BODY HOMEOSTASIS AND THE ENDOCANNABINOID SYSTEM AND CANNABIDIOL IN SPORTS PERFORMANCE

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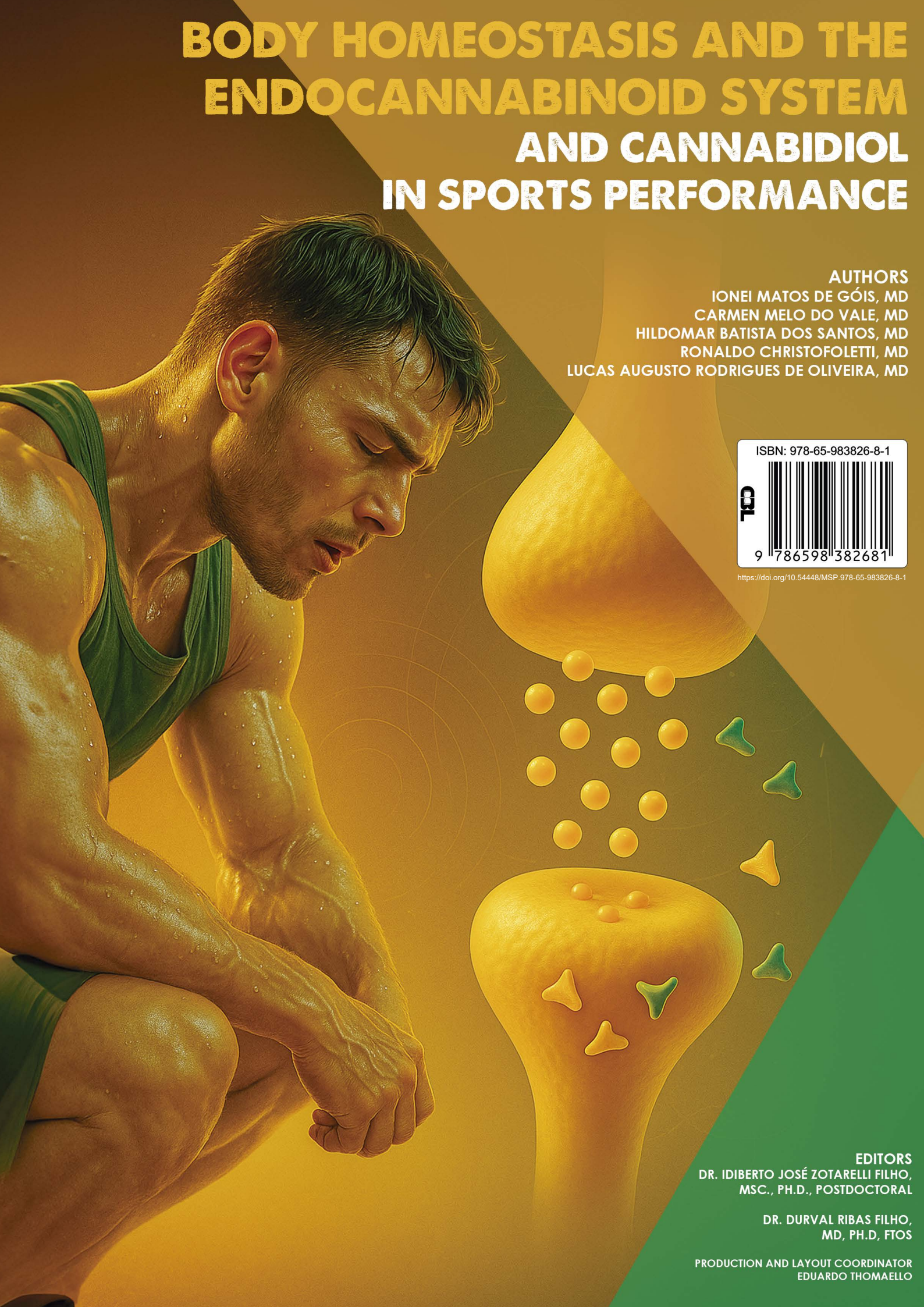
ISBN: 978-65-983826-8-1



https://doi.org/10.54448/MSP.978-65-983826-8-1

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https://doi.org/10.54448/MSP.978-65-983826-8-4

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https://doi.org/10.54448/MSP.978-65-983826-8-1

Dados Internacionais de Catalogação na Publicação (CIP)
(Câmara Brasileira do Livro, SP, Brasil)

Body homeostasis and the endocannabinoid system and cannabidiol in sports performance [livro eletrônico] / Ionei Matos de Góis...[et al.] ; production and layout coordinator Eduardo Thomaello ; edited by Idiberto José Zotarelli Filho, Durval Ribas Filho. -- São José do Rio Preto, SP : MetaScience Press, 2025.
PDF

Outros autores: Carmen Melo do Vale, Hildomar Batista dos Santos, Ronaldo Christofoletti, Lucas Augusto Rodrigues de Oliveira.
Bibliografia.
ISBN 978-65-983826-8-1

1. Canabinóides 2. Esportes 3. Esportes - Aspectos nutricionais 4. Nutrição I. Góis, Ionei Matos de. II. Vale, Carmen Melo do. III. Santos, Hildomar Batista dos. IV. Christofoletti, Ronaldo. V. Oliveira, Lucas Augusto Rodrigues de. VI. Thomaello, Eduardo. VII. Zotarelli Filho, Idiberto José. VIII. Ribas Filho, Durval.

25-288681 CDD-613.2024796

Índices para catálogo sistemático:

1. Nutrição esportiva 613.2024796

Eliane de Freitas Leite - Bibliotecária - CRB 8/8415

https://doi.org/10.54448/MSP.978-65-983826-8-1

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MetaScience Press
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https://doi.org/10.54448/MSP.978-65-983826-8-1

Scientific Publisher

https://zotarellifilhoscientificworks.com/

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ISBN: 978-65-983826-8-1

https://doi.org/10.54448/MSP.978-65-983826-8-1

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https://doi.org/10.54448/MSP.978-65-983826-8-1

Preface

Dear Reader

The International Association of Athletics Federations recognizes the importance of nutritional practices in optimizing an athlete's well-being and performance. In this regard, periodized guidelines can be provided for the appropriate type, quantity, and timing of food and fluid intake to promote optimal health and performance in different training and competition scenarios. The most common examples of supplements are caffeine, bicarbonate, beta-alanine, nitrate, creatine, glutamine, and iron ions. Dietary supplements offer ergogenic aids in an attempt to increase energy, improve recovery, modulate body composition, and control muscle acidity (number of free protons, acidosis), enabling improved performance. Increasing beta-alanine availability through dietary supplementation, combined with training, can improve athlete performance. $NaHCO_3$ was effective in improving short-term, high-intensity exercise capacity. Glutamine is involved in several biological functions, such as nucleotide synthesis, cell proliferation, regulation of protein synthesis and degradation, energy production, glycogenesis, ammonia detoxification, and maintenance of acid-base balance. Finally, cannabidiol has been reported to exert a range of physiological, biochemical, and psychological effects with the potential to benefit athletes. For example, there is preliminary evidence supporting cannabidiol's anti-inflammatory, neuroprotective, analgesic, and anxiolytic actions, and the possibility that it may protect against gastrointestinal damage associated with inflammation and promote healing of traumatic skeletal injuries.

https://doi.org/10.54448/MSP.978-65-983826-8-1

SUMMARY

https://doi.org/10.54448/MSP.978-65-983826-8-1

INTRODUCTION

Many of the established positive health benefits of exercise have been documented by landmark discoveries in the field of exercise physiology. These investigations typically assess performance thresholds or exercise-induced health benefits [1]. Several important discoveries have been informed by the study of highly trained athletes. Recent advances have been made in skeletal muscle metabolism and personalized exercise regimens [1]. In this context, regular physical training combined with dietary supplements has broad health benefits by positively impacting nearly every organ system in the body [2].

In this sense, dietary supplements offer ergogenic aids by attempting to increase energy, improve recovery, modulate body composition, and control muscle acidity (free proton count, acidosis), enabling improved performance [3,4]. For example, β-alanine (beta-alanine) (BA) is a non-essential amino acid that can be synthesized in the liver and obtained from the diet, particularly from white meat (poultry and fish) and red meat [5]. Endogenous BA synthesis derives from the degradation of the nitrogenous bases pyrimidines, thymine, cytosine, and uracil, and its transport to skeletal muscle is dependent on sodium and chloride ions [6,7].

In this context, it is in skeletal muscle that BA plays its role as an intermediary for the synthesis of carnosine, a dipeptide (histidine and BA) responsible for reducing fatigue and buffering muscles against acidosis [8-11]. Furthermore, muscle carnosine content is also influenced by muscle contraction and increases with muscle tension [12]. Increasing BA availability through dietary supplementation, combined with training, can improve the performance of athletes performing high-intensity exercise by increasing muscle buffering capacity (reducing acidity) [13-16]. In this regard, the duration of supplementation between studies appears to generally vary between 4 and 10 weeks, and

https://doi.org/10.54448/MSP.978-65-983826-8-1

doses are distributed throughout the day, making the effect of BA supplementation on exercise still controversial [14,17].

Furthermore, over the past 3 decades, research has investigated the potential of sodium bicarbonate ingestion ($NaHCO_3$ alkalosis and subsequent physical enhancement) for metabolic induction [18]. Early research reported that $NaHCO_3$ was effective in improving short-term, high-intensity exercise capacity [19-21], while more recent studies have demonstrated that $NaHCO_3$ can also improve performance during aerobic endurance and prolonged, intermittent, high-intensity exercise [22,23]. In this regard, the performance-enhancing effects of $NaHCO_3$ are largely associated with the degree of metabolic alkalosis [24]. During high-intensity exercise, increased alkalosis attenuates the rate of free proton (H+) increase in the sarcoplasm, thus reducing competition at the ionizable binding sites of the actin/myosin complex, as well as the sarcoplasmic reticulum delay in Ca2+ release and absorption, delaying the onset of fatigue [25-27].

In this context, the International Association of Athletics Federations recognizes the importance of nutritional practices in optimizing an athlete's well-being and performance. In this regard, periodized guidelines can be provided for the appropriate type, quantity, and timing of food and fluid intake to promote optimal health and performance in different training and competition scenarios. Therefore, the use of medical supplements to address nutrient deficiencies or sports foods to help athletes achieve nutritional goals is well-known. The most common examples of supplements are caffeine, bicarbonate, beta-alanine, nitrate, creatine, and glutamine [18].

As another example, glutamine is an essential amino acid widely used in sports nutrition, particularly for its immunomodulatory role. Glutamine plays several other biological functions, such as cell proliferation, energy production, glycogenesis, ammonia buffering, and maintenance of acid-base balance. Therefore, this amino acid has

https://doi.org/10.54448/MSP.978-65-983826-8-1

been investigated in sports nutrition beyond its effect on the immune system, attributing various properties to glutamine, such as an anti-fatigue role [28].

Also, iron is a highly significant trace mineral for endurance athletes. Iron is critical for optimal athletic performance due to its role in energy metabolism, oxygen transport, and acid-base balance. Endurance athletes are at increased risk of suboptimal iron levels, with potential negative consequences on performance, due to the combination of increased iron requirements and inadequate dietary intake. Mechanisms that explain the increased risk of iron deficiency in endurance athletes include exercise-associated inflammation and the release of hepcidin in iron sequestration. Information is presented on screening athletes for iron deficiency, and suggestions are provided for increasing iron intake through dietary modification or iron supplementation [29].

Additionally, cannabidiol (CBD) was identified 50 years ago and has effects that impact mood, sensation, perception, tension, appetite, and pain [30]. Furthermore, CBD has shown anxiolytic, antipsychotic, neuroprotective, anti-inflammatory, and antiemetic properties [31,32]. However, growing interest in the substance as a 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-arachidonoylglycerol (2-AG)), and enzymes as part of the endocannabinoid system (ECS) in the brain [33]. 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. Outside the brain, the ECS system is one of the crucial modulating factors of the autonomic nervous system, the immune system, the endocrine system, the gastrointestinal tract, the reproductive system, and the microcirculation [34].

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Endocannabinoids are one of the most important systems controlling excitatory and inhibitory neurotransmission, as well as neuroplasticity [34]. They serve as retrograde signaling messengers at GABAergic and glutamatergic synapses, as well as modulators of postsynaptic transmission, interacting with other neurotransmitters, including dopamine. Endocannabinoids also participate in the modulation of the hypothalamic-pituitary-adrenal (HPA) axis and stress regulation. The synthesis of cannabinoid receptor agonists and antagonists, anandamide uptake blockers, and inhibitors of endocannabinoid degradation has opened new treatment strategies [35].

In the sports field, 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, 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 global regulatory status of CBD is complex, and this compound remains a controlled substance in many countries. Interestingly, however, the World Anti-Doping Agency removed CBD from the list of prohibited substances, both in-, and out-of-competition, in 2018. This recent WADA decision leaves the door open for the use of CBD by athletes. Preclinical studies suggest that CBD may be useful for athletes due to its anti-inflammatory, analgesic, anxiolytic, and neuroprotective properties, as well as its influence on the sleep-wake cycle. Unfortunately, little clinical data are available on CBD in the context of exercise [36].

Therefore, this e-book aimed to present and discuss the main approaches and importance of body homeostasis and the endocannabinoid system and cannabidiol in sports performance.

https://doi.org/10.54448/MSP.978-65-983826-8-1

CHAPTER I

Elements of Acid-Base Balance - Henderson-Hasselbalch vs. Physicochemical Approach

Both exercise and hypoxia cause complex alterations in acid-base homeostasis. A randomized, prospective, crossover study investigated whether, during intense physical exercise in normoxia and hypoxia, the modified physicochemical approach offers a better understanding of changes in acid-base homeostasis than the traditional Henderson-Hasselbalch approach. Nineteen healthy men completed an exercise test to voluntary fatigue on a bicycle ergometer on two different study days, once during normoxia and once during normobaric hypoxia (12% oxygen, equivalent to an altitude of 4,500 m). Arterial blood gas (ABG) was collected during and after the exercise test and analyzed according to the modified physicochemical and Henderson-Hasselbalch approaches, respectively. Peak power decreased from 287 ± 9 watts in normoxia to 213 ± 6 watts in hypoxia (-26%, $p<0.001$). Exercise decreased arterial pH to 7.21 ± 0.01 and 7.27 ± 0.02 ($p<0.001$) during normoxia and hypoxia, respectively, and increased plasma lactate to 16.8 ± 0.8 and 17.5 ± 0.9 mmol/L ($p<0.001$) [1-3].

While the Henderson-Hasselbalch approach identified lactate as the primary factor responsible for non-respiratory acidosis, the modified physicochemical approach additionally identified strong ions (i.e., plasma electrolytes, organic acid ions) and non-volatile weak acids (i.e., albumin, phosphate ion species) as important contributors. Therefore, the Henderson-Hasselbalch approach can serve as a basis for screening for acid-base disturbances, but the modified physicochemical approach offers more details about the complex changes in acid-base status during exercise in normoxia and hypoxia [37].

https://doi.org/10.54448/MSP.978-65-983826-8-1

CHAPTER II

β-Alanine

Physical exercise leads to metabolic changes that affect acid-base balance in skeletal muscle and other tissues. Nutrition is one of the factors that can influence the body's acid-base balance. Maintaining alkaline conditions in the body is important not only for health and athletic performance during training, but also during competition [38].

As a corollary to scientific developments, the International Society of Sports Nutrition (ISSN) has shown that four weeks of beta-alanine supplementation (4-6g per day) significantly increases muscle carnosine concentrations. Furthermore, beta-alanine supplementation currently appears to be safe in healthy populations at recommended doses. However, the only reported side effect is paresthesia, but studies indicate that this can be mitigated by using smaller, divided doses (1.6g) or using a sustained-release formula. Furthermore, daily supplementation with 4 to 6 g of beta-alanine for at least 2 to 4 weeks has been shown to improve exercise performance, with the most pronounced effects in open-ended/time trial tasks lasting 1 to 4 minutes. Beta-alanine mitigates neuromuscular fatigue, particularly in older individuals, and preliminary evidence suggests that beta-alanine may improve tactical performance. Furthermore, combining beta-alanine with other supplements may be advantageous when beta-alanine supplementation is high enough (4 to 6 g per day) and long enough (minimum 4 weeks) [38].

In this regard, a study determined dietary acid-base balance in high-performance competitive athletes and evaluated the effect of the athletes' actual diets on net endogenous acid production, muscle mass, and body mineral content during four one-year Olympic cycles. The study participants were high-performance athletes aged 18.1 ±

https://doi.org/10.54448/MSP.978-65-983826-8-1

3.3 years (n=323). Body composition measurements were performed using bioimpedance analysis. In 10.2% of athletes, net endogenous acid production exceeded 100 mEq/day, averaging 126.1 ± 32.7 mEq/day. Higher net endogenous acid production in athletes is associated with lower muscle mass (1.2% of body weight, $p < 0.001$) but does not affect body mineral status (0.01% of body weight, $p = 0.073$). Overall, 25-30% of high-performance athletes consume high-protein diets (2.0–4.8 g kg-1.day-1), leading to dietary acid-base imbalance and excessive endogenous acid production. It is recommended to increase calcium intake to 1500 mg per day. In exceptional cases, periodized nutrition for athletes may involve diets supplemented with bicarbonate and/or BA supplements [39].

In this scenario, an eight-week, double-blind, randomized, crossover study investigated the effect of supplementation with the intracellular buffering agent BA versus the extracellular buffering agent (alkaline phosphatase-ALK), combined with the usual treatment of branched-chain amino acids (BCAAs) and creatine malate (MCT) under natural training conditions. Thirty-one elite athletes (11 sprinters and 20 endurance athletes) participated in the study. After BA-ALK-BCAA and MCT supplementation, total fat-free mass increased in sprinters. No other differences were found in body composition, respiratory parameters, aerobic capacity, blood lactate concentration, or hematologic indices after BA-ALK-BCAA and MCT/ALK-BA-BCAA and MCT supplementation. The peak post-exercise blood ammonia (NH3) concentration decreased in both groups after BA-ALK-BCAA and MCT supplementation. Furthermore, lower NH3 concentrations were observed in endurance athletes in the post-exercise recovery period. Combined BCAA, MCT, and BA supplementation is more effective than combined BCAA, MCT, and ALK supplementation for an increase in fat-free mass and exercise adaptation, but not for improving aerobic capacity [40].

https://doi.org/10.54448/MSP.978-65-983826-8-1

In this context, despite the widespread use of beta-alanine, the understanding of potential adverse effects is limited. Therefore, a systematic review and risk assessment meta-analysis was performed. A total of 101 human and 50 animal studies were included. Paresthesia was the only reported side effect and had an estimated OR of 8.9 with supplementation relative to placebo. Beta-alanine supplementation in animals caused a small increase in circulating alanine aminotransferase concentration, although mean data remained within clinical reference ranges. Meta-analysis of human data showed no main effect of beta-alanine supplementation on skeletal muscle taurine or histidine concentration. Therefore, the results indicate that beta-alanine supplementation did not adversely affect [41].

Furthermore, a randomized, double-blind, placebo-controlled study investigated the influence of beta-alanine supplementation during a high-intensity interval training (HIIT) program on repeated sprint performance (RSA). Eighteen men performed an incremental run-to-exhaustion (TINC) test at baseline and followed by a 4-week HIIT (10 × 1-min runs at 90% of maximum speed TINC [1-min recovery]). Participants were then randomized into two groups and performed a 6-week HIIT combined with 6.4 g/day of BA or dextrose supplementation (placebo group). The results showed that BA supplementation during HIIT increased muscle carnosine and attenuated neuromuscular fatigue, which may contribute to improved RSA performance. Furthermore, the improvement in muscle carnosine content induced by beta-alanine supplementation may have contributed to an attenuation of central fatigue during repeated sprints [42].

https://doi.org/10.54448/MSP.978-65-983826-8-1

CHAPTER III

Sodium bicarbonate ($NaHCO_3$)

The degree of metabolic alkalosis is altered by the dosage and timing of $NaHCO_3$ ingestion. However, although research has investigated the types of exercise that are enhanced by $NaHCO_3$ ingestion, research is still limited on the dosage and timing of $NaHCO_3$ ingestion that optimizes metabolic alkalosis and the factors associated with ergogenic effects. Doses between 0.2 and 0.3 g kg-1 have ineffective performance outcomes [43-47].

For example, Horswill et al. [48] reported that dosages of 0.2 g kg-1 or less were ineffective in improving sprint performance, while McNaughton [49] reported that, with dosages of 0.1, 0.2, 0.3, 0.4, and 0.5 g kg-1, only 0.1 g kg-1 did not significantly increase sprint performance. Doses of 0.15 and 0.20 g kg-1 have also been reported by other researchers to improve performance [50,51].

Additionally, the time between $NaHCO_3$ ingestion and the onset of exercise varied from 1 to 2 hours. A limitation of this approach is that the time course of the level of metabolic alkalosis may differ between various dosages, and therefore, the ergogenic effect of $NaHCO_3$ may be moderated by the interaction of dosage with the timing of ingestion before the onset of exercise. Two studies investigated the time course of metabolic alkalosis and reported that peak alkalosis occurred between 60 and 90 minutes [52,53]. However, these studies used only a dosage of 0.3 g kg-1.

One study observed that over 2 hours after ingestion of a single bolus of $NaHCO_3$ at concentrations of 0.1, 0.2, or 0.3 g kg-1, the blood buffering capacity was, on average, significantly higher for the 0.2 and 0.3 g kg-1 doses than for the 0.1 g kg-1 dose. However, there was no significant difference between the 0.2 and 0.3 g kg-1 doses. The blood

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buffering profile was relatively constant between 60 and 120 minutes after dosing for the 0.2 g kg-1 dose, while the 0.3 g kg-1 dose, on average, peaked at 65 minutes and declined slowly thereafter [52].

There are several studies that incorporated the 0.2 g kg-1 dose into their methodological application, but also began the exercise trial at 60 minutes or longer [48,49]. This difference between the peak timing of the two doses (0.2 and 0.3 g kg-1) and the onset of exercise may explain some of the ambiguous findings regarding the efficacy of $NaHCO_3$ loading. To maximize the ergogenic potential of 0.2 g kg-1, exercise should begin as early as 50 minutes after $NaHCO_3$ administration [54].

Although a single bolus of 0.3 g kg-1 of $NaHCO_3$ ingested 1 to 2 hours before exercise has been the most common, other studies have used variations of this protocol in attempts to further increase ergogenic potential. Bishop and Claudius [55] implemented a “stacking” protocol, in which the overall $NaHCO_3$ load remained 0.3 g kg-1 or greater but was divided into smaller doses (0.2 g kg-1 ingested at time 0 and again 70 min after the initial ingestion, with exercise beginning at 90 minutes). Still, two other studies incorporated the stacking concept, yet instead of hours before exercise, they involved several days of NaHCO3 loading before assessing exercise performance [56,57].

In this context, the sustained elevation of $NaHCO_3$ observed up to 4 hours after ingestion was similar to the levels produced by doses of 0.2 and 0.3 g kg-1 at 120 minutes, suggesting that "stacking" may be an effective practice for up to 4 hours after ingestion. This has important practical implications not only in terms of increasing the extracellular blood buffering potential but also in terms of being more tolerable for individuals unfamiliar with $NaHCO_3$ loading or those susceptible to gastrointestinal disturbances [58].

https://doi.org/10.54448/MSP.978-65-983826-8-1

While much research has investigated the types of exercise that are enhanced by $NaHCO_3$ ingestion, research is limited on the dosage and timing of ingestion that optimizes the associated ergogenic effects. Thus, one study investigated the effects of various $NaHCO_3$ loading protocols on the time-dependent blood buffering profile. Eight male volunteers (age, 22.4 +/- 5.7 years; height, 179.8 +/- 9.6 cm; body mass, 76.3 +/- 14.1 kg) completed Part A, alkalosis measurements over 120 minutes after ingestion of various single dosages of $NaHCO_3$ (0.3 g kg-1, 0.2 g kg-1, 0.1 g kg-1, and placebo); and Part B, similar profiles after alternative $NaHCO_3$ loading protocols (single morning dosage [SMD], single evening dosage [SED], and dosages ingested on 3 consecutive nights [CED]). In Part A, blood buffering in the 0.1 g kg-1 condition was significantly lower than in the 0.2 g kg-1 and 0.3 g kg-1 conditions ($p<0.002$), but there were no significant differences between the 0.2 g kg-1 and 0.3 g kg-1 conditions ($p=0.34$). Although blood buffering was relatively constant in the 0.1 and 0.2 conditions, it was significantly higher at 60 minutes than at 100 minutes and 120 minutes in the 0.3 g kg-1 condition ($p<0.05$). The results of Part B are as follows. Blood buffering for SMD was significantly higher than for SED and CED ($p<0.05$). Blood buffering in the SMD condition was significantly lower at 17:00 hours than at 11:00 hours ($p=0.007$). Single doses of 0.2 and 0.3 g kg-1 of NaHCO3 appeared to be most effective in increasing blood buffering capacity. The 0.2 g kg-1 dose is best ingested 40 to 50 minutes before exercise, and the 0.3 g kg-1 dose 60 minutes before exercise [58].

A randomized, placebo-controlled study examined the effects of $NaHCO_3$ and a lactate supplement during a 40 km cycling event. Seven men aged 45, 22.3 ± 3.3 years, with a mean height of 182.5 ± 6.5 cm, and a body mass of 79.2 ± 6.3 kg, completed five 40 km cycling events. Subjects ingested (a) 300 mg per kg body mass $NaHCO_3$, (b) 45 mg per kg sodium chloride as a placebo, (c) 1115 mg lactate, or (d) plain flour as a placebo

https://doi.org/10.54448/MSP.978-65-983826-8-1

for the lactate assay 60 min before exercise. $NaHCO_3$ ingestion induced significant changes in all baseline variables (all $p < 0.05$); no significant changes were observed after lactate ingestion ($p > 0.05$) [59].

https://doi.org/10.54448/MSP.978-65-983826-8-1

CHAPTER IV

Glutamine

Glutamine is a neutral five-carbon amino acid with a molecular weight of 146.15 g/mol and is considered the most abundant free amino acid in the human body [60]. In adult humans fasting overnight, normal blood glutamine levels are 550–750 μmol/L [61], accounting for over 20% of total blood amino acids [62]. In skeletal muscle, glutamine comprises 50–60% of total free amino acids and is considered the most synthesized amino acid in human muscle, especially in slow-twitch muscles, which contain glutamine concentrations three times higher than in fast-twitch muscles [62,63]. Thus, skeletal muscle releases glutamine into the circulation at high rates, approximately 50 mmol per hour in the fed state [61].

In this context, organs can be classified as glutamine producers or consumers. Skeletal muscles, lungs, liver, brain, and adipose tissue exhibit high glutamine synthetase activity (an enzyme that synthesizes glutamine from ammonia and glutamate in the presence of adenosine triphosphate) and are considered glutamine producers. Leukocytes, enterocytes, colonocytes, thymocytes, fibroblasts, endothelial cells, and renal tubular cells exhibit high glutaminase activity (an enzyme that hydrolyzes glutamine, converting it to glutamate and ammonia) and are classified as glutamine consumers [64-68].

Glutamine is also involved in several biological functions, such as nucleotide synthesis, cell proliferation, regulation of protein synthesis and degradation, energy production, glycogenesis, ammonia detoxification, and maintenance of acid-base balance, among others. Furthermore, this amino acid regulates the expression of several genes associated with metabolism and activates several intracellular signaling pathways [69]. Nutritionally, glutamine is considered conditionally essential because, in catabolic

https://doi.org/10.54448/MSP.978-65-983826-8-1

situations such as clinical trauma, burns, sepsis, and prolonged and exhaustive exercise, endogenous glutamine synthesis may not be sufficient to meet the body's needs, leading to glutamine deficiency [64,65].

Moreover, glutamine metabolism has been investigated during and after physical exercise, and it has been observed that blood glutamine responds differently depending on the duration of the exercise. Short-term exercise increases muscle glutamine release and its blood concentrations, whereas, in long-duration and exhaustive exercise, such as marathon running, muscle glutamine synthesis is insufficient to meet the body's needs for this amino acid, decreasing blood glutamine concentrations [70,71]. This decrease is transient and appears to last 6–9 h after a marathon, and is accompanied by a 30–40% drop in muscle glutamine or its precursors, such as glutamate [71]. However, some studies have shown that even after exhaustive exercise, blood glutamine does not change [72].

Also, decreased glutamine availability is associated with immune system disturbances and an increased incidence of infections. Dos Santos et al. [73] observed, in an experimental model (rats), that exhaustive exercise induces an increase in macrophage functionality (phagocytosis and H_2O_2 production), as well as an increase in glutamine consumption and metabolism in these cells, indicating the importance of glutamine for macrophage regulation in the post-training period. Regarding glutamine supplementation, evidence indicates that plasma glutamine, in response to glutamine supplementation, increases markedly within 30 minutes of supplementation, returning to baseline levels approximately 2 hours after glutamine administration. Furthermore, doses of 20-30g of glutamine have been reported to be tolerated and pose no harm to humans [35].

Because this amino acid exerts a wide variety of biological activities, glutamine has been investigated in sports nutrition beyond its effect on the immune system, attributing various properties to this amino acid, such as an anti-fatigue role. Thus,

https://doi.org/10.54448/MSP.978-65-983826-8-1

glutamine supplementation appears to increase muscle glycogen synthesis and reduce exercise-induced ammonia accumulation, especially when administered for long periods (more than 5 consecutive days). However, with regard to glycogen synthesis, further research is needed to establish a greater effect of glutamine compared to supplements containing carbohydrate or creatine monohydrate [66].

Glutamine supplementation appears to attenuate markers of muscle damage, such as blood cholesterol and HDL levels. These aforementioned properties of glutamine are especially interesting for athletes who engage in exhaustive and prolonged exercise. Despite improving some markers of fatigue, glutamine supplementation appears to have limited effects on physical performance. Supplements containing glutamine combined with several other nutrients appear to have ergogenic effects. However, these properties cannot be attributed solely to glutamine [67].

Despite this, the ergogenic potential of this amino acid is still not fully understood. Therefore, a literature review study addressed the main properties by which glutamine could delay fatigue, as well as the effects of glutamine supplementation, alone or combined with other nutrients, on markers of fatigue and performance in the context of physical exercise. Fifty-five studies met the inclusion criteria and were evaluated in this integrative literature review. Most of the studies observed that glutamine supplementation improved some markers of fatigue, such as increased glycogen synthesis and reduced ammonia accumulation, but this intervention did not increase physical performance. Thus, despite improving some fatigue parameters, glutamine supplementation appears to have limited effects on performance [35].

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CHAPTER V

Iron

The high clinical prevalence of iron deficiency among endurance athletes, especially female athletes, is significant because of the significant impact it can have on health and performance. Dietary modification, rather than supplementation, is the preferred strategy for ensuring adequate intake among athletes because high doses of iron have the potential to be dangerously toxic. Therefore, the use of supplemental iron to correct a deficiency should only be undertaken under the supervision of a healthcare professional to monitor the efficacy and safety of the treatment [74].

Iron is essential for oxidative metabolism and is therefore especially important for endurance athletes whose athletic performance depends on high aerobic capacity [74,75], given that hemoglobin and myoglobin bind oxygen through the porphyrin ring of the heme group. Hemoglobin in red blood cells transports oxygen from the lungs to exercising skeletal muscles. Electron transport in the respiratory chain, which is the final step in the oxidative synthesis of ATP, depends on heme-containing cytochromes (a, a3, b, b5, c, c1) and non-heme iron-sulfur enzymes (NADH dehydrogenase, succinate dehydrogenase, and ubiquinone-cytochrome c reductase) [76].

Thus, iron deficiency impairs ATP production and increases the reliance on anaerobic glucose metabolism to produce ATP, effectively reducing endurance capacity. As a corollary, iron has the potential to affect maximal and submaximal exercise capacity. Endurance athletes are at increased risk of iron deficiency for a number of reasons, including inadequate intake, low bioavailability, and increased losses [75,76].

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CHAPTER VI

Main Scientific Evidence – Cannabidiol (CBD)

A narrative review explored various physiological and psychological effects of CBD that may be relevant to the context of sport and/or exercise and identified key areas for future research. Because there are no direct studies on CBD and sports performance, the evidence for this narrative review was obtained from preclinical studies and a limited number of clinical trials in non-athlete populations. Preclinical studies have observed robust anti-inflammatory, neuroprotective, and analgesic effects of CBD in animal models [77,78].

Preliminary preclinical evidence also suggests that CBD may protect against gastrointestinal damage associated with inflammation and promote 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 "stress-inducing" situations and in individuals with anxiety disorders. Although 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 may 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 [77].

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 [78,79]. This exercise-induced muscle damage (EIMD) impairs muscle function and initiates an inflammatory response [79]. Although

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inflammation is an integral part of EIMD repair, regeneration, and adaptation, excessive inflammation can contribute to prolonged muscle soreness and delayed functional recovery [80].

In this sense, CBD modulates inflammatory processes [81]. In preclinical models of acute inflammation, CBD has been reported to attenuate the accumulation of immune cells (e.g., neutrophils, lymphocytes, macrophages) [82–85], stimulate the production of anti-inflammatory cytokines (e.g., interleukin (IL)-4, IL-10) [86,87], and inhibit the production of pro-inflammatory cytokines (e.g., IL-1β, IL-6, IL-8, tumor necrosis factor (TNF)-α) and reactive oxygen species [88]. Models demonstrating these effects include lung injury induced by chemical treatment and hypoxia-ischemia (HI); liver injury induced by ischemia-reperfusion and alcohol feeding, myocardial and renal ischemia-reperfusion injuries, surgically induced oral lesions, chemically induced osteoarthritis, spinal cord contusion injury, and colitis [89]. Anti-inflammatory effects are generally observed at higher doses of CBD in vivo (e.g., ≥10 mg kg-1); although, lower doses (e.g., ~1.5 mg kg-1) have indicated efficacy in some studies. However, research investigating the effects of CBD on inflammation in humans is limited and inconclusive [89].

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 pro-inflammatory markers (i.e., IL-6 receptors, TNF-α, TNF-β1, and inducible nitric oxide synthase) in the gastrocnemius and diaphragm of dystrophic mdx mice (a mouse model of Duchenne muscular dystrophy) [90]. 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, were also reported with this dose. Lower, but still relatively high, doses of CBD (20–40 mg kg-1 d-1) did not produce functional benefits [91].

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Also, CBD is widely marketed to athletes for effects such as decreased anxiety, extinction of fear memory, anti-inflammatory properties, pain relief, and post-exercise recovery. CBD products, specifically non-medicinal, so-called full-spectrum cannabis extracts, can contain significant levels of these substances, but also tetrahydrocannabinol (THC) contamination (>2.5 mg/day in >30% of products on the German market), potentially leading to positive drug tests. Labeled claims about CBD content and the absence of THC are often false and misleading. Exposure to psychoactive THC can result in adverse effects on cognition, and the safety profile of CBD relative to its toxicity is a controversial topic of discussion. For these reasons, the use of over-the-counter CBD products is currently discouraged [92].

As scientific evidence, a study investigated the effect of CBD oil on the perception of muscle soreness, inflammation, and strength performance after eccentric elbow flexor exercise (ECE). Thirteen untrained men (mean ± SD age: 21.85 ± 2.73) performed 6 sets of 10 maximal isokinetic ECC muscle actions of the elbow flexors as part of a double-blind, crossover design. Non-invasive measures (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) conditions separated by 2 weeks. Results showed no condition x time interaction or main effect of condition ($p>0.05$) 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 noninvasive markers of muscle damage in the upper extremity [92].

A study determined whether there are age-related differences in cannabis use patterns and subjective effects in adult athletes. Age was over 21 years. Of the 1,161

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participants, 301 (26%) athletes currently used cannabis. Younger athletes reported significantly more adverse and positive subjective effects of cannabis compared to older athletes. Younger athletes used cannabis concurrently with exercise more frequently than older athletes and consumed edibles, vaporized, and smoked more than older athletes. Therefore, patterns of cannabis use and subjective effects of cannabis are age-related. Concerns about cannabis misuse and abuse in athletes may be exaggerated, as the potential benefits (improved sleep, decreased anxiety, reduced pain) outweigh the adverse effects (increased anxiety, increased appetite, difficulty concentrating) [93].

In this context, the effects of chronic cannabis use on physiological parameters of athletic performance are investigated to determine whether it negatively impacts athletic performance, improves performance, potentially through enhanced recovery, or has no effect. Resting heart rate was the only physiological measure that differed significantly between groups, and in only 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. The included studies did not assess other elements, such as recovery or endurance. Therefore, no evidence of ergogenic or ergolytic effects of chronic cannabis consumption was observed [94].

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CHAPTER VII

Regulatory Measures - World Anti-Doping Agency

According to World Anti-Doping Agency (WADA) regulations, the use of cannabinoids is prohibited in competition, except for the use of 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-9-carboxy-tetrahydrocannabinol-9-carboxylic acid (carboxy-THC) in urine at a concentration greater than 180 ng/mL. All other cannabinoids are reported as AAA when identified, except CBD, which has been explicitly excluded from the cannabinoid class on the WADA Prohibited List since 2018. However, because CBD isolated from cannabis plants may contain additional minor cannabinoids, the permitted use of CBD may lead to unintentional violations of anti-doping regulations [95].

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 limit of identification were determined. Variable patterns of cannabinoids or their metabolites were observed in the urine samples, especially when full-spectrum CBD products were consumed. The presence of minor cannabinoids or their metabolites in the urine sample of an athlete in competition poses a substantial risk of anti-doping rule violation [95].

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Finally, review studies 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 an athletic population. For acute pain, two small randomized, double-blind, crossover studies found no immediate effect of cannabinoid therapy. More robust evidence exists for the treatment of chronic pain conditions through meta-analyses and systematic reviews. Cannabinoid therapy has moderate efficacy as a treatment for some chronic pain conditions. Investigations covered a broad spectrum of chronic pain conditions, including neuropathic, musculoskeletal, inflammatory, and central pain, and revealed pain reduction and improved quality of life with limited adverse effects. For concussion, the evidence is based on preclinical in vitro and animal models revealing possible neuroprotective effects, as well as two clinical studies involving the presence of cannabinoids for concussion (some related to sports), but there are currently no high-quality trials evaluating the efficacy of cannabinoid treatment. Thus, while there are several biochemical explanations for the use of cannabinoid therapy through ECS modulation for various medical problems affecting athletes, physician recommendations should be extrapolated from the majority of research done in the non-athletic population. The lack of high-quality clinical evidence, coupled with inconsistent federal and state laws, as well as purity concerns with cannabis-based products, makes it difficult for sports medicine clinicians to broadly recommend cannabinoid therapies at this time [96-102].

https://doi.org/10.54448/MSP.978-65-983826-8-1

FINAL CONSIDERATIONS

The International Association of Athletics Federations recognizes the importance of nutritional practices in optimizing an athlete's well-being and performance. In this regard, periodized guidelines can be provided for the appropriate type, quantity, and timing of food and fluid intake to promote optimal health and performance in different training and competition scenarios. Therefore, the use of medical supplements to address nutrient deficiencies, or sports foods to help athletes achieve nutritional goals, is well-known. The most common examples of supplements are caffeine, bicarbonate, beta-alanine, nitrate, creatine, glutamine, and iron ions.

Dietary supplements offer ergogenic aids by attempting to increase energy, improve recovery, modulate body composition, and control muscle acidity (number of free protons, acidosis), enabling improved performance. As a first example, increasing beta-alanine availability through dietary supplementation, combined with training, can improve the performance of athletes performing high-intensity exercise by increasing muscle buffering capacity (reducing acidity). As another example, early research reported that $NaHCO_3$ was effective in improving short-duration, high-intensity exercise capacity, while more recent studies have shown that $NaHCO_3$ can also improve performance during aerobic endurance and prolonged, high-intensity intermittent exercise.

Glutamine is also involved in several biological functions, such as nucleotide synthesis, cell proliferation, regulation of protein synthesis and degradation, energy production, glycogenesis, ammonia detoxification, and maintenance of acid-base balance, among others. Furthermore, this amino acid regulates the expression of several genes associated with metabolism and activates several intracellular signaling pathways. Glutamine metabolism has been investigated during and after physical exercise, and it has been observed that blood glutamine responds differently depending on the duration

https://doi.org/10.54448/MSP.978-65-983826-8-1

of exercise.

Also, iron is essential for oxidative metabolism and is therefore especially important for endurance athletes whose athletic performance depends on high aerobic capacity. Cannabidiol has been reported to exert a range of physiological, biochemical, and psychological effects with the potential to benefit athletes. For example, there is preliminary evidence supporting CBD's anti-inflammatory, neuroprotective, analgesic, and anxiolytic actions, and the possibility that it may protect against gastrointestinal damage associated with inflammation and promote healing of traumatic skeletal injuries. 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 studies directly investigating CBD and athletic performance are lacking.

https://doi.org/10.54448/MSP.978-65-983826-8-1

Acknowledgment

Not applicable.

Ethical Approval

Not applicable.

Informed Consent

Not applicable.

Funding

Not applicable.

Data Sharing Statement

No additional data are available.

Conflict of Interest

The authors declare no conflict of interest.

Similarity Check

It was applied by Ithenticate[@].

Application of Artificial Intelligence (AI)

Not applicable.

Peer Review Process

It was performed.

https://doi.org/10.54448/MSP.978-65-983826-8-4

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https://doi.org/10.54448/MSP.978-65-983826-8-1

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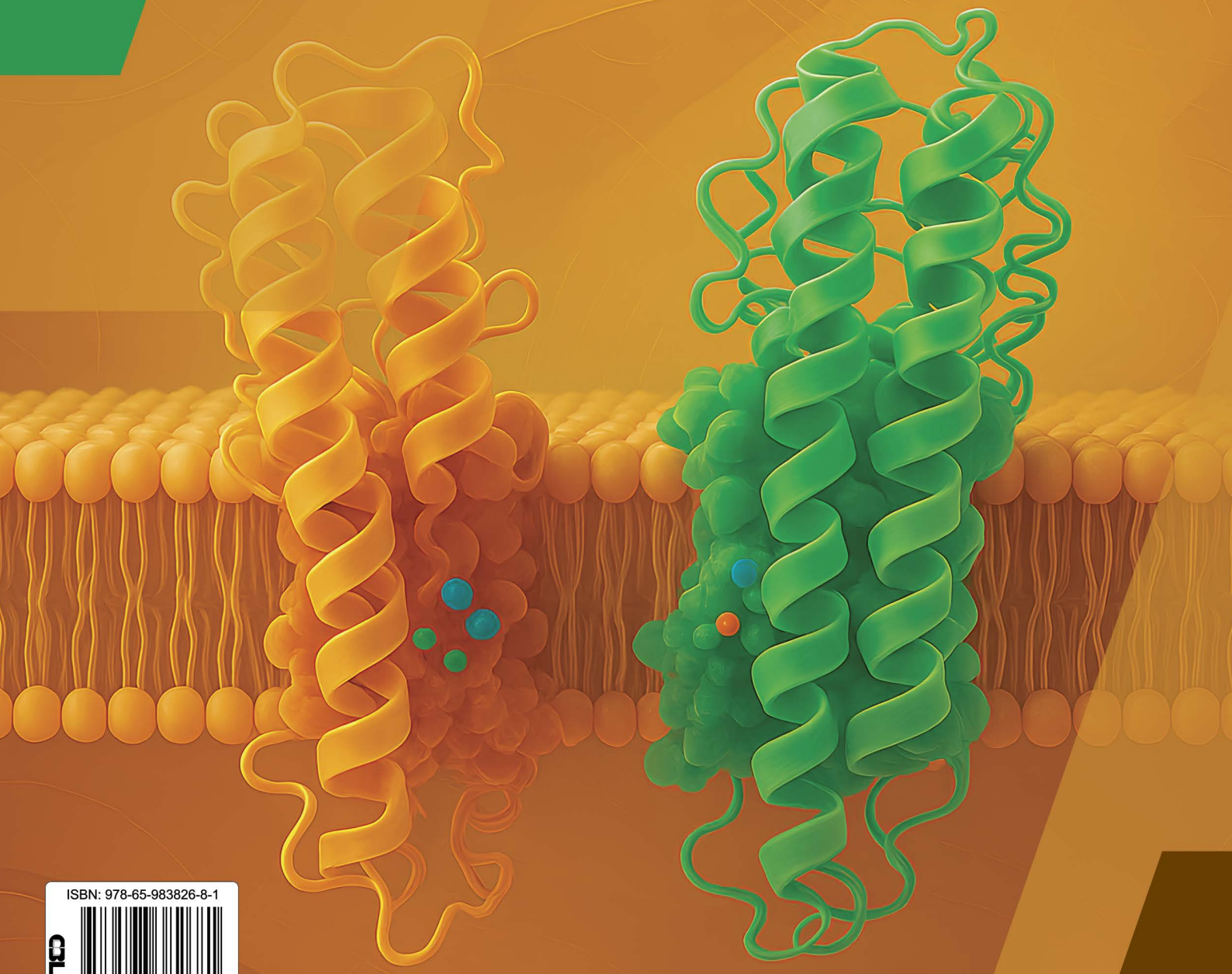
https://doi.org/10.54448/MSP.978-65-983826-8-1

BODY HOMEOSTASIS AND THE ENDOCANNABINOID SYSTEM AND CANNABIDIOL IN SPORTS PERFORMANCE



ISBN: 978-65-983826-8-1

https://doi.org/10.54448/MSP.978-65-983826-8-1

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