47.5% carbohydrates (only 4% from sucrose), 25% protein fiber and micronutrients, and 10% moisture; Lab Diet 5001, United States) to act as controls (n = 7). The sample size was determined based on previous studies with high fat/high sucrose and control rats [31,37]. Rats had a 2-week acclimation period before allocating them to groups. Rats were housed in pairs at 21 °C on a 12:12 light-dark cycle in the same room and had access to diet and water ad libitum. All rats were purchased from the University of Calgary Life and Environmental Sciences Animal Resource Centre. The initial and final weight of the rats is provided in the Supplementary materials (Table S1).

2.1. Histology processing

At 24 weeks of age, rats were anesthetized using 5% isoflurane, then euthanized by severing the aorta and vena cava. Both knee joints were harvested, fixed in a 10% neutral buffered formalin solution for 4 weeks, then decalcified in Cal-x-II (Fisher Scientific, Canada) for 4 weeks Joints

Table 2
Non-Typical OA score using modified Mankin Score and modified Osteoarthritis Research Society International grading system. The table can be copied or printed for use in the lab as a scoresheet.

ID#		Tibia		Femur	
Compartment of bone scored		Medial	Lateral	Medial	Lateral
Local Thickening/size of proteoglycan-rich area extending into bone	**Score**				
Mild (0.063–0.63 μm^2)	1				
Medium (0.64–0.94 μm^2)	2				
Moderate (0.95–1.25 μm^2)	3				
Large (>1.25 μm^2)	4				
Fragmentation of calcified cartilage and tidemak (disruptive/cavities/degenration)					
Minimal to mild focal/small,few cavities	1				
Marked focal, multiple/med size cavities	2				
Severe, multifocal areas/larger size cavities	3				
Partial Tears/flaps along length of TM					
Involving <1/4 length of TM	4				
Involving up to 1/2 length of TM	5				
Involving up to 3/4 length of TM	6				
Involving >3/4 length of TM	7				
Subjacent bone defect: severity complete tears/flap along full length of tidemark					
Loose body formation off Femur	8				
Collapse on Tibia into epiphysis	8				
Bone changes: area of epiphysis involved (fibrotic tissue + cysts/cavities)					
Minimal focal changes involving <1/3	1				
Mild to moderate, involving up to 1/3	2				
Marked changes involving up to 2/3	3				
Severe changes involving all of epiphysis	4				
Subjacent Bone Cyst/Cavities Severity					
Cyst-Full	1				
Cyst-Partial remnants/debris	2				
Cavity- empty	3				
Total Non-typical score	19				

were dehydrated in alcohol, cleared in xylene, and infiltrated with paraffin using a Leica TP1020 automated processor. Knee joints were then embedded in a mix of Paraffin wax. 10 μm serial sagittal sections were obtained and alternate slides, 100 μm apart were stained with Hematoxylin, fast green, and Safranin-O using Leica Autostainer XL (Leica Biosystems, Nussloch, Germany). All joints were alphabetically code for blind scoring.

2.2. Scoring system

Our new scoring system is divided into 3 categories, Typical score, Non-Typical score, and Total OA score. Two observers scored the joints independently twice: All stained slides were reviewed, and sections were selected for scoring based on characteristic lesions observed. Lesions were separated into two categories, based on whether they showed classic, typical chondropathy or non-typical bone and cartilage lesions as observed in Sprague Dawley rats. Both compartments: medial and lateral tibia plateau and femoral condyle, were evaluated as well as synovial lining and meniscus.

2.2.1. Typical score

Classical histopathological changes in the cartilage reported to occur in rat OA models were assessed in four quadrants of the knee joints; medial femur, medial tibia, lateral femur, and lateral tibia using a modified Mankin scoring system ranging from 0 to −14 points: Cartilage structure was evaluated using the range of (0–6), a score of 0–3 was used to assess chondrocyte changes. Matrix staining, indicating proteoglycan loss, was evaluated with a score range of 0–3, and a range of 0–2 for tidemark integrity. Fig. 1 represents lesions assessed under the Typical OA score. Detailed criteria to assess cartilage integrity are provided in Table 1. The maximum possible modified Mankin score for these typical lesions in each quadrant is 14. The scores for all 4 quadrants were then summed. The original Mankin scoring system [45] is provided in the supplementary materials (Table S2). Modifications to Mankin scoring were performed to capture all changes observed based on our model.

2.2.2. Non-typical score

The non-typical score is comprised of two scores: The first score captures changes in the cartilage above the tidemark using a modified Mankin scoring system, 0–14, as described above. A score of 6 was assigned for cartilage structure when a complete plate failure was observed. The second score was used to evaluate changes observed along the calcified cartilage and tidemark and included the size of local thickening, fragmentation, and degeneration. Spontaneous lesions in the subchondral bone, subjacent to the cartilage were scored separately, and similarly to the criteria described by Keto et al. [40–43]. Here the OARSI histological bone score was used [44] as a guide to develop a more detailed assessment of subchondral lesions based on observation of changes seen in our model.

Four pathological changes were identified around the tidemark and in the subchondral bone: (i) local thickening of the cartilage at the tidemark that protruded into the subchondral bone was assigned 0–4 based on the size. Fig. 2 shows local thickening with different sizes. (ii) cavities and fissures in the calcified zone and along the tidemark (Fig. S1), where the cartilage sometimes detached from the subchondral bone (Fig. S2), assigned 0–8 points. This criterion evaluated the length of cartilage detachment from the tidemark due to the fissures formed at the basal layer of the thickened cartilage (A score of 8 was given when a complete flap tear occurred forming a loose body in the femur (Fig. 3) or a complete collapse of the cartilage into the subchondral bone, as observed in the tibia (Fig. 4A). (iii) fibrotic tissue in the subchondral bone lesion was assessed with 0–4 points based on the area of the epiphysis occupied, and (iv) cavities and cysts in the subchondral lesion were assessed as 0–3 for each based on the severity. (Fig. 4B). Table 2 shows the detailed criteria used for scoring these Non-typical lesions. The final score was determined by the sum of the cartilage score and the subjacent bone score and ranged from 0 to 33, Fig. S3 presents a detailed example of the evaluation of Non-typical score in the femur with the individual score of each criterion in the cartilage, tidemark, and subchondral bone changes. The same four quadrants used for assessing the typical OA score were also used to assess the Non-typical score.

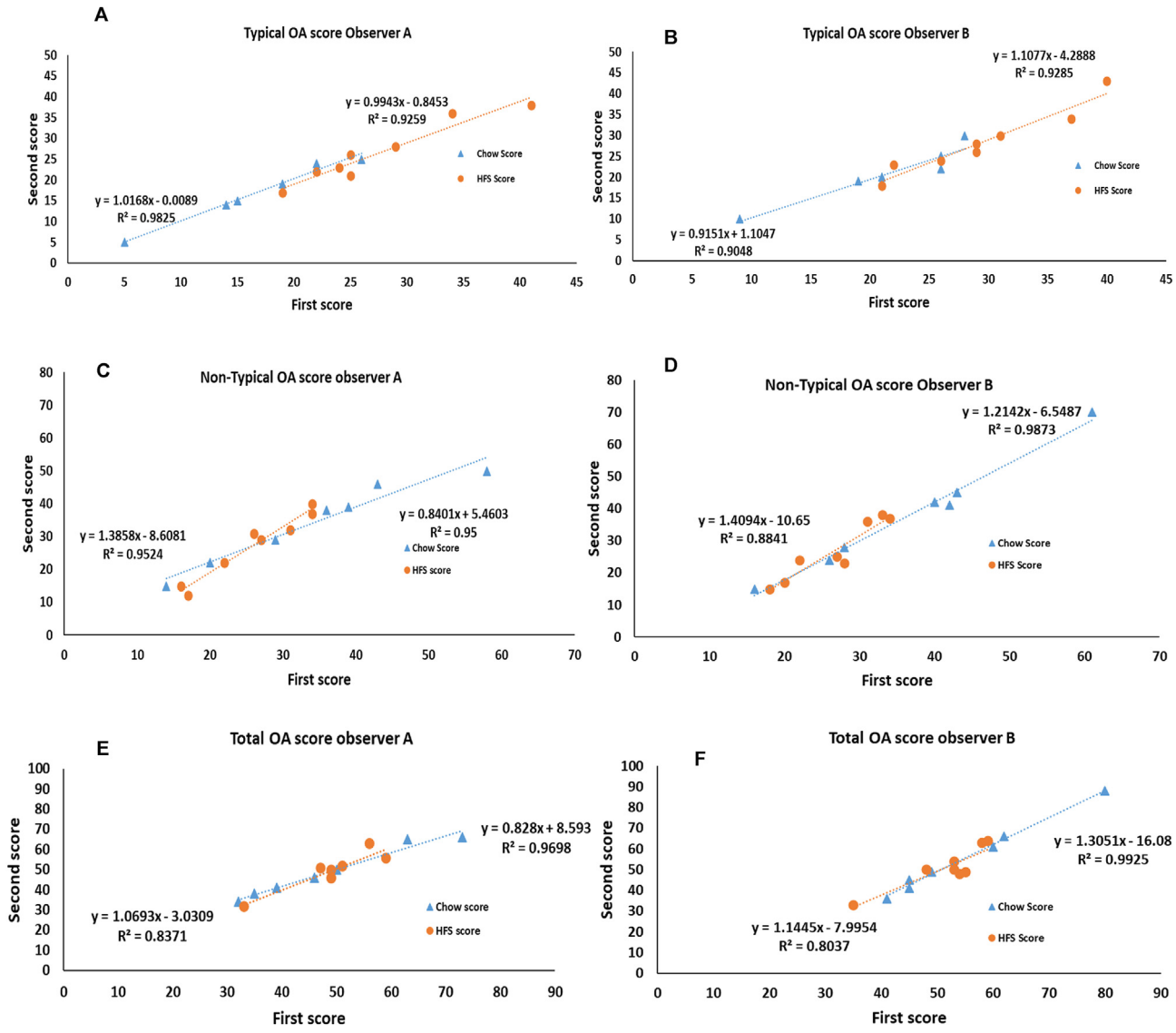


Fig. 5. Intra-observer correlation analysis between the first and the second score for observers A and B for Typical OA score (A, and B), Non-typical OA score (C, and D), and Total OA score (E, and F).

2.2.3. *Total OA score*

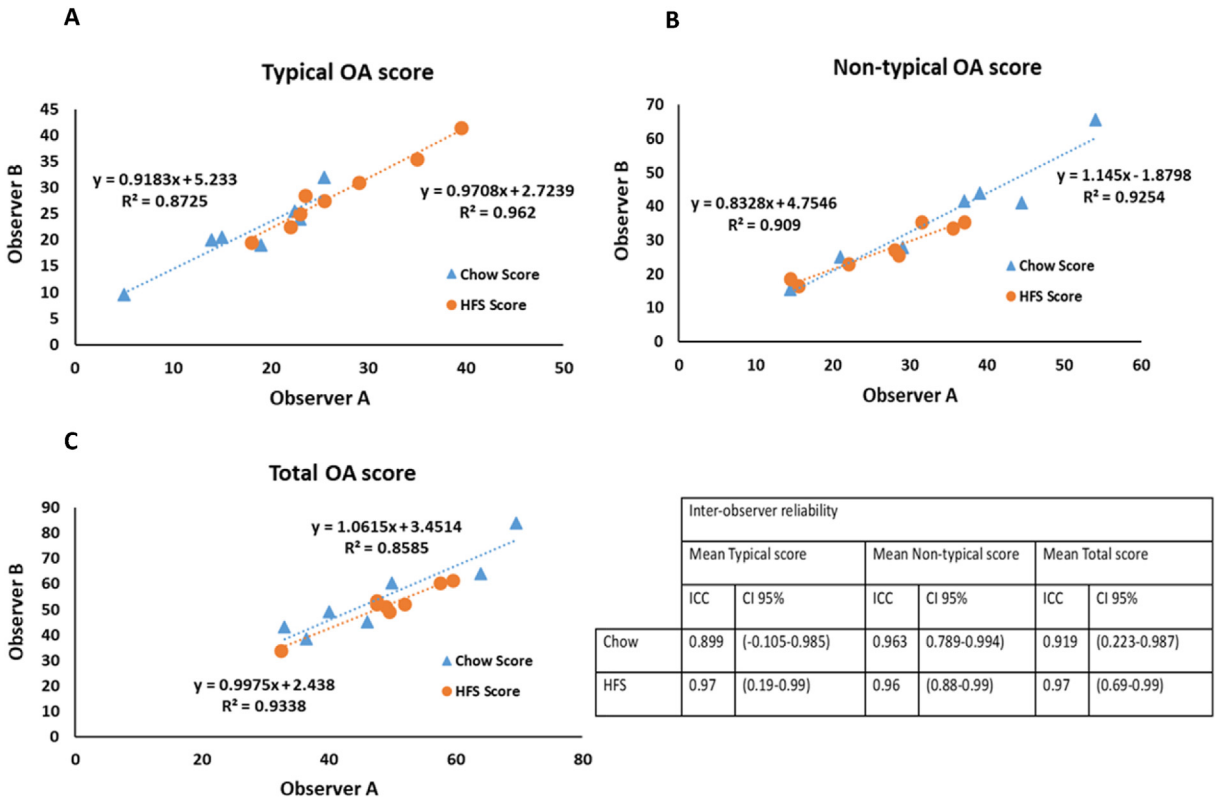
The total OA score is an overall evaluation of the histopathological changes in the rat knee joint and was defined as the sum of (i) the classical OA lesions in the cartilage represented by the Typical OA score and (ii) the spontaneous thickening around the tidemark, fragmentation, and subchondral bone lesions with the changes in the cartilage just above the subchondral bone represented by the Non-typical score. Synovial membrane cell thickening (0–3) and meniscal damage in the inner white zone (0–3) were then added to yield the final total score (Table S3 supplementary material). The individual scores for each rat are provided in the supplementary materials (Table S4). No osteophytes were observed in this rat model of OA, as such no score was entered for this parameter. This could easily be added to adapt to a different model, where osteophytes are observed.

2.3. *Statistical analysis*

To validate the new histological scoring system, the intra-observer reliability was assessed using Pearson's correlation coefficient analyses between the first and the second score for each observer. The mean of the first and the second scores was calculated. Inter-observer reliability was determined using the calculated mean for each observer using the intraclass correlation coefficient. This was calculated using a two-way mixed effect analysis of variance, type two-way mixed, average measure. Mann-Whitney-U tests were used to compare typical OA, non-typical OA, and total OA knee joint scores between the chow group and the HFS group animals. Comparisons were made using IBM SPSS statistic V26 (IBM SPSS, Armonk, NY, USA). Significance was accepted at $p < 0.05$, two-tailed *t*-test.

3. Results

Two groups of male Sprague Dawley rats from a diet-induced obesity model were used to evaluate the new osteoarthritis scoring system with the aim to separate classical OA Joint degeneration from spontaneous and naturally occurring subchondral bone lesions. Two independent observers scored histological images from the HFS group rats and the Chow control group rats two times on separate occasions.



Inter-observer reliability						
	Mean Typical score		Mean Non-typical score		Mean Total score	
	ICC	CI 95%	ICC	CI 95%	ICC	CI 95%
Chow	0.899	(-0.105-0.985)	0.963	0.789-0.994)	0.919	(0.223-0.987)
HFS	0.97	(0.19-0.99)	0.96	(0.88-0.99)	0.97	(0.69-0.99)

Fig. 6. Inter-observer reliability and the intraclass correlation coefficient between observers A and B for chow-fed and HFS-fed rats for Typical score (A), Non-typical score (B), and Total OA score (C).

3.1. Intra-observer variability

Intra-observer variability analysis of the same set of histological images for the HFS and the Chow group animals showed high reproducibility between the first and second scores for observer A and observer B in Typical OA, Non-typical OA, and Total OA scores. For the Typical OA score, the intra-observer correlation coefficients were 0.98 and 0.93 for the chow and HFS group for observer A, respectively (Fig. 5A), and 0.90 and 0.93 for the chow and HFS group for observer B, respectively (Fig. 5B). The intra-observer correlation coefficients for the Non-typical score for the HFS and Chow groups were 0.95 and 0.95 for observer A (Figs. 5C), 0.99 and 0.88 for observer B, respectively (Fig. 5D). The intra-observer correlation coefficients for observers A and B in Total OA scores were 0.84, 0.97 (Figs. 5E), 0.80 and 0.99 (Fig. 5F) for the HFS and Chow animals, respectively.

The mean of the first and the second scores for observers A and B were calculated and used to assess inter-observer reproducibility. The ICCs between observers A and B for typical, nontypical, and total OA scores were 0.89, 0.96, and 0.92, for the Chow group, respectively, and 0.97, 0.97, and 0.96 for the HFS group, respectively. Fig. 6 shows the ICCs for the three scoring categories with a 95% confidence interval.

Two groups of rats were used to evaluate the new scoring system, a Chow-fed group and a HFS-fed group. Both observers A and B identified a significant increase in cartilage degeneration represented by a higher Typical OA score in the knee joints of the HFS group animals compared to the chow group animals ($p = 0.04$, and $p = 0.04$, respectively, Fig. 7A). Rats from the HFS and chow groups had similar bone lesions indicated by the close Non-typical scores between the two groups (Fig. 7B). The total OA score calculated by observers A and B showed no difference in bone and cartilage degeneration between the HFS and the chow group animals (Fig. 7C).

4. Discussion

A new scoring system for male Sprague Dawley rats was developed that distinguished between classical OA degeneration reported in preclinical models of OA using rats and mice, and age-related spontaneous lesions including thickening around the tidemark, fragmentation, and subchondral bone lesions observed in Sprague Dawley rats. The new scoring system is comprised of three categories: Typical, Non-typical, and Total OA score. A modified Mankin scoring system was used to establish these categories. The typical OA scoring system evaluates the severity of the histopathological changes observed in the superficial, middle, and deep zones of the articular cartilage. By using this score, a significant increase in knee joint scores was observed in the rats from the HFS group compared to the rats from the Chow group. These results agree with previous studies showing that feeding rats a high fat/high sucrose diet-induced metabolic knee joint OA [19,20,32,37]. The high intra- and inter-reproducibility between the first and second score and between the first and the second rater for the HFS and the Chow group animals indicates the effectiveness of using the typical OA score not just in the diet-induced obesity model, but for other rat models of secondary-induced joint degeneration.

In the Non-typical scoring approach, the modification applied to the OARSI scoring system can be used to evaluate mild and severe thickening around the tidemark, fragmentation of the calcified cartilage, and subchondral bone lesions. Four stages, determined by the sizes of the local thickening: proteoglycanrich area protruding into the subchondral bone were identified. The severity of the fissures and the extent of tears along the tidemark, and 3 stages of cysts tocavity formation in the bone, were also evaluated. This non-typical score provides a more detailed assessment of the Non-typical lesions observed not only in the femur, as described by Kato et al. [31] but also in the tibia. Both HFS and Chow rats

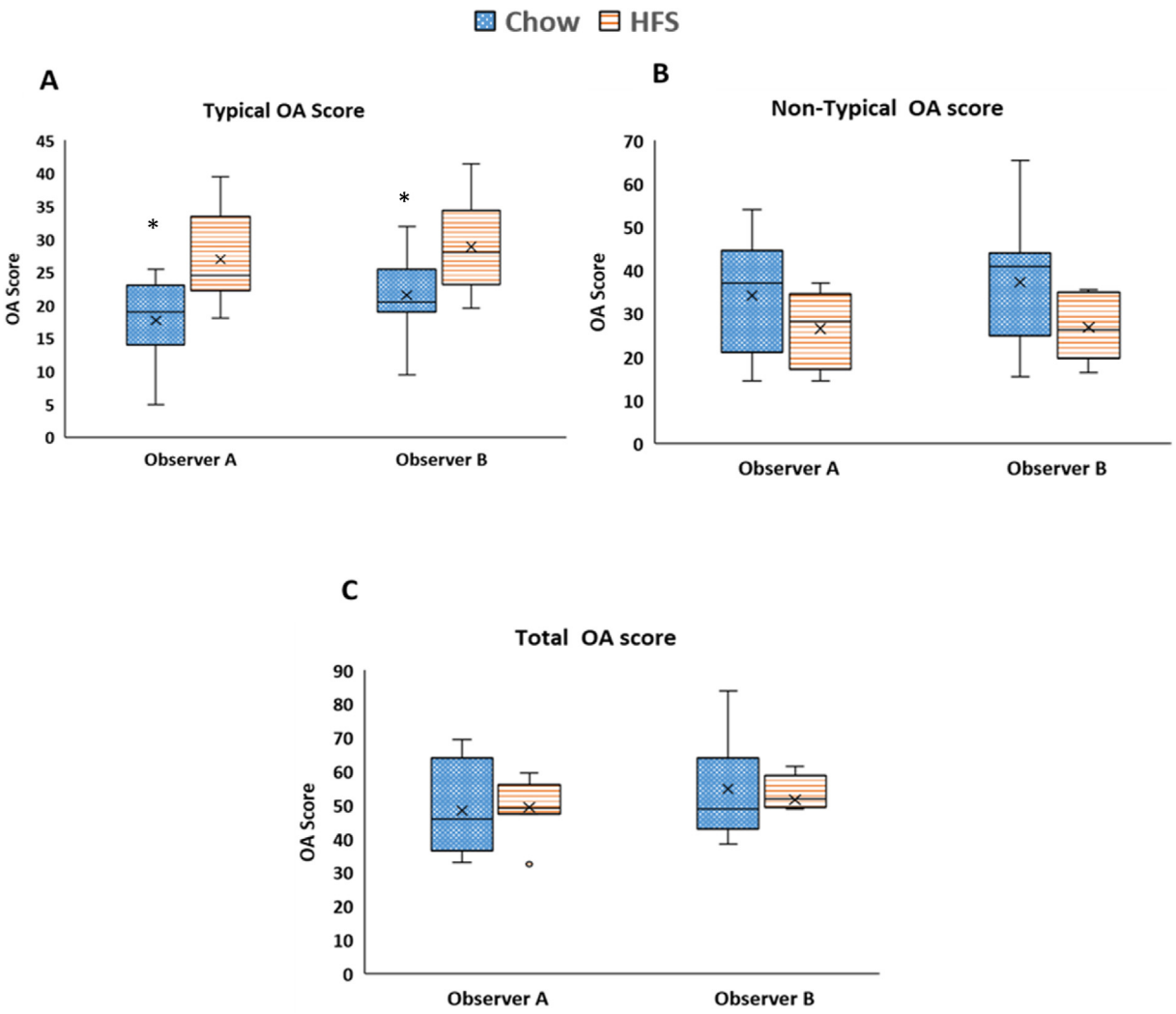


Fig. 7. Joint degeneration represented by OA score. A: Typical OA score for chow-fed rats and HFS-fed rats. B: Non-typical OA score for chow-fed rats and HFS-fed rats. C: Total OA score for chow-fed rats and HFS-fed rats.* indicates a significant difference compared to the HFS group.

showed the development of lesions along the tidemark and in the subchondral bone in the medial and lateral tibia, which, in the most severe cases, produced a collapse of the cartilage into the epiphysis of the tibia, forming a cavitation at the surface of the cartilage (Fig. 4A). No significant differences in Non-typical scores were detected between the Chow-fed control and HFS-fed rats suggesting that these lesions are not linked to the HFS diet, and consequently, they should not be classified as a feature of the diet-induced obesity model.

The cartilage above the subchondral bone lesions was scored using the same criteria employed in the Typical OA scoring system. Interestingly, the articular cartilage of both tibia and femur showed no classical erosion or degradation and maintained its proteoglycan content. However obvious tears along the tidemark resulting in plate failure and collapse of cartilage were seen. There was no difference between the Nontypical knee joint scores of HFS and the Chow-fed rats, suggesting that the development of the Non-typical lesions was independent of the diet.

The total OA score represents the total histopathological changes observed in the knee joints, including the typical OA lesions and the Non-typical lesions. The total score follows the classic scoring methods used to evaluate OA in pre-clinical models of induced knee joint OA [31]. No significant difference was found in the total knee joint OA score between the HFS rats and the Chow-fed rats, indicating that the severity of the Non-typical lesions masked the true mild to moderate changes in the articular cartilage associated with obesity and metabolic syndrome, therefore rendering the total OA score inaccurate and impractical for the evaluation of knee joint OA secondary to an intervention.

Joints with severe subchondral bone lesions are considered unhealthy. They have similar features to joints with severe OA in terms of loss or separation of the articular cartilage, changes of the articulating bones, and inflammation of the synovium [46]. However, the development of these bone lesions differs from the commonly accepted notion of the development of OA. The early stages of OA are characterized by discontinuity of the articular cartilage surface, fibrillation, and vertical fissure extending no deeper than the mid-zone [47]. Later stages of OA are associated with erosion of the articular surfaces, tissue fibrillation, and an appreciable narrowing of the joint space [48]. The early stages of the spontaneous Non-typical lesions are characterized by a thickening of the articular cartilage that protrudes into the subchondral bone, the formation of fissures along the tidemark which, at a later stage, start to form complete cartilage flaps in the femur [40]or a total collapse of the tibia as observed in this study. The mechanism by which these Non-typical lesions develop in the Sprague-Dawley rats is not fully understood. Genetic and environmental influences, biomechanical factors, such as exercise, and molecular alterations in matrix production have been suggested to be risk factors for the development of osteochondrosis in horses [49], similar to the lesions seen here. A sedentary lifestyle has been reported to increase the odds of developing bone lesions in male Sprague Dawley rats [39]. Rats performing aerobic and resistance

training for 12 weeks had significantly fewer subchondral bone lesions compared to chow and HFS-fed rats [39]. Moreover, male Sprague Dawley rats are thought to have a higher incidence of developing osteochondrosis than female rats [40].

The new scoring system provides an accurate and detailed assessment of cartilage and bone changes observed in the diet-induced obesity model of OA and spontaneous Non-typical lesions in knee joints of male Sprague-Dawley rats. Separating the typical changes from the non-typical ones enabled us to detect the differences in cartilage structure between the Chow control and the HFS experimental group animals, thus allowing for the identification of the specific effects of the high fat/high sucrose diet on knee joint OA.

In a previous study, Rios et al. reported that Sprague Dawley rats fed a high fat/high sucrose diet for 14 weeks had similar "OA-like damage" as the chow-fed group. Moderate aerobic exercise, and/or a 10% prebiotic fiber supplementation diet did not mitigate the effect of the high fat/high sucrose diet on the knee joints [50] in these animals. However, the OA-like damage referred to in that paper included the Non-typical lesions, the local thickening and collapsing of the articular cartilage, and the development of flaps and fibrotic lesions subjacent to the thickened cartilage, which are known to become more severe in aged rats [40]. Perhaps if the same joints had been evaluated using the new scoring system proposed in this study, identifying the HFS specific changes to the knee joints, the results generated, and their subsequent interpretation might have been different and in agreement with the results presented here.

Use of the new scoring system with other rat strains, and various genetic hybrids of Sprague-Dawley rats could lead to a new understanding of the molecular and cellular basis for the various elements of this new scoring system. Furthermore, this new approach may also provide an improved understanding of how diet can impact the integrity of adult joints and those of the very young with rapidly growing bones and joints.

4.1. Limitation

A limitation of this study is the small number of animals used to evaluate the new scoring method. We observed a trend of decreasing non-typical scores with a simultaneous trend of increasing typical scores in the HFS group. However, the number of animals was not sufficient to reach the threshold set for statistical significance. Currently, the proposed scoring method is being used to assess shoulder and knee joint degeneration in a new group of male and female Sprague Dawley rats to increase the statistical power of the current observations and to determine potential sex-related differences. A second limitation was the lack of pain and behavioral assessments of the rats, thus possibly being able to relate the structural differences observed histologically with clinical symptoms of knee joint OA and Non-typical lesions. The third limitation of this study is that both observers were trained in the same lab and thus the reliability of the scoring may be smaller if repeated in other laboratories. However, with training in another laboratory, intra-rater and inter-rater reliability should be similar to that reported in our manuscript.

Author contributions

Nada Abughazaleh was responsible for data collection, data analysis, interpretation of data, drafting the manuscript, revising the manuscript, and approving the final submitted version of the manuscript under Walter Herzog's supervision. Ruth-Anne Seerattan was responsible for providing the second score (observer B) and revising the manuscript. David A. Hart and Raylene A. Reimer contributed to interpretation of data, critically revising the manuscript, and approving the final submitted version. Walter Herzog contributed to study design, interpretation of the data, critically revising the manuscript, and approving the final submission.

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Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ocarto.2024.100521.

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