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Changes in novel anti-infalmmatory cytokine concetration in the bood of endurance and race horses at different levels of training

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ABSTRACT

Several anti-inflammatory cytokines have been proposed as markers for exercise monitoring in humans such as the interleukin 1 receptor agonist (IL-ra), or interleukin 13 (IL-13). Equine athletes may be considered a model for human exercise physiology research, however there is a lack of such studies of this species. Thus, we decided to examine the changes of IL-1ra and IL-13 in serum concentration during aerobic (endurance) and anaerobic (race) exercise in horses of different fitness levels in comparison with the well-known anti-inflammatory cytokine interleukin 10 (IL-10). The group of endurance horses (n = 13) consisted of animals competing over 100 (n = 7) and 120 km (n = 6) rides. The group of racehorses (n = 18) consisted of trained (n = 9) and untrained (n = 9) animals. The blood samples were obtained before and after the exercise. The ELISA test was performed to evaluate the changes of IL-1ra, IL-13 and IL-10 during different types of exercise. In endurance horses there was an increase in IL-13 ($p = 0.0012$) after the 100 km ride and in IL-1ra ($p = 0.0411$) after the 120 km ride. In race horses there was a higher IL-13 basal serum concentration in the untrained group, as well as a decrease of IL-13 after exercise ($p = 0.0188$). In trained racehorses there was an increase in IL-1ra ($p < 0.0001$) and IL-13 after exercise ($p = 0.0028$). In conclusion, the reaction of IL-1ra and IL-13 to different types of exercise differ from each other. Thus, in future, they may be helpful in monitoring the fitness of horses, however more research is needed.

1. Introduction

Long-distance endurance is similar in race horses and human athletes [1–3]. In both instances, they have to adapt their metabolism, aerobic and/or anaerobic capacity and muscle strength to short- or long-term effort. Although the training regimen differs between horses and sports people in several aspects, the adaptational changes are similar. Thus, equine athletes may be considered models for human exercise physiology research. Many analogies, or indeed parallels, have been documented in both species [4–6]. The immunological reaction during exercise, as well as the hormonal reaction, seem to be similar in both species [1–3]. In addition, superficial digital flexor tendon injuries in equine athletes are some of the most well-accepted animal models for exercise-induced Achilles tendon injury [5]. The changes during exercise very often are functionally and clinically equivalent energy-storing structures for which no other equally appropriate analogues exist [5]. In addition, studies of the effects of exercise in humans have certain limitations, such as the stress of professional work which may influence obtained results, whereas horses are involved only in physical training.

Regular physical training is necessary for many adaptational changes, such as the immunological response to an exercise [7,8]. The exact role of the influence of exercise on the immune systems of humans and animals is unclear. It is documented that exhaustive and prolonged exercise can result in immunosuppression, known as the "open window" theory [8,9]. On the other hand, the American College of Sports Medicine (ACSM) initiated the global health program called Exercise is Medicine® (EIM), whose main goal is to make physical activity assessment and promotion a standard in clinical care. Studies in exercise physiology mostly monitor the changes in

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the concentration of blood cytokines in both species [2,10,11] as well as cellular reactions [3]. During physical activity, muscles spasm, and accompanied by damage to the muscle fibers, initiate an inflammatory response to exercise (IRE). Pro-inflammatory cytokines, like a tumor necrosis factor α (TNF-α) and interleukin-1β (IL-1β), activate neutrophils and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzymes, which in turn enhance production of reactive oxygen species (ROS) and free radicals, which influence the metabolism. The inflammatory process is crucial for tissue remodeling during physical activity [12,13]. However, during conditioning concentration of pro-inflammatory mediators mostly type 1 (ex. IL-1 and TNFα) drop with increasing efficiency of the organism, whereas there is an increase in cytokines such as interleukin-6 (IL-6) and interleukin-10 (IL-10) of anti-inflammatory properties [2,8]. Anti-inflammatory mediators are actively involved in processes of repairing muscle tissue after effort [14]. On the other hand, there are no studies in horses connected with changes of other novel anti-inflammatory cytokine concentration, such as interleukin-1 receptor antagonist (IL-1ra) and interleukin-13 (IL-13), which may be key factors in adaptation to increasing workloads during physical training. In human studies, it is documented that IL-13 is an exercise-induced regulator of endurance capacity, whereas IL-1ra competitively inhibits the local inflammatory effects of IL-1 [15,16].

Insufficient preparation for exercise may immediately cause injuries, such as bone fractures or ruptures of muscles and tendons, and the elimination of competition which may relate to initiation of the acute phase response (APR) [17,18]. To reduce the risk of injuries during exercise in both human and equine athletes, it is crucial to find biomarkers to prevent orthopedic injuries. In a study connected with the reasons for endurance horse eliminations during competition, lameness was the most frequent cause, 69.2 % of all eliminations and 31.8 % of all started horses [19]. Proximal metacarpal pain, subsequently hoof pain and fetlock region pain, are the most common reasons for lameness [20]. In racehorses, the prevalence of fatigue injury of the third metacarpal bone affects 30–70 % of thoroughbred horses in the first months of race training [21]. Unfortunately, a high proportion of horses, which have suffered orthopedic injuries during intense exercise do not come back to training and competition.

The balance between pro-and anti-inflammatory cytokines is essential. However, the changes in cytokines, such as the IL-1, IL-4, IL-6, IL-10, TNFα and INFγ, blood concentration under exercise (endurance as well as race) are well known and most of them agree with each other [2,4,6,11,16,26]. Thus, to avoid the generation of the same results once again, the evaluation of novel cytokines such as IL-1ra and IL-13 in comparison to well-known cytokines such as IL-10 was performed. Thus, to better understand the adaptive process, the aim of the study was to evaluate the changes in IL-10, IL-13, and IL-1ra blood concentration before and after exercise in horses after long and short-duration exercise at different fitness levels.

2. Materials and methods

2.1. Animals

Two groups of horses were enrolled in the study: endurance horses (n = 13) and racehorses (n = 18). The racehorses were thoroughbreds and the endurance horses were Arabians.

Endurance horse group: for this study thirteen (n = 13) privately-owned healthy endurance horses, aged 9–11 years, were selected. All of them were participating in endurance racing at different distances: the first group of horses (n = 7) competed over 100 km 100 km distance (4 mares, 2 geldings, 1 stallion), while the second group of horses (n = 6) competed over 120 km distance (2 mares and 4 geldings). They were fed with the standard diet designed for endurance horses, hay (7.5 kg/horse), oats, oils and a special concentrate for endurance horses (2–4 kg/horse). The concentrated feed and roughage were given three times per day.

They were trained during daily sessions with the exercise-load depending on the horse's condition and increasing with time; the sessions with a high exercise-workload were performed every 14–20 days.

Racehorse group: the racehorse (n = 18) were divided into two groups: (n = 9) well-trained after 1–2 training seasons with good performance (3–4 years old), and (n = 9) untrained horses (2–3 years old) at the beginning of their race training. All the racehorses were trained by one trainer and housed in the same stable on straw under the same environmental conditions. They were fed with the standard diet designed for racehorses (oats 5.5 kg/horse, meadow hay 7.5 kg/horse and a special concentrate for performance horses). The concentrated feed and roughage were given three times per day. The horses exercised on sand for 800 m at a speed of about 800 m/min. For untrained horses, it was the first training session with a gallop.

The training sessions involved daily sessions including galloping on the sand for 800–1000 m. The sessions with high exercise-load were performed every 2 days in cycles of 5 days a week.

All horses in both groups passed veterinary health checks which included heart rate, mucous membranes (colour and moisture), dehydration (measured as the time it takes for a pinched skin-fold over the point of the shoulder to flatten), gut sounds, muscle condition, and regularity of gait (evaluated in a trot). Additionally, a basal morphological and biochemical blood examination before and after exercise was performed and no pathologies were observed.

2.2. Samples

Peripheral blood was gathered from the jugular vein into sterile K2-ethylenediaminetetraacetic acid (K2-EDTA) tubes for hematological tests and sterile dry tubes for serum analyses using the BD Vaccutainer system (BD, USA). The tubes were centrifuged (3000 x *g*, 15 min) and the serum was isolated and stored at −80 C for further analyses.

Blood samples were collected before starting exercise (1 h before feeding), and at 30 min the effort, as a part of standard veterinary diagnostic procedures. Thus, no approval of the Local Commission for Ethics in Animal Experiments was required, according to Polish legal regulations and European directive EU/2010/6.

2.3. Procedures

To determine cytokine concentration: IL-1ra, IL-10, IL-13 the available immunoenzymatic commercial assay dedicated for equine species was used - ELISA test (BT Lab, UK). The used enzyme immunoassay has an intra-assay precision CV % < 8 % and an inter-assay precision CV % < 10 % and was validated for use with horses' serum samples according to the manufacturer's instruction. The absorbance was measured by Multiscan Reader (Labsystem, Helsinki, Finland) using a Genesis V3.00 software program.

2.4. Statistical analysis

A statistical analysis was performed using the non-parametric Mann-Whitney Test - GraphPad Prism 9.4.0 (GraphPad Software Inc., La Jolla, CA, USA). All mean values are presented using standard deviation or standard error. Differences at $p < 0.05$ were considered significant.

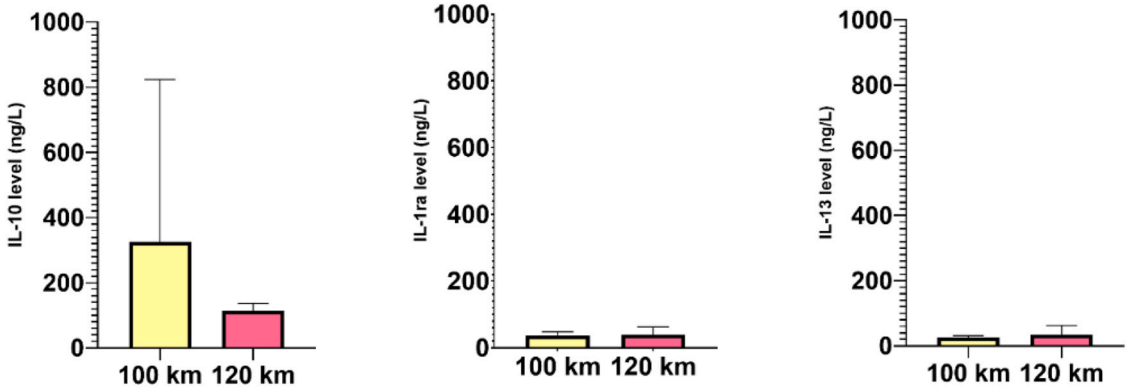

Fig. 1. Comparison of IL-10, IL1-ra and IL-13 basal serum concentration before 100 and 120 km competition in endurance horses. * p < 0.05.

3. Results

3.1. Endurance horses

There were no differences between the basal levels of IL-1ra, IL-13, and IL-10 (Fig. 1). There was an average increase in the IL-13 level by 57.22 ng/L (p = 0,0012) after the 100 km ride,whereas there was no statistically significant difference in IL-13 serum concentration after the 120 km ride. The average increase in the IL-1ra level of 12,75 ng/L (p = 0,0411) was detected after the 120 km endurance ride. However, there was no change in IL-1ra blood concentration after 100 km endurance ride as well as IL-10 in both groups (Fig. 2).

3.2. Race horses

There was a higher IL-13 basal serum concentration in untrained race horses - an average of 14,96 ng/L (p = 0,0142) (Fig. 3). In untrained racehorses there was an average decrease in IL-13 serum concentration of15,29 ng/L after exercise (p = 0,0188). In trained racehorses there was an average increase in IL-1ra of 39,49 ng/L (p < 0,0001) and an increase in IL-13 of 40,11 ng/L after exercise (p = 0,0028) (Fig. 4).

4. Discussion

During physical activity, muscle spasms accompanied by damage to the muscle fibers, initiate IRE and successive secretion of

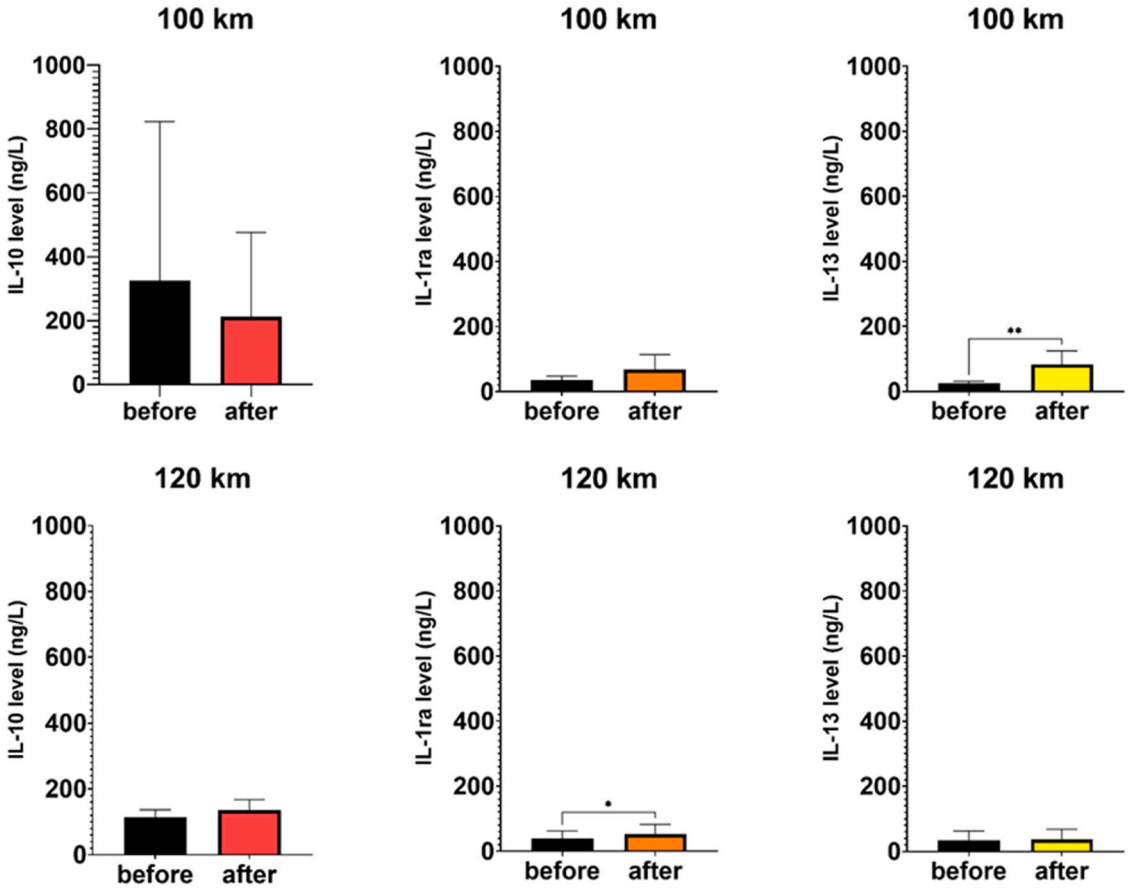

Fig. 2. Comparison of IL-10, IL-1ra and IL-13 serum concentration before and after 100 and 120 km competition in endurance horses. * p < 0.05; **p < 0.01.

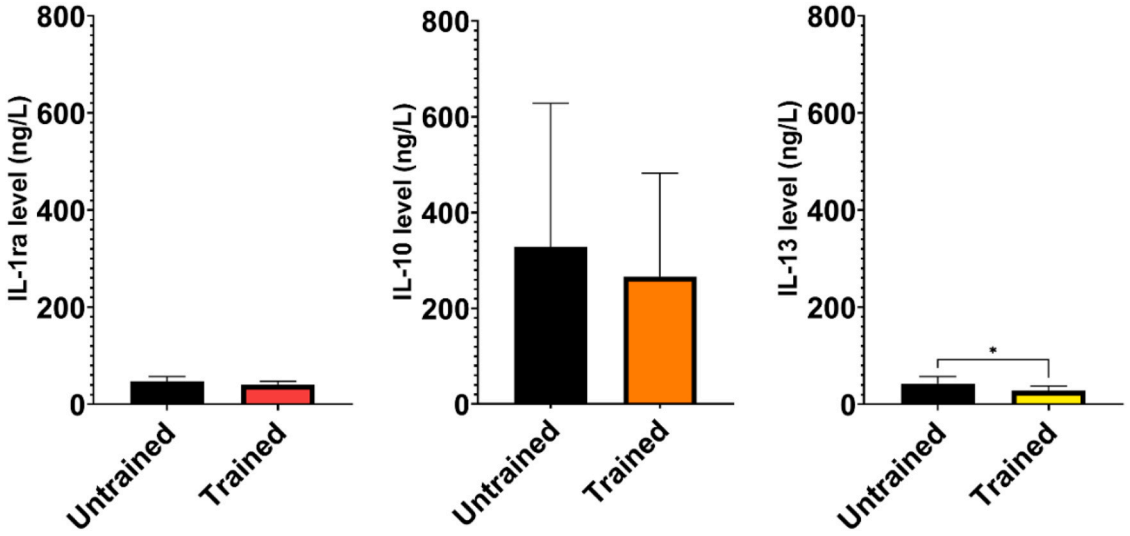

Fig. 3. Comparison of IL-1ra, IL-10 and IL-13 basal serum concentration before exercise in untrained and trained race horses. * $p < 0.05$.

cytokines. The inflammatory process is necessary for regeneration and remodeling of the musculature during sports training [22]. Satellite cells hold the potential for regenerating muscles as they release a platelet-derived growth factor (PDGF) and IL-6, which promote proliferation and cell differentiation in the healing area [23]. The PDGF, TNFα, and IL-1β stimulate the production of ROS. Anti-inflammatory cytokines like IL-1ra, IL-4, IL-10, and in addition IL-6 block the activity of pro-inflammatory mediators such as TNFα and IL-1β, and therefore inhibit ROS generation [24,25]. An equilibrium between pro- and anti-inflammatory cytokines is crucial for preventing excessive accumulation of ROS, improving cell metabolism and is a guarantee of complete reconstruction of skeletal muscles [13].

It has been documented that exercise-induced APR in horses in regular training is accompanied by a strong anti-inflammatory response, which prevents clinical disorders after intense exercise

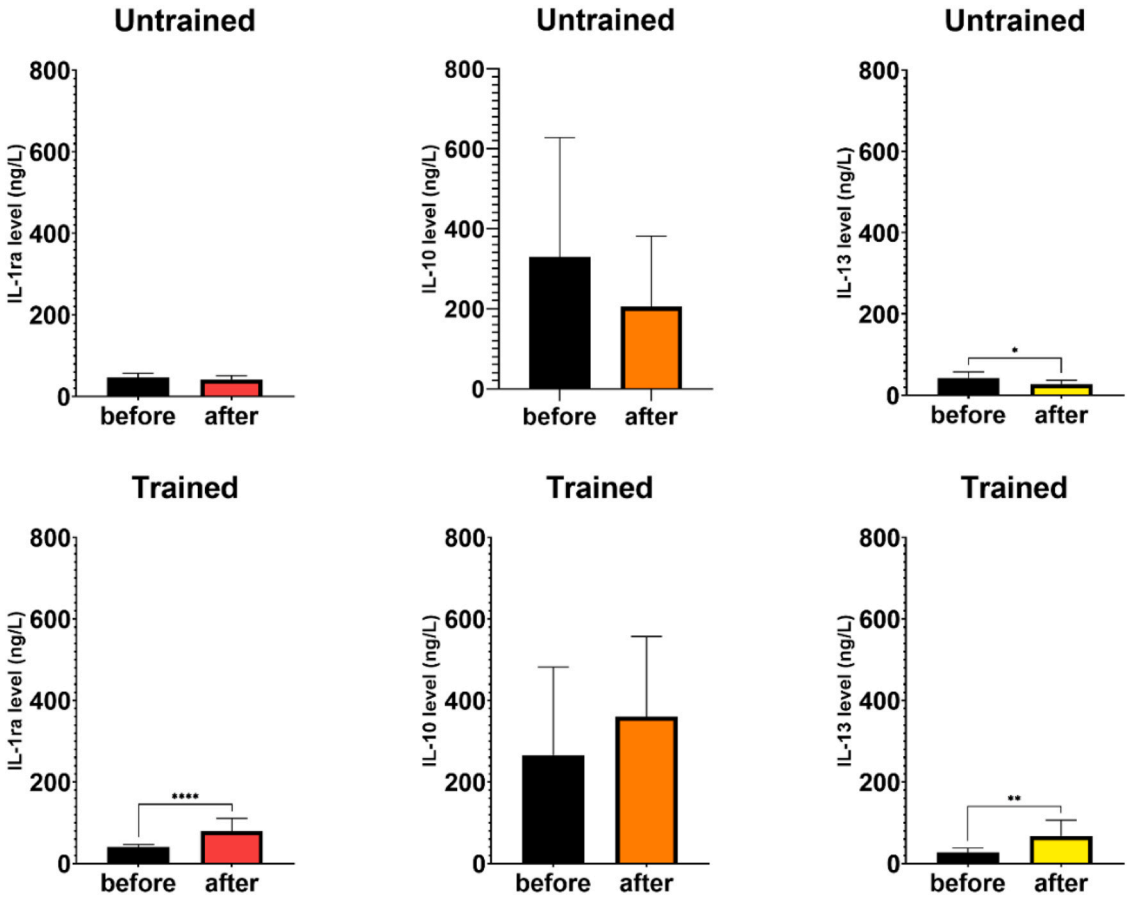

Fig. 4. Comparison of IL-1ra, IL-10 and IL-13 serum concentration before and after exercise in untrained and trained race horses. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

[11,18,26]. IL-10 is synthesized mostly by T cells (in particular T helper cells- Th2) and macrophages, but many studies have shown that IL-10 is also produced by myocytes [27]. IL-10 is one of the strongest anti- inflammatory cytokines, which minimizes the consequences of inflammatory processes through inhibiting the synthesis of pro-inflammatory mediators, including TNFα and IL-1β. Moreover, IL-10 stimulates production of IL-1 receptor antagonist (IL-1ra) [13,28]. Thus, we decided to evaluate the changes in IL-1ra and IL-13 concentration in comparison to well-known anti-inflammatory cytokines such as IL-10 [11,18,29]. In humans, in master sprinters, and master endurance athletes (25-years' experience) the level of IL-10 increased after training [30]. Similarly, in elite endurance horses (120–160 km) the IL-10 blood concentration increased considerably after the ride [11]. In our study, IL-10 concentration was unchanged in all groups after the exercise. It may relate to a weaker conditioning process because horses which participated in the longest distances (120–160 km) in the previous study were at a higher level of training in comparison to our examined groups (100 and 120 km). In racehorses the type of effort is different to endurance horses. In one study, it was documented that in well-trained racehorses, leukocytes produce high amounts of IL-10 [6] after exercise. However, it was after several days of cell culturing and mitogen stimulation. Thus, in vivo and in vitro studies sometimes may be hard to compare.

Interleukin 1 receptor antagonist (IL-1ra) is produced essentially by monocytes and macrophages [28,31]. In humans, it has been demonstrated that the release of anti-inflammatory cytokines, such as IL-1ra, is also stimulated by IL-6, which is the most important myokine produced by muscle fibers [32]. IL-1ra inhibits the production of the pro-inflammatory cytokines released during exercise-induced muscle damage by bonding with IL-1Rl bonding site, which is the same for IL-1α and IL-1β. In this way, it blocks the action IL-1 [28,33]. Thus, IL-1ra plays an anti-inflammatory role in acute and chronic inflammation. Dysfunction within production and function of IL-1ra results in development of inflammatory disease symptoms, thus this cytokine might be used in the treatment of tendonitis in horses [33]. In addition, a study during which mice were injected with IL-1ra and IL-1β showed that they lead to improvement of running performance [34], which may suggest a positive effect on physical performance. Similar results were obtained in regularly trained racehorses [35]. In our study, IL-1ra blood concentration was increased in more advanced (120 km) endurance groups and in trained racehorses after exercise in comparison to horses at lower levels of training. In addition, there is a chance IL-1ra could be a potential biomarker of overtraining and risk of injuries. In one of the studies, IL-1ra expression was significantly lower in catastrophically injured racehorses in comparison to non-injured control subjects [36]. However, it was suggested that IL-1ra reduces insulin sensitivity in rats through a muscle-specific decrease in glucose uptake [37]. In obese humans, markedly increased plasma levels of IL-1ra might contribute to the development of insulin resistance [38]. However, IL-1ra correlation with the insulin resistance in obesity is probably connected with high levels of IL-1. On the other hand, a deficiency of IL-1ra yields an abnormal lipid metabolism after feeding mice an atherogenic diet [39]. Thus, during exercise, IL-ra is probably released to balance the immune system, rather than being strongly influential on glucose and lipid metabolism.

To the Author's best knowledge, until this point, no research on changes of IL-13 concentration after effort has been performed on horses. Study on mice demonstrated that in adaptation to long-lasting exercise, IL-13 plays a very significant role, affecting metabolic changes. IL-13 is produced by type 2 lymphoid cells (ILC2) situated in the muscle. The metabolic efficiency of the muscle has to be improved to meet increased energy demand. For this purpose, IL-13 reprograms metabolism to reduce usage of glycogen, promote mitochondrial respiration, and fatty acid oxidation [40]. IL-13 activates mitochondrial ETC complex Stat3-dependent, beyond mitochondrial IL-13-Stat3 interaction with two nuclear receptors, which control fat catabolism and mitochondrial respiration in muscle during exercise [41]. In addition, exercise influences an IL-13-dependent gene signature for fatty acid metabolism and the tricarboxylic acid cycle in muscles [16]. However, nowadays there are only few publications about IL-13 changes during exercise in humans and mice [16,41], and there is a lack of them in horses. In our study, the IL-13 blood concentration was increased in endurance horses at lower levels of training (100 km) which may relate to stimulation of the adaptational reactions to prepare these animals for higher levels of aerobic exercise. Increased production of IL-13 during exercise in horses at lower fitness level could represent a strategy implemented by the organism to limit pro-inflammatory reactions to exercise-induced muscle damage [42,43]. A similar thesis was proposed by a very recent study in humans and mice, published in Science in which increased levels of IL-13 during endurance exercise was present not only in muscle tissue, but also in the plasma [16]. Thus, IL-13 may have systemic effects on other systems in the body. It opens the potential for new therapeutic opportunities for IL-13 to treat human diseases [40]. By promoting muscle mitochondrial biogenesis and increased oxidative capacity associated with improved glucose tolerance in response to endurance training, IL-13 may enhance muscle repair and function in patients who have suffered significant muscle injury, or muscle wasting following illness.

In racehorses there was an increase of IL-13 in trained horses and a decrease in untrained groups after exercise. Thus, in anaerobic exercise, the IL-13 stimulation differs, and the post-exercise level of IL-13 may be connected with the fitness level. In humans, the college cross-country runners and American football players had significantly higher IL-13 than normal-weight sedentary men [16]. It is possible, the IL-13 release to the bloodstream is delayed in racehorses in comparison to endurance ones. The race exercise is a short-lasting reaction, and the IL-13 synthesis may occur after several hours. However, in our study the blood samples were obtained during standard veterinary procedures, thus other time points of blood sampling were not performed. On the other hand, the basal level of IL-13 in untrained racehorses was higher than in trained ones, which may indicate a higher reserve in the bloodstream. However, more research is needed to confirm this statement and evaluate the exact role of this cytokine in horses.

The main limitation of the study is that measurements of cytokine muscle expression were not performed, and neither were muscle biopsies in race and endurance horses during the racing season. Thus, only blood samples were obtained which were collected as part of the routine horse hematological evaluation. Only excess peripheral blood was used for this study. We are aware that very often the muscle cells are a major source of cytokines during exercise. However, these responses are highly specific to the exercise protocol and physiological strain (duration, nature, and intensity) and the type of cytokine. It was stated that a primary source of IL-13 is tissue resident type 2 innate lymphoid cells (ILC2s), and skeletal muscle may serve only as an additional source of IL-13 activated by endurance exercise [16]. In addition, in the mentioned study, IL-13 mRNA was not detectable in mouse primary myotubes, but only in enriched in Percoll-isolated immune and stromal cells from muscle lysate. This indicates that the main source of IL-13 should be considered blood. In addition, myokines act in an autocrine and paracrine fashion locally on skeletal muscle, but may also act in an endocrine fashion by communicating with a variety of other tissue types and it is mostly via blood [44].

5. Conclusions

This study builds on a growing body of evidence supporting an integral role for the immune system during exercise. We confirmed that IL-13 and IL-1ra serum concentrations changed during different types of exercise in horses. This is the first research concerning the blood concentration of these anti-inflammatory cytokines in horses throughout physical activity. In endurance and racehorses at a lower level of training, the anti-inflammatory response connected with IL-ra release was less expressed,whereas the IL-13 response was different in aerobic and anaerobic types of exercise. Thus, further research is needed because the exact role of IL-13 in horses is still unclear. However, these findings increase awareness of the importance of the cytokine response during different types of exercise and their future potential therapeutic roles in muscle diseases.

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CRediT authorship contribution statement

Urszula Plisak: Formal analysis, Investigation, Writing – original draft. **Jarosław Szczepaniak**: Data curation, Writing – review & editing, Visualization. **Magdalena Żmigrodzka**: Writing – review & editing. **Beata Giercuszkiewicz-Hecold:** Resources. **Olga Witkowska-Piłaszewicz:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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Institutional review board statement

Samples collected from horses were a part of standard veterinary diagnostic procedures according to Polish legal regulations (art 1.2 (5) Ust. z dnia 15 stycznia 2015 r. o ochronie zwierzat wykorzystywanych do celów naukowych lub edukacyjnych, Dz.U.2018.0.1207 (Resolution on the animals protection used for scientific and educational purposes); the European directive EU/2010/63 approval of the Local Commission for Ethics in Animal Experiments was not required.

Informed consent statement

Not applicable.

References

[1] Knechtle B, Nikolaidis PT. Physiology and pathophysiology in ultra-marathon running. Front Physiol 2018;9:634.
[2] Witkowska-Piłaszewicz O, Bąska P, Czopowicz M, Żmigrodzka M, Szarska E, Szczepaniak J, et al. 2019a: anti-inflammatory state in Arabian horse introduced to the endurance training. Animals 2019;9:616.
[3] Witkowska-Piłaszewicz O, Grzędzicka J, Seń J, Czopowicz M, Żmigrodzka M, Winnicka A, et al. 2021a: Stress Response after race and endurance training sessions and competitions in Arabian horses. Prev Vet Med 2021;188:105265.
[4] Page AE, Steward JC, Fielding CL, Horohov DW. The effect of a 160-kilometer competitive endurance ride on inflammatory marker mRNA expression in horses. J. Equine Vet. Sci. 2019;79:45–9.
[5] Patterson-Kane JC, Rich T. Achilles tendon injuries in elite athletes: lessons in pathophysiology from their equine counterparts. ILAR J 2014;55:86–99.
[6] Witkowska-Piłaszewicz O, Pingwara R, Winnicka A. The effect of physical training on peripheral blood mononuclear cell ex vivo proliferation, differentiation, activity, and reactive oxygen species production in racehorses. Antioxidants ((Basel)) 2020:9,1155.
[7] Cappelli K, Amadori M, Mecocci S, Miglio A, Antognoni MT, Razzuoli E. Immune Response In Young Thoroughbred Racehorses Under Training. Animals 2020;10:1809.
[8] Gleeson M, Bishop NC, Stensel DJ, Lindley MR, Mastana SS, Nimmo MA. The anti-inflammatory effects of exercise: Mechanisms and implications for the prevention and treatment of disease. Nat. Rev. Immunol. 2011;11:607–15.
[9] Nieman DC, Wentz LM. The compelling link between physical activity and the body's defense system. J. Sport Health Sci 2019;8:201–17.
[10] Fallon KE. The acute phase response and exercise: the ultramarathon as prototype exercise. Clin J Sport Med 2001;1:38–43.
[11] Miglio A, Cappelli K, Capomaccio S, Mecocci S, Silvestrelli M, Antognoni MT. Metabolic and biomolecular changes induced by incremental long-term training in young thoroughbred racehorses during first workout season. Animals ((Basel)) 2020;10:317.
[12] Kinnunen S, Atalay M, Hyyppa S, Lehmuskero A, Hanninen O, Oksala N. Effects of prolonged exercise on oxidative stress and antioxidant defense in endurance horse. J Sports Sci Med 2005;4:415–21.
[13] Zembroń-Łacny A, Ostapiuk-Karolczuk J. Udział cytokin w metabolizmie mięśni szkieletowych. Sport Wyczynowy 2008;10–12:95–107.
[14] Pedersen BK. Muscular interleukin-6 and its role as an energy sensor. Med. Sci. Sports Exerc 2012;44:392–6.
[15] Arend WP, Gabay C. Physiologic role of interleukin-1 receptor antagonist. Arthritis Res 2000;4:245–8.
[16] Knudsen NH, Stanya KJ, Hyde AL, Chalom MM, Alexander RK, Liou Y-H, et al. Interleukin-13 metabolic conditioning of muscle to endurance exercise. Science 2020;1:368.
[17] Hines MT, Schott II HC, Bayly WM, Leroux AJ. Exercise and Immunity: a review with emphasis on the Horse. J. Vet. Intern. Med 1996;10:280–9.
[18] Witkowska-Piłaszewicz O, Bąska P, Czopowicz M, Żmigrodzka M, Szczepaniak J, Szarska E, et al. 2019b. Changes in Serum Amyloid A (SAA) concentration in arabian endurance horses during first training season. Animals 2019;6:330.
[19] Nagy A, Murray JK, Dyson S. Elimination from elite endurance riders in nine countries: a preliminary study. Equine Vet J Suppl 2010;38:637–43.
[20] Nagy A, Dyson SJ, Murray JK. A veterinary review of endurance riding as an international competitive sport. Vet J 2012;3:288–93.
[21] Wilsher S, Allen WR, Wood JL. Factors associated with failure of thoroughbred horses to train and race. Equine Vet J 2006;38:113–8.
[22] Peake JM, Neubauer O, Della Gatta PA, Nosaka K. Muscle damage and inflammation during recovery from exercise. J Appl Physiol (1985) 2017;3:559–70.
[23] Relaix F, Zammit PS. Satellite cells are essential for skeletal muscle regeneration: the cell on the edge returns centre stage. Development. 2012;16:2845–56.
[24] Waldemer-Streyer RJ, Kim D, Chen J. Muscle cell-derived cytokines in skeletal muscle regeneration. FEBS J 2022:24. https://doi.org/10.1111/febs.16372
[25] Ms SAB, Waldman HS, Krings BM, Lamberth J, Smith JW, McAllister MJ. Effect of curcumin supplementation on exercise-induced oxidative stress, inflammation, muscle damage, and muscle soreness. J Diet Suppl 2020;4:401–14.
[26] Horohov D, Sinatra S, Raj MD, Jankowit S, Betancourt A, Bloomer RJ. The effect of exercise and nutritional supplementation on proinflammatory cytokine expression in young racehorses during training. J. Equine Vet. Sci. 2012;32:805–15.
[27] Lavin KM, Perkins RK, Jemiolo B, Raue U, Trappe SW, Trappe TA. Effects of aging and lifelong aerobic exercise on basal and exercise-induced inflammation. J Appl Physiol (1985) 2020;1:87–99.
[28] Jurkowski MK, Bobek-Billewicz B, Ćwiklińska-Jurkowska M. Wysiłek fizyczny i cytokiny. Medycyna Sportowa 2006;22:245–52.
[29] de Siqueira RF, Fernandes WR. Post-ride inflammatory markers in endurance horses. Ciência Rural 2016;07:1256–61.
[30] Rosa TS, Neves RVP, Deus LA, Sousa CV, da Silva AS, de Souza MK, et al. Sprint and endurance training in relation to redox balance, inflammatory status and biomarkers of aging in master athletes. Nitric Oxide 2020;1:42–51.
[31] Dayer JM. Evidence for the biological modulation of IL-1 activity: the role of IL-1Ra. Clin Exp Rheumatol 2002;20:14–20.
[32] Peake JM, Suzuki K, Hordern M, Wilson G, Nosaka K, Coombes JS. Plasma cytokine changes in relation to exercise intensity and muscle damage. Eur J Appl Physiol 2005;9:514–21.
[33] Wilkin T, Baoutina A, Hamilton N. Equine performance genes and the future of doping in horseracing. Drug Test Anal 2017;9:1456–71.
[34] Carmichael MD, Davis JM, Murphy EA, Brown AS, Carson JA, Mayer EP, et al. Role of brain IL-1 beta on fatigue after exercise- induced muscle damage. Am J Physiol Regul Integr Comp Physiol 2006;291:R1344–8.
[35] Arfuso F, Giudice E, Panzera M, Rizzo M, Fazio F, Piccione G, et al. Interleukin-1Ra (Il-1Ra) and serum cortisol level relationship in horse as dynamic adaptive response during physical exercise. Vet Immunol Immunopathol 2022;243:110368.

[36] Page AE, Adam E, Arthur R, Barker V, Franklin F, Friedman R, et al. Expression of select mRNA in Thoroughbreds with catastrophic racing injuries. Equine Vet J 2021;1:63–73.
[37] Isoda K, Sawada S, Ayaori M, Matsuki T, Horai R, Kagata Y, et al. Deficiency of interleukin-1 receptor antagonist deteriorates fatty liver and cholesterol metabolism in hypercholesterolemic mice. J Biol Chem 2005;280:7002–9.
[38] Luotola K. IL-1 receptor antagonist (IL-1Ra) levels and management of metabolic disorders. Nutrients 2022;14:3422.
[39] Böni-Schnetzler M, Boller S, Debray S, Bouzakri K, Meier DT, Prazak R, et al. Free fatty acids induce a proinflammatory response in islets via the abundantly expressed interleukin-1 receptor. I. Endocrinology 2009;150:5218–29.
[40] Webb LM, Tait Wojno ED. Exercising immunity: interleukin-13 flexes muscle. Immunity 2020;6:902–4.
[41] Knudsen, N.H. Molecular mechanisms of metabolic adaptation to endurance exercise. Harvard University Library. Cambridge, Massachusetts., 2018, http://nrs.harvard.edu/urn-3:HUL.InstRepos:39947172.
[42] Piccione G, Giannetto C, Bruschetta D, Congiu F, Arfuso F, Giudice E. Influence of exercise and dietary omega-3 oil 293 supplementation on interleukin 1-ra serum concentrations in standardbred horses. Anim. Prod. Sci. 2019;2:232–5.
[43] Colahan PT, Kollias-Bakert C, Leutenegger CM, Jones JH. Does training affect mRNA transciption for cytokine production in circulating leucocytes? Equine Vet. J. Suppl 2002;34:154–8.
[44] Schnyder S, Handschin C. Skeletal muscle as an endocrine organ: PGC-1α, myokines and exercise. Bone 2015;80:115–25.