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REVIEW ARTICLE

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Major aspects of cannabidiol in the interaction with microRNAs and exosomes in the modulation of inflammatory and immunological processes in athletes: a systematic review

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Abstract

Introduction: The correct interaction between elements of the endocannabinoid system plays an important role in the development of the central nervous system. Clinical and preclinical studies suggest that cannabidiol (CBD) may be useful for athletes due to its anti-inflammatory, analgesic, anxiolytic, and neuroprotective properties and its influence on the sleep-wake cycle. In addition, a series of implications for epigenetic processes have also been proven, through changes in the expression of microRNAs responsible for modulating the immune and inflammatory systems. **Objective:** It was to develop a systematic review study to highlight the main aspects of cannabidiol in the interaction with microRNAs and exosomes in the modulation of inflammatory and immunological processes in athletes. **Methods:** The systematic review rules of the PRISMA Platform were followed. The research was carried out from February to April 2023 in Scopus, PubMed, Science Direct, Scielo, and Google Scholar databases. The quality of the studies was based on the GRADE instrument and the risk of bias was analyzed according to the Cochrane instrument. **Results and Conclusion:** A total of 228 articles were found, and 84 articles were evaluated in full and 60 were included and developed in this systematic review study. Considering the Cochrane tool for risk of bias, the overall assessment resulted in 20 studies with a high risk of bias and 90 studies that did not meet GRADE. CBD has been reported to exert a range of physiological, biochemical, and psychological effects with the potential to benefit human health. For example, there is preliminary supporting evidence for the anti-inflammatory, neuroprotective, analgesic, and anxiolytic actions of CBD and the possibility that it may protect against gastrointestinal damage associated with inflammation and promote the healing of traumatic skeletal injuries. The combination of Δ9-THC and CBD can alter the activity of microRNAs responsible for increasing the biosynthesis of inflammatory mediators, leading to a reduction in the inflammatory profile. However, it is important to recognize that these findings are very preliminary, sometimes inconsistent, and largely derived from preclinical studies. These studies are limited in their generalizability to athletes and often administer high doses of CBD. The central observation is that there is a lack of studies that directly investigate CBD and sports performance.

Keywords: Sports nutrition. Cannabidiol. Cannabis. microRNAs. Exosomes. Metabolism. Inflammatory processes. Immunological processes. Athletes.

Introduction

Cannabidiol (CBD) was identified 50 years ago and has effects that impact mood, sensation, perception,

tension, appetite, and pain **[1]**. Also, CBD has shown anxiolytic, antipsychotic, neuroprotective, anti-inflammatory, and antiemetic properties **[2,3]**. However, the growing interest in the substance as medicine was renewed in the 1990s, with the discovery of cannabinoid receptors 1 and 2 (CB1 and CB2, respectively), endogenous ligands (endocannabinoids, N-arachidonoylethanolamine (anandamide/AEA), and 2 -arachidonoyl-glycerol (2-AG)) and enzymes as part of the endocannabinoid system (ECS) in the brain **[4]**.

In this scenario, the correct interaction between all these ECS elements plays an important role in the development of the central nervous system (CNS), synaptic plasticity, motor control, memory, cognition, stress, emotional responses, reward and motivated behavior, appetite, pain, development and homeostasis **[5]**. Outside the brain, the ECS system is one of the crucial modulating factors in the autonomic nervous system, the immune system, the endocrine system, the gastrointestinal tract, the reproductive system, and microcirculation **[5]**.

In this regard, endocannabinoids are one of the most important systems controlling excitatory and inhibitory neurotransmission as well as neuroplasticity **[5]**. They serve as retrograde signaling messengers at GABAnergic and glutamatergic synapses, as well as modulators of postsynaptic transmission, interacting with other neurotransmitters, including dopamine. Endocannabinoids also participate in hypothalamic-pituitaryadrenal (HPA) axis modulation and stress regulation. The synthesis of cannabinoid receptor agonists and antagonists, anandamide uptake blockers, and anandamide endocannabinoid degradation inhibitors have opened up new treatment strategies **[6]**.

In the realm of sports, cannabis has been banned by the World Anti-Doping Agency (WADA) in all competitive sports since 2004. The few studies on exercise and cannabis have focused on the main compound, ie Δ9-tetrahydrocannabinol. CBD is another well-known phytocannabinoid present in dried or heated cannabis preparations. Unlike Δ9-tetrahydrocannabinol, CBD is not intoxicating but exhibits interesting pharmacological properties for medical use. The worldwide regulatory status of CBD is complex and this compound is still a controlled substance in many countries. Interestingly, however, the World Anti-Doping Agency has removed CBD from the list of prohibited substances, in or out of competition since 2018. This recent decision by WADA leaves the door open for the use of CBD by athletes **[7]**.

Preclinical studies suggest that CBD may be useful for athletes due to its antiinflammatory, analgesic, anxiolytic, and neuroprotective properties and its influence on the sleep-wake cycle. Unfortunately, little clinical data is available on CBD in the context of exercise. In addition, a series of implications for epigenetic processes have also been proven, through changes in the expression of microRNAs responsible for modulating the immune and inflammatory systems **[7,8]**.

Therefore, the present study aimed to develop a systematic review study to highlight the main aspects of cannabidiol in the interaction with microRNAs and exosomes in the modulation of inflammatory and immunological processes in athletes.

Methods

Study Design

The systematic review rules of the PRISMA Platform were followed. Available at: www.prisma-statement.org/). Accessed: 05/22/2023.

Data Sources and Research Strategy

The search strategies for this systematic review were based on the keywords (MeSH Terms): *Sports nutrition. Cannabidiol. Cannabis. microRNAs. Exosomes. Metabolism. Inflammatory processes. Immunological processes. Athletes.* The research was carried out from February to April 2023 in Scopus, PubMed, Science Direct, Scielo, and Google Scholar databases. In addition, a combination of keywords with the Booleans "OR", "AND" and the operator "NOT" were used to target scientific articles of interest.

Quality of Studies and Risk of Bias

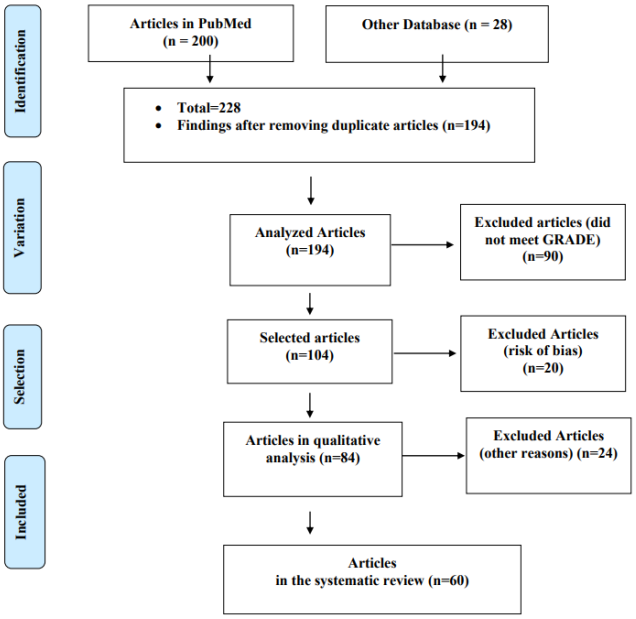
The quality of the studies was based on the GRADE instrument and the risk of bias was analyzed according to the Cochrane instrument.

Results and Discussion

Summary of Findings

A total of 228 articles were found. Initially, duplication of articles was excluded. After this process, the abstracts were evaluated and a new exclusion was performed, removing the articles that did not include the theme of this article, resulting in 104 articles. A total of 84 articles were evaluated in full and 60 were included and developed in this systematic review study (Figure 1). Considering the Cochrane tool for risk of bias, the overall assessment resulted in 20 studies with a high risk of bias and 90 studies that did not meet GRADE.

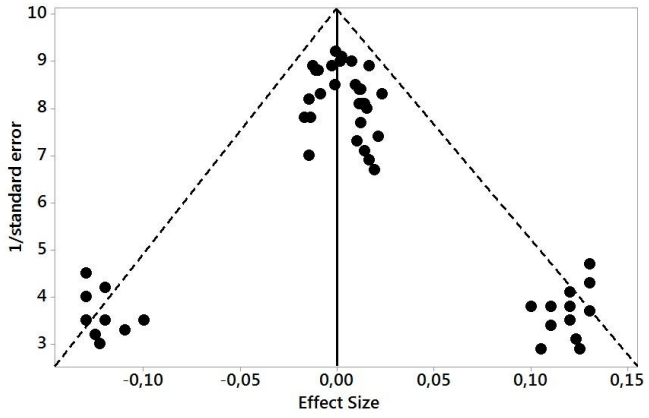
Figure 1. Flowchart - Article Selection Process.



Source: Own authorship.

Figure 2 presents the results of the risk of bias in the studies through the Funnel Plot. This graph showed symmetrical behavior, not suggesting a significant risk of bias, both between studies with small sample sizes (lower precision) that are shown at the bottom of the graph and in studies with large sample sizes that are displayed in the upper region.

Figure 2. The symmetrical funnel plot does not suggest a risk of bias among the small sample size studies that are shown at the bottom of the plot. High confidence and high recommendation studies are shown above the graph (NTotal=60 studies evaluated in full in the systematic review).



Source: Own authorship.

Main Scientific Evidence - Cannabidiol and microRNAs/Exosomes

One of the systems that have been intensively studied in recent years is the endocannabinoid signaling pathway, as a series of important interactions between cannabinoid receptors and biochemical pathways have been clarified. Furthermore, some important implications on inflammation and the immune system that are induced by the activity of cannabinoid receptors stimulated by delta-9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD) were observed. One of the most important is the ability to reduce the biosynthesis of pro-inflammatory mediators and the modulation of immune mechanisms. Different studies have reported that cannabinoids can reduce oxidative stress at mitochondrial and cellular levels. There are important mechanisms modulated by the endocannabinoid signaling pathway, as well as molecular and cellular connections **[9]**.

Recent studies have shown the involvement of specific endocannabinoid receptors, such as the endocannabinoid receptor CB1 and the CB2 receptor, as well as their connection with important processes in sepsis, such as the immune response, inflammatory response and redox activity **[10]**. In addition, a series of implications for epigenetic processes have also been proven, through changes in the expression of microRNAs that are responsible for modulating the immune and inflammatory systems

[8].

In this sense, by stimulating CB1 and CB2 receptors through cannabinoids, such as delta-9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD), important changes occur in the main biochemical and cellular mechanisms **[11]**, with effects on the inflammatory profile, immune response, metabolism, and metabolic state. Different research groups have also shown the impact of cannabinoids on the expression of microRNAs and the mechanisms of transcription and genetic modulation of cellular processes **[12]**.

In this scenario, it is highlighted that the molecular segment involved in the modulation of the immune response and the inflammatory cascade is represented by the expression of microRNAs **[13]**. The specific molecular activity of microRNAs in sepsis is complex, with numerous interactions being observed between Toll-Like receptors (TLRs) and some other specific biological signals, such as nuclear factor kappa-lightchain-enhancer of activated B cells (NF- κB), mechanisms of autophagy, and apoptosis. The activity of TLRs is mediated in many cases by the expression of microRNAs, with subsequent modulation of molecular and biological mechanisms for the production of inflammatory mediators **[13]**.

Based on this, it can be defined that microRNAs are non-coding single-stranded RNAs that contain between 19 and 25 nucleotides **[14]**. Its biosynthesis begins in

the cell nucleus through the action of RNA polymerase II on specific microRNA genes. After these reactions, the first forms of microRNAs are obtained, (pri-microRNA) which in the end lead to the formation of mature species of microRNAs **[14,15]**.

The mature species are released from the cell as exosomes, apoptotic bodies, or high-density lipoproteins, becoming one of the intercellular communication pathways, as well as a pathway for the modulation of specific biochemical and biological processes **[16-21]**. These epigenetic mechanisms are also involved in the modulation of the cannabinoid system. Furthermore, recent studies have proven the existence of certain links between THC/CBD activity and CB1 and CB2 receptor response by modulating microRNA expression **[22-26]**.

Authors Juknat et al. **[27]** conducted a study on he interactions between CB1 and CB2 receptors with microRNAs after activation of Δ9-THC and CBD. To simulate proinflammatory conditions, they stimulated BV-2 microglial cells with lipopolysaccharide (LPS) and subsequently analyzed the effects induced by Δ9-THC on microRNA expression. There was a significant increase in the expression of microRNA-21, microRNA-146a, and microRNA-155, closely linked to the biochemical pathways of TLRs and NF-κB. Regarding CBD activity, they observed a decrease in microRNA-146a and microRNA-155 expressions, as well as an increase in microRNA-34a expression. A similar study performed by Yang et al. **[28]** showed a decrease in the expression of microRNA-17, microRNA-92, microRNA-421, and microRNA-374b, induced by the action of Δ9-THC.

Furthermore, studies have shown that Δ9-THC modulates and reduces the biosynthesis of cytokines mediated by T cells (Th1), as well as the expression of TNF-α and IFN-γ mediated by microRNAs **[29-32]**. Added to this, Rao et al. **[33]** studied the effects induced by Δ9-THC on the inflammatory profile and the activity of staphylococcal enterotoxin B (SEB). They showed a 100% increase in survival rate in mice that received Δ9-THC treatment, in contrast to the control group, where mortality was 100%. Regarding the expression of microRNAs, they reported alterations in the activity of microRNA-17-92 and microRNA-18a.

The authors Chiarlone et al. **[34]** also reported the involvement of let-7d in the biochemical pathways that activate CB1 receptors. Al-Cghezi et al. investigated the effects of decreasing neuroinflammation induced by Δ9-THC and CBD in the context of multiple sclerosis. In their experimental study, they reported a decrease in neuroinflammation through inhibition of Th17 and Th1 cell activity. The combination of Δ9-THC and CBD led to a decrease in CD4+ T cell activity and a decrease in IL-1β, FoxP3, and STAT5b concentrations. Decreased expression was observed for microRNA21a-5p, microRNA-122-5p, microRNA-31-5p, microRNA-14a-5p, microRNA-150-5p, microRNA-27b-5p and microRNA-155-5p, and an increase in microRNA-706-5p and microRNA-7116 levels. Therefore, the combination of Δ9-THC and CBD can alter the activity of microRNAs responsible for increasing the biosynthesis of inflammatory mediators, leading to a reduction in the inflammatory profile.

Also, in the study by Sido et al. **[35]**, it was demonstrated that the administration of THC (20 mg/kg) to C57BL/6 mice led to a modified expression of the biochemical pathways involved in the immune modulation and the inflammatory profile. There was a decrease in microRNA-21 expression that directly leads to slower immunological differentiation of Th17 cells. Increased levels of microRNA-29b have been linked to the inhibition of IFN-γ expression. Al-Ghezi et al. **[36]** conducted a study on the effects induced by THC and CBD (10 mg THC and 10 mg CBD) on specific neuronal inflammation of multiple sclerosis. There was a decrease in the expression of microRNA-27b-5p, microRNA-155-5p, microRNA-150-5p, microRNA-146a-5p, microRNA-1225p, microRNA-21a-5p, microRNA-31-5p, and an increase in the expression of microRNA-706-5p and microRNA-7116. These changes were achieved by administering CBD/THC to CD4+ T cells. Key elements in modulating inflammation are specific molecules such as SOCS1 and FOXP3. Therefore, THC + CBD decreases neuroinflammation, and this effect is related to the modulation of microRNA expression.

In this context, three metabolites isolated from hemp seeds - cannabidiolic acid, N-trans-caffeoyl-tyramine, and cannabisin B - were examined for their ability to alter the expression levels of microRNAs in human neural cells. Thus, cultured SH-SY5Y cells were treated with the three compounds, and their microRNA content was characterized. As a result, 31 microRNAs underwent major changes in expression, being at least doubled or halved by treatments. A computational analysis of the biological pathways affected by these microRNAs showed that some are implicated in neural functions such as axon guidance, hippocampal signaling, and neurotrophin signaling. Of these, miR-708-5p, miR-181a-5p, miR-190a-5p, miR-199a-5p, and miR-143-3p are known to be involved in Alzheimer's disease and it is expected that their expression changes improve the function **[37]**.

In this sense, it is also highlighted that microRNAs play a critical role in modulating the response of immune cells to stimuli. Cannabinoids are known to exert

beneficial actions such as neuroprotection and immunosuppressive activities. Using lipopolysaccharide (LPS) to stimulate BV-2 microglial cells, we examined the role of cannabinoids in miRNA expression. MiRNA sequencing analysis revealed that 31 miRNAs were differentially modulated by LPS and by cannabinoid treatments. Furthermore, it was found that at the concentration tested, CBD has a greater effect than THC on the expression of most miRNAs studied. The results link the effects of LPS and cannabinoids to inflammatory signaling pathways. LPS increased the expression of proinflammatory miRNAs associated with Toll-like receptor (TLR) and NF-κB signaling, including miR-21, miR-146a, and miR-155, whereas CBD inhibited LPS-stimulated expression of miR -146a and miR -155. Furthermore, CBD upregulated miR-34a, known to be involved in multiple pathways including Rb/E2f cell cycle and Notch-Dll1 signaling. The results show that both CBD and THC reduced the expression of the LPSregulated Notch ligand Dll1. MiR-155 and miR-34a are considered to be redox-sensitive miRNAs that regulate Nrf2-driven gene expression. Nrf2-mediated expression of redoxdependent genes defines a Mox-like phenotype in CBD-treated BV-2 cells **[38]**.

One study evaluated the impact of two doses of CBD oil on inflammation (IL-6), performance, and pain after an eccentric loading protocol in athletes. Participants (n=4) participated in three conditions (placebo, low dose, and high dose) in this randomized, counterbalanced design. Each condition took 72 hours to complete, with a 1-week washout period between conditions. At the beginning of each week, participants underwent a loading protocol of six sets of ten eccentric-only repetitions in the biceps curl. Participants consumed placebo capsules, low dose (2mg/kg), or high dose (10mg/kg) CBD oil immediately after the session and continued every twelve hours for 48 hours. Venipunctures were performed before exercise and repeated 24, 48, and 72 hours after exercise. Blood samples were centrifuged for 15 minutes in lithium heparin gel vacutainers. Plasma was separated from the cells and stored at -80° until analysis. Samples were analyzed using an IL-6 immunometric assay (ELISA). There were no differences in inflammation across conditions or over time, handgrip strength across conditions or over time, or biceps curl strength across conditions or over time. There were no differences in pain between conditions, but there was a difference over time. However, there was a visible increase in IL-6 48 (4.88 ± 6.53) and 72 hours (3.12 ± 4.26) postexercise in the placebo condition, which was not observed in the low dose condition (48: 0.35 ± 2.22; 72: 1.34 ± 5.6) and high (48: 1.34 ± 1.34; 72: -0.79 ± 5.34) **[39]**.

Chronic musculoskeletal pain (MSK) is one of the most prevalent causes that bring patients to the doctor's office. The most common disorders that affect MSK structures are osteoarthritis, rheumatoid arthritis, back pain, and myofascial pain syndrome, all of which are responsible for severe pain and physical disability. While there are many known management strategies currently in practice, herbal compounds have recently begun to rise in the medical community, especially cannabidiol (CBD). CBD plays vital roles in human health that go far beyond the classic immunomodulatory, antiinflammatory, and antinociceptive properties. Recent studies have shown that CBD also improves cell proliferation and migration, especially in mesenchymal stem cells. Based on this, a review study analyzed the therapeutic potential of CBD in the context of regenerative medicine. Numerous studies listed in the literature indicate that CBD has a significant ability to modulate mammalian tissue to attenuate and reverse the notorious hallmarks of chronic musculoskeletal disorders (MSDs). Most of the research included in this review reports common findings, such as immunomodulation and stimulation of cell activity associated with tissue regeneration, especially in human mesenchymal stem cells. CBD is considered safe and well tolerated, as no serious adverse effects have been reported **[40]**.

A narrative review study explored various physiological and psychological effects of CBD that may be relevant to the sport and/or exercise context and identified key areas for future research. As there are no direct studies on CBD and sports performance, evidence for this narrative review was drawn from preclinical studies and a limited number of clinical trials in non-athletic populations. Preclinical studies have observed robust anti-inflammatory, neuroprotective, and analgesic effects of CBD in animal models. Preliminary preclinical evidence also suggests that CBD may protect against gastrointestinal damage associated with inflammation and promote the healing of traumatic skeletal injuries. However, more research is needed to confirm these observations. Early-stage clinical studies suggest that CBD may be anxiolytic in "stressinducing" situations and in individuals with anxiety disorders. While some case reports indicate that CBD improves sleep, robust evidence is currently lacking. Cognitive function and thermoregulation appear to be unaffected by CBD, while effects on food intake, metabolic function, cardiovascular function, and infection require further study. CBD can exert a range of physiological, biochemical, and psychological effects with the potential to benefit athletes. However, studies in athletic

populations are needed to better define the usefulness of CBD in supporting athletic performance **[41]**.

In this context, exercise, particularly when strenuous, unfamiliar, and/or involving an eccentric component, can cause ultrastructural damage to skeletal muscle myofibrils and the surrounding extracellular matrix **[42,43]**. This exercise-induced muscle damage (EIMD) impairs muscle function and initiates an inflammatory response **[43]**. Although inflammation is an integral part of EIMDM repair, regeneration, and adaptation, excessive inflammation can contribute to prolonged muscle soreness and delayed functional recovery **[44]**.

In this sense, CBD modulates inflammatory processes **[45]**. In preclinical models of acute inflammation, CBD has been reported to attenuate the accumulation of immune cells (e.g. neutrophils, lymphocytes, macrophages) **[46-49]**, stimulate the production of anti-inflammatory cytokines (e.g. interleukin (IL) -4, IL -10) **[50,51]** and inhibit the production of pro-inflammatory cytokines (eg, IL-1β, IL-6, IL-8, tumor necrosis factor (TNF) -α) and reactive species of oxygen **[52]**. Models demonstrating these effects include chemical treatment-induced lung injury and hypoxia-ischemia (HI); ischemiareperfusion and alcohol-feeding-induced liver injury, myocardial and renal ischemiareperfusion injuries, surgically induced oral injuries, chemically induced osteoarthritis, spinal cord contusion injury, and colitis **[53]**.

Anti-inflammatory effects are generally seen at higher doses of CBD in vivo (eg, ≥ 10 mg.kg-1); although, lower doses (eg, ~1.5 mg • kg-1) have shown efficacy in some studies. However, research investigating the effects of CBD on inflammation in humans is limited and inconclusive **[52,53]**.

In terms of muscle-specific inflammation, a preclinical study investigated the effect of high-dose CBD (i.e., 60 mg•kg-1•d-1) on the transcription and synthesis of proinflammatory markers (i.e., receptors of IL-6, TNF-α, TNF-β1, and inducible nitric oxide synthase) in the gastrocnemius and diaphragm of MDX dystrophic mice (a mouse model of Duchenne muscular dystrophy). In this investigation, CBD attenuated the mRNA expression of each marker and reduced plasma concentrations of IL-6 and TNFα. Improvements in muscle strength and coordination, as well as reductions in tissue degeneration, have also been reported at this dose. Lower but still relatively high doses of CBD (20–40 mg • kg-1 • d-1) had no functional benefit **[54]**.

Still, CBD is widely marketed to athletes due to effects such as decreased anxiety, extinction of fear memory, anti-inflammatory properties, pain relief, and post-exercise recovery. Specifically non-medicinal CBD products, so-called full-spectrum cannabis extracts, can contain significant levels of these substances, but also tetrahydrocannabinol (THC) contaminations (> 2.5 mg/day in > 30% of products on the German market) potentially leading to positive doping tests. Labeled claims about CBD content and the absence of THC are often false and misleading. Contaminations with psychoactive THC can result in adverse effects on cognition and, in general, the safety profile of CBD concerning its toxicity is a controversial topic of discussion. For these reasons, the use of over-the-counter CBD products is currently advised against **[55]**.

As scientific evidence, one study investigated the effect of cannabidiol oil (CBD) on the perception of muscle pain, inflammation, and strength performance after eccentric elbow flexor (EEC) exercise. Thirteen untrained males (mean ± SD age: 21.85 ± 2.73) performed 6 sets of 10 isokinetic maximal CCS muscle actions of the elbow flexors as a part of a double-blind crossover design. Non-invasive measurements (perceived pain, arm circumference, suspension joint angle (JA), and peak torque (PT)) were performed PRE,

POST, 24 hours, 48 hours, and 72 hours after EEC. All subjects completed the supplement (CBD: 150 mg POST, 24-h, 48-h) and placebo (PLC: POST, 24-h, 48-h) condition separated by 2 weeks. As a result, there was no condition x time interaction or condition main effect ($p>0.05$) for perceived pain, arm circumference, JA, or PT. There were main effects for the time of perceived pain. Thus, the current dose of 150 mg of CBD oil at POST, 24 hours, and 48 hours did not affect non-invasive markers of upper extremity muscle damage **[56]**.

Yet, one study determined whether there are age-related differences in cannabis use patterns and subjective effects in adult athletes. The age was above 21 years. Of the 1161 participants, 301 (26%) athletes were currently using cannabis. Younger athletes compared to older athletes reported significantly more adverse and positive subjective effects of cannabis. Younger athletes used cannabis concurrently with exercise more often than older athletes and consumed edibles, vaped, and smoked more than older athletes. Therefore, age-related patterns of cannabis use and subjective effects of cannabis were found. Concerns about cannabis misuse and abuse in athletes may be overblown with the potential benefits (improved sleep, decreased anxiety, less pain) outweighing the adverse effects (increased anxiety, increased appetite, difficulty concentrating) **[57]**.

Still in this context, the effects of chronic cannabis use on the physiological parameters of athletic performance are investigated to determine whether it

negatively affects athletic performance, whether it improves performance, potentially through enhanced recovery, or whether it has no effect. Resting heart rate was the only physiological measure that differed significantly between groups and only in one of the four studies included here. The strongest predictors of athletic performance (VO_2 Max and performance) were not significantly different between groups in any of the included studies. Chronic cannabis use had no significant effect on athletic performance. Included studies did not assess other elements such as recovery or endurance. Therefore, no evidence of ergogenic or ergolytic effects of chronic cannabis use was observed **[58]**.

Regulatory Measures

According to the regulations of the World Anti-Doping Agency (WADA), the use of cannabinoids is prohibited in competition, except for the use of cannabidiol (CBD). For an adverse analytical finding (AAA) in doping control, cannabinoid misuse is based on the identification of the pharmacologically inactive metabolite 11-nor-delta-9carboxy-tetrahydrocannabinol-9-carboxylic acid (carboxy-THC) in the urine in a higher concentration of 180 ng/mL. All other cannabinoids are reported as AAA when identified, except CBD which has been explicitly excluded from the class of cannabinoids on the WADA Prohibited List since 2018. However, because CBD isolated from cannabis plants may contain additional minor cannabinoids, the permissible use of CBD can lead to unintentional violations of anti-doping regulations **[59]**.

Therefore, an assay for the detection of 16 cannabinoids in human urine was established. Sample preparation consisted of enzymatic hydrolysis of the glucuronide conjugates, liquid-liquid extraction, trimethylsilylation, and analysis by gas chromatography/tandem mass spectrometry (GC-MS/MS). Urine samples from CBD users, as well as specimens obtained from CBD administration studies conducted with 15 commercially available CBD products, were analyzed and assay characteristics such as selectivity, reproducibility of detection at the minimum required performance level, limit of detection, and identification limit were determined. Variable patterns of cannabinoids or their metabolites were observed in urine samples, especially when full-spectrum CBD products were consumed. The presence of minor cannabinoids or their metabolites in the urine sample of a competitive athlete poses a substantial risk of an anti-doping rule violation **[59]**.

In this context, another recent literature review study revealed that there are limited high-quality studies on the use of cannabinoids for acute pain, chronic pain, or concussion. None of the trials involving cannabinoids included the athletic population. For acute pain, 2 small randomized double-blind crossover studies did not conclude any immediate effect of cannabinoid therapy. There is more robust evidence for the treatment of chronic pain conditions through meta-analyses and systemic reviews. Cannabinoid therapy is moderately effective as a treatment for some chronic pain conditions. Investigations have included a broad spectrum of chronic pain conditions, including neuropathic, musculoskeletal, inflammatory, and central pain conditions, and have revealed pain reduction and quality of life improvement with limited adverse effects. For concussion, the evidence is based on preclinical in vitro and animal models revealing possible neuroprotective effects, as well as 2 clinical studies involving the presence of cannabinoids for concussion (some sports related), but there are no high-quality trials evaluating efficacy. of cannabinoid treatment at this time. Thus, while there are several biochemical explanations for the use of cannabinoid therapy via endocannabinoid system modulation for various medical conditions that affect athletes, physicians' recommendations must be extrapolated from most research done in the non-athletic population. The lack of high-quality clinical evidence, along with inconsistent federal and state laws, as well as purity issues with cannabis-based products, make it difficult for the sports medicine clinician to broadly recommend cannabinoid therapeutics at this time **[60]**.

Conclusion

CBD has been reported to exert a range of physiological, biochemical, and psychological effects with the potential to benefit human health. For example, there is preliminary supporting evidence for the anti-inflammatory, neuroprotective, analgesic, and anxiolytic actions of CBD and the possibility that it may protect against gastrointestinal damage associated with inflammation and promote the healing of traumatic skeletal injuries. The combination of ∆9-THC and CBD can alter the activity of microRNAs responsible for increasing the biosynthesis of inflammatory mediators, leading to a reduction in the inflammatory profile. However, it is important to recognize that these findings are very preliminary, sometimes inconsistent, and largely derived from preclinical studies. These studies are limited in their generalizability to athletes and often administer high doses of CBD. The central observation is that there is a lack of studies that directly investigate CBD and sports performance.

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