

The Effect of Physical Activity on Cytokine Production: A Systematic Parallel Study of Horse and Human Athletes

Ifeoluwa Peace Oladapo^{1,2}, Abolaji Samson Olagunju³, Olalekan Chris Akinsulie², Eduarda Proença¹, Ursula Yaeko Yoshitani¹, and Marcelo Beltrão Molento^{1,*}

¹Laboratory of Veterinary Clinical Parasitology, Federal University of Paraná, Paraná, 80.035-050, Brazil

²College of Veterinary Medicine, Washington State University, Washington, 99163, United States

³Laboratory Cell and Molecular Biology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, 05508-000, Brazil

* Author to whom any correspondence should be addressed; Email: molento@ufpr.br

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Abstract

Cytokines are glycoproteins responsible for cellular communication, influenced by exercise. Their levels vary depending on the intensity and type of physical activity in both humans and horses. This review aimed to assess the effects of various types and intensities of exercise on the production of pro- and anti-inflammatory cytokines. A systematic search (2000–2022) was performed using databases such as PubMed, Scopus, and Web of Science. Studies involving horses ($n = 3$) and/or humans ($n = 21$) undergoing physical activity were selected, resulting in 24 eligible articles. IL-6, TNF- α , and IL-10 levels increased after high-intensity exercise in both species, while moderate-intensity activity yielded more variable results. Some cytokines, such as IL-1 β and IL-17, exhibited species-specific differences. Cytokine production is influenced by exercise intensity and duration. The observed differences between horses and humans suggest physiological divergences, warranting further research in the field of exercise immunology and other related disciplines. As a potential outcome of this work, we propose that horses could serve as an appropriate translational animal model for human athletes in exercise immunology, justifying a more comprehensive comparative approach.

Keywords

Cytokines; pro-inflammatory cytokines; anti-inflammatory cytokines; physical activity; exercise; equids; human physiology

1. Introduction

Humans and horses have a unique relationship, not only due to their shared cultural and historical backgrounds, but also because of their natural athleticism [1]. This similarity has led researchers to consider the horse as a reference species for comparative studies in human exercise physiology, including immunology (both innate and under stress) and disease responses [2,3]. Conversely, knowledge gained from human medicine often serves as a starting point for research in veterinary exercise medicine [4]. The importance of exercise for both species is evidenced by the numerous studies exploring the production of cytokines involved in the response to moderate activity and/or strenuous exercise

[4–6]. These studies have demonstrated that oxidative stress during exercise is a physiological phenomenon common among exercising mammals [6–8].

The effect of physical activity on cytokine (interleukins; ILs) production has been an area of growing interest in recent years, with extensive research conducted in humans and a limited number of studies conducted in horses. Cytokines, a large family of intercellular signaling molecules, control immunological and inflammatory responses [9]. They are soluble glycoproteins that the body's immune and non-immune cells, organs, and organ systems produce and use to communicate with one another. Numerous cells can

produce cytokines, which typically act in tissues at extremely low concentrations in an autocrine or paracrine manner (**Figure 1**). Inflammatory cytokines, which comprise both pro- and anti-inflammatory mediators, are altered by various stressors, including physical exercise [10]. Localized inflammation is the typical physiological defensive reaction to initial tissue (muscle, joint, and connective tissue) damage [9]. The release of cytokines into the circulation due to a heightened response can be pathogenic, self-destructive, and occasionally lethal to the host [9].

Hypercytokinemia, also known as a cytokine storm, is a condition characterized by the systemic release of cytokines and is often referred to as the systemic inflammatory response syndrome (SIRS). Pro-inflammatory cytokine overproduction damages several organs and is linked to severe acute insults, including severe trauma, thermal injury, ischemia-reperfusion injury, septic shock, and systemic infections [11]. Moreover, physical activity can have varying effects on local and systemic cytokine production in both humans (athletes/obese, older/younger) and horses (athletes, younger/older, mares/foals), with the impact being dependent on the type of activity (aerobic/anaerobic) and duration (30 minutes to more than 6 hours) [2,12].

Moderate exercise helps maintain a healthy body weight, reducing the risk of obesity. In contrast, too little or excessive activity has been associated with chronic disorders such as diabetes, cardiovascular diseases, certain cancers, chronic respiratory diseases, and other serious health problems [13,14]. Additionally, individuals with highly inactive lifestyles frequently experience low-grade chronic inflammation, which often includes immunological components [15]. Regular exercise promotes the development of a balanced pro-inflammatory and anti-inflammatory environment in both younger and older humans [16,17] and younger and older horses [18,19], resulting in reduced basal inflammation and increased concentrations of both pro-inflammatory and anti-inflammatory cytokines [20–22]. It is thought that the recurrent acute inflammatory and subsequent anti-inflammatory cytokine responses after recovery from single bouts of exercise cause these long-term alterations in circulating cytokines [23].

Exercise elevates the levels of the pro-inflammatory cytokines IL-6, TNF- α , and IL-1 β in humans, triggering an acute inflammatory response [24–26]. Similarly, it has been reported to raise the systemic mRNA expression of TNF- α and IL-1 β in horses [27–29]. Overtraining, which is linked to muscle, skeletal, and joint injuries, is considered the primary culprit behind this exercise-induced inflammatory response in humans [30] and horses [31].

It is known that the type of activity that involves greater participation of different muscle groups, as well as the type of ergometer, generates different cytokine responses. The majority of the sport-specific interventions in humans included in this study were ergometer cycling, downhill running, various sports, and swimming. As a result of physical activity or exercise (at least 30 minutes/day), leukocytosis is commonly induced in humans [10,32–34] and in horses [10,35,36].

The cytokines released into the blood after exercise have received much attention in understanding the underlying

mechanisms of these events. Indeed, several studies [37–40] have consistently demonstrated that IL-1Ra, IL-6, IL-8, and IL-10 levels significantly increase after endurance activities (e.g., marathons) lasting from 1 to more than 24 hours. Excessive exercise may compromise the immune system. Although advancements have been made, many questions remain unanswered regarding the processes that link the types and levels of an individual's activity to immunological responses and illness. The impact of regular physical exercise on cytokine expression has yet to be thoroughly examined.

A brief overview of various cells expressing different cytokines is provided in **Figure 1**. The figure shows the different cytokines that are produced during exercise, their functions, and the pathways through which they act, showing the four main subsets of T cells (Th1, Th2, Th17, and TFH). Th1 cells secrete cytokines such as interferon-gamma (IFN- γ) and tumor necrosis factor-alpha (TNF- α), which promote inflammation and help fight intracellular pathogens. Th2 cells secrete cytokines such as interleukin-4 (IL-4) and IL-13, which help fight extracellular pathogens. Th17 cells secrete cytokines such as IL-17, which promote inflammation and help fight extracellular pathogens. TFH cells secrete cytokines such as IL-21, which help regulate the function of B cells, another type of immune cell that produces antibodies.

Comparative studies of cytokine production in humans and horses can provide valuable insights into the evolutionary and physiological basis of these responses, as well as their potential therapeutic implications for both human and veterinary medicine. By investigating the effect of physical activity on cytokine production in parallel studies of human and horse athletes, this study aims to contribute to our understanding of the immune adaptations to exercise and their potential implications for improving athletic performance and injury recovery in both species. In this review, we examined the impact of physical activity or exercise training on cytokines in humans and horses (adults, young, and obese). In general, the present systematic review supports the idea that exercise can stimulate both pro-inflammatory and anti-inflammatory cytokine responses.

2. Materials and Methods

2.1. Protocol for the Systematic Review

This systematic review was structured in accordance with the Preferred Reporting Items for Systematic Reviews (PRISMA) protocol (**Figure 2**). The Problem, Intervention, Comparison, and Outcome (PICO) model was used to formulate keywords and facilitate the literature search. The "P" (Population), which includes humans and animals focusing on horses; "I" (Intervention) is physical activity/exercise; "C" (Comparison) is a comparison between the trained group and the control group or between aerobic training and resistance training; and "O" (Outcome) is the effect of the training on the production of cytokine(s). This review is also based on a thorough systematic literature search of electronic databases for research examining the relationship between physical activity and cytokine production in humans and horses, published between 2000 and 2022.

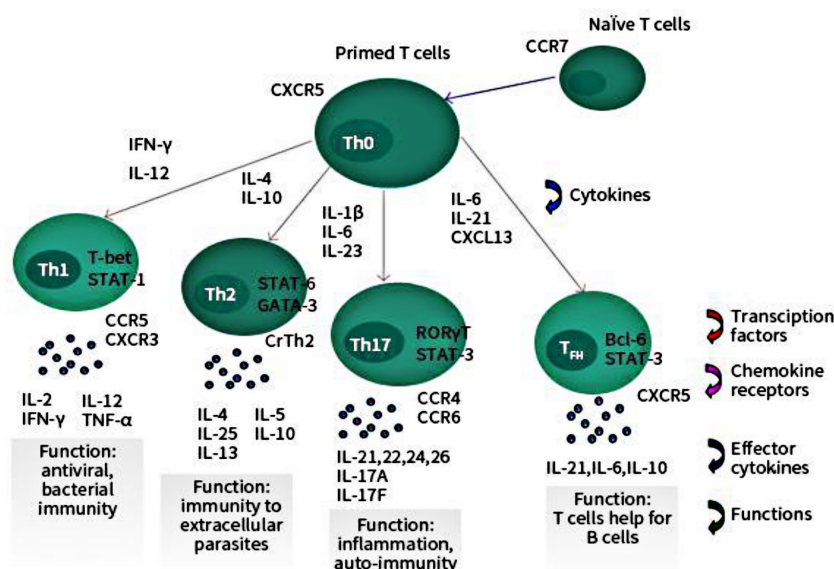


Figure 1: A schematic representation of different exercise-induced cytokine secretion, their functions, pathways, and effects. Naïve T cells, upon activation by an antigen, give rise to Th0, which differentiates into T cell subsets (Th1, Th2, Th17, and TFH) that secrete different cytokines, thereby participating in various signaling mechanisms. Naïve T cell (CD4+): This represents an inactive T cell that has not yet encountered an antigen. Upon encountering an antigen-presenting cell (such as a dendritic cell) that presents an antigen to it, the naïve T cell gets activated. After activation, the naïve T cell becomes an undifferentiated T helper cell, commonly referred to as Th0. Th0 then differentiates into various T helper cell subsets depending on the signals it receives. Th0 can differentiate into Th1 under specific signals. Th1 cells express chemokine receptors CCR5 and CXCR3, allowing them to migrate to sites of inflammation. Th1 cells primarily secrete cytokines such as Interleukin-2 (IL-2) and Interferon-gamma (IFN- γ). IL-2 helps in the activation and proliferation of T cells, while IFN- γ stimulates immune responses against intracellular pathogens. Th0 also differentiates into Th2 under specific signals. Th2 cells express chemokine receptors CCR4 and CCR6. Th2 cells secrete cytokines such as Interleukin-4 (IL-4), Interleukin-5 (IL-5), Interleukin-13 (IL-13), and Interleukin-10 (IL-10). These cytokines are involved in promoting allergic responses, activating B cells for antibody production, and enhancing the function of eosinophils. Th0 also differentiates into Th17 under specific signals. Th17 cells express chemokine receptors CCR6 and CXCR5. Th17 cells secrete cytokines such as Interleukin-17 (IL-17), Interleukin-21 (IL-21), and Interleukin-22 (IL-22). These cytokines play a role in promoting inflammation, neutrophil recruitment, and defense against certain bacterial and fungal infections. Th0 also differentiates into TFH under specific signals. TFH cells express the chemokine receptor CXCR5. TFH cells secrete cytokines such as Interleukin-21 (IL-21), Interleukin-4 (IL-4), and Interleukin-10 (IL-10). TFH cells play a crucial role in providing help to B cells within germinal centers, promoting antibody production, and facilitating the formation of memory B cells.

2.2. Eligibility Criteria

The study was carried out between 2000 and 2022. Only studies that fulfilled the following criteria were included:

1. Articles that were original and based on scientific research.
2. The reported study had an experimental design on human subjects, horses, or experimental/laboratory animals.
3. There was clear information about the training or exercise intervention.
4. The study investigated the levels of cytokines, either pro- or anti-inflammatory, or both, after an exercise or training intervention.

2.3. Information and Search Strategy

The databases included in the search were Scopus (<https://scopus.com>), SciELO (www.scielo.br), Web of Science (<https://webofknowledge.com>), PubMed (<https://pubmed.ncbi.nlm.nih.gov/>), and ScienceDirect (<https://sciencedirect.com>), using the terms: ("physical activities" OR "exercise"

"OR" "training") AND ("cytokine production" OR "cytokine") AND ("human athlete" OR "animals horse-equids") AND ("2000–2022") as keywords. The search was conducted in October 2022 (first record). This search strategy identified 4,450 published articles (Figure 2). Two reviewers independently screened each article based on its title, keywords, and abstract to ensure that all relevant studies were captured.

The inclusion criteria were: English language, access to full texts, studies examining the effects of physical activity in experimental animals, humans, and horses/equids, and assessment of both pro-inflammatory and anti-inflammatory effects. After excluding duplicate studies, 24 studies were included in the review. A large number of studies (4,426) were excluded because they were not original scientific articles, there was no clear information on training or exercise interventions, participants were not healthy, they received medications in addition to physical activity, or the studies fell outside the search period (2000–2022). Studies included and excluded were reviewed separately by two researchers. The selected papers were then examined for methodological quality and classified based on individual levels of evidence.

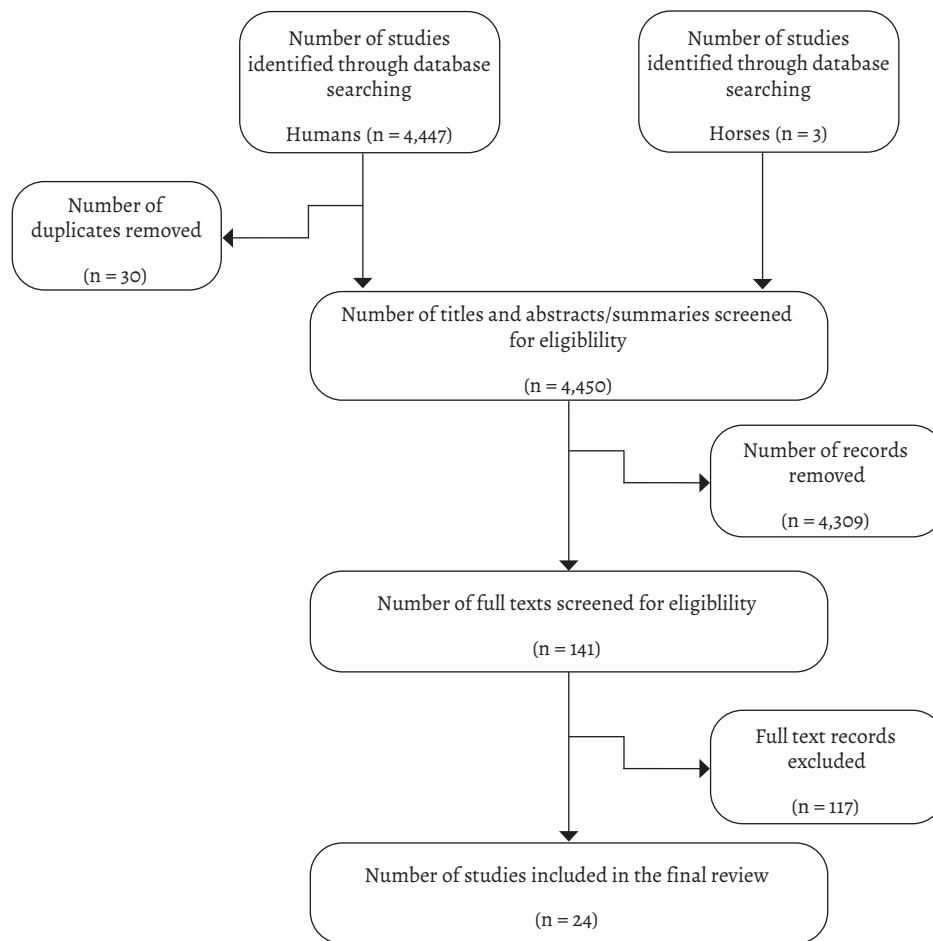


Figure 2: PRISMA flowchart for the process of selecting eligible articles.

2.4. Study Record, Data Items, and Outcomes

Information on the title, abstract, and keywords was acquired in the Research Information System (RIS) format and imported into Mendeley software, after which duplicate documents were excluded. The title and abstract were examined, and the inclusion criteria were reviewed during the article screening process. One author (IPO) analyzed the selected documents, excluding those that did not meet the inclusion requirements. Two additional authors verified the information (ASO and OCA). Data on the concentration of cytokines (pro- and anti-inflammatory) were obtained from the full text for reporting purposes. The Mendeley program was used to examine the exported keywords. Subsequently, data from Mendeley were exported in RIS format, including authors, titles, abstracts, and keywords.

3. Results

3.1. Quantitative Analysis

A total of 4,450 studies were identified after the search (after removing duplicates). Of these, 4,426 studies did not fulfill the eligibility criteria and were excluded. After applying the inclusion and exclusion criteria, 24 articles were deemed eligible for inclusion in this review. Three (3) studies focused on the effects of physical activity (exercise) on cytokine production in horses. In contrast, twenty (20) studies focused on the impact of physical activity (exercise) on cytokine production in humans and laboratory animal models, and one

(1) study conducted a comparative analysis between horses and humans.

3.2. Study Characteristics

The included studies (Figure 3) reported the effect of physical activity on pro- and anti-inflammatory cytokine levels. Of the 24 studies, six focused on laboratory animals (mice and rats), three on horses, and fifteen on humans. The human participants enrolled were between 18 and 81 years old, while the experimental laboratory mice were between 5 and 8 weeks old. The horse participants enrolled were between 3 and 8 years old. Participants from all 24 studies were generally healthy.

The exercise intervention was highly variable, ranging from resistance training to aerobic endurance, and from anaerobic endurance to various activities such as swimming, treadmill walking, and running. The duration of the exercise intervention was also highly variable, ranging from 4 days to 5 months. The studies collected blood samples at varying times (immediately, 30 min, and 1, 3, 6, and 24 h) after exercise (Table 1).

From the full texts, data regarding the study region were extracted and summarized. The majority of the studies were conducted in Brazil (n = 8), followed by Iran (n = 2), Japan (n = 2), South Korea (n = 2), and the United States (n = 2). Although relevant to the literature, no study assessed cytokine production using *in vitro*-stimulated immune cells.

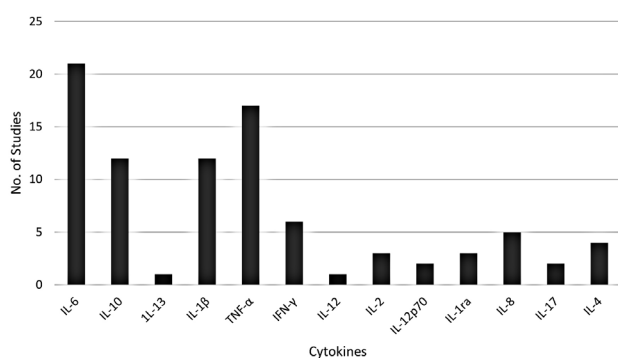


Figure 3: Number of references (n = 24) for studies on specific cytokines related to exercise in humans and horses.

3.3. Pro-Inflammatory Cytokines

A total of 24 studies were included after reviewing the articles (Figure 3). Among these studies, 12 examined the levels of IL-1β [41–52], 17 examined the level of TNF-α [41–58], five examined the level of IFN-γ [51–59], one examined the level of IL-12 [59], three examined the level of IL-2 [50,52,54], 21 examined the level of IL-6 [6,10,25,41–48,50–54,56,57,60], two examined the level of IL-12p₇₀ [50,51], five examined the level of IL-8 [50,51,55,56], two examined the level of IL-17 [41,52], and one study examined the level of IL-12p35 [59].

3.4. Anti-Inflammatory Cytokines

Among the included studies, 12 examined the level of IL-10 [10,41,45,48–55,60], one examined the level of IL-13 [45], four examined the level of IL-4 [50,52,54,59], and three examined IL-1ra [10,44,50].

3.5. Effect of Exercise on Cytokine Production in Humans

Concerning IL-6 levels, lower values were reported in participants exposed to moderate-intensity exercise (921.3 ± 228.7) compared to those in the control group (1429.6 ± 270.4) [10,25,41,44,46,51] (Figure 4). Higher values were reported in participants exposed to high-intensity exercise (1364.0 ± 164.0) compared to those in the control group (901.8 ± 198.2) [10,42,50,57,58].

For serum IL-1β levels, higher values were reported in participants exposed to moderate-intensity exercise (479.4 ± 4.0) compared to those in the control group (372.0 ± 5.0) [44,46,51]. Lower values were reported in participants exposed to high-intensity exercise (108.3 ± 41.7) compared to those in the control group (321.88 ± 48.0) [45,48,49].

For serum IFN-γ levels, no significant differences were reported in participants exposed to moderate-intensity exercise (42.6 ± 1.0) compared to those in the control group (43.3 ± 1.2) [41,53,54]. A similar result was reported for participants exposed to high-intensity exercise (1.4 ± 0.5) compared to those in the control group (1.5 ± 0.5) [50].

For serum IL-17 levels, no significant differences were reported in participants exposed to moderate-intensity exercise (3.4 ± 0.1) compared to those in the control group (3.5 ± 0.1) (Figure 4).

For serum IL-4 levels, no significant differences were reported in participants exposed to moderate-intensity exercise (4.8 ± 0.04) compared to those in the control group (4.9 ± 0.02) [54]. Higher values were reported in participants exposed to high-intensity exercise (4.9 ± 0.6) compared to those in the control group (3.5 ± 0.8) [50].

For serum IL-1Ra levels, no significant differences were reported in participants exposed to high-intensity exercise (0.03 ± 0.1) compared to those in the control group (0.04 ± 0.1) [10,50] (Figure 4).

For serum IL-10 levels, lower values were reported in participants exposed to moderate-intensity exercise (212.3 ± 7.7) compared to those in the control group (242.84 ± 8.16) [51,53]. Higher values were reported in participants exposed to high-intensity exercise (113.4 ± 37.0) compared to those in the control group (27.7 ± 25.3) [10,45,48–50,55].

For serum TNF-α levels, higher values were reported in participants exposed to moderate-intensity exercise (154.5 ± 1.0) [41,51] and high-intensity exercise (364.0 ± 60.0) [45,48,49,55,57,58], compared to those in the control groups (145.3 ± 1.0 and 228.0 ± 62.2 , respectively).

For serum IL-2 levels, no significant differences were reported in participants exposed to high-intensity exercise (7.6 ± 0.3) compared to those in the control group (7.7 ± 0.5) [50].

For serum IL-8 levels, higher values were reported in participants exposed to moderate-intensity exercise (54.5 ± 10.5) [43] and high-intensity exercise (83.3 ± 26.7) [10,50,55], compared to the control groups (27.5 ± 12.5 and 31.0 ± 24.1 , respectively) (Figure 4).

3.6. The Effect of Frequency and Duration of Exercise on Cytokine Production in Horses

Concerning IL-6 levels, higher values were reported in horses exposed to strenuous exercise (142.0 ± 5.0) compared to those in the control group (132.0 ± 4.1) [6,47,52] (Figure 5). For serum IL-10 levels, lower values were reported in horses exposed to strenuous exercise (226.3 ± 5.0) compared to those in the control group (236.6 ± 5.4) [6]. For serum IL-1β, no significant differences were reported in horses exposed to strenuous exercise (41.8 ± 0.4) compared to those in the control group (41.1 ± 0.3) [47,52].

For serum TNF-α, lower values were reported in horses exposed to strenuous exercise (124.2 ± 0.5) compared to those in the control group (136.7 ± 1.8) [6]. For serum IFN-γ, lower values were reported in horses exposed to strenuous exercise (39 ± 1.2) compared to those in the control group (42 ± 1.5) [52,59]. For serum IL-17, lower values were reported in horses exposed to strenuous exercise (54.0 ± 1.5) compared to those in the control group (57.0 ± 1.5) [52]. For serum IL-4, lower values were reported in horses exposed to strenuous exercise (36.0 ± 0.5) compared to those in the control group (37.0 ± 0.5) [52,59] (Figure 5).

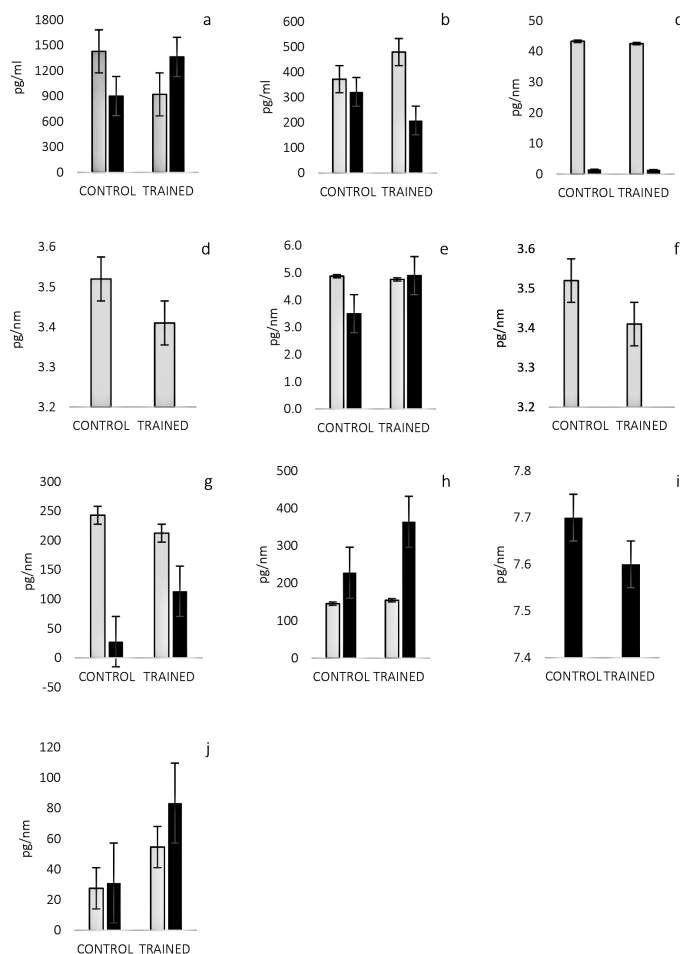


Figure 4: Human serum concentrations of (a) interleukin-6 (IL-6), (b) interleukin-1 β (IL-1 β), (c) interferon-gamma (IFN- γ), (d) interleukin-17 (IL-17), (e) interleukin-4 (IL-4), (f) interleukin-1Ra (IL-1Ra), (g) interleukin-10 (IL-10), (h) tumor necrosis factor-alpha (TNF- α), (i) interleukin-2 (IL-2), and (j) interleukin-8 (IL-8). Grey bars indicate moderate exercise intensity; black bars indicate high exercise intensity.

4. Discussion

The duration and intensity of physical activities have been reported to alter cytokine levels [61]. Additionally, moderate exercise loads reduce blood leptin levels, a hormone that helps maintain healthy calorie burning [62]. This adipocytokine, known as leptin, is known to increase IL-6 protein and its mRNA expression, thereby causing inflammation [12]. As a result, the data suggest that moderate exercise, such as treadmill training, lowers IL-6 levels. This finding is consistent with the study [62], which found that Zucker rats subjected to moderate but regular exercise experienced a substantial reduction in IL-6 compared to rats that did not exercise.

In contrast, the present review also found an increase in IL-6 levels among humans and horses involved in endurance training or marathons [6]—suggesting a possible cytokine response similarity between species. This could be because the IL-6 level in the bloodstream is significantly enhanced 30 minutes after the start of physical activity. In the selected articles, IL-6 concentration was measured immediately after the exercise intervention and not during the exercise. Thus, after running a 42 km marathon in humans or an endurance test for horses, the maximal IL-6 level was measured immediately afterward, showing a 100-fold increase. Although

more equine-based studies are necessary, the kinetics of exercise-induced IL-6 may be closely related to the duration of the exercise for both species.

It was also found that participants subjected to physical training for 2–4 km or performing aerobic training on treadmills for 30–60 min (moderate intensity) had an increase in IL-1 β , a pro-inflammatory cytokine. Moreover, a decrease in IL-1 β was observed in humans subjected to physical training of 42 km or endurance training on treadmills for 2–6 hours (high-intensity exercise) (Figure 6). In contrast, we reported an increase in IL-1 β in horses subjected to high-intensity physical training (Figure 7). These varying results may be due to species-specific differences in the immune system's response to exercise, with horses exhibiting a distinct pattern of cytokine production compared to humans [13]. Additionally, the type and duration of the exercise protocol used in the studies differ between humans and equines, which could contribute to the differing responses. Finally, the age of the horses and humans could contribute to the observed differences in the cytokine response. For example, older individuals exhibit a blunted cytokine response to exercise compared to younger individuals, which may have a greater impact on the IL-1 β response observed in the human study.

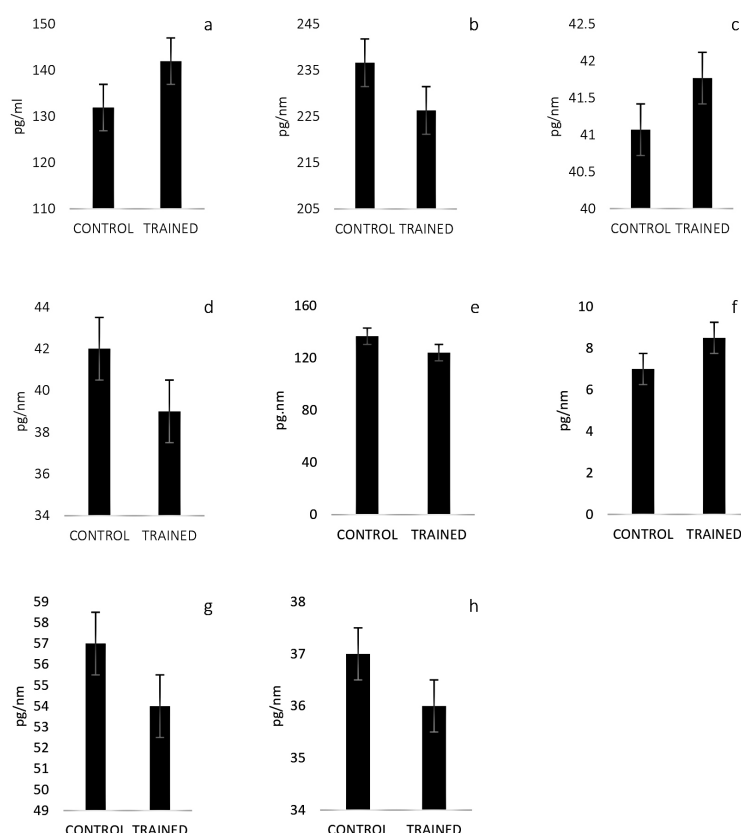


Figure 5: Horse serum concentrations of (a) interleukin-6 (IL-6), (b) interleukin-10 (IL-10), (c) interleukin-1 β (IL-1 β), (d) interferon-gamma (IFN- γ), (e) tumor necrosis factor-alpha (TNF- α), (f) interleukin-12 (IL-12), (g) interleukin-17 (IL-17), and (h) interleukin-4 (IL-4). Black bar: high-intensity exercise.

There were, however, no significant changes in the pro-inflammatory cytokines, including IFN- γ , IL-17, and IL-12, among all human participants and horses subjected to high-intensity exercise (**Figure 6** and **Figure 7**). However, there was a decrease in IL-17 among participants who underwent moderate-intensity exercise. The reduction in IL-17 levels among participants who engaged in moderate-intensity exercise could be attributed to several factors, including exercise duration and intensity. A subset of immune cells called T-helper 17 (Th17) cells primarily produces IL-17. These cells play a crucial role in regulating immune responses, particularly in the context of inflammation and autoimmune diseases. Moderate-intensity exercise has been associated with anti-inflammatory effects, while high-intensity exercise can lead to a more pronounced stress response and inflammatory state. The decrease in IL-17 levels observed in participants who engaged in moderate-intensity exercise could be indicative of the downregulation of Th17 cell activity. Another possible explanation is that moderate-intensity exercise promotes the release of anti-inflammatory cytokines and enhances the activity of regulatory T cells (Tregs). Tregs help maintain immune balance by suppressing excessive inflammation. The increase in anti-inflammatory factors and the regulatory activity of Tregs may counteract the production of IL-17, resulting in a decreased level.

In this review, an increase in anti-inflammatory IL-4 levels was also reported among participants exposed to high-intensity exercise (**Figure 6**). In contrast, there were no signifi-

cant changes in IL-4 levels among horses exposed to high-intensity exercise. The contrasting findings between humans and horses regarding IL-4 levels in response to high-intensity exercise can be attributed to several factors, as humans and horses have distinct immune system regulations and responses. IL-4, which is primarily associated with Th2 immune responses, may have different regulatory mechanisms in horses compared to humans. Secondly, the physiological responses to exercise differ between the two species. Humans and horses exhibit distinct muscle fiber types, energy metabolism, and aerobic capacities, which can impact their immune responses to exercise and subsequent cytokine production, including IL-4. Lastly, species-specific adaptations and genetic differences can lead to unique responses to exercise. Horses, which have been selectively bred for endurance and specific athletic abilities, may exhibit a different immune response profile compared to humans.

For the anti-inflammatory IL-1Ra level, which was reported only in human participants, there was no significant difference in the cytokine level among all participants involved in high-intensity exercise (marathon) (**Figure 6**). However, a decrease in IL-1Ra levels was observed among participants exposed to moderate-intensity exercise. The contrasting findings in IL-1Ra levels between high-intensity exercise (marathon) and moderate-intensity exercise among human participants can be attributed to the varying physiological responses and inflammatory processes associated with different exercise intensities. High-intensity exercise induces a

robust inflammatory response, leading to the release of various cytokines, including IL-1Ra, which acts as an antagonist to IL-1, a pro-inflammatory cytokine. During high-intensity exercise, IL-1Ra levels may remain stable or unchanged as the body releases IL-1Ra to counterbalance the elevated levels of IL-1. Consequently, no significant difference in IL-1Ra levels is likely to be observed among participants involved in high-intensity exercise.

In contrast, moderate-intensity exercise typically elicits a less pronounced inflammatory response due to lower mechanical stress and metabolic demands. The decrease in IL-1Ra levels among participants exposed to moderate-intensity exercise could be attributed to the relatively lower demand for IL-1Ra to counterbalance the inflammatory process. It is essential to acknowledge that individual variations and other factors may impact the observed cytokine responses, and additional research is required to fully elucidate the underlying mechanisms of IL-1Ra level changes in response to varying exercise intensities. There was no reported effect of exercise on the IL-1Ra level in horses, as no author reported this in any of the studies that passed the inclusion criteria. However, a study by [63] reported that after strenuous exercise in horses, the concentration of the cytokine IL-1Ra was reduced.

Physical exercise plays a crucial role in enhancing health benefits by increasing the production of IL-10. Interleukin-10 is a cytokine that plays a critical role, with potent anti-inflammatory properties, when produced during and after exercise. IL-10 helps the host's immune response to pathogens, preventing tissue damage. In this review, the findings showed that all participants involved in the marathon had an increased level of the anti-inflammatory cytokine IL-10 [10,49,50,55]. This could be a result of the magnitude of the exercise, as an increase in IL-10 levels is associated with active muscle mass and, therefore, exercise intensity in humans [64] (Figure 6). However, other exercises, ranging from swimming to squatting, either reduced IL-10 levels or showed no significant difference between the IL-10 cytokine level before and after the exercise. This data is consistent with the findings of the current investigation,

supporting the idea that the pattern and magnitude of the IL-10 response vary with the type of exercise. The IL-10 response in this study is similar to that previously reported by [13], who found that marathon training significantly increased levels of IL-10 in all groups relative to their baseline values (10–to 20-fold).

In contrast to humans, a decreased IL-10 level has been reported in horses, which may be a result of the prolonged training duration (5 months) in horses (Figure 7). This result is consistent with [65], who found that plasma IL-10 levels were significantly increased after 6 months of training in horses compared to baseline levels. The authors suggested that this increase may reflect a training-induced anti-inflammatory response that helps to maintain immune homeostasis in the horses [65].

TNF- α is a classic pro-inflammatory cytokine released in response to cellular damage. Low-intensity exercise in untrained individuals upregulates the body's inflammatory cytokine response, which is thought to increase with continued exercise, such as marathon running [66]. Fatigue and increased metabolic load work together to further activate circulating monocytes, causing them to generate and release pro-inflammatory cytokines [67]. Furthermore, cytokines released in peripheral tissues might exacerbate localized inflammatory pathways in muscles and joints, leading to an intensified systemic response. As a result, the extent of systemic elevations in TNF- α is related to the intensity, type, and duration of exercise [63]. TNF- α gene expression has been observed to increase for up to 24h after several exercise regimens (graded, interval, and repeated sprint exercise tests) in Standardbred mares. In this review, an increase in the TNF- α level was reported among humans and horses exposed to high-intensity exercise (Figure 6 and Figure 7). There was an increase in participants exposed to the treadmill (75% intensity VO₂), with reduced levels after 2 h. There was also an increase in the IL-8 level in all human participants, irrespective of the exercise type. These findings support previous studies [68,69]. Moreover, there was an increase in horses exposed to high-intensity exercise.

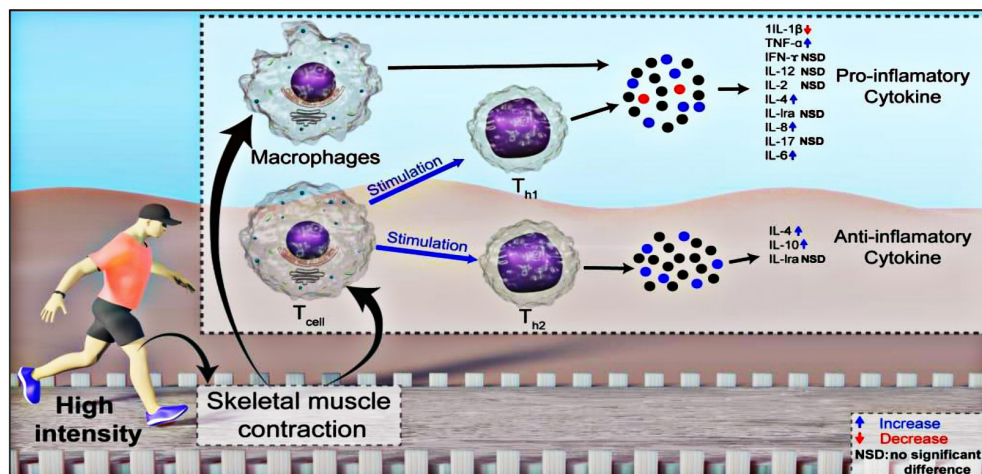


Figure 6: Cascade effect of high-intensity exercise on human skeletal muscle, cell stimulus, and cytokine production (increase, decrease, no significant difference).

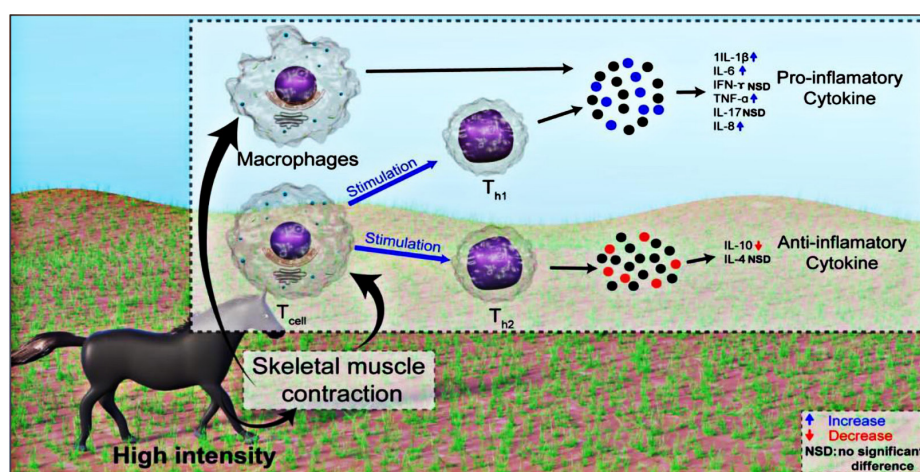


Figure 7: Cascade effect of high-intensity exercise on horse skeletal muscle, cell stimulus, and cytokine production (increase, decrease, no significant difference).

Peripheral blood mononuclear cells (PBMCs) can also be used in preclinical research due to their role in immune responses (i.e., diseases and inflammatory processes), including IL-6, IL-4, and IL-1 β , more recently [6,59,70]. In horses, *ex vivo* stimulation of PBMCs with lipopolysaccharide (LPS) has been employed to assess cytokine production, showing significant increases in TNF- α , IL-1 β , IL-6, and IL-8, which could be attenuated by treatment with silibinin [71]. Recent advances in monoclonal antibody development have enabled the quantification of mature IL-1 β secreted by PBMCs following LPS stimulation, with CD14 $^{+}$ monocytes identified as the predominant cellular source [72]. Additionally, stimulation with PMA/ionomycin has been used to investigate TNF- α expression, demonstrating its production not only by monocytes but also by CD4 $^{+}$ and CD8 $^{+}$ T lymphocytes [70]. Collectively, these findings highlight the applicability of equine PBMCs as a robust *ex vivo* model for characterizing cytokine responsiveness.

Oxidative stress, mediated by the production of reactive oxygen species (ROS), is also a key regulatory mechanism of cytokine gene expression, primarily through the activation of transcription factors such as NF- κ B and AP-1. In horses, recent studies have demonstrated this link, showing that antioxidant interventions can modulate cytokine responses under stress conditions. For example, astaxanthin supplementation in Thoroughbred racehorses reduced ROS formation and attenuated the exercise-induced increase of pro-inflammatory cytokines [73]. At the same time, treatment with chlorogenic acid decreased ROS production in immune cells, accompanied by an increase in IL-10 and a reduction in TNF- α , IL-6, IL-1 β , and IL-8 [74]. Moreover, in immunization models for antivenom production, horses with stronger immune responses exhibited concomitant increases in oxidative stress markers and anti-inflammatory cytokines, such as IL-4 and IL-10, suggesting that redox modulation can influence cytokine profiles during adaptive responses [75]. These findings underscore the importance of considering oxidative stress mechanisms in conjunction with *ex vivo* stimulation data when interpreting cytokine production in horses.

Clenbuterol (β 2-adrenoceptor agonist) was evaluated for its effect on weight loss. Although it is prohibited in humans for anti-doping control, clenbuterol is popular in bodybuilding and physio-culture sports. The substance inhibits the inflammatory process. The cells from 14 untrained and 14 well-trained racehorses were collected after acute exercise and cultured with clenbuterol. The expression of CD4, CD8, FoxP3, CD14, MHC II, and CD5, in addition to IL-1 β , IL-4, IL-6, IL-10, IL-17, IFN- γ , and TNF- α , was assessed. β 2-adrenoceptor stimulation led to enhanced anti-inflammatory properties in well-trained horses, as did low doses in untrained animals. In contrast, higher doses of clenbuterol created a pro-inflammatory environment in untrained horses [76].

The use of constant immunological markers to monitor cell and organ damage in advance of moderate- and high-intensity training is an area of ongoing research. While the development and implementation of such markers are still in progress, there is potential for both human and equine athletes to benefit from these tools in optimizing their training and performance. In the context of human athletes, various studies have explored the use of immunological markers to assess the impact of training, predict performance, and monitor the overall health status of athletes. For example, markers such as cytokine levels (including interleukins) and other immune cell parameters have been investigated to understand the inflammatory response and potential tissue damage associated with intense exercise [58]. By tracking these markers over time, trainers and athletes can gain insights into the training load, recovery patterns, and potential risks of overtraining or injury. In the field of equine training, similar investigations are being carried out to identify markers that can provide insights into the health and performance of horses. The focus is on understanding how exercise-induced stress, inflammation, and other physiological responses impact horses' overall well-being and athletic potential. While specific markers in horse training have not been mentioned in the provided context, ongoing research aims to uncover reliable indicators that can assist trainers and horse owners in optimizing training protocols and health management (i.e., kinship) [77]. Moreover, the use of the horse as a close model in translational studies to understand human physiology and immunity will shed light on preclinical and clinical trials [3].

Table 1: Summary of inclusion studies on exercise-induced cytokine production in humans and horses.

Ref.	Population, age, and location	Exercise and duration	Cytokines quantified	Biological sample and sampling time	Techniques
[41]	35 rats, 60 days, Brazil	Swimming, an aerobic and resistance training (moderate intensity) for 16 weeks	IFN- γ , IL-6, TNF- α , and IL-10	Serum, before and immediately after exercise	ELISA
[42]	43 overweight men, 25 years, Iran	Endurance training (aerobic running), resistance training, and concurrent training for 8 weeks	IL-1 β , IL-6, and TNF- α	Serum, before and immediately after exercise	ELISA
[60]	26 elderly women, above 60 years	Strengthening exercise, balancing, and walking exercise programs for 8 weeks	IL-6	Serum, before and immediately after exercise	ELISA
[43]	19 male, 23–25 years, South Korea	Treadmill for 1 hour at 75% intensity VO ₂ for 6 weeks	IL-1 β , IL-6, IL-8, and TNF- α	Serum, before and immediately after exercise, 30 minutes, and 2h after exercise	ELISA
[61]	Young male, 18–30 years, Canada	Eccentric exercise bouts for 4 days	IL-1 β , IL-6, and IL-10	Serum, before exercise, immediately after exercise, and 24h after exercise	ELISA
[53]	Male Swiss mice, 5–6 weeks old, Brazil	Progressive swimming for 5 days	IFN- γ , TNF- α , IL-10, IL-6, and IL-12p70	Plasma, before the exercise, immediately after exercise, and 3h after exercise	Cytometric bead array kit
[54]	12 elderly women, above 60 years, Brazil	Stretching, resistance, and isotonic exercise in circuits using weights for 16 weeks	IL-2, IL-4, IL-6, IL-10, IL-17, TNF- α , and IFN- γ	Plasma, before and immediately after exercise	Cytometric bead array kit
[44]	32 elderly women, above 60 years, Brazil	Aerobic, resistance, and stretch exercise at moderate intensity for 24 weeks	IL-1 β , IL-1Ra, and IL-6	Serum, before and immediately after exercise	ELISA
[45]	15 young males, 18–30 years, Brazil	Acute strength exercise (acute-SE) for 3 months	IL-6, IL-10, IL-1 β , TNF- α , and IL-13	Plasma, before, immediately after exercise, and 30 minutes after exercise	Bio-Plex Pro Human cytokine kit
[46]	28 rats, 8 weeks old, Iran	Endurance training and downhill running, treadmill for 8 weeks	IL-6, IL-1 β , and TNF- α	Serum, before and immediately after exercise	ELISA
[59]	11 horses, 4–8 years, USA	Strenuous exercise (Treadmill) for 10–12 weeks	IL-4, IFN- γ , and IL-12p35	DNA, before and immediately after exercise	Real-time PCR
[47]	8 horses, 3–4 years, Belgium	Strenuous exercise (Treadmill) for 1 month	TNF- α , IFN- β , IL-6, and IL-1 β	DNA, before and immediately after exercise	Real-time PCR
[48]	39 young Wistar rats, Minas Gerais, Brazil	Treadmill with high intensity for 6 weeks	IL-1 β , IL-6, TNF- α , and IL-10	Serum, before and immediately after exercise	ELISA
[49]	15 male Wistar rats, Sao Paulo, Brazil	Endurance training, Treadmill running for 8 weeks	IL-1 β , TNF- α , and IL-10	Serum, before and immediately after exercise	ELISA
[55]	18 master athletes, 53–64 years, Portugal	Marathon	IL-10, IL-8, and TNF- α	Serum, before and immediately after exercise	ELISA
[50]	16 male marathoners, Japan	Marathon for 8 weeks	IL-1 β , IL-4, IL-2, IL-8, IFN- γ , IL-10, IFN- α , TNF- α , IL-1Ra, TGF- β IL-6, and IL-12	Serum, before and immediately after exercise	ELISA

Ref.	Population, age, and location	Exercise and duration	Cytokines quantified	Biological sample and sampling time	Techniques
[51]	Male and female rowers	Water rowing workouts, running, and an ergometer for 8 weeks	IL-1 β , IL-6, IL-8, IL-10, IL-12p70, and TNF- α	Plasma, before, immediately after exercise, 30 minutes after exercise, 3 hours, and 6 hours post-training	Cytometric bead array human kit
[4]	8 trained male athletes, 25–28 years, and 8 trained horses, 6–8 years, Italy	Endurance races and training	IL-6	DNA, before and immediately after exercise	Real-time PCR
[52]	9 Arabian horses, Poland	Endurance training for 5 months	IL-1 β , IL-2, IL-4, IL-6, IL-10, IL-17, IFN- γ , and TNF- α	Serum, before and immediately after exercise	Immuno-enzymatic commercial assay for equine species
[62]	31 males, 24–26 years	Cycle ergometer	IL-6	DNA, before and immediately after exercise	Real-time PCR
[56]	22 male athletes, 22–25 years	Focused fitness for 5 days a week	TNF- α and IL-6	Serum, before and immediately after exercise	Linco Plex (ELISA-species-specific radioimmunoassay kit)
[63]	45 athletes/marathoners, average age of 25 years, USA	Marathon	IL-1Ra, IL-6, IL-8 and IL-10	Plasma, before and immediately after exercise	ELISA
[57]	15 male marathoners, 20–60 years	Marathon	Plasma TNF- α and IL-6	Plasma, before and immediately after exercise	Solid-phase, two-sided chemiluminescent assay on the Immulite system before and after the race
[58]	18 male, 19 years	Resistance exercise	IL-6 and TNF- α	Serum, before and immediately after exercise	ELISA

The incorporation of artificial intelligence (AI) can significantly enhance the analysis and interpretation of the gathered data in both human and equine contexts. AI algorithms can handle large datasets, identify patterns, and generate models that associate variables such as immunological markers, training protocols, physical conditions, and performance outcomes. This can facilitate sound evaluations, help predict optimal training strategies, and provide valuable insights into the complex interplay between immunological responses, training adaptations, and performance outcomes. In equine immunology, clustering and multivariate approaches have already been employed to capture immune dynamics. Principal component analysis and hierarchical clustering of 22 inflammation-related genes in peripheral blood leukocytes revealed distinct transcriptional signatures differentiating local and systemic inflammatory responses following LPS challenge [78]. Similarly, single-cell RNA sequencing coupled with unsupervised clustering resolved over 30 immune cell subpopulations in equine PB-MCs, including a predominance of T-bet⁺ B cells, thereby establishing a high-resolution immune cell atlas [3]. These findings highlight the feasibility of advanced clustering methods and suggest that their integration with machine

learning techniques may enable predictive modeling of cytokine-driven immune responses in horses. It is important to note that while the potential benefits of constant immunological markers and AI-based analysis are promising, further research, validation, and refinement of these approaches are still required before widespread implementation can occur. Moreover, as more data are gathered, the protocol can associate these variables and execute a large number of possibilities to produce sound evaluations. Nonetheless, the ongoing advancements in these fields hold promise for the future of training optimization, performance enhancement, and genetics in humans and horses.

5. Conclusion

The present findings suggest that cytokine levels, whether anti-inflammatory or pro-inflammatory, depend solely on the intensity and duration of exercise in both humans and horses. However, further studies are needed to compare these responses with immune system disorders and other exercise-induced cytokines. The use of cell-type-specific and longitudinal studies should be complemented by transcriptomic analysis to better determine their role in horse physiology, pathogenesis, and breeding.

6. Limitations

A significant limitation of this systematic review is the methodological heterogeneity in cytokine measurement approaches across the included studies. Studies varied in their analytical methods, with some quantifying mRNA expression in tissue samples while others measured circulating protein concentrations in serum or plasma. This distinction is critical because mRNA expression levels do not necessarily correlate with protein secretion due to post-transcriptional and post-translational regulatory mechanisms. The temporal dynamics also differ, with mRNA typically peaking earlier than the corresponding protein levels. Additionally, tissue-specific mRNA measurements cannot be directly compared to systemic protein concentrations, as local tissue expression may not reflect circulating levels.

Additionally, the lack of standardized timing for cytokine measurements significantly impacts the interpretation of results. Different cytokines follow distinct temporal release patterns that were not adequately captured in most studies. IL-6, for instance, typically peaks during exercise or within 30 minutes post-exercise and then rapidly declines, while TNF- α demonstrates a more delayed peak occurring 2–6 hours post-exercise. Anti-inflammatory cytokines, such as IL-10, exhibit biphasic responses, characterized by an early peak followed by a sustained elevation that can persist for over 24 hours. IL-1 β shows variable timing patterns depending on exercise intensity and duration. The majority of included studies measured cytokines "immediately after the exercise intervention rather than during exercise," creating several interpretive challenges. For cytokines that peak during exercise (such as IL-6), immediate post-exercise measurements may capture declining rather than peak concentrations, potentially underestimating the true magnitude of the response. Conversely, delayed-response cytokines might appear unchanged if measured too early, leading to false-negative results regarding anti-inflammatory responses. Single-time-point measurements cannot capture the dynamic interplay between pro- and anti-inflammatory responses, which is essential for understanding the complete immunological response to exercise.

Authors' Contributions

IPO: Conceptualization, literature review, data analysis, writing. ASO and OCA: Validation, review, and editing. EP and UYY: Literature review and critical review. MBM: Supervision, critical review, and final approval of the manuscript.

Data Availability

All data analyzed in this review are available in the published literature and are listed in the reference section.

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Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical Approval

This article is a systematic review and does not involve the collection of original data from humans or animals; therefore, ethical approval was not required.

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