Original Article

Effect of Moderate Treadmill Exercise on hip Osteoarthritis in Male and Female Wistar Rats

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Received: 06 March, 2017; Accepted: 16 July, 2017

Abstract

Background: Osteoarthritis (OA) is a slowly progressive degenerative disease characterized by gradual loss of articular cartilage. The influence of excessive running load on the development of knee OA was investigated in male Wistar rats. This study was done to test the sex-related difference in the risk of OA of the hip joint after moderate running exercise.

Materials and Methods: Forty male and female Wistar rats were randomly assigned to four equal groups (2 male and 2 female groups) in a same condition. Ten of each sex were selected as control groups and kept separately while running exercises were performed in remained 20 male and female rats using a motor treadmill to motivate rats to run daily distances of 1 km at 5 days/week within six weeks. The treadmill incline was zero. On day 43, all control and training animals were killed and the hip articular cartilage and its synovial layer were evaluated microscopically.

Results: The appearance of hip articular cartilage was normal and similar in all male, female and also male running groups while female running group showed some changes. Obtained results showed a mild OA only in the female running group. There wasn't seen synovitis in both male and female running groups in comparison with control groups ($P>0.05$).

Conclusion: This study showed that the development of hip OA may be related to the sex differences as seen in the knee OA previously.

Keywords: Osteoarthritis, Hip joint, Exercise, Sex, Rat

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Please cite this article as: Naeini R M, Sahebalzamani M, Nazem M N. Effect of moderate treadmill exercise on hip osteoarthritis in male and female Wistar rats. Novel Biomed. 2017;5(4):177-84.

Introduction

Movement training is the very important way to improve quality, different training mode and intensity can lead to different effects to the human body, while unreasonable movement training can even induce injury. Both excessive motion of the joint[1] and excessive exposure of load on the joint are known clinically and experimentally to cause degeneration or damage of the articular cartilage[2,3].

The term "osteoarthritis" (OA) is used to represent a heterogeneous group of joint disorders in patients presenting with joint pain and stiffness. OA is a disease with a truly formidable impact. As the most common form of arthritis, it accounts for more dependency in walking, stair climbing, and other lower extremity tasks than any other disease, especially in the elderly[4].

History of an injury to a joint, particularly at the knee

and hip, is associated with an increased risk for osteoarthritis in cross-sectional and case–control studies[5]. To date, prospective studies have examined the relation between history of joint injury and OA in middle-aged persons and senior citizens[6,7], but not in young adults. However, many athletic injuries occur in high school and college. For example, hip injuries are reported to be common among hockey players and are receiving increased attention in the literature[8,9,10,11]. In addition, joint trauma may be a more common cause of osteoarthritis than has been previously recognized[12].

Running (in both humans and animal models) has been thought to be a very eligible and popular physical activity for many years; however, the repetitive nature of running can lead to overuse injuries in the weighted joint[1,13]. The character of this activity is the repeatability, which can contribute to the gradual accumulation of knee cartilage damage due to insufficient recovery time between the demands theoretically, further lead to the overuse injuries of cartilage[1].

Osteoarthritis of the hip is categorized as primary (idiopathic) or secondary (systemic or localized) disease. Risk factors for primary osteoarthritis of the hip include old age, high bone mass, a genetic predisposition for the disease, increased body-mass index, participation in weight-bearing sports (e.g., running at an elite level), and occupations that require prolonged standing, lifting, or moving of heavy objects[14-17]. Polymorphisms and signaling pathways involved with the development and metabolism of bone and cartilage have also been linked to the risk of osteoarthritis[18-20].

Some studies have investigated the effect of high-intensity running on the knee joint OA[1,21-23] while there is not enough information about this physical activity in the hip joint. In this search, based on previous studies[1,21-23] we built a model of cartilage overuse injury in the rat knee joint (moderate OA) by the daily high-intensity running, and then evaluated the effects of this high-intensity exercise on the hip joint.

Methods

All procedures involving the experimental use of animals were approved by the Animal Ethics Committee, a branch of the Research Council of the Veterinary School at Shahid Bahonar University, Kerman Province, Iran.

Twenty, 9–10-week old, male Wistar rats, and also twenty 10-11-week old, female Wistar rats, initially weighing 190 to 200 g, were randomly assigned to four equal groups (2 male and 2 female groups) and housed in metabolic cages with free access to commercial rodent diet and water. All rats were allowed a 5-day adaptation period in a room with controlled conditions (temperature 22–25 °C and humidity 60–70%) before starting the experiment. This protocol was performed, in accordance with the international guiding principles for biomedical researchers involving laboratory animals, at the Veterinary Medicine Faculty, Shahid Bahonar University of Kerman, Iran.

Ten of each sex were selected as control groups and kept separately. Running exercises were performed in remained 16 male and female Wistar rats. One week after adapting period, both male and female groups commenced 6 weeks of exercise on a motor-driven treadmill (Model T510, DRI Co., Taoyuan, Taiwan). There was a wind stimulus at the end of it. The speed was set at 18-20 m/min and the rats ran on the treadmill for 60 minutes each day for 5 days of every week. On the other word, the running load was set at 1000 m/day for a 5 day/week program. The treadmill incline was zero. This intensity was considered a moderate level of exercise[24].

On day 43, animals were killed by cervical dislocation under anesthesia. After necropsy, the heads of the right femur were separated of acetabulum immediately after sacrifice and examined grossly. Then left hip joints without separation of acetabulum (including cartilage and subchondral bone) were floated in 10% paraformaldehyde solution in order to light microscope histological observation. On day 10, all samples for light microscope histological observation were decalcified with 10% formic acid decalcifying fluid; after being embedded in paraffin, 5μm sections were cut at the mid of femoral head near the fovea capitis perpendicularly and stained with hematoxylin-eosin method finally.

The histological sections were evaluated using the Mankin scheme[25]. We only differed safranin-O staining with hematoxylin-eosin method. In order to

histological grading of synovial layer changes, Krenn et al's method was used[26]. Both Mankin and synovial evaluated parameters and also grading of them are mentioned in Tables 1 and 2.

Results were expressed as Means±SE using the software SPSS 16 (Statistical Package for the Social Sciences, version 16, SPSS; Chicago, USA).

Results

The synovial layer in both male and female control groups was normal. There was a not seen multi-layer cell or inflammatory cells in these groups. Male examples in running group except 3 ones, showed a normal condition as males in the control group. Two male examples showed just an increase in their layers up to 3 layers and also a mild filtration of plasma cells was seen in one of them (Table 1).

At sacrifice, all right hip joints were opened and examined for gross morphological degeneration to the articular cartilage, inflammation and osteophytosis. Cartilage in both male and female control groups appeared glossy and translucent, with a smooth surface. Differences in the findings between male controls with male running group could not be observed (Table 3).

At 6-week, in the female running group, the synovium of joint appeared slightly coarse and inspissate only in 4 female examples in running groups, and with a mild hyperemia dropsy display (Figure 1). In addition, based on grading by Krenn et al. (2002), the grading of synovial layer in female and male running groups were 1±0.47 and 0.4±0.22 respectively (Table 1). In addition, both running groups showed no synovitis as normal groups. On the other hand, superficial cartilage irregularity in the femur head could be found only in 7 female examples of running group (Figure 2). Osteophytes were not seen in any of the hip joints, and no other gross abnormalities were noted in any of the hip joint cartilages (Table 2).

Based on obtaining results, we suggest that moderate running exercise may lead to a mild osteoarthritis in the hip joint of some females after 6 weeks.

Discussion

The joint is a complex organ, made up of periarticular and subchondral bone, articular cartilage, synovial membrane, joint capsule, and periarticular musculature. Deleterious effects of trauma that compromise the structural integrity of one or more of these joint constituents are implicated in the development of osteoarthritis [27]. Harmful forces inflicted on a joint during an injury lead to cartilage breakdown, trabecular microfracture, and bone remodeling [28,29].

The pathogenesis of osteoarthritis is not completely understood. Osteoarthritis most likely begins with degradation of the articular cartilage in a localized, nonuniform manner. This process is followed by a subsequent thickening of the subchondral bone, new bony outgrowths at joint margins (referred to as osteophytes), and mild-to-moderate synovial inflammation. The initiating events that lead to osteoarthritis are not clearly established, but are probably due to abnormal signals that alter the chondrocyte phenotype so that it synthesizes proteins that degrade the matrix and causes the joint to degenerate[30].

There is a relationship between a rather subtle deformity of the proximal femur, which is called the ‘‘tilt deformity’’ to the subsequent development of osteoarthritis (OA) of the hip[31]. The new theory first required an extensive set of exclusion criteria: inflammatory diseases of the hip (such as rheumatoid arthritis, ankylosing spondylitis, Reiter’s syndrome, or lupus) as well as calcium pyrophosphate disease diffuse idiopathic skeletal hyperostosis, gout, and hemochromatosis. Also excluded were osteonecrosis and fractures around the joint, including fracture of the acetabulum, femoral head, or femoral neck. Similarly, cases of damage to the cartilage from infection or resulting from penetration of a fixation device into the joint space were excluded. This set of exclusions was necessary to narrow the definition of hip OA to those causes that arise within the joint itself without extraneous inflammatory, traumatic, and metabolic causes, such as OA that can develop after an earlier septic damage or after rheumatoid arthritis has damaged the joint at an earlier time and has burned out as an active rheumatoid process[31].

Table 1: Synovial layer grading system of osteoarthritis and obtained results in male and female examples*..

Category	Grade	Male (example number)											Female (example number)									
I. Enlargement of the synovial lining cell layer		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
a:The lining cells form one layer	**0**		*	*		*	*	*	*	*	*					*		*	*	*	*	*
b: The lining cells form 2–3 layers	**1**	*			*								*		*		*					
c: The lining cells form 4–5 layers, few multinucleated cells might occur	**2**													*								
d:The lining cells form more than 5 layers, the lining might be ulcerated and multinucleated cells might occur	**3**																					
II. Density of the resident cells																						
a: The synovial stroma shows normal cellularity	**0**	*	*	*		*	*	*	*	*		*				*	*	*	*	*	*	*
b: The cellularity is slightly increased	**1**				*								*	*	*							
c: The cellularity is moderately increased, multinucleated cells might occur	**2**																					
d: The cellularity is greatly increased, multinucleated giant cells, pannus formation and rheumatoid granulomas might occur	**3**																					
III. Inflammatory infiltrate																						
a: No inflammatory infiltrate	**0**	*	*	*	*	*	*	*	*			*				*		*	*	*	*	*
b: Few mostly perivascular situated lymphocytes or plasma cells	**1**									*			*	*		*						
c: Numerous lymphocytes or plasma cells, sometimes forming follicle-like aggregates	**2**																					
d: Dense band like inflammatory infiltrate or numerous large follicle-like aggregates	**3**																					
Total	**9**	**1**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**1**	**0**		**3**	**4**	**2**	**1**	**0**	**0**	**0**	**0**	**0**	**0**

Cross-sectional studies have shown that a history of joint injury is related to knee and hip osteoarthritis[32,33]. When the exposure (injury) and outcome (osteoarthritis) are assessed at the same point in time, it is difficult to discern which one occurred first. Injury may result from an unsteady, mechanically impaired knee and may not predate the development of osteoarthritis[34].

One theory of pathogenesis for osteoarthritis is that excessive body weight and the associated increased stress on joints induce the transformation of passive hip joint laxity to functional hip joint laxity, thereby initiating osteoarthritis[35]. Excessive body weight has been determined as a risk factor for development of osteoarthritis in humans, guinea pigs, mice, and dogs[36]. In this study, we showed a same moderate exercise in males and females with the same body weight may lead to a mild OA only in the hip of females.

It is reported in the literature that women have a higher prevalence of knee and certain types of hand OA than men, particularly after 50 years of age[37], whereas the evidence for a sex difference in hip OA is conflicting from individual study results[38].

Jones et al. (2000) previously reported that males had significantly more knee cartilage than females in healthy children, even after adjustment for other confounders and concluded that sex-related differences in cartilage development might be one explanation for sex variation in knee OA observed in later life [39]. Ding et al. (2003) reported that men had significantly larger patellar and femoral cartilage volume than women, independently of body and bone size. Tibial cartilage volume was larger in men, but became non-significant after adjustment for body and bone size[40]. Faber et al. (2001) reported sex differences of similar magnitude in tibial and femoral but not patellar cartilage volume in young healthy subjects. These differences became non-significant after adjustment for body weight and height[41].

Based on results that showed by Ding et al. (2003), the sex differences appeared, in part, to be mediated by the body and bone size, but not physical activity [40]. Furthermore, these differences became larger after the age of 50 suggesting that the sex differences are due to both cartilage development and cartilage loss in later

Table 2: Mankin histological and histochemical grading system of osteoarthritis and obtained results in male and female examples.

Category	Grade	Male (example number)										Female (example number)									
I. Structure		1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
a. Normal	**0**	*	*	*	*	*	*	*	*	*	*				*	*	*	*	*	*	*
b. Surface irregularity	**1**											*		*							
c. Pannus and surface irregularity	**2**												*								
d. Clefts to transitional zone	**3**																				
e. Clefts to radial zone	**4**																				
f. Clefts to calcified zone	**5**																				
g. Complete disorganization	**6**																				
II. Cells																					
a. Normal	**0**	*	*			*	*	*	*	*	*					*	*		*	*	*
b. Diffuse hypercellularity	**1**			*	*							*		*	*			*			
c. Cloning	**2**												*								
d. Hypocellularity	**3**																				
III. Safranin-O staining■																					
a. Normal	**0**	*	*			*	*	*	*	*						*			*	*	
b. Slight reduction	**1**			*	*						*	*	*	*	*		*	*			*
c. Moderate reduction	**2**																				
d. Severe reduction	**3**																				
e. No dye noted	**4**																				
IV. Tidemark integrity	**1**																				
a. Intact	**0**	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
b. Crossed by blood vessels	**1**																				
Total	**14**	**0**	**0**	**2**	**2**	**0**	**0**	**0**	**0**	**0**	**1**	**3**	**5**	**3**	**2**	**0**	**1**	**2**	**0**	**0**	**1**

■ In this study H&E staining was used instead of Safranin-0 staining.

life[42]. In contrast, in a sample of 18 young subjects, Faber et al. (2001) reported that men had 20–47% higher knee cartilage volume, and that the sex differences became non-significant at all sites after adjustment for body weight and height[41]. We did not measure cartilage thickness or hip cartilage volume.

In order to explain the difference between the male and female's cartilage volume, the most likely candidates are sex hormones and growth factors. Oestrogen, progesterone and testosterone receptors are present in human fetal cartilaginous tissue[43], and androgens can stimulate human chondrocyte proliferation as well as collagen and proteoglycan synthesis[44]. Hormone replacement therapy has been associated with higher cartilage volume in post-menopausal women[45]. In addition, recent evidence suggested that there was a positive association between knee cartilage volume and serum testosterone at tibial cartilage sites, which only reached statistical significance for medial tibial cartilage where serum testosterone explained up to 8% of the variation in cartilage volume[46]. Similarly, growth factors such as transforming growth factor-ɑ and insulin-like growth factor-1 play important roles in articular cartilage formation and proteoglycan synthesis[47]. We suggest that these factors may effect on the hip cartilage volume in the men and females.

When running, the change of the loading on the surface of articular cartilage is relatively slow, and the loading proportion on the cartilage matrix and matrix deformation degree are great. Cartilage structure deformation mainly depends on matrix liquid flowing out. The collagen framework deforming slowly, related liquid flowing thoroughly, stress distributing in a balance mode, all of these prevent partial hypertension from happening. For this reason, damage of cartilage matrix structure is relatively slight in exercise of excess running. However, when running,

Table 3: Average (Mean±SE) of obtained results in Mankin and synovial gradings.

	Male		Female	
	Control	**Runner**	**Control**	**Runner**
Mankin	0^{a}	0.5 ± 0.27^{a}	0^{a}	1.7 ± 0.52^{b}
Synovial Layer*	0^{a}	0.4 ± 0.22^{a}	0^{a}	1 ± 0.47^{b}

^{a,b} Means in a row without a common superscript are different ($P < 0.05$)
0*: Sum 0-1 = No synovitis (Normal); Sum 2–4 = Low-grade synovitis; Sum 5–9 = High-grade synovitis

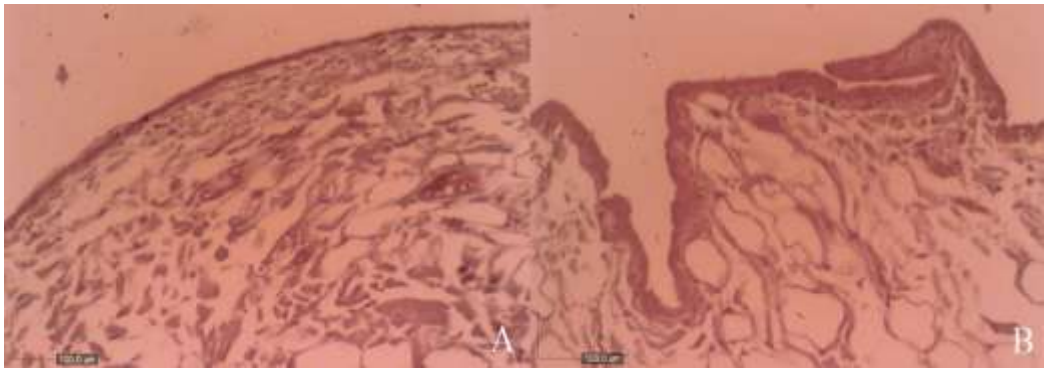

Figure 1. Synovial layer cells in control (A) and female running (B) groups. There is more than one layer cell (and also less than four layer cell) in the female running group compare to the one layer cell in control group (H&E, magnification ×80 and ×200 in A and B respectively).

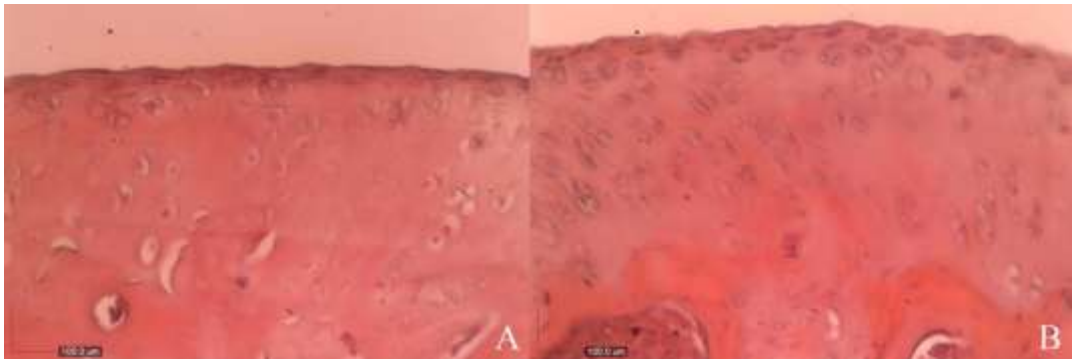

Figure 2. Articular cartilage surface in control (A) and female running (B) groups. There is surface irregularities, some diffuse hypercellularity and slight reduction in the staining in the female running group compare to the control one that is evaluated as mild OA (H&E, magnification ×200).

much liquid drains out from cartilage matrix, collagen framework deforms lastingly, and spatial structure of cartilage matrix changes greatly, which lead to great deforming force effecting on the chondrocyte embedded in the matrix. Therefore, Qi and Changlin (2008) considered that damage of chondrocyte is the main aspect in the cartilage injury induced by excess running. This view had been proved in their study: dead cell ratio had a significant elevation after 4 weeks of high-intensity running training, and much degenerated chondrocytes with deformed nucleus, expanded endoplasmic reticulum, and increased heterochromatin were found in observation with transmission electron microscope[1].

Conclusion

Excessive and repetitive use of joint, may cause partial load and attrition increasing on the articular cartilage surface, stimulates the inflammatory reaction, or even cartilage tissue degeneration. In this research, the male and female rats were trained in moderate-intensity running mode, and observed the some mild pathological changes of articular cartilage in the hip joint at 6-week only in the female running group. Mankin grades is a common used scoring standard for the evaluation of cartilage injury degree, which grades the examples for structure, matrix dyeing, tidemark

integrity, and cellularity. This study showed Mankin grades in female running group at 6-week was significantly higher than female control group, while there was no difference between male running and control groups. Evaluation the synovial layer and Mankin grade in two male and female running groups showed moderate running exercise could induce a mild cartilage injury only in the female's hip joint directly.

Acknowledgment

This research was financially supported by the research council of Shahid Bahonar University of Kerman (No: 1.7.1395). The authors would like to thank Mr. Mazhab Jaafari for providing histological laboratory methods and histology slides. The authors declared no conflict of interests.

References

1. Qi Ch, Changlin H. Effects of different mode high intensity movement training on articular cartilage in histology – A randomized controlled trial on rabbit knee. Biology of Sport. 2008;25(4):371-86.
2. Chen CT, Bhargava M, Lin PM, Torzilli PA. Time, stress, and location dependent chondrocyte death and collagen damage in cyclically loaded articular cartilage. Journal of Orthopedic Research. 2003;21:888-98.
3. Loening AM., James IE, Levenston ME, Badger AM, Frank EH, Kurz B, et al. Injurious mechanical compression of bovine articular cartilage induces chondrocyte apoptosis. Archives Biochemistry Biophysics. 2000;381:205-12.
4. Lane NE. Osteoarthritis of the Hip. The New England Journal of Medicine. 2007;357:1413-21.
5. Roddy E, Zhang W, Doherty M. Aerobic walking or strengthening exercise for osteoarthritis of the knee? A systematic review. Annual Rheumatology Disease. 2005;64:544-8.
6. Giannoni P, Siegrist M, Hunziker EB, Wong M. The mechanosensitivity of cartilage oligomeric matrix protein (COMP). Biorheology. 2003;40:101-9.
7. Murata M, Bonassar LJ, Wright M, Mankin HJ, Towle CA. A role for the interleukin-1 receptor in the pathway linking static mechanical compression to decreased proteoglycan synthesis in surface articular cartilage. Archives Biochemistry Biophysics. 2003;413:229-33.
8. Bizzini M, Notzli HP, Maffiuletti NA. Femoroacetabular impingement in professional ice hockey players: a case series of 5 athletes after open surgical decompression of the hip. American Journal of Sports Medicine. 2007;35:1955-9.
9. Philippon MJ, Weiss DR, Kuppersmith DA, Briggs KK, Hay CJ. Arthroscopic labral repair and treatment of femoroacetabular impingement in professional hockey players. American Journal of Sports Medicine. 2010;38:99-104.
10. Torry MR, Schenker ML, Martin HD, Hogoboom D, Philippon MJ. Neuromuscular hip biomechanics and pathology in the athlete. Clinical Sports Medicine. 2006; 25:179-97.
11. Tyler TF, Nicholas SJ, Campbell RJ, Donellan S, McHugh MP. The effectiveness of a preseason exercise program to prevent adductor muscle strains in professional ice hockey players. American Journal of Sports Medicine 2002; 30:680-683.
12. Valhmu WB, Raia FJ. Myo-inositol 1,4,5-trisphosphate and Ca2þ/calmodulin-dependent factors mediate transduction of compression-induced signals in bovine articular chondrocytes. Biochem J. 2002;361:689-96.
13. Gerberich SG, Luhmann S, Finke C, Priest JD, Beard BJ. Analysis of severe injuries associated with volleyball activities. Physician Sports medicine. 1987;15:75-9.
14. Lievense AM, Bierma-Zeinstra SA, Verhagen AP, Verhaar JAS, Koes BW. Influence of work on the development of osteoarthritis of the hip: a systematic review. Journal of Rheumatology. 2001;28:2520-8.
15. Lievense AM, Bierma-Zeinstra SA, Verhagen AP, Bernsen RM, Verhaar JAS, Koes BW. Influence of sporting activities on the development of osteoarthritis of the hip: a systematic review. Arthritis Rheumatology. 2003;49:228-36.
16. Lievense AM, Bierma-Zeinstra SA, Verhagen AP, Verhaar JAS, Koes BW. Influence of hip dysplasia on the development of osteoarthritis of the hip. Annual Rheumatology Disease. 2004;63:621-6.
17. Lane NE, Lin P, Christiansen L, Gore LR, Williams EN, Hochberg MC, et al. Association of mild acetabular dysplasia with an increased risk of incident hip osteoarthritis in elderly white women: the Study of Osteoporotic Fractures. Arthritis Rheumatology. 2000;43:400-4.
18. Lane NE, Lian K, Nevitt MC, Zemuda JM, Lui L, Wang J, et al. Frizzled-related protein variants are risk factors for hip osteoarthritis. Arthritis Rheumatology. 2006;54:1246-54.
19. Lian K, Zmuda JM, Nevitt MC, Lui L, Hochberg MC , Greene D, et al. Type I collagen alpha1 Sp1 transcription factor binding site polymorphism is associated with reduced risk of hip osteoarthritis defined by severe joint space narrowing in elderly women. Arthritis Rheumatology. 2005;52:1431-6.
20. Loughlin J. The genetic epidemiology of human primary osteoarthritis: current status. Expert Reviews in Molecular Medicine Journal. 2005;7:1-12.
21. Kaiki G, Tsuji H, Yonezawa T, Sekido H, Takano T, Yamashita S, Hirano N, Sano A. Osteoarthrosis induced by intra-articular hydrogen peroxide injection and running load. Journal of Orthopedic Research. 1990;8:731-40.
22. Lovasz G, Llinas A, Benya P, Bodey B, McKellop HA, Luck Jr JV, Sarmiento A. Effects of valgus tibial angulation on cartilage degeneration in the rabbit knee. Journal of Orthopedic Researh. 1995;13:846-53.
23. Pap G, Eberhardt R, Sturmer I, Machner A, Schwarzberg H, Roessner A, Neumann W. Development of osteoarthritis in the knee joints of Wistar rats after strenuous running exercise in a running wheel by intracranial self-stimulation. Pathology Research and Practice. 1998;194:41-7.
24. Iwamoto J, Takeda T, Ichimura S: Effect of exercise on tibial and lumbar vertebral bone mass in mature osteopenic rats: bone

histomorphometry study. Journal of Orthopaedics Science. 1998;3(5):257-63.
25. Mankin HJ, Dorfman H, Lippiello L, Zarins A. Biochemical and metabolic abnormalities in articular cartilage from osteoarthritic human hips II. Correlation of morphology with biochemical and metabolic data. The Journal of Bone and Joint Surgery. American Volume. 1971;53:523–37.
26. Krenn V, Morawietz L, Häupl T, Neidel J, Petersen I, König A. Grading of chronic synovitis – A histopathological grading system for molecular and diagnostic pathology. Pathology Research Practice. 2002;198(5):317-25.
27. Yamamoto K, Shishido T, Masaoka T, Imakiire A. Morphological studies on the ageing and osteoarthritis of the articular cartilage in C57 black mice. Journal of Orthopedic Surgery. 2005;13:8-18.
28. Galois L, Etienne S, Grossin L, Watrin-Pinzano A, Cournil-Henrionnet C, Loeuille D, et al. Dose-response relationship for exercise on severity of experimental osteoarthritis in rats: a pilot study. Osteoarthritis Cartilage. 2004;12:779-86.
29. Roos EM, Dahlberg L. Positive effects of moderate exercise on glycosaminoglycan content in knee cartilage. Arthritis Rheumatology. 2005;52:3507-14.
30. DiCesare PE, Abramson SB. Pathogenesis of osteoarthritis. In: Harris ED, Budd RC, Firestein GS, eds. Kelley's text-book of rheumatology. 7th ed. Vol. 2. Philadelphia: Elsevier/Saunders. 2005:1493-513.
31. Ganz R, Leunig M, Leunig-Ganz K, Harris WH. The etiology of osteoarthritis of the hip, an integrated mechanical concept. Clinical Orthopedic Related Research. 2008;466:264–72.
32. Guzman RE, Evans M, Bove S, Morenko B, Kilgore K. Monoiodoacetate-induced histologic changes in subchondral bone and articular cartilage of rat femorotibial joints: an animal model of osteoarthritis. Toxicology Pathology. 2003;31:619-24.
33. Leandro CG, Levada AD, Hirabara SM, Manhaes-De-Castro R, De-Castro CB, Curi R, et al. A program of moderate physical training for Wistar rats based on maximal oxygen consumption. Journal of Strength Conditioning Research. 2007;21:751-6.
34. Cifuentes DJ, Rocha LG, Silva LA, Brito AC, Rueff-Barroso CR, Porto LC, et al. Decrease in oxidative stress and histological changes induced by physical exercise calibrated in rats with osteoarthritis induced by monosodium iodoacetate. Osteoarthritis and Cartilage. 2010;18:1088-95.
35. Kapatkin AS, Fordyce HH, Mayhew PD, Smith GK. Canine hip dysplasia: the disease and its diagnosis. Compendium on Continuing Education for the Practicing Veterinarian. 2002;24:526–38.
36. Smith GK, Paster ER, Powers MY, Lawler DF, Biery DN, Shofer FS, et al. Lifelong diet restriction and radiographic evidence of osteoarthritis of the hip joint in dogs. Journal of the American Veterinary Medical Association. 2006;229(5):690-3.
37. Sowers M. Epidemiology of risk factors for osteoarthritis: systemic factors. Current Opinion Rheumatology 2001;13(5):447-51.
38. Srikanth VK, Fryer B.Math JL, Zhai G, Winzenberg TM, Hosmer D, Jones G. A meta-analysis of sex differences prevalence, incidence and severity of osteoarthritis. OsteoArthritis and Cartilage. 2005;13:769-81.
39. Jones G, Glisson M, Hynes K, Cicuttini F. Sex and site differences in cartilage development: a possible explanation for variations in knee osteoarthritis in later life. Arthritis Rheumatology. 2000;43:2543–8.
40. Ding C, Cicuttini F, Scott F, Glisson M, Jones G. Sex differences in knee cartilage volume in adults: role of body and bone size, age and physical activity. Rheumatology. 2003;42:1317–23.
41. Faber SC, Eckstein F, Lukasz S, Muhlbauer R, Hohe J, Englmeier KH, et al. Sex differences in knee joint cartilage thickness, volume and articular surface areas: assessment with quantitative three-dimensional MR imaging. Skeletal Radiology. 2001;30:144–50.
42. Pierce CM, Laprade RF, Wahoff M, O'Brien L, Philippon MJ. Ice hockey goaltender rehabilitation, including on-ice progression, after arthroscopic hip surgery for femoroacetabular impingement. Iournal of Orthopaedic and Sports Physical Therapy. 2013;43(3):129-41.
43. Ben-Hur H, Thole HH, Mashiah A, Insler V, Berman V, Shezen E, et al. Estrogen, progesterone and testosterone receptors in human fetal cartilaginous tissue: immunohistochemical studies. Calcified Tissue International. 1997;60:520–6.
44. Franchimont P, Bassleer C. Effects of hormones and local growth factors on articular chondrocyte metabolism. Journal of Rheumatology. 1991;27(Suppl.):68–70.
45. Wluka AE, Davis SR, Bailey M, Stuckey SL, Cicuttini FM. Users of oestrogen replacement therapy have more knee cartilage than non-users. Annual Rheumatology Disease. 2001;60:332–6.
46. Cicuttini F, Wluka A, Baily M. Factors affecting knee cartilage volume in healthy men. Osteoarthritis Cartilage. 2002;10(Suppl. A):S59.
47. van der Kraan PM, Buma P, van Kuppevelt T, van den Berg WB. Interaction of chondrocytes, extracellular matrix and growth factors: relevance for articular cartilage tissue engineering. Osteoarthritis Cartilage. 2002;10:631–7.