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Pre- and post-exercise ADAMTS-4 and ADAMTS-5 Levels in Concur Horses

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Abstract

A disintegrin-like and metalloproteinase with thrombospondin motifs (ADAMTS) proteinase family play an important role in many physiological and physiopathological processes such as the maintenance of locomotor system health in sport horses. In this study, we aimed to determine the changes of ADAMTS-4 and ADAMTS-5 levels in concour horses before and after exercise.
The Oldenburg and Selle Français horse-breed types which are healthy, 6-15 years old, around 650-750 kg, and distinct genders were used (n=10). Following the physical examinations, the horses were subjected to 50 minutes of regular exercise program. Blood samples were collected into anticoagulant-free tubes in order to determine ADAMTS-4 and ADAMTS-5 mRNA expression and ELISA levels before and after exercise.
There were no differences were observed statistically on ADAMTS-4, neither mRNA expression in spite of 25% downregulated, nor at the ELISA levels. On the other hand, ADAMTS-5 mRNA expression were upregulated 3.88 fold ($p<0.01$), as well ELISA level significantly increased as 1.2 fold ($p<0.01$).
In conclusion, the increase of the serum ADAMTS-5 levels may be one of the potential biomarkers in the joint disorders. However, it is necessary to investigate more extensively to clarify its action with clinical evidence.

Key Words: ADAMTS, aggrecanase, equine exercise, metalloproteinase

Konkur Atlarında Egzersiz Öncesi ve Sonrası ADAMTS-4 ve ADAMTS-5 Düzeyleri

Öz

A disintegrin-like and metalloproteinase with thrombospondin motifs (ADAMTS) proteinaz ailesi, spor atlarında lokomotor sistem sağlığının korunması gibi birçok fizyolojik ve fizyopatolojik olayda önemli rol oynamaktadır. Bu çalışmada, yarış atlarında egzersiz öncesi ve sonrasındaki ADAMTS-4 ve ADAMTS-5 düzeylerinin belirlenmesi amaçlanmıştır.
Bu amaçla, sağlıklı, 6-15 yaşları arasında, 650-750 kg ağırlığında, farklı cinsiyetlerden, Oldenburg ve Selle Français ırkı yarış atları (n=10) kullanıldı. Atların fiziksel muayeneleri ardından, 50 dakikalık standart egzersiz programı uygulandı. Egzersiz öncesi ve sonrasında ADAMTS-4 ve ADAMTS-5 mRNA ekspresyonlarını ve ELİSA düzeyleri ölçülmek üzere antikoagulansız tüplere kan örnekleri alındı.
ADAMTS-4 mRNA ekspresyonu %25 oranında azalmasına rağmen istatistiki olarak anlamlı bulunmadı ve ELİSA düzeyinde de değişim gözlenmedi. ADAMTS-5 mRNA ekspresyonu 3.88 kat arttı ($p<0.01$) ve ELİSA düzeyi de anlamlı olarak 1.2 kat arttı ($p<0.01$).
Sonuç olarak, serum ADAMTS-5 düzeylerindeki artışın, atlarda eklem hastalıklarının da potansiyel biyolojik belirteçlerinden biri olabileceği ve etkilerini netleştirmek için klinik bulgularla desteklenen daha detaylı araştırmaların yapılması gerektiği kanısına varıldı.

Anahtar Kelimeler: ADAMTS, agrekanaz, at egzersiz, metalloproteinaz

INTRODUCTION

Sports horses are often challenged to prepare for individual competitions in different disciplines, such as show jumping, dressage, and endurance. Although maintenance, the feeding and training programs vary according to discipline; the factors that the muscles, joints, ligaments, tendons, and hoofs of the horses are subjected to anatomically are increased due to the intensity of the training and competitions. This makes sports horses more prone to orthopedic problems (1-3). Spontaneous joint diseases, osteoarthritis (OA), laminitis, tendinitis and, synovitis are the main problems faced by sport horses, resulting in loss of performance and consequently the end of competitive life (1, 3, 4). Treatment of joint disorders is long, costly, and laborious. Besides, associated diseases after joint discomfort also cause major economic losses for the breeder. Studies show that 60% of laminitis formed in horses is caused by OA (5).

Articular cartilage structurally composed of partially chondrocyte cells and a large number of extracellular matrix components. Many macromolecules have been identified in

cartilage tissue, including collagen fibrils, aggregate proteoglycans, and glycoproteins. Although joint damage is caused by oxidative metabolism-induced free radicals and hypoxic conditions, the main reason is the increase in proteolytic enzymes. Matrix metalloproteinases (MMPs), pro- and anti-inflammatory cytokines interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α) and retinoic acid are the main biomarkers recommended for the diagnosis of joint damage (6). IL-1 and TNF-α play an important role in the physiopathology of many diseases ranging from periodontal diseases (7) to cancer (8) and it is emphasized that they can be considered as biomarkers in the literature. However, MMPs actively involved in cartilage destruction during the progression of joint disease are involved in normal anabolic and catabolic metabolism (turnover) of the aggregate and IL-1 and TNF-α do not show tissue-specific distribution, the potential to be used as an early biomarker in diagnosis is weakening especially in aseptic degenerative joint diseases. Indeed, Billinghurst et al. (9) reported an increase in TNF-α levels in acute joint inflammation, whereas this increase did not occur in the case of chronic arthropathy. Jouglin et al. (10) also reported that they could not find any correlation between TNF-α activity and cartilage tissue damage and therefore both authors stated that TNF-α is limited in its use as a biomarker for arthritis. A biomarker that can be used with 100 percent accuracy for the early diagnosis of horses' orthopedic disorders has not yet been determined.

There are nineteen known types of ADAMTS proteinases family and it is one of the important biomarkers that can be evaluated in the early diagnosis of diseases due to their roles in the physiopathological mechanisms of many diseases such as cancer, arthritis, and atherosclerosis (11-15). ADAMTS-4 and ADAMTS-5 have been reported to play an important role in the pathogenesis of osteoarthritis in humans and various animals following their first molecular purification and cloning (12, 15, 16). Although their expression has been detected in many tissues such as the brain, heart, and lung, the main function is the degradation of aggregate in cartilage tissue. ADAMTS-4 and ADAMTS-5, which are discovered as aggrecanase-1 (17) and aggrecanase-2 (16), are distributed in horses' hoof lamina (18) and joints (19) and are expressed more in cartilage tissue than other tissues (20). Due to the characteristics of the ADAMTS family members, it has been shown that they are the most suitable candidate molecules to be specific biomarkers for various diseases (11, 14, 21-23).

In this study, it was aimed to determine the genetic and enzymatic changes in serum ADAMTS-4 and ADAMTS-5 levels before and after exercise in concour horses competing professionally in the discipline of showjumping.

MATERIALS AND METHODS

Animal Material

To investigate the alteration of ADAMTS-4 and ADAMTS-5 levels, 6-15 years old and 650-750 kg weight Oldenburg and Selle Français breeding, gelding and mare horses (n=10) used for the study. The study was conducted with the ethics committee permission given (Date: 21/10/2016 Number: 78543580/9) by Cukurova University Faculty of Medicine, Experimental Medicine Research and Application Center and with the permissions obtained from horse owners. Horses with joint disorders and laminitis in recent years eliminated from the study.

Exercise Program and Sample Collection

Sport horses involved in the study fed by ~ 6 kg of concentrated feed (Optima Yem, Turkey) per day, and Cosequine® Original (Nutramaxlabs, England) was used as a feed additive for morning and evening scales. Water requirements met as ad libitum and horses held in the individual horse boxes at 18-20°C. Routine physical and laboratory examinations were performed in terms of lameness and any infectious or non-infectious disorder. Then the standard exercise program reported in Table 1 was applied by the same rider to horses which evaluated as healthy. Blood samples collected from the vena jugularis in order to measure serum ADAMTS-4 and ADAMTS-5 levels before and after exercise. Blood samples were centrifuged at 3000 rpm for 10 minutes and the obtained serum stored at -20°C until analysis.

Table 1. Exercise program of horses

Exercise Type	Duration
Walk	10 min
Trot	10 min
Jumping	20 min
Relaxing & Cooling	10 min
TOTAL	50 min

Determination of ADAMTS-4 and ADAMTS-5 Enzyme Activities

Enzyme activities of ADAMTS-4 and ADAMTS-5 were detected by using horse specific enzyme-linked immunosorbent assay (ELISA) kits (Sunred Bio, China) according to the procedures of commercial kits as described by Will et al. (24), and the results were determined by the spectrophotometer. Absorbance was read at λ = 450 nm with a reference filter at λ = 620 nm by ELISA device (Thermo Scientific, MultiscanGO).

Determination of Serum ADAMTS-4 and ADAMTS-5 mRNA Expressions

Alteration in mRNA expressions of ADAMTS-4 and ADAMTS-5 genes were investigated by quantative Real Time PCR (qRT-PCR). Total RNA was isolated from horse blood samples by using qiazol as described manufactural protocols. The qualitative and quantitative analyses of Total RNA were determined by nanodrops and purity of the samples was controlled by agarose gel electrophoresis. cDNA from the obtained RNAs was synthesized using iScript cDNA synthesis kit (Bio-Rad, USA). 20 µL of reaction mixture contains 2 ng total RNA, 1 µL of reverse transcriptase and 4 µl of 5X iScript reaction mixture. The reaction mixture was incubated at 25ºC for 5 minutes, 46°C for 20 min and 95°C for 1 min. The synthesized cDNAs were stored at -20°C to until qRT-PCR studies.

qRT-PCR studies were performed using Bio-Rad CFX Connect (Bio-Rad, USA). 25 µL of the reaction mixture contains 12.5 µL of 2X SYBR Green qPCR Master mix, 0.4 µL of reverse and forward primers and 2 µL of cDNA. As a house keeping gene, GAPDH was used. Primer sequences of ADAMTS-4 and ADAMTS-5 design were shown on Table 2 and Table 3. qRT-PCR cycle was set as initial denaturation at 95ºC for 5 min, then 40 cycles of denaturation at 95 °C for 15 sec, annealing step at 60 °C for 30 sec. The changes in mRNA expression of ADAMTS-4 and ADAMTS-5 were calculated by using $2^{-\Delta\Delta Ct}$ method.

Table 2. ADAMTS-4 Primary Sequence

Forward:	5'-AGCTCAACGGTAGTGTCGTG-3'
Reverse:	5'-GCCATAACTGTCAGCAGGTA-3'

Table 3. ADAMTS-5 Primary Sequence

Forward:	5'-GGCATCATTCATGTGACACC-3'
Reverse:	5'-TTCTGTGATGGTGGCCGAGG-3'

Statistical Analysis

The values obtained from the study were statistically analyzed by GraphPad Prism 8.0 software (San Diego, USA). The differences among groups were evaluated by paired t-test and confidence interval was accepted as 95%.

RESULTS

Serum ELISA results of sports horses, before (BE) and after exercise (AE) are presented in Table 4. Accordingly, ADAMTS-4 levels were measured as BE: 76.27 ± 12.46 ng/ml and AE: 82.60 ± 10.43 ng/ml, respectively and no statistical change was observed (Figure 1). In contrast, serum ADAMTS-5 levels were measured as 1.70 ± 0.18 ng/ml and ES: 1.97 ± 0.19 ng/ml, respectively, and increased significantly by 1.2 times (Figure 2, p<0.01).

Table 4. ADAMTS-4 and ADAMTS-5 levels before and after exercise (n=10, x̄±SEM).

	ADAMTS-4 (ng/ml)	**ADAMTS-5 (ng/ml)**
Before Exercise (BE)	76.27±12.46	1.70±0.18[b]
After Exercise (AE)	82.60±10.43	1.97±0.19[a]

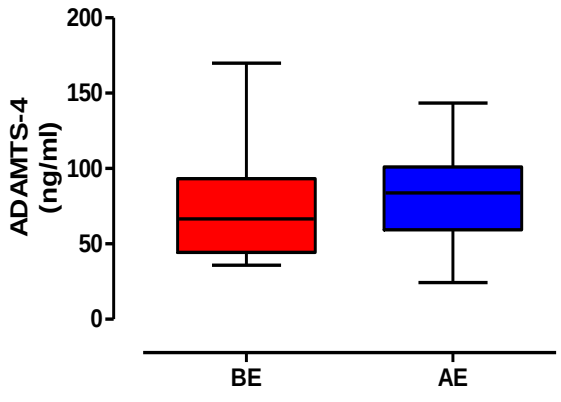

Figure 1. Serum ADAMTS-4 levels (x̄±SEM, p=0.39).

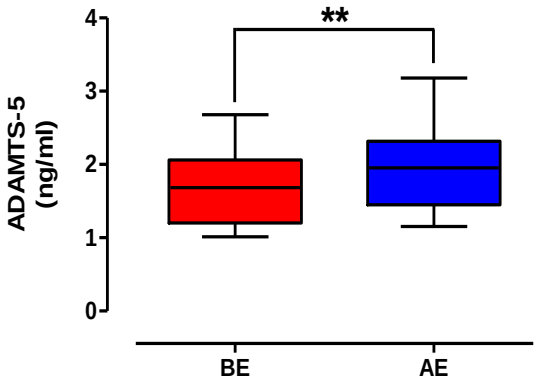

Figure 2. Serum ADAMTS-5 levels (x̄±SEM, p=0.0032).

Serum mRNA levels of ADAMTS-4 and ADAMTS-5 were determined by the qRT-PCR method after RNA isolation and cDNA synthesis in the samples obtained before and after exercise (Figure 3). Accordingly, although the ADAMTS-4 level decreased after the exercise by 25% compared to the pre-exercise group, the data obtained were not statistically significant. ADAMTS-5 level increased 3.88 times after exercise and it was found to be significant (p<0.01).

DISCUSSION AND CONCLUSION

The showjumping process consists of four stages. These are positioning, take-off, landing, and braking. The hind legs of the jumping horses do 0.71 J / kg during jumping, which is 4.5 times the size of a normal walking horse (25), during landing, approximately 10 kN load is applied to the musculus flexor digitalis superficial (MFDS) and musculus flexor digitalis profundus (MFDP) tendons (26). This is why the MFDS and MFDP tendon injuries in the forelimb is more common in jumping horses (1). Egenvall et al. (4), examined 263 elite concur horses, been rode by 31 riders from 4 different countries and reported that 126 horses (48%) lost days during two racing seasons due to orthopedic problems. Thus, the correct biomarkers are crucial to thoroughly perceive articular damage in sport horses on the early stage.

In our study, the ADAMTS-4 serum levels slightly increased but this did not important statistical (Figure 1), on the other hand ADAMTS-5 level increased significantly (Figure 2) after regular exercise program (Table 1) as presented on the Table 4. Exercise is considered to be an effective factor for PBMC infiltration into tissues (27). In vivo and in vitro studies in horses, humans, rats and mices show that exercise enhances synovial macrophage infiltration which regulates proinflammatory cytokines (28-31). It is reported that ADAMTS-4 is mediated by TNF-α, IL-1 and nuclear factor-kappa B (NFκB) released from synovial macrophages, while the regulation of ADAMTS-5 is reported to be independent of the aforementioned cytokines (32). Horses are also used as model organisms for aseptic arthritis which is caused by amphotericin B, an antifungal agent. Amphotericin B enhances peripheral blood mononuclear cell (PBMC) infiltration in human (33), mouse (34), horse (13), and donkeys (35) and regulates the release of proteases responsible for cartilage destruction by IL-1 and TNF-α secreted from these cells,

resulting in cartilage destruction. Ma et al. (36), reported regular increases in levels of IL-1, TNF-α, MMP-2, MMP-3, MMP-9, MMP-13 and ADAMTS -5 in a 9-week study in which they administered OA by administering a single dose of 25 mg/ml amphotericin B. According to routine clinical examinations, neither orthopedic nor septic or aseptic problems were not found of the sport horses used in our study. In this regard, the slight increasing of ADAMTS-4 level could be explain by exercise induced PBMC infiltration, however the ADAMTS-5 level need to clarify on molecular stage.

Besides, McGivney et al. (37) in their study of transcriptional adaptation after exercise in thoroughbred horses reported that ribosomal genes were downregulated immediately after exercise, leading to inhibition of protein synthesis and mechanosensation, muscle hypertrophy, repair and remodeling genes. Also, the polymorphisms on the Adamts5 gene caused susceptibility to ECM involved in the development of knee OA in humans (38, 39).

In this study, it was suggested that the significant increase in ADAMTS-5 mRNA levels may be associated with the usual joint and muscle metabolic cycle as a result of adaptation to exercise following the literature. However, 25% inhibition of ADAMTS-4 mRNA level is an issue that needs to be examined in depth at the molecular level of cytokine-dependent ADAMTS aggrecanase activity in acute response to exercise. The transcriptional responses of ADAMTS-4 and ADAMTS-5 reveal that both aggrecanases follow different pathways in response to exercise.

Additionally, an important footnote related to our observations after complete to the assays was that the horses which have got higher ADAMTS-5 but not -4 serum levels had called the local veterinarian by an orthopedic problem into two or three weeks. There is several limitations need to be addressed in this study. First, this is not an experimentally induced study, thus hard to take permission from horse owners for the sampled blood twice in one hour. Second, the uncontrollable feeds and additives of horses are restricted to statistical explanations of ADAMTS' and other possible interactions. Third, the lack of synovial fluid did not allow to compare the levels with the serum.

ADAMTS aggrecanases plays a critical role in the follow-up of horses' joint and muscle health. Indeed, cytokines and chemokines secreted from mononuclear cells act as regulators of joint degeneration. According to the results of this study, it was concluded that ADAMTS-4 and -5 would to activate by different pathways during exercise. However, there is a need for further transcriptional and translational studies supported by radiological and ultrasonographic findings to elucidate the molecular mechanism of the relationship with ADAMTS aggrecanases of pro- and anti-inflammatory cytokines, peripheral and synovial mononuclear cells and especially synovial macrophages in horses.

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CONFLICT OF INTEREST STATEMENT

SK designed the study. CE performed exercise protocols and collected the blood samples from concour horses, SK and SK performed the analysis and statistics. All authors discussed the results and commented on the manuscript.

REFERENCES

1. Murray RC, Dyson SJ, Tranquille C, Adams V. (2006). Association of Type of Sport and Performance Level with Anatomical Site of Orthopaedic Injury Diagnosis. Equine Vet J Suppl. (36): 411-416. doi:10.1111/j.2042-3306.2006.tb05578.x.
2. Lönnell AC, Bröjer J, Nostell K, et al. (2014). Variation in Training Regimens in Professional Showjumping Yards. Equine Vet J. 46(2): 233-238. doi:10.1111/evj.12126.
3. Yamada ALM, Pinheiro M, Marsiglia MF, et al. (2020). Ultrasound and Clinical Findings in the Metacarpophalangeal Joint Assessment of Show Jumping Horses in Training. J Vet Sci. 21.
4. Egenvall A, Tranquille CA, Lonnell AC, et al. (2013). Days-Lost to Training and Competition in Relation to Workload in 263 Elite Show-Jumping Horses in Four European Countries. Prev Vet Med. 112(3-4): 387-400. doi:10.1016/j.prevetmed.2013.09.013.
5. McIlwraith CW, Frisbie DD, Kawcak CE. (2012). The Horse as a Model of Naturally Occurring Osteoarthritis. Bone Joint Res. 1(11): 297-309. doi:10.1302/2046-3758.111.2000132.
6. Chimenti MS, Triggianese P, Conigliaro P, et al. (2015). The Interplay between Inflammation and Metabolism in Rheumatoid Arthritis. Cell Death Dis. 6: e1887. doi:10.1038/cddis.2015.246.
7. Gomes FI, Aragao MG, Barbosa FC, et al. (2016). Inflammatory Cytokines Interleukin-1beta and Tumour Necrosis Factor-Alpha - Novel Biomarkers for the Detection of Periodontal Diseases: A Literature Review. J Oral Maxillofac Res. 7(2): e2. doi:10.5037/jomr.2016.7202.
8. Baker KJ, Houston A, Brint E. (2019). Il-1 Family Members in Cancer; Two Sides to Every Story. Front Immunol. 10: 1197. doi:10.3389/fimmu.2019.01197.
9. Billinghurst RC, Fretz PB, Gordon JR. (1995). Induction of Intra-Articular Tumour Necrosis Factor During Acute Inflammatory Responses in Equine Arthritis. Equine Vet J. 27(3): 208-216.
10. Jouglin M, Robert C, Valette JP, et al. (2000). Metalloproteinases and Tumor Necrosis Factor-Alpha Activities in Synovial Fluids of Horses: Correlation with Articular Cartilage Alterations. Vet Res. 31(5): 507-515. doi:10.1051/vetres:2000136.
11. Kuno K, Kanada N, Nakashima E, et al. (1997). Molecular Cloning of a Gene Encoding a New Type of Metalloproteinase-Disintegrin Family Protein with Thrombospondin Motifs as an Inflammation Associated Gene. J Biol Chem. 272(1): 556-562.
12. Tortorella MD, Burn TC, Pratta MA, et al. (1999). Purification and Cloning of Aggrecanase-1: A Member of the Adamts Family of Proteins. Science. 284(5420): 1664-1666. doi:DOI 10.1126/science.284.5420.1664.
13. Marttinen PH, Raulo SM, Suominen MM, Tulamo RM. (2006). Changes in Mmp-2 and -9 Activity and Mmp-8 Reactivity after

Amphotericin B Induced Synovitis and Treatment with Bufexamac. J Vet Med A Physiol Pathol Clin Med. 53(6): 311-318. doi:10.1111/j.1439-0442.2006.00837.x.

14. Majumdar MK, Askew R, Schelling S, et al. (2007). Double-Knockout of Adamts-4 and Adamts-5 in Mice Results in Physiologically Normal Animals and Prevents the Progression of Osteoarthritis. Arthritis Rheum. 56(11): 3670-3674. doi:10.1002/art.23027.
15. Kandir S. (2020). Adamts Proteases: Potential Biomarkers and Novel Therapeutic Targets for Cartilage Health. In Equine Science. (Ruthland C ed). pp. 91-105. IntechOpen, London. doi:10.5772/intechopen.93046
16. Abbaszade I, Liu RQ, Yang F, et al. (1999). Cloning and Characterization of Adamts11, an Aggrecanase from the Adamts Family. J Biol Chem. 274(33): 23443-23450. doi:10.1074/jbc.274.33.23443.
17. Tortorella MD, Burn TC, Pratta MA, et al. (1999). Purification and Cloning of Aggrecanase-1: A Member of the Adamts Family of Proteins. Science. 284(5420): 1664-1666. doi:10.1126/science.284.5420.1664.
18. Pawlak E, Wang L, Johnson PJ, et al. (2012). Distribution and Processing of a Disintegrin and Metalloproteinase with Thrombospondin Motifs-4, Aggrecan, Versican, and Hyaluronan in Equine Digital Laminae. Am J Vet Res. 73(7): 1035-1046. doi:10.2460/ajvr.73.7.1035.
19. Peffers MJ, Thornton DJ, Clegg PD. (2016). Characterization of Neopeptides in Equine Articular Cartilage Degradation. J Orthop Res. 34(1): 106-120. doi:10.1002/jor.22963.
20. Moon J-W, Ahn K, Bae J-H, et al. (2012). Mrna Sequence Analysis and Quantitative Expression of the Adamts4 Gene in the Thoroughbred Horse. Genes & Genomics. 34(4): 441-445. doi:10.1007/s13258-011-0204-0.
21. Huang K, Wu LD. (2008). Aggrecanase and Aggrecan Degradation in Osteoarthritis: A Review. J Int Med Res. 36(6): 1149-1160. doi:Doi 10.1177/147323000803600601.
22. Coyne MJ, Cousin H, Loftus JP, et al. (2009). Cloning and Expression of Adam-Related Metalloproteases in Equine Laminitis. Vet Immunol Immunop. 129(3-4): 231-241. doi:10.1016/j.vetimm.2008.11.022.
23. Velasco J, Li J, DiPietro L, et al. (2011). Adamts5 Deletion Blocks Murine Dermal Repair through Cd44-Mediated Aggrecan Accumulation and Modulation of Transforming Growth Factor Beta1 (Tgfbeta1) Signaling. J Biol Chem. 286(29): 26016-26027. doi:10.1074/jbc.M110.208694.
24. Will H, Dettloff M, Bendzko P, Sveshnikov P. (2005). A Quantitative Assay for Aggrecanase Activity. J Biomol Tech. 16(4): 459-472.
25. Dutto DJ, Hoyt DF, Clayton HM, Cogger EA, Wickler SJ. (2004). Moments and Power Generated by the Horse (Equus Caballus) Hind Limb During Jumping. J Exp Biol. 207(Pt 4): 667-674. doi:10.1242/jeb.00808.
26. Meershoek LS, Schamhardt HC, Roepstorff L, Johnston C. (2001). Forelimb Tendon Loading During Jump Landings and the Influence of Fence Height. Equine Vet J Suppl. (33): 6-10.
27. Krumrych W, Gołda R, Gołyński M, Markiewicz H, Buzała M. (2018). Effect of Physical Exercise on Cortisol Concentration and Neutrophil Oxygen Metabolism in Peripheral Blood of Horses. 18(1): 53. doi:https://doi.org/10.1515/aoas-2017-0019.
28. Woods J, Lu Q, Ceddia MA, Lowder T. (2000). Special Feature for the Olympics: Effects of Exercise on the Immune System: Exercise-Induced Modulation of Macrophage Function. Immunol Cell Biol. 78(5): 545-553. doi:10.1111/j.1440-1711.2000.t01-9-.x.
29. Gomez-Aristizabal A, Gandhi R, Mahomed NN, Marshall KW, Viswanathan S. (2019). Synovial Fluid Monocyte/Macrophage Subsets and Their Correlation to Patient-Reported Outcomes in Osteoarthritic Patients: A Cohort Study. Arthritis Res Ther. 21(1): 26. doi:10.1186/s13075-018-1798-2.
30. Tu J, Hong W, Zhang P, et al. (2018). Ontology and Function of Fibroblast-Like and Macrophage-Like Synoviocytes: How Do They Talk to Each Other and Can They Be Targeted for Rheumatoid Arthritis Therapy? Front Immunol. 9: 1467. doi:10.3389/fimmu.2018.01467.
31. Nees TA, Rosshirt N, Zhang JA, et al. (2019). Synovial Cytokines Significantly Correlate with Osteoarthritis-Related Knee Pain and Disability: Inflammatory Mediators of Potential Clinical Relevance. J Clin Med. 8(9). doi:10.3390/jcm8091343.
32. Bondeson J, Wainwright S, Hughes C, Caterson B. (2008). The Regulation of the Adamts4 and Adamts5 Aggrecanases in Osteoarthritis: A Review. Clin Exp Rheumatol. 26(1): 139-145.
33. Vonk AG, Netea MG, Denecker NE, et al. (1998). Modulation of the Pro- and Anti-Inflammatory Cytokine Balance by Amphotericin B. J Antimicrob Chemother. 42(4): 469-474.
34. Tokuda Y, Tsuji M, Yamazaki M, et al. (1993). Augmentation of Murine Tumor Necrosis Factor Production by Amphotericin B in Vitro and in Vivo. Antimicrob Agents Chemother. 37(10): 2228-2230.
35. Mokbel AN, El Tookhy OS, Shamaa AA, et al. (2011). Homing and Reparative Effect of Intra-Articular Injection of Autologus Mesenchymal Stem Cells in Osteoarthritic Animal Model. BMC Musculoskelet Disord. 12: 259. doi:10.1186/1471-2474-12-259.
36. Ma TW, Li Y, Wang GY, et al. (2017). Changes in Synovial Fluid Biomarkers after Experimental Equine Osteoarthritis. J Vet Res. 61(4): 503-508. doi:10.1515/jvetres-2017-0056.
37. McGivney BA, Eivers SS, MacHugh DE, et al. (2009). Transcriptional Adaptations Following Exercise in Thoroughbred Horse Skeletal Muscle Highlights Molecular Mechanisms That Lead to Muscle Hypertrophy. Bmc Genomics. 10. doi:10.1186/1471-2164-10-638.
38. Zhou X, Jiang L, Zhang Y, et al. (2019). Genetic Variation of Aggrecanase-2 (Adamts5) in Susceptibility to Osteoarthritis. Braz J Med Biol Res. 52(2): e8109-e8109. doi:10.1590/1414-431X20188109.
39. Canbek U, Imerci A, Kara M, et al. (2016). Polymorphisms in Adamts4 and Adamts5 Are Not Linked to Susceptibility to Knee Osteoarthritis in the Turkish Population. Genetics and molecular research : GMR. 15(3). doi:10.4238/gmr.15038264.

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