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**Document**

# CLINICAL PRACTICE GUIDELINES FOR SPORTS NUTRITION: BRAZILIAN SPORTS NUTRITION ASSOCIATION

## DIRETRIZES DE PRÁTICA CLÍNICA PARA NUTRIÇÃO ESPORTIVA: ASSOCIAÇÃO BRASILEIRA DE NUTRIÇÃO ESPORTIVA

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**RESUMO**
Considerando o crescimento da nutrição esportiva no mundo e no Brasil, nosso objetivo foi elaborar uma diretriz de prática clínica sobre nutrição e exercício físico para otimizar as práticas baseadas em evidências. Trata-se de uma diretriz de prática clínica elaborada com base nas recomendações propostas pelo sistema GRADE. A busca foi padronizada considerando atletas adultos como população e o desempenho físico como desfecho de interesse. Esta diretriz recomenda que o consumo de carboidratos seja ajustado de acordo com a demanda energética, respeitando a quantidade total e os ajustes feitos antes, durante e após o exercício físico quando necessários. Além disso, o consumo de proteínas e lipídios deve ser adequado para desfechos como força, massa muscular e perfil inflamatório, respectivamente. A diretriz também recomenda o monitoramento e a correção de deficiências de micronutrientes ao longo do tempo. Os suplementos ergogênicos (p. ex., cafeína, creatina, beta-alanina, bicarbonato de sódio e nitrato) são recomendados em casos específicos, sendo fundamental avaliar as características do atleta e do esporte praticado. Contudo, a diretriz não recomenda o uso de suplementos alimentares com descrições incertas dos compostos, principalmente pelo risco de doping. Finalmente, esta diretriz recomenda que, para atletas com necessidades especiais, a avaliação nutricional seja feita respeitando as particularidades dessa população.
**Palavras-chave:** Ciências da Nutrição Esportiva. Exercício Físico. Guia de Prática Clínica.

**ABSTRACT**
Considering the growth of sports nutrition worldwide and in Brazil, our goal was to develop a clinical practice guideline on nutrition and physical exercise to optimize evidence-based practices. This clinical practice guideline was developed based on

the recommendations proposed by the GRADE system. The search was standardized considering adult athletes as the population and physical performance as the outcome of interest. This guideline recommends that carbohydrate intake be adjusted according to energy demand, respecting the total amount, with adjustments made before, during, and after physical exercise as needed. Additionally, protein and lipid intake should be adequate for outcomes such as strength, muscle mass, and inflammatory profile, respectively. The guideline also recommends monitoring and correcting micronutrient deficiencies over time. Ergogenic supplements (e.g., caffeine, creatine, beta-alanine, sodium bicarbonate, and nitrate) are recommended in specific cases, with a fundamental assessment of the athlete's characteristics and the sport practiced. However, the guideline does not recommend the use of dietary supplements with uncertain descriptions of their compounds, primarily due to the risk of doping. Finally, this guideline recommends that for athletes with special needs, nutritional assessment should consider the particularities of this population.
**Keywords:** Sports Nutritional Sciences. Exercise. Practice Guideline.

## Introduction

The sports nutrition guidelines from the Brazilian Sports Nutrition Association (ABNE) were organized to establish interventions related to food and nutrition to optimize exercise-related performance. This document describes details on nutritional evaluation, which includes the assessment of food intake, total daily energy expenditure (TDEE), energy availability (EA), biomarkers, and body composition. Likewise, we inserted information concerning macronutrients, micronutrients, dietary supplements, hydration, electrolytes, doping, and the nutritional assessment of athletes living with specific requirements.

ABNE comprehends that the dynamics of nutritional assessment and dietary-related clinical practice for athletes is complex and require specific tools and essential valuable skills. Therefore, recommendations always need to be aligned with the patient's reality. When considering dietary management, it is essential to consider factors involving eating habits, traditions, and regionality and respect individual needs and features. This document represents the evolution of sports nutrition in Brazil and brings the most current information on nutrition applied to physical exercise. These guidelines were developed to offer up-to-date and high-quality evidence-based nutrition information to nutritionists and health professionals working with physical exercise and sport.

To verify the effect of dietary-related interventions, we chose published scientific articles that presented the most appropriate methodological design, such as randomized clinical trials (RCTs), double-blind and controlled by a placebo or control group. Still, systematic reviews with meta-analysis (SRMA) were evaluated. Therefore, if available in the SRMA, the examination of the risk of bias of primary studies was used as a criterion for analyzing the methodological quality of the studies. For other parameters such as energy intake, energy expenditure, EA, and body composition, observational studies were used analyzed. It is essential to highlight that some methodological limitations are indissoluble in nutrition applied to physical exercise. Data derived from RCTs, especially double-blind and placebo-controlled trials, are only obtainable if the studies compare single nutrients or non-nutrients (e.g., creatine, caffeine, iron, etc.)[1]. Thus, studies that evaluate the effect of dietary interventions are unlikely to have an identical control group[1].

Although the nutritional composition of different foods is similar, they have different matrix. Similarly, people have varied eating habits and food-related histories, depending on the different regions of the country. Therefore, all these variations may be the result of biases in data analysis[2,3]. Despite methodological variations, RCTs are considered the gold standard in the evidence pyramid. Case studies, cross-sectional studies, and longitudinal observational studies, especially in the areas of nutrition and physical exercise, have been little applied to identify the effect of interventions, which places them as less of a priority in the hierarchy of scientific evidence for decision-making.

Finally, although personal experiences are essential for evidence-based practices, they present low level of scientific evidence, mainly because they are dipped in biases, which can generate misinterpretations[4]. Still, a significant challenge is managing patient preferences, which may be antagonistic to scientific evidence. Thus, it is vital to align patient expectations, avoiding overestimation of interventions associated effects and damage to other areas of health care[4].

The pillars of evidence-based practices were: (i) the best scientific evidence available, (ii) professional's clinical experience, (iii) patient preference[2, 3]. It is essential to analyze that previously published sports nutrition consensus focused on the discussion of nutrients applied to physical exercise[5, 6]. However, we reiterate that the nutritionist who will monitor this individual is responsible for translating nutritional recommendations into dietary recommendations. The concept of evidence-based practices has generated essential changes in the paradigm in contemporary healthcare. However, it is still possible to identify several barriers that limit the application of this approach, such as (i) little knowledge about the pillars of practices based on scientific evidence, (ii) difficulty in tracking, interpreting, and applying knowledge obtained from the literature scientific in clinical practice; (iii) difficulty in assimilating a high content of available scientific information[2, 3]. Based on this reflection, the preparation of this document is justified as an essential source of scientific information for the professional practice of Sports Nutritionists.

As such, we aimed: (i) to discuss the main topics related to nutritional assessment of physically active people and high-performance athletes; (ii) to describe the effect of macronutrients and micronutrients on the physical performance of physically active people and high-performance athletes; (iii) to discuss the adverse effects of hypohydration on physical performance and the importance of hydration and electrolyte intake; (iv) to present the effects of dietary supplements on physical performance; (v) to discuss the particularities related to the assessment of body composition and energy expenditure, as well as the dietary management of particular populations that practice physical exercise.

## Methods

This Guideline was designed according to the Grading of Recommendations, Assessment, Development, and Evaluations (GRADE) system, whose primary purpose is to grade the quality of evidence and the strength of recommendations for the most diverse health outcomes[7-11].

Initially, the ABNE scientific committee selected researchers and clinicians with previous experience in the topics studied. From this selection, three committees were organized, namely: (i) the search committee, (ii) the writing committee and (iii) the review committee. These committees were developed so that each step was handled carefully. Then, meetings were held with the authors to align and standardize the methodological procedures necessary to create practical Guidelines with high level of scientific evidence.

### *Search procedures*

Groups composed of clinicians and researchers from each area formulated the research questions. The search was carried out considering the acromion P (people), I/E (intervention and exposure), C (control group), O (outcome), and S (study type), selecting terms and keywords according to the Health Sciences Descriptors (DeCS) and Medical Subject Headings (MeSH)[12]. Cochrane recommendations for searching scientific articles were followed[13]. The search was standardized, considering adult athletes as a population and physical performance as the primary outcome. Similarly, for some topics (e.g., protein intake), body composition was considered as the outcome. The search was carried out using the Medical Literature Analysis and Retrieval System Online (MEDLINE) database. Finally, when necessary, the handsearching process was adopted[14, 15].

The search committee was composed of internal and external ABNE members. They systematically carried out searches to identify scientific articles derived from each research question. The search committee then sent the articles to the writing committee, which was responsible for preparing each topic of this Guideline.

*Establishment of the level of evidence*

The level of evidence and strength of recommendation were graded in accordance with the GRADE criteria, which proposes the transparent establishment of the systematized methods necessary for the development of scientific evidence syntheses[10, 11]. The writing committee was responsible for separating, interpreting, and carefully evaluating the quality of the scientific articles identified. Authors were instructed to evaluate articles according to commonly used risk of bias assessment tools[16].

The GRADE system proposes four levels of evidence: **Very low**—The actual effect is probably very different from the estimated effect; **Low**—The actual effect may be different from the estimated effect; **Moderate**—The authors believe that the actual effect is probably close to the estimated effect; **High**—The authors are very confident that the actual effect is similar to the estimated effect[7-9]. The evidence analysis considered the risk of bias, imprecision, and inconsistency of the results, as well as their directness. Finally, publication bias was considered[7-11].

After identifying the articles, reading, interpreting, establishing the level of evidence (e.g., low, moderate or high), and writing the documents, the topic authors established the strength of recommendation, which could be strong or weak. Strong recommendation: The benefits clearly overlap the risks and demands for most, if not all, individuals. Weak recommendation: Benefits and risks are closely balanced and uncertain10. **Figure 1** illustrates the evidence pyramid adapted from Murad et al.[17]. The sinuosities represent that, regardless of the type of scientific study, the methodological quality is varied. Thus, even RCTs can present low or high methodological quality.

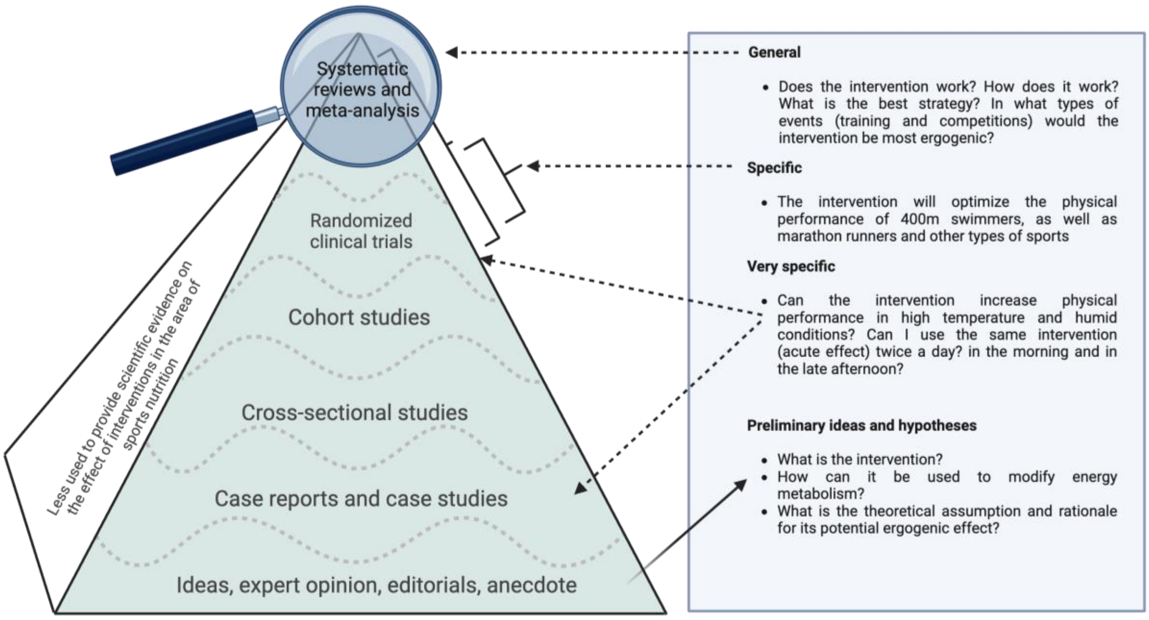


**Figure 1.** Different types of evidence can be applied to decisions made about the effectiveness of dietary and nutritional interventions to improve physical performance.
**Source**: Adapted from Murad et al. [17]

After preparing the documents, the scientific committee received the prepared texts and sent them to the review committee, which is made up of external researchers with expertise in each area. The review committee returned the document with considerations to the scientific committee, which adjusted the texts along with the writing committee. After this stage, the final version in Portuguese was obtained, which was then translated and adapted into the English version.

Regarding the gradation of the level of evidence and the establishment of the strength of recommendation, several discussions are carried out by researchers in the field of sports science. Therefore, it is essential to highlight that many interventions may present small effect size (ES) and intervals reliable with a small variability, which indicates a small but precise effect; other interventions, however, present higher ES, but with high variability, indicating imprecision of the result[13, 18]. The potential disparity in the results was considered when establishing the level of evidence.

Despite possible small ES, the physical performance outcome needs to be interpreted with caution, especially when coming from high-level athletes. For example, minor effects may be significant for a competitive athlete and irrelevant for a recreational practitioner[18]. Similarly, interventions are subject to variation due to the variability of physical capacity. Consequently, people with different levels of physical capacity may respond differently to the same intervention[18].

Moreover, external and ecological validity extends beyond internal validity. Studies with excellent internal validity may need to present adequate external validity[19]. For instance, the physical performance tests used for performance assessment may need to present adequate reliability and validity. Such a limitation can make it difficult to detect small but significant changes in physical performance that are relevant to real life[20].

Sport nutrition-related studies showed a relatively small sample size, which limits the generalization of results to all physical exercise practitioners or high-performance athletes[21]. For example, mainly sport nutrition-related studies were developed with males, while studies with females are scarce[22-24]. Furthermore, our Guidelines focus mainly on adults since information about nutrition applied to physical exercise for children, adolescents, and aged people is scarce.

Finally, discussions about sports science consider the need to reinterpret scientific data. Recently, the adoption of a comprehensive interpretation of statistical results has been advocated. This approach would involve focusing on estimating effects rather than statistical significance and focusing on the uncertainty surrounding the estimated effect (e.g., the size of the confidence interval). This estimation approach would avoid the problem of oversimplifying results into significance and non-significance[21]. Therefore, we emphasize that our Guidelines were developed considering previous scientific publications from the American College of Sports Medicine and the International Olympic Committee[5, 6, 25, 26].

## Concepts about physical activity, physical exercise and sport

Considering nutrition-related science, sports nutrition is responsible for nutrition applied to sports and physical exercise. However, the concepts of nutrition applied to sports are often also replicated in situations of regular physical activity and even non-competitive physical exercise. The terms Physical Activity, Physical Exercise, and Sports are often used as synonyms; however, there are significant differences among them. Thus, an adequate understanding of these concepts is fundamental to the satisfactory interpretation of this document.

While physical activities comprise any body movement, generally spontaneous, that generates a higher energy expenditure than at rest (e.g., domestic activities or commuting), physical exercises are characterized by being carried out in a planned manner, the goals of which are well-defined and may be related to the improvement or maintenance of sports performance[27]. Sport is comprehended as competitive human activities or social participation in which formal rules and standards of behavior must be followed to identify the best performances.

This document aims to shed light on the influence of various nutritional approaches on the motor capabilities developed through physical exercise and expressed in different sports. By understanding the motor predominance of different sports, we can classify them into categories such as endurance, strength, and intermittent sports. This classification underscores the significant role of nutrition in shaping the performance of athletes, making us feel enlightened about the impact of nutrition on sports performance[28].

Endurance and ultra-endurance sports (e.g., running, cycling, open water swimming) refer to sports whose aerobic resistance - the predominance of aerobic metabolism - is the predominant conditioning component; exercise intensity is equivalent to 75 to 85% of maximum oxygen consumption ($\dot{V}O_{2max}$)[29]. The difference between endurance and ultra-endurance sports is the time until completion; while the first one must last up to 6 hours, the other must take its participants to compete for more than 6 hours[30].

For strength sports (e.g., weightlifting, powerlifting, throwing, and sprinting), the execution time is very short – a few seconds – and the ability to generate force and power is fundamental to the success of sport. Therefore, it is essential in these modalities that the alactic anaerobic metabolism (adenosine triphosphate [ATP]-creatine phosphate [CP]) is very well developed, as well as the speed of movement[31].

There are also sports in which lactic anaerobic metabolism is highly solicited (e.g., half-distance running, middle-distance swimming) since the time required to execute them is just a few minutes. In these cases, it is essential to develop energy efficiency resulting from glucose metabolism[28, 32].

Intermittent sports (e.g., all team sports) are characterized by alternating high and low-intensity movements associated with the execution of complex, sport-specific skills. Generally, intermittent modalities take place for a period that varies between 1 and 2 hours and depend on the optimal combination of the efficiency of aerobic and anaerobic metabolism[33].

Finally, regardless of the type of physical exercise or sport, we consider three categories to define the individual's level, as follows: recreational athletes - those individuals involved in recreational or leisure sports; competitive athletes: individuals who exercise and train regularly for the purpose of participating in official sporting competitions, at any level; elite and professional athletes: a group of competitive athletes who achieve athletic excellence and generally compete at an international level[34].

## Athlete-related nutritional evaluation

### Food intake

Assessing the food intake of sportspeople and athletes is of paramount importance. It serves to verify the discrepancy between current energy and nutritional intake and physical exercise energetic and nutritional demands[35]. Moreover, it is crucial for calculating EA. There are various methods, both retrospective and prospective, to assess food intake (e.g., 24-hour recall [24hR], dietary history, food frequency questionnaire [FFQ] and food diary)[35]. However, these dietary surveys have their limitations, particularly when compared to Double Labeled Water (DLW), the gold standard method for assessing total energy expenditure[35, 36]. A recent systematic review revealed that the assessment of energy intake by R24h underestimated energy intake by 8 to 30%, primarily for women[36]. In athletes, the assessment of food intake presents additional challenges, such as variations in intake depending on training, the size of food portions, and the wide use of dietary supplements[37].

The underestimation of food intake by athletes is ~19% (0.4 to 36%) compared to DLW. The ES of the difference between the value derived from dietary surveys and DLW was 1.006

(95% CI: -1.3 – -0.7), reinforcing that, among athletes, the difference between the dietary survey report and the DLW objective measurement is high[37].

Considering that the 24hR is similarly applied to athletes compared to the general population, problems of underestimation are expected. This underestimation can be mitigated if more than one method is used. For example, 24hR associated with dietary history, which, in the case of athletes, directs questions about nutrition to the moments before and after training[35]. Furthermore, the multiple-step method (MSM) favors a more accurate reporting of dietary intake and should be applied to improve the assessment of dietary intake, minimizing underestimation. The MSM consists of (i) conducting a quick list of foods eaten the previous day or in the previous 24 hours; (ii) checking potentially forgotten food; (iii) checking the situation and moment of ingestion; (iv) cycle of details for each meal, quantities and types of food; (v) final evaluation of all meals[38-40]. Thus, the application of 24hR associated with dietary history or other methods can increase the reliability of food intake data with less error and underestimation. Likewise, the use of MSM contributes to better quality of data extracted from athletes.

***This guideline strongly recommends using the 24-hour recall (24hR) combined with dietary history as the primary method for assessing athletes' dietary intake. Additionally, the use of photographic tools and the multiple-pass method is advised to enhance the accuracy of the information obtained and, consequently, improve the effectiveness of the proposed intervention. The level of evidence supporting these recommendations is moderate, and the strength of the recommendation is strong.***

## Energy expenditure

The TDEE is a crucial concept in understanding human metabolism. It is the sum of various components, including Resting Energy Expenditure (REE) (also known as Resting Metabolic Rate [RMR] or Basal Metabolic Rate [BMR]), Non-Exercise Energy Thermogenesis (NEET), Energy Expenditure from Physical Exercise (EEE), and the Thermal Effect of Food (TEF). Understanding REE is vital to comprehending the complexities of human energy balance, especially in the context of physical exercise and body composition[41]. The reference standard instrument of REE measure is the indirect calorimetry (IC) at rest, especially after a night of fasting, in the morning, with a controlled temperature (between 22 and 26 ºC), avoiding possible factors that modify oxygen consumption (e.g., body movements with more significant energy expenditure [climbing stairs], physical exercise, diet, environmental stress, etc.). It is expected that REE suffers minor influences derived from travel to the laboratory and other environmental stresses that are difficult to control[41].

Given the impossibility of using REE measured by IC, the use of predictive equations makes it possible to estimate REE. However, prediction equations present several problems and limitations, mainly when they depend on body composition parameters, which will require more accurate methods; otherwise, the error derived from a body composition predictive equation may maximize the error in the REE estimate. A recent SRMA evaluated the accuracy of predictive equations for REE among recreational and high-performance athletes[41]. The most studied equations were Cunninghan version 1980 (lean mass; 21 studies)[42] and Harris and Benedict (age, body mass and height; 21 studies)[43]. The accuracy assessment revealed that the equations of Cunninghan version 1980 (lean mass), Harris and Benedict (age, body mass and height), Cunninghan version 1991 (fat-free mass)[44], De Lorenzo (age, body mass and height)[45], and Ten-Haaf (age, body mass and height)[46] presented non-statistically significant differences compared to the IC measurement.

The Cunningham equation version 1980 showed an ES of 0.15 (95% CI: - 0.26 – 0.57; $I^2$: 93%; 846 evaluated; mean difference of 1 kcal/24h), the Harris and Benedict equation showed an ES of -0.14 (95% CI: - 0.53 – 0.25; $I^2$: 93%; 892 evaluated; mean difference of -50.4 kcal/24h), Cunninghan version 1991 presented an ES of 0.31 (95% CI: -0.09 – 0.70; $I^2$: 80%; 307 evaluated; mean difference of 45.2 kcal/24h), De Lorenzo presented an ES of 0.15 (95% CI: - 0.27 – 0.56; $I^2$: 86%; 359 evaluated; mean difference of 13.7 kcal/24h) and Ten-Haaf presented an ES of 0.04 (95% CI: - 0.16 – 0.23; $I^2$ : 0%; 204 evaluated; average difference of 6.6 kcal/24h). Despite the trivial/minor ES for the Cunninghan equations versions 1980 and 1991, Harris and Benedict and De Lorenzo, the authors point to the high heterogeneity observed. Thus, they reinforce that the Ten-Haaf equation presented low heterogeneity and ES.

It is crucial to emphasize that the Ten-Haaf equation presents simple anthropometric-related parameters (age, body mass, and height) and offers a high level of precision in estimation. In contrast, Cunningham's equations require lean body mass (1980) and fat-free mass (1991), which leads to the REE estimative being more complex than the Ten-Haaf equation. Depending on the method used to obtain these parameters (e.g., DXA vs. anthropometry), the accuracy of the estimates may be compromised. In summary, among the prediction equations, the Ten-Haaf equation (age, body mass, and height) emerges as the most accurate and least heterogeneous, providing a reliable tool for estimation. However, the Harris, Benedict, and Cunninghan equations from the 1980 and 1991 versions can also be considered.

The assessment of NEEE and EEE in a practical setting is a complex task due to the variety of available methods. The Academy of Nutrition and Dietetics and the ACSM (2016) recommend the use of the activity factor (AF), which ranges between 1.8 and 2.4 for physically active individuals. The physical activity compendium, published in 2024 and updated in the 1993, 2000, and 2011 versions, provides a list of metabolic equivalents (MET) for estimating various activities, including activities of daily living and a wide range of physical exercises at different intensities. One MET represents the amount of oxygen consumed by the body under resting conditions, and is defined as 3.5 mL $O_2$/k/min or ~1 kcal/kg/h. The compendium categorizes activities and physical exercises into sedentary behavior (1 to 1.5 MET), low-intensity physical activity (1.6 to 2.9 MET), moderate-intensity physical activity (3.0 to 5.9 MET) and high-intensity physical activity (≥ 6 MET). However, categorizing METs by fixed intensity can lead to inaccurate estimates[47].

***This Guideline underscores the crucial role of quantifying total energy expenditure in guiding dietary planning for physically active individuals and high-performance athletes. The Harris and Benedict, Cunninghan (1980 and 1991), and Ten-Haaf equations, despite showing lower ES compared to indirect calorimetry, are still valuable tools. Among them, only the Ten-Haaf equation demonstrated low heterogeneity, further highlighting its reliability. Despite the inconsistencies, the use of activity factors and METs to estimate the energy expenditure of physical activities and physical exercise remains the best way to quantify the energy demand of this population. This recommendation, with a moderate level of evidence and strong recommendation strength, underscores the significance of your work in this field.***

## Relative Energy Deficiency in Sport (REDs)

The term Relative Energy Deficiency in Sport (REDs) is attributed to the syndrome of compromised physiological and psychological functioning with multifactorial etiology, experienced by female and male athletes and triggered by exposure to problematic (prolonged and severe) low energy availability (LEA), which may be accompanied (or not) by eating disorders[48]. Harmful outcomes include, but are not limited to, changes in energy metabolism, reproductive function, musculoskeletal health, immunity, glycogen synthesis, and cardiovascular and hematologic health, which can individually and synergistically lead to wellness problems, increased risk of injury, and decreased sports performance[49].

The definition of REDs comes from 40 years of studies related to the Female Athlete Triad (FAT) but with essential expansions. REDs affects both men and women and include clinical manifestations in different physiological systems, in addition to the reproductive and musculoskeletal systems affected by FAT[50].

The primary disorders observed in female athletes living with REDs include hormonal changes, such as dysregulation of sexual hormones, which can lead to menstrual changes such as amenorrhea or oligomenorrhea, changes in the thyroid gland and the hormone cortisol, and low bone mineral density (BMD); changes in appetite, reduction in REE and ferritin levels, growth retardation, some psychological disorders, dyslipidemia and hypotension, abdominal distension and constipation, and immunosuppression[48-51].

For LEA analysis, the EA must be evaluated. It is calculated by subtracting the exercise energy expenditure (EEE) from the energy intake (IE); the result of this subtraction must be divided by the fat-free mass (FFM)[52].

$$(EA = [EI\ (kcal/day) - EEE\ (kcal/day)]/\ FFM\ (kg))$$

**Source**: Mountjoy et al.[50]

An EA of 45 kcal/kg FFM/day has been indicated as ideal for maintaining physiological functions in women, while ≤ 30 kcal/kg FFM/day has been considered a cutoff point for diagnosing LEA. This cutoff was suggested due to studies that described a reduction in hormones in sedentary women when reaching an EA below the cutoff point[53]. However, advances in scientific knowledge on LEA and REDs have suggested that EA ≤ 30 kcal/kg FFM/day in association with other diagnostic criteria (described in **Chart 1**) is considered as a physiological response that can vary according to age and stage of sexual maturation. It is also supposedly influenced by inter-individual variability, derived from genetic factors, which predisposes to a greater capacity for adaptation of some women, allowing them to continue to have normal menstrual cycles despite LEA[54]. In the 2023 International Olympic Committee consensus, the authors named this condition as "Adaptable LEA", in which exposure to LEA occurs for a short period and does not affect (or affects very little) long-term health, well-being, and physical performance[49].

**Chart 1.** REDs consequences on performance and health.

| **Potential consequences for performance (Aerobic and anaerobic)** | **Potential consequences for health - Damages/changes in** |
|---|---|
| Reduced endurance performance | Menstrual function |
| Increased risk of injury | Bone health |
| Reduced muscle strength | Endocrine function |
| Reduced glycogen levels | Metabolic function |
| Reduced Coordination | Hematological Function |
| Reduced concentration | Psychological/mental function |
| Reduced judgment capacity | Cardiovascular function |
| Reduced adaptive response to training | Gastrointestinal function |
| Increased irritability | Immune function |
| Increase in depression | Growth and development |

**Source:** Adapted from Mountjoy et al. [48,49]

For male biological athletes, discussions about REDs and cutoff points for EA classification are initial[55-57]. For men, the hypothesis is that they need more severe energy restrictions to develop REDs-related symptoms[55-57]. It appears to be ~9 to 25 kcal/kg FFM/day, lower than the female cutoff. In fact, there is evidence that most men can sustain lower ED before physiological and psychological disturbances manifest. However, problematic LEA can occur in male athletes and is associated with adverse effects on the hypothalamic-pituitary-gonadal (HPG) axis and associated hormones, changes in metabolic hormones, deficiencies in immune function, detriments to bone health, as well as negative results in physical performance and decreased FFM. Two emerging potential indicators of REDs in men are low libido and decreased morning erections, which have been identified as consequences of LEA[56].

Given the potential disturbances in various physiological systems, it is expected that there will be direct or indirect consequences on the sports performance of athletes affected by LEA, regardless of the type, volume or intensity of physical exercise. The mechanisms potentially involved are of multiple natures, such as depletion of muscle and liver glycogen reserves, lower rate of skeletal muscle protein synthesis, and iron deficiency, which can cause significant impairments in endurance capacity, muscle strength, coordination, concentration, judgment and adaptive response to training, as well as compromised emotional state, characterized by an increase in depressive symptoms and high irritability[58]. The greater risk of injuries could also significantly affect physical performance, due to absences from training sessions[59].

Despite the existence of several mechanisms that may potentially explain the relationship between LEA and physical performance decline, there are few studies that have tested this association[60, 61]. This lack of research is a significant gap in our understanding. The difficulties of standardizing physical performance indicators in different scenarios of sports training and competitive events, as well as the challenges in diagnosing LEA, may be the main reasons for the scarcity of this type of study. Clearly, further investigations, including robust protocols with random allocation of athletes to intervention groups, are urgently needed to provide further evidence and explanation of the effects of LEA on adaptations to training and sports performance[61]. Studies that investigated LEA and REDs are often cross-sectional in nature, which limits data extrapolation and reliability to clearly establish a causality relationship. Therefore, in addition to intervention studies, prospective longitudinal cohorts that monitor athletes for periods sufficient to identify REDs are necessary to understand, above all, the temporality of this phenomenon.

It is believed that athletes at the highest risk of LEA are those involved mainly in acrobatic and endurance sports, due to the power/body mass relationship, as well as those engaged in combat sports and aesthetic sports[62]. Despite the potential increased risk in these groups, athletes of all modalities must be monitored. In addition to situations in which a reduction in body mass is desired, LEA can also appear unintentionally, resulting from a significant increase in training volume without due adjustment of the energy consumption, and difficulties with food intake during training and competition sessions, generally related to gastrointestinal discomfort[58]. Studies that evaluated the prevalence of LEA found 23 and 79.5% among women and 15 to 70% among men; this variation is generated by the lack of a singular definitive diagnosis, mistaken use of LEA and REDs as interchangeable terms, lack of standardization and precision of research methods (e.g., imprecise measurements of EA components), variation in physiological demands among study populations, and volunteering biases of study participants[63].

Diagnosis of REDs is challenging due to the complexity of the syndrome. Burke et al.[64] described the main obstacles to calculating DE, with emphasis on (a) underreporting errors arising from the use of food records; (b) uncertainties about the number of days to be evaluated to reflect the caloric intake of athletes in different phases of training periodization; (c) difficulties in assessing energy expenditure resulting from (i) physical exercise in more complex sports, since the use of heart rate monitors, global position system (GPS), gas analyzers, and accelerometers seems to be more viable in cyclical sports; or (ii) energetic compensations and metabolic adaptations to physical exercise that can compromise the estimate of energy expenditure during physical exercise; (d) standardization of methods for assessing body composition, since access to dual emission X-ray absorptiometry (DXA) is reduced besides possible discrepancies between different machines and techniques. Concerning body composition assessment, anthropometry has been adopted in many studies; however, this assessment technique is more suitable for evaluating discrete physical changes over a given period. Furthermore, as it is a doubly indirect method, several discussions are made about the precision and accuracy of the prediction equations. In summary, significant validity and reliability errors occur during the process, culminating in inadequacies of 300 to 600 kcal/day. This suggests that to diagnose, prevent, or treat REDs, it is necessary to interpret EA in conjunction with clinical manifestations and indicators of physical performance decline, as described in **Chart 2**.

**Chart 2.** Important clinical characteristics to observe in sportspeople and athletes susceptible to REDs.

| |
|---|
| Eating habits and caloric intake |
| Body image |
| Recent changes in body mass |
| Recent increases in training |
| Disordered eating (restriction or self-induced vomiting) |
| Previous stress fractures |
| Use of medications |
| Use of dietary supplements |
| Menstrual history in women (e.g., menarche and menstrual cycle frequency) |
| Duration of fatigue or low athletic performance |
| Increased resting heart rate |
| Sleep pattern (e.g., sleep latency, sleep duration, sleep quality, and sleep efficiency) |
| Athlete's knowledge of “energy balance” |
| **Physical examination** |
| Height, body mass, BMI; consider orthostatic evaluation (blood pressure and pulse), body fat percentage |
| Physical signs of eating disorders: lanugo, enlarged parotid gland, hypercarotenemia, Russell's sign (calluses on the knuckles) |
| Cardiac: bradycardia, arrhythmia |
| Mucous membranes: pale, dry |
| Skin: acne, male-pattern hirsutism |
| Tanner staging (assessment of sexual maturation status) |
| Musculoskeletal injury assessment |
| **Additional tests** |
| Basic laboratory tests may include a complete blood count and a comprehensive metabolic panel, with special attention to potassium |
| ECG: evaluate for arrhythmias or prolonged QT interval in athletes with eating disorders |
| DXA to assess BMD for athletes with previous or current bone stress injuries or eating disorders |
| Athletes with previous or current bone stress injuries or eating disorders |

| |
|---|
| Additional laboratory evaluation may be indicated depending on the specific clinical presentation of the athlete (significant fatigue/poor performance, previous bone injury, secondary amenorrhea, or severe hypogonadotropic hypogonadism). |
| Possible tests to consider thyroid hormones (e.g., TSH, T3, and T4), gonadotropins, prolactin, vitamin levels, and a pregnancy test in women. |
| **Legend:** BMI: Body Mass Index; ECG: Electrocardiogram; DXA: Dual-energy X-ray Absorptiometry; BMD: Bone Mineral Density; TSH: Thyroid-Stimulating Hormone; T3: Triiodothyronine; T4: Thyroxine. |

**Source:** Adapted from Mountjoy et al. [48,49] and Statuta et al.[65]

Notably, the study conducted by Torstveit et al.[52] is significant as it suggests that the traditional 24-hour period for assessing EA and its effects on health and performance may overlook real-time endocrine responses due to changes in energy intake and expenditure. The authors propose a more suitable intraday assessment at 1-hour intervals. However, the feasibility of this type of assessment in non-laboratory training scenarios needs to be verified.

In an attempt to associate LEA with health outcomes and physical performance, screening tools have been proposed for adult athletes, such as the Relative Energy Deficiency in Sport Clinical Assessment Tool (REDs-CAT) original version66 and the updated version – REDs-CAT2[49], as well as the Low Energy Availability in Females Questionnaire (LEAF-Q)[67], whose original version was translated, culturally adapted, and validated for Brazilian athletes[68]. This tool was designed to identifying the risk of LEA[63]; however, it is limited in terms of gender[69] and age group[70]. The Low Energy Availability in Male Athletes (LEAF-M)[71], which includes other questions not included in the LEAF-Q, is in the development and validation process.

The 2023 International Olympic Committee consensus on REDs[49] outlines a three-step process for diagnosis. ***Step 1*** involves the initial identification of at-risk athletes through the implementation of REDs screening questionnaires validated for specific populations and clinical interviews. These methods, while less sensitive and objective, are cost-effective and easy to implement. ***Step 2*** includes the use of assessment instruments such as REDs-CAT2 severity/risk assessment and stratification with sports participation guidelines. These tools are based on the accumulation of several primary and secondary risk indicators, resulting in the stratification of an athlete's severity and risk according to the colors green, yellow, orange or red, as illustrated in **Chart 3** and **Chart 4**. ***Step 3*** is the diagnosis, which is made by a specialist doctor and includes the development of a treatment plan, ideally in collaboration with a multidisciplinary team.

**Chart 3.** RED severity/risk stratification according to the International Olympic Committee, 2023.

| **Green** | | | |
|---|---|---|---|
| **Severity/risk** | **Clinical criteria** | **Treatment** | **Recommendation for training and competitions** |
| None or very low | 0 primary indicators;<br>≤ 1 secondary indicators | No need | No limitations to train or compete |
| **Yellow** | | | |
| Light | 0 primary indicator and ≥ 2 secondary indicators<br>1 primary indicator and ≤ 2 secondary indicators<br>2 primary indicators and ≤ 1 secondary indicator | Regular monitoring at regular intervals | No limitations to train or compete |
| **Orange** | | | |
| Moderate to high | 1 primary indicator and ≥ 3 secondary indicators<br>2 primary indicators and ≥ 2 secondary indicators<br>3 primary indicators and ≤ 1 secondary indicator | Treatment, monthly monitoring | Some aspects of training and competitions may be modified |
| **Red** | | | |
| Very high/Extreme | 3 primary indicators and ≥ 2 secondary indicators or ≥ 4 primary indicators | Immediate treatment (hospitalization) required for daily monitoring up to monthly intervals depending on severity | Significant changes in training and competitions are necessary and, in most cases, interruption of training and/or competitions is necessary. |

**Source:** Adapted from Mountjoy et al. [48,49]

**Chart 4**. Primary, secondary indicators and potential risk factors derived from assessment tools and severity/risk for REDs according to the International Olympic Committee, 2023.

| **Severe primary indicators (Count as 2 primary criteria)** |
|---|
| Primary amenorrhea (females: primary amenorrhea is indicated when there has been failure of menstruation by age 15 years in the presence of normal secondary sexual development (two standard deviations [SD] above the mean of 13 years), or within 5 years of breast development if this occurs before 10 years); or prolonged secondary amenorrhea (absence of 12 or more consecutive menstrual cycles) due to functional hypothalamic amenorrhea |
| Clinically low free or total testosterone (men: below reference values) |
| **Primary indicators** |
| Secondary amenorrhea (women: absence of 3 to 11 consecutive menstrual cycles) caused by functional hypothalamic amenorrhea |
| Subclinically low total or free testosterone (men: within the lowest 25% (quartile) of the reference range) |
| Subclinical or clinically low total or free T3 (within or below the lowest 25% (quartile) of the reference range) |
| History of ≥ 1 high-risk bone stress injury (femoral neck, sacrum, pelvis) or ≥ 2 low-risk bone stress injuries (all other bone stress injury locations) in the past 2 years or absence of ≥ 6 months of training due to bone stress injuries in the past 2 years |
| Premenopausal women and men <50 years of age: bone mineral density Z-score* <−1 at the lumbar spine, total hip, or femoral neck or a decrease in bone mineral density Z-score on previous testing. |
| Children/adolescents: Bone mineral density Z-score* <− 1 at the lumbar spine or whole body (except head) or a decrease in bone mineral density Z-score from previous testing (may occur due to bone loss or inadequate bone accrual) |
| A negative deviation from a pediatric or adolescent athlete's previous growth trajectory (height and/or body mass) |
| An elevated global EDE-Q score (>2.30 in women; >1.68 in men) and/or clinically diagnosed DSM-5-defined eating disorder (only one primary indicator for one or both outcomes) |
| **Secondary indicators** |
| Oligomenorrhea caused by functional hypothalamic amenorrhea (>35 days between periods for a maximum of 8 periods/year) |
| History of 1 low-risk bone stress injury (see definition of high versus low risk above) in the previous 2 years and absence of <6 months of training due to bone stress injuries in the previous 2 years |
| Elevated total cholesterol or LDL cholesterol (above the reference value) |
| Clinically diagnosed depression and/or anxiety (only a secondary indicator for one or both outcomes) |
| **Potential indicators (unscored, emerging) †** |
| Subclinically or clinically low IGF-1 (within or below the lowest 25% (quartile) of the reference value) |
| Clinically low blood glucose (below reference value) |
| Clinically low blood insulin (below reference value) |
| Chronic or sudden decline in indicators of iron status (e.g., ferritin, iron, transferrin) and/or hemoglobin |
| Lack of ovulation (via urinary ovulation detection) |
| Elevated morning resting or 24-hour urine cortisol (above reference range or significant change for an individual) |
| Urinary incontinence (women) |

| |
|---|
| Gastrointestinal or hepatic dysfunction/adverse gastrointestinal symptoms at rest and during exercise |
| Reduced or low RMR < 30 kcal/kg FFM/day or measured (indirect calorimetry)/predicted (predictive equation) RMR ratio < 0.90 |
| Reduced or low libido/sexual desire (especially in men) and decreased morning erections |
| Symptomatic orthostatic hypotension |
| Bradycardia (HR < 40 beats per min in adult athletes; HR < 50 beats per min in adolescent athletes) |
| Low systolic or diastolic BP (<90/60 mmHg) |
| Sleep disorders |
| Psychological symptoms (e.g., increased stress, anxiety, mood swings, body dissatisfaction, and/or body dysmorphia) |
| Exercise dependence/addiction |
| Low BMI |
| **Legend:** EDE-Q: Eating Disorder Examination Questionnaire; DSM-5: Diagnostic and Statistical Manual of Mental Disorders; LDL-c: Low-density lipoprotein cholesterol; IGF-1: Insulin-like growth factor; FFM: Fat-free mass; RMR: Resting metabolic rate; HR: Heart rate; BP: Blood pressure. Each indicator above requires consideration of a non-LEA-mediated differential diagnosis. All indicators apply to both women and men unless otherwise noted. Menstrual cycle status and endogenous sex hormone levels cannot be accurately assessed in athletes taking medications that alter sex hormones (e.g., hormonal contraceptives), and indicators of thyroid hormone status cannot be accurately assessed in athletes taking thyroid medications. All laboratory values should be interpreted according to age- and sex-appropriate, laboratory-specific reference values. Most RED data and associated thresholds were established in premenopausal/andropausal adults, unless otherwise indicated. Note: This tool should not be used in isolation or solely for diagnosis, as each indicator requires clinical consideration of a non-LEA-mediated differential diagnosis. Furthermore, the tool is less reliable in situations where it is impossible to assess all indicators (e.g., menstrual cycle status in women using hormonal contraception). This tool is not a substitute for professional clinical diagnosis, counseling, and/or treatment by a team led by physicians with expertise in health and RED performance. Adolescent refers to <18 years of age. *Bone mineral density assessed by DXA at ≤6 months. In some situations, the use of a Z-score from another skeletal site may be warranted (e.g., distal 1/3 of the radius when other sites cannot be measured or including proximal femoral measurements in some older adolescents (>15 years) for whom longitudinal BMD monitoring into adulthood is indicated). A true decrease in BMD (identified in previous testing) is ideally assessed by comparison with the individual facility's DXA LSC based on the facility's calculated coefficient of variation (%CV). As established by the International Society for Clinical Densitometry, at a minimum, the difference should be 5.3%, 5.0%, and 6.9% for spine, hip, and femoral neck to detect a clinical change. †Potential predictors are intentionally vague in quantification, pending further research to more precisely quantify parameters and cutoffs. |

**Source:** Adapted from Mountjoy et al. [48,49]

Considering possible difficulties inherent to the application of protocols such as REDs-CAT and LEAF-Q, research groups have suggested specific investigations to look for signs of REDs. Souza et al.[53] suggested the association of EA calculation with the evaluation of some indicators capable of predicting the beginning of suppression of reproductive function in women, such as (a) the evolution of body mass and its composition, (b) the presence of risky eating behaviors; (c) changes in the concentration of estradiol and progesterone in athletes who are not users of contraceptives; (d) monitoring of REE, comparing the REE measured by IC with the REE predicted by equations such as those of Cunningham[42,44], Harris and Benedict[43] and Ten-Haaf [46]. In this case, the risk of LEA is suggested if the measured REE/predicted REE ratio is <0.9[54]. Furthermore, eating disorders are suspected as underlying causes of REDs. In that case, the Brief Eating Disorder in Athletes Questionnaire (BEDA-Q) can be a useful screening tool, as can other protocols described[72,73].

It is noteworthy that factors other than LEA can cause clinical outcomes similar to those described in REDs. For example, an athlete who presents a reduction in thyroid hormones resulting from an autoimmune disease does not necessarily present LEA. Therefore, a clinical evaluation must be carried out to exclude other possible causes[74]. It is also of particular interest that specific screening tools are still needed for males and for different age groups, especially for adolescent and elderly athletes[63]. In short, considering the importance of REDs consequences (as they affect health and physical performance), evaluating this condition and predisposing factors is essential, as well as proper prevention, diagnosis, and treatment.

***This Guideline recommends screening for REDs through EA calculation and validated questionnaires. If available, check the primary and secondary criteria established by the International Olympic Committee to maximize screening and risk stratification. The level of evidence is moderate, and the recommendation is strong.***

## Body composition

Body composition assessment is an essential tool for health professionals to prescribe and monitor dietary planning and sports training program more assertively and safely.

Thus, several evaluation methods and techniques have been proposed with the aim of splitting body mass not only into fat mass (FM) and FFM but also into their subcomponents, with high precision[75, 76]. More than 30 years ago, Wang et al.[77] proposed a new approach to organizing research on body composition, dividing it into five levels: atomic, molecular, cellular, organ/tissue, and total body. Each level presents different components, which can be organized into compartments and divided into models with two, three, and four compartments[78]. The four-compartment model (4C) is recognized as the “gold standard” due to its more significant degree of sensitivity to the interindividual variability of FFM, so all other models should be validated based on it. However, the need for laboratories, equipment, and procedures with high operational costs limits their use only to research situations[79]. This model involves measuring body mass, total body volume, total body water (TCA), and bone mineral.

In contrast, two-compartment models (2C) divide the body into FM and FFM and constitute the most used approach to estimate body composition in adults, in clinical practice and in field situations. In these models, regression equations are used to estimate body components, with anthropometry and bioimpedance analysis (BIA) being the techniques for which most equations have been developed. In view of the logistical and operational issues related to the 4C model, the development and cross-validation of many regression equations have been carried out using DXA as a reference standard, and it is worth highlighting that this technique is based on a three-compartment model (3C), which measures bone mineral content, fat mass, and lean and soft tissue mass[80]. In these Guidelines, we present the techniques that demonstrate the best cost/benefit ratio for use in clinical and field practice, considering their validity for evaluating athletes and physical exercise practitioners.

## Body composition, physical exercise, and sport

The relationship between aspects of body composition and athletic performance is already well-established for different sports[81-84]. In the same way, the quantities and distribution of body components demonstrate an association between physical exercise practice and health status[85-89]. In this context, the choice of technique and predictive models is crucial for professionals, so we present in **Chart 5** the advantages and disadvantages of each technique in clinical practice and in **Figure 2** a flowchart to assist in the most appropriate choice according to the characteristics of the individual or group to be evaluated and the available technical conditions. One of the primary challenges in the application of the techniques outlined in **Chart 5** is the requirement for valid predictive equations for the subject or group under evaluation. It requires the use of equations developed and/or validated for the Brazilian population, both for anthropometry/skinfold thickness[90-95] and for BIA[96-100]. Equations developed in other countries are typically employed for specific sports[101-105].

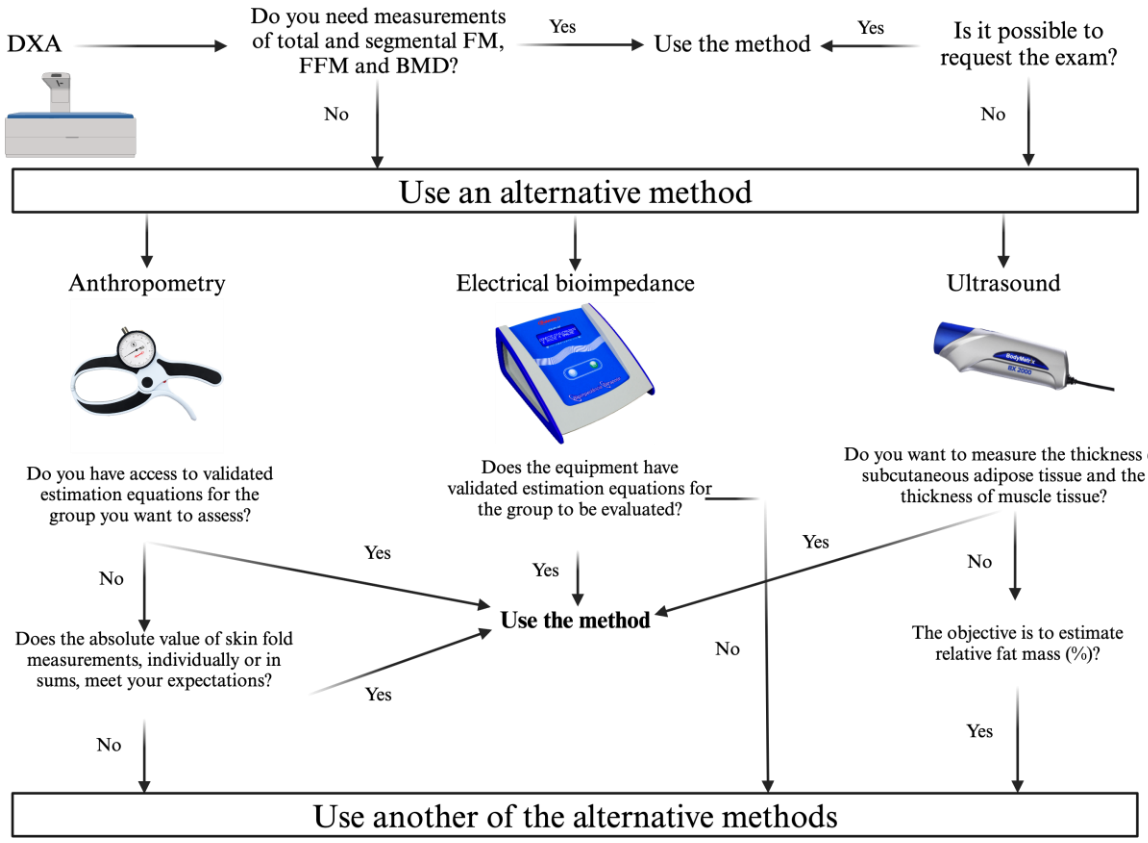


**Figure 2.** Algorithm for decision-making regarding the technique to be used in clinical or field settings.
**Legend:** DXA: dual-energy X-ray absorptiometry; FM: fat mass; FFM: fat-free mass; BMD: bone mineral density.
**Source**: The authors.

It is crucial to emphasize that the professional's role in using the algorithm presented in **Figure 2** is of utmost importance. It is assumed that the professional has received adequate training. In the case of using predictive equations to measure skinfold thickness, the measurement standard must comply with that proposed by the author of the equation or as indicated in the original article. In relation to the BIA, the entire pre-test protocol must be carried out by the person being evaluated. Thus, regardless of the instrument, the choice of equation determines the validity in predicting body composition; that is, BIA and anthropometry can be used interchangeably, allowing valid estimates of body components as long as sport-specific equations are applied[106].

***This Guideline recommends evaluating the body composition of athletes and high-performance athletes to monitor body compartments over time, especially to verify potential changes related to energy supply and demand, as well as adaptations imposed by physical training. When it comes to alternative methods (e.g., Anthropometry and BIA), the evaluator must recognize the appropriate procedures, as well as know how to select the most appropriate predictive equation for the individual and population being evaluated. Level of evidence: moderate; recommendation: strong.***

**Chart 5.** Body composition assessment techniques most applicable in the clinical practice of nutritionists.

| Technique | Measure/Estimate | Advantages | Disadvantages |
|---|---|---|---|
| Anthropometry Skinfold Thickness | Anthropometric measurements for estimating fat mass and lean mass | Low cost, relatively simple and portable | Skinfold caliper opening limit Need for validated equations for the subject or group to be assessed |
| BIA or BIS | Measurement of ACT for estimation of fat-free mass Measurement of intracellular and extracellular water | Relatively low cost, non-invasive, fast and portable | Need for prior preparation of those being evaluated Need for validated equations for the subject or group to be evaluated, which are not available in most equipment |
| Ultrasound (portable) | Measurement of the thickness of subcutaneous adipose tissue and muscle tissue Estimation of fat mass | Relatively low cost, non-invasive, fast and portable | Need for validated equations for the subject or group to be evaluated, which are not available in the equipment |
| Three-dimensional photonic scanner | Measurement of total and regional volumes Estimation of fat mass | Non-invasive, highly accurate anthropometric measurements and body volumes | High cost Not portable Few devices available in Brazil |

**Legend:** BIA: Bioelectrical impedance; BIS: Bioelectrical impedance spectroscopy; ACT: Total body water
**Source:** Adapted from Ackland et al.[81]

## Biomarkers

During different training periods (e.g., general preparation period, specific period, competitive period, and transition), athletes are exposed to different types of stimuli, dynamic in volume and intensity. These factors can, over time, affect health status and physical performance. In this context, the competitive calendar of some athletes has become increasingly complex, with several national and international competitions enabling the early emergence of problems associated with physical exercise[107-109].

Different physiological effects induced by physical exercise are already known. For beneficial adaptive responses to occur (e.g., increase in skeletal muscle strength, improvement in physical capacity, increase in physical performance), the workload must be appropriately manipulated; otherwise, unwanted effects may occur (e.g., non-functional overreaching) due to the accumulated effect of stress induced by physical exercise[110]. Furthermore, the high energy demand associated with insufficient intake of energy, macronutrients, and micronutrients can predispose or exacerbate adverse health conditions for the athlete[111].

Understanding the importance of monitoring athletes throughout a season and checking the parameters capable of revealing their health status and physical performance is crucial. In this regard, methods that can monitor external and internal load can be of great value to the clinical practice. Among them, blood biomarkers stand out, as they aid in monitoring biological processes, whether in health, pathological conditions, or in response to exposure to some stimuli[112].

It is important to note that the use of biomarkers for decision-making must be preceded by adequate validation. To serve as diagnostic criteria for a specific condition, such parameters need to be evaluated in depth for their ability to distinguish people who do or do not have the condition[113]. Reference gold standard methods for the specific condition must be used to assess sensitivity and specificity of the parameter[114].

Despite these parameters, it is possible that they may not be sufficient to discriminate who has that specific condition in the real world. Therefore, positive and negative predictive values are applied and reflect the proportion of positive and negative results that are true positives and true negatives, respectively[113]. These statistical metrics in sports science, however, still need to be further explored. Due to statistical particularities, the validity of markers is much discussed, considering that some markers may not reliably represent the athlete's condition. In contrast, other markers may be non-specific and be altered for reasons other than those ones associated with physical exercise. However, despite limitations on the interpretation of blood biomarkers, "non-blood" markers also suffer from critical limitations. For example, questionnaires can be subjective and do not express the athlete's actual condition. At the same time, physical performance tests can affect the physical training routine. If there is no adequate methodological structure to apply them, the data can suggest conditions that are far from reality. Finally, the parameter evaluated may present high variability between sports, which makes it challenging to understand the athlete's condition[113].

Blood biochemical markers need to be stable and little affected by intervening factors, such as diet and circadian rhythm[113]. Furthermore, they need to be easy to collect so that they can be frequently measured over time, facilitating athlete monitoring[113, 115]. However, to date, there need to be robust reference values for frequently used parameters, which hinders interpretation by some of the professionals who accompany the athletes because of the high variability in volume and intensity between sports. Furthermore, factors not associated with the individual can generate variability, especially pre-analytical factors, such as (i) time of day, (ii) collection procedure, and (iii) centrifugation of biological material. Other factors associated with the individual can also generate noise, such as (i) posture, (ii) hydration status, (iii) period and day of the menstrual cycle, (iv) fasting time, and (v) psychological stress[113,115]. Biochemical markers such as lactate, urea, myoglobin, and creatine kinase are often used to monitor the status of athletes[112].

Lactate, a pivotal marker in athlete monitoring, plays a crucial role in energy generation, particularly during high-intensity physical exercise. It plays role in regenerating nicotinamide adenine dinucleotide ($NAD^+$) to maintain glycolysis, buffering $H^+$ ions, and serving as a gluconeogenic molecule underscores its importance. The increase in lactate levels in response to intense physical exercise can indicate a high energy demand, a key aspect in athlete performance evaluation[112,116].

Urea, a marker representing the excretion form of nitrogen, presents a challenge in athlete monitoring. Under catabolic conditions, such as long-term physical exercise, maintaining nitrogen balance can be difficult, especially if protein intake is not adequately considered. While evaluating the nitrogen excretion rate can provide insights into protein balance, the reliability of its blood measurement is questionable. Some authors propose measuring urinary urea and sweat, but the practicality of obtaining these biological samples is a hurdle. Previous studies have found values that make it hard to establish a response pattern, leading to potentially contradictory results[112,117].

Myoglobin, a protein present in the heart and skeletal muscle, is responsible for transporting oxygen to the site of oxidation of energy substrates. Skeletal muscle damage induced by physical exercise increases the permeability of myocytes, leading to the extravasation of intracellular substances into the extracellular environment (e.g., blood). As it is a small molecule, its appearance in the blood and its clearance by the kidneys is relatively quick. Considering that myoglobin levels increase after physical exercise and due to its damaging effect on the kidneys, monitoring it can be essential. However, despite the increase in response to physical exercise, there are no reference values and, therefore, it is not possible to distinguish increases in myoglobin associated with physical exercise adaptations from increases that determine skeletal muscle damage[112, 118].

Leukocytes (white blood cells) are measured as a response parameter to physical exercise, mainly because their high levels reveal an increase in some immunological response. However, increases in leukocytes may not necessarily determine a cellular immune response.

Long-term physical exercise, especially endurance exercise, increases the levels of leukocytes in the blood. In this sense, leukocytes are influenced by the adrenaline and cortisol, hormones that increase in response to physical exercise for metabolic control and, therefore, are affected by energy availability. For example, physical exercises performed with lower skeletal muscle and liver glycogen stores can optimize the increase in these hormones and, consequently, impact blood leukocyte levels. Despite the increase in leukocytes in response to physical exercise, subcategories (e.g., lymphocytes, granulocytes, and monocytes) do not increase similarly. Therefore, leukocytosis induced by physical exercise requires interpretations based on the different behavior of each subcategory of leukocytes. It is essential to consider that, in conditions of low ED, athletes are more susceptible to opportunistic infections, which can impact leukocyte levels[112, 119, 120].

Finally, C-reactive protein, an acute-phase protein, is considered a marker of inflammation produced in the liver, and its increase may be associated with infectious conditions. As mentioned, LEA conditions can increase susceptibility to opportunistic infections. In addition to C-reactive protein, others seem to emerge as potential markers for identifying changes associated with physical exercise (e.g., enzymes, cytokines, chaperones, or other markers)[112, 115, 118].

In short, despite the different parameters exposed above, there are still many doubts about the degree of evidence, applicability, validity, variability, and influencing factors of the main biochemical parameters associated with physical exercise. **Chart 6**, adapted from Haller et al.[112], presents the main parameters associated with physical exercise, confounding factors, available reference values, interpretation, and degree of reproducibility.

**Chart 6.** Biomarkers evaluated to determine the health status of athletes

| **System** | **Marker** | **Sample** | **Confusers** | **Reference values** | **Interpretation for high levels** | **Reproducibility** |
|---|---|---|---|---|---|---|
| SkM | CK | Serum, plasma or capillary (earlobe or finger) | Myocardial damage, glutamine intake and circadian rhythm | F: 30 to 135 UL;<br>M: 55 to 170 UL;<br>Athlete Reference Ranges<br>F: 47 to 513 UL<br>M: 82 to 1083 UL | High mechanical or metabolic load | Good reproducibility (ICC = 0.9) |
| SkM | Myo-globin | Serum, plasma or capillary (earlobe or finger) | Myocardial damage, glutamine ingestion, kidney disease | M: <85 ng/mL | High mechanical or metabolic load | Good reproducibility (ICC: 0.75) |
| SkM | Lactate | Capillary (earlobe or finger), whole blood or plasma | Carbohydrates, collection site, protocol | 0.3 to 1.5 mmol/L | High mechanical or metabolic load (high glycolytic flux) | Good reproducibility (ICC: 0.75 – 0.99) |
| Immune | Leuko-cytes | Whole blood | Inflammatory conditions (e.g., infections, chronic | 4 to 11 $10^9$ L | Infections and inflammation | Moderate reproducibility (ICC: 0.51 – 0.74) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | inflammation, injuries) | | | |
| Metabolism | Urea | Serum, plasma or capillary (earlobe or finger) | Protein and fluid intake, sweat, plasma volume and lean mass | F: 4 to 6 mmol/L M: 5 to 7 mmol/L Blood nitrogen: 8 to 26 mg/dL | Protein catabolism | N/A |
| Inflammation | CRP | Capillary (finger), serum, whole blood or plasma | Inflammatory conditions, acute and chronic diseases, and diet | < 10 mg/dL | Inflammation induced by exercise, infections or non-infectious diseases | Moderate reproducibility (ICC: 0.71) |

**Legend:** SkM: skeletal muscle; CK: creatine kinase; F: female; M: male; mg: milligrams; dL: deciliter; ICC: intraclass correlation coefficient.
**Source:** Adapted from Haller et al.[112]

***This Guideline recommends evaluating biomarkers to monitor external and internal training loads in sportspeople and high-performance athletes. However, to date, there is a notable absence of more specific biochemical parameters, as well as sensitivity and specificity assessment for various biomarkers, which hinders their applicability and relevance in the short-, medium-, and long-term. Therefore, their use should be cautious and aligned with other nutritional assessment parameters. Level of evidence: weak; recommendation: strong.***

## Macronutrients and physical performance

### Carbohydrates

Carbohydrates, key players in skeletal muscle metabolism, have held this role for over a century[121]. The interplay between the volume and intensity of physical exercise determines the preferred energy substrate for the production of ATP[122]. In high-intensity physical exercise, the contribution of muscle glycogen is paramount. However, as the volume of physical exercise increases, glucose and fatty acids uptake rises to sustain ATP production[122]. The need for blood glucose increases and it is provided primarily by the liver, due to increased glycogenolysis and gluconeogenesis during physical exercise. Yet, during prolonged physical exercise, glucose uptake by skeletal muscle becomes greater than its liver production. In this context, carbohydrate intake becomes a critical factor in maintaining glucose uptake and oxidation by skeletal muscle[122]. Carbohydrates, beyond their energetic function, exert significant effects on physical performance, influencing athlete’s concentration, cognitive control, hormonal regulation, and the immune system's activity[123-127].

Considering the importance of carbohydrates as a source of energy, as well as their participation in different functions of the human body, adequate carbohydrate intake is essential for physical exercise, especially for high-performance athletes. Despite the importance of carbohydrates, achieving the recommendation is complex, and therefore, nutritional and dietary management must be individualized according to the athlete and athlete's competitive planning. Individual objectives must be taken into consideration when managing carbohydrates, especially when there is a need to modify body composition[5]. It is important to emphasize that despite the noteworthiness of carbohydrates for physical exercise, physiological, psychological, social, and cultural factors negatively affect their intake, mainly due to the fear that some athletes have of modifying their body composition[124-131]. Carbohydrate recommendations can be divided into: (i) total for the day, (ii) before physical exercise, (iii) during physical exercise, and (iv) after physical exercise[5].

## Recommended carbohydrates per day

Carbohydrates, regardless of the characteristics of physical exercise, will always be the most essential macronutrient for meeting total energy needs. Previous studies published by Burke et al.[124, 132] continue to establish the main carbohydrate recommendations for people who practice physical exercise and high-performance athletes. By evaluating a series of essential aspects involving carbohydrate intake (e.g., volume, intensity, number of workouts per day, time for recovery between training sessions, and energy expenditure from physical exercise), it is possible to manage recommendations for different situations.

The authors categorize carbohydrate intake by the intensity and volume of physical exercise. For the first category, which comprises lower-intensity exercises, 3 to 5 g of carbohydrates per kg of body mass/day are recommended. For physical exercises lasting approximately 1 hour, the recommendation is 5 to 7 g/kg/day. For endurance physical exercises, with a volume of 1 to 3 hours/day, the recommendation is 6 to 10 g/kg/day. Finally, for moderate or high-intensity physical exercise lasting ≥ 4 h/day, the recommendation is 8 to 12 g/kg/day[5, 124, 132].

## The authors suggest several vital recommendations for carbohydrate intake, including:

1) For more intense exercises, in which glycogen depletion will be more significant, carbohydrate intake must be adequate to restore depleted stores;
2) Goals for daily carbohydrate intake are based on body mass and exercise load;
3) Despite the intake suggestion by guidelines, the adequacy of carbohydrate intake must be adjusted according to the athletes' general objectives and training feedback;
4) When the period between physical exercise sessions is less than 8 hours, athletes should ingest carbohydrates as quickly as possible after the first session, probably with a rapid glycemic response, to optimize the recovery of glycogen stores;
5) To recover glycogen, opting for more than one meal (fraction) after physical exercise can optimize the recovery of muscle glycogen;
6) During more extended recovery periods (e.g., 24 h), the types of carbohydrates, intake pattern, and time between meals and carbohydrate-rich intermeal snacks can be chosen according to what is more practical and pleasurable for the athlete, and meals should be proposed according to potential health conditions related to carbohydrate metabolism (e.g., presence of glycemic, lipid or gastrointestinal disorders);
7) Foods containing carbohydrates that promote a rapid glycemic response are more critical when the time between training sessions is less than 8 hours. However, the type of carbohydrate selected seems to be less critical when the recovery time is longer;
8) Restrictive practices related to nutrition, such as extreme calorie restriction or elimination of certain food groups, mainly targeting body composition modification, can negatively affect total carbohydrate intake, resulting in worse recovery of muscle glycogen stores between training sessions;
9) Although there are slight differences in glycogen storage throughout the menstrual cycle, women can store glycogen as effectively as men;
10) Acute intake recommendations are related to competitive situations. However, it is suggested that strategies used in competitive periods be tested throughout the season to avoid gastrointestinal problems during competition[5,124, 132].

Finally, nutritional periodization, especially of carbohydrates, must be in perfect alignment with training periodization. [5,124, 132] This ensures that the distribution of carbohydrates is constantly adapted to the training phase and the characteristics of the sport, empowering the audience with the knowledge of how to optimize their nutrition for their training needs[5,124, 132].

**Carbohydrate recommendation before physical exercise**

Carbohydrate intake before physical exercise aims to reduce the depletion of muscle glycogen during physical exercise and maintain the availability of exogenous glucose for oxidation. The potential positive effect of carbohydrate intake before physical exercise depends on its the total volume, being more evident in physical exercises lasting > 1 to 2 h.

Regarding the effect of carbohydrate intake before physical exercise on sports performance, there was a small but positive effect (ES: 0.2; 95% CI: 0 – 0.30) for continuous physical exercise, predominantly aerobic, according to the data published by Aird et al.[133]. However, in the subgroup analysis, when physical exercise was of short duration, positive effects were not identified (ES: 0.0; 95% CI: -0.3 to 0.2). Thus, only long-term physical exercise benefits from carbohydrate intake (ES: 0.3; 95% CI: 0.1 – 0.5). In this review, carbohydrate intake happened in a time frame of 30 min and four hours. Furthermore, the meals tested varied in terms of composition, although carbohydrates were the predominant macronutrient. It is essential to highlight that the studies were conducted with untrained and trained people, with overnight fasting times between 10 and 12 hours, and, finally, they were carried out mainly with adult men.

For long-term endurance physical exercises, glycogen loading/saturation/super-compensation protocols are proposed. These protocols are suggested based on the concept of muscle glycogen recovery, which was discussed in the 1960s and 1970s. It occurs in approximately 24 hours, especially if the energy demand derived from physical exercise is reduced[5, 134-136]. It is suggested that carbohydrate intake should be increased 1 to 2 days before the target test, in amounts ranging between 8 and 12 g of carbohydrates/kg of body mass per day[5, 124]. Although the practice of carbohydrate loading is familiar, the natural ergogenic effect of these strategies needs to be clarified. Furthermore, increasing glycogen stores also increases muscle glycogenolysis rates, which can reduce the effect of carbohydrate loading by anticipating carbohydrate depletion[137, 138].

An SRMA was explicitly considered to evaluate the effect of carbohydrate intake on physical strength exercise[139]. For the total physical exercise session, the authors observed a positive effect of carbohydrate intake before strength physical exercise (ES: 0.61; 95% CI: 0.11 – 1.11). However, the authors point to a low level of evidence based on GRADE. Subgroup analyses revealed positive effect for physical exercise sessions with a volume greater than 45 min (ES: 1.02; 95% CI: 0.07 – 1.97). For strength physical exercise sessions lasting < 45 min, no positive effects of carbohydrate intake were observed (ES:0.23; 95% CI: -0.21 – 0.67). Subgroup analyses suggest a low to moderate level of evidence, with dependence on the fasting time before starting the physical exercise session.

Other nutrients contained in the meal that precede physical exercise must be taken into consideration, mainly due to the possibility of promoting gastrointestinal disorders, which can worsen physical performance. Although the effect of physical exercise is small on the absorption of water and carbohydrates, for other nutrients (e.g., fiber, proteins, and lipids), this caution must be exercised. As proposed by de Oliveira, Burini and Jeukendrup[140], some guidelines can be followed, such as: (1) avoid foods rich in fiber the day or even days before the competition; (2) for the athlete in training, diet with fiber in adequate amounts will help maintain regular bowel function; (3) avoid foods rich in fructose (in particular drinks that contain exclusively fructose); (4) avoid hypohydration, as it can worsen gastrointestinal symptoms; (5) start training well hydrated; (6) make sure to test the nutritional plan several times before the day of the test/competition. This will allow the athlete to discover what works and what does not work and reduce the chances of developing gastrointestinal symptoms.

**Glycemic index of carbohydrates before physical exercise**

The intake of different types of carbohydrates before physical exercise has been evaluated over the last few years. Meals/carbohydrates that have a low glycemic index (low glycemic response) can provide a lower insulin peak and, therefore, maintain higher rates of lipolysis (breakdown of triacylglycerols into free fatty acids) and beta-oxidation of fatty acids during physical exercise. This mechanism could help maintain EA in situations where carbohydrates cannot be ingested during physical exercise[5]. An SRMA evaluated the effects of pre-meal glycemic index (≥ 70 vs. ≤ 55) on physical performance[141]. The studies were predominantly conducted with trained athletes, and carbohydrate intake before physical exercise varied between 0.18 and 2 g/kg, 30 to 210 min before physical exercise. For time trial tests, it was found that the low glycemic index meal did not optimize physical performance (ES:-0.18; 95% CI: -0.58 – 0.22). The same result was verified for submaximal physical exercise protocols associated with time trial tests (ES:-0.17; 95% CI: -0.55 – 0.22) and time to exhaustion (ES:-0.36; CI 95%: -0.93 – 0.22). Another SRMA conducted the same assessment and presented discordant results[142]. However, the authors point to the low methodological quality of the studies included and the need for more robust studies. A recent clinical trial did not show a positive effect in the same comparison after 12 hours of fasting, with the meal being taken 45 minutes before physical exercise[143].

***This Guideline emphasizes the importance of individual adjustments in carbohydrate intake before physical exercise. It recommends the ingestion of carbohydrates before long-term physical exercise to improve physical performance, while positive effects in short-term physical exercise are not evidenced. These effects depend on the muscle and liver glycogen stores before the start of physical exercise. Therefore, an assessment of the training routine and carbohydrate availability must be made. The type of carbohydrate and/or glycemic response of the meal has little or no effect on physical performance. The concern, however, should be with the potential gastrointestinal discomfort generated by the meal. Therefore, individual adjustments must be made to avoid gastrointestinal disorders during training or competitions. This approach empowers the individual to take control of their dietary choices and optimize their physical performance. Level of evidence: moderate; recommendation strength: Strong.***

**Carbohydrate recommendation during physical exercise**

**Up to 1 hour long**

Regarding the duration of physical exercise, it should be noted that there is divergence in the literature regarding the need for carbohydrate intake in physical exercise sessions lasting < 90 min, especially between 60 and 75 min, even if at high intensity. It is believed that, in these cases, carbohydrate ergogenic effect is dependent on glycogen stores before the start of physical exercise[144-148]. Recently, Ramonas et al.[149] found that in a glycogen-depleted condition, carbohydrate intake before and during physical exercise resulted in improved physical performance. These findings suggest that considering two physical exercise sessions on the same day, which is a standard pattern in the routine of many athletes, even if the second workout is shorter, carbohydrate intake (around 6% solution; 60 g/h) can exert a positive effect on physical performance.

Furthermore, in these situations, mouthwash with carbohydrates has been highlighted for contributing to physical performance and avoiding gastrointestinal discomfort. Recent

SRMA demonstrated that mouth rinsing with carbohydrate drinks increases physical performance when compared to the use of placebo[148, 150, 151]. For example, the review conducted by Brietzke et al.[148] showed that for the average power outcome, mouthwash with carbohydrates improved physical performance (ES: 0.25; 95% CI: 0.04 – 0.46). However, for the variable time of physical exercise, there was no positive effect (ES: -0.13; 95% CI: -0.36 – 0.10).

The SRMA of Hartley et al.[150] showed that rinsing with maltodextrin improved physical performance compared to the placebo substance (ES: 0.15; 95% CI: 0.04 – 0.27), although a more demanding analysis did not observe a positive effect (ES: 0.17; 95% CI: -0.01 – 0.34). Among the rinsing times evaluated (e.g., 5 to 40 s), only 10 s had a positive effect (ES: 0.22; 95% CI: 0.05 – 0.39). Furthermore, when categorized by the types of protocols tested (e.g., cycling, strength training, running, vertical jumping, etc.), no positive effects on physical performance were observed with carbohydrate rinsing. The majority of studies presented a low risk of bias.

**For physical exercises with volume greater than 60 – 75 minutes**

For physical exercises lasting more than 75 min, an intake of 30 to 60 g/h is recommended. For exercises lasting more than 2.5 h, up to 90 g/h of carbohydrates from mixed sources (e.g., e.g., glucose and fructose) is recommended. This combination of monosaccharides is necessary due to the sodium-dependent glucose transporter (SGLT-1) glucose absorption limit per minute[146]. It is also noteworthy that the longer the exercise duration, the greater the need to adjust carbohydrate-offering strategies throughout the test to optimize physical performance and reduce the risk of gastrointestinal discomfort[140, 146]. Bourdas et al.[152] evaluated in depth the effects of carbohydrate intake during physical exercise to optimize physical performance. The authors, however, noted that the risk of bias is high with regard to (1) the participant inclusion process, (2) sample power analysis, (3) measurement reliability, and (4) measurement validity.

The mean effect of carbohydrate intake during physical exercise was moderate (ES: 0.43; 95% CI: 0.35 – 0.51). The authors also emphasize that the positive effects persist regardless of age, biological sex, and level of cardiorespiratory fitness. Positive effects were demonstrated in physical exercises lasting 1 to 2 hours (ES: 0.41; 95% CI: 0.27 – 0.55) and 2 to 4 hours (ES: 0.51; 95% CI: 0 .40 – .62). However, physical exercises in which duration was less than one hour did not show an improvement in physical performance with carbohydrate supplementation (ES: 0.15; 95% CI: -0.13 – 0.43). The interesting result of this study was observed for physical exercises with duration greater than four hours, in which effects were also not statistically significant (ES: 0.19; 95% CI: -0.16 – 0.55). This is due to the lower dependence on glucose in prolonged physical exercises, in which intensity is lower and lipid metabolism is predominant[152].

Recently, it has been discussed whether an additional CHO intake, with values between 100 and 120 g/h during more extended physical exercises, can optimize physical performance. However, the effect of doses > 100 g/h was not significant (ES: 0.17; 95% CI: -0.23 – 0.57) compared to amounts between 81 and 100 g/h of physical exercise (ES: 0.82; 95% CI: 0.31 – 1.34)[152]. The concentration of carbohydrate drinks during physical exercise should vary between 6 – 8%, depending on gastric emptying and intestinal absorption. The concentration of the drink, as well as the type of carbohydrate, impacts gastric emptying and intestinal absorption. For example, a solution containing sucrose (8%; 251 mOsm/kg) has faster gastric emptying than glucose at the same concentration (8%; 470 mOsm/kg)[153]. Isotonic drinks with concentrations of 6 - 8% and different types of carbohydrates (e.g., maltodextrin and sucrose) have adequate gastric emptying capacity and fluid absorption in the intestine. Thus, the concentration between 6 – 8%, composed especially of glucose and fructose (ratio 2:1), remains sufficient to optimize physical performance, including favoring a better osmotic gradient for water absorption (see topic on hydration)[154].

Despite scientific advances in carbohydrate intake during physical exercise, there are no differences between intake patterns (e.g., every 10 min vs. 20 min vs. 30 min vs. 40 min, etc.). Therefore, in this context, it is still necessary to evaluate the best intake logistics according to each sport modality, taking into account the gastrointestinal tolerance of each individual. Despite concerns about carbohydrate intake, several studies have evaluated the average intake per hour of physical exercise in different sports. These studies observed intakes varying between 14.9 and 62.2 g/h or 0.27 and 0.64 g/kg/h[128-130, 152, 155, 156]. Therefore, the intake observed among sportspeople and athletes is still below compared to current recommendations[5].

***This Guideline recommends the intake of carbohydrates, especially those that are easily digestible and absorbed (e.g., maltodextrin, sucrose, glucose + fructose), during long-term physical exercise to improve physical performance. It is essential to consider the concentration (around 6 to 8%) and the logistics of ingestion according to the sport. Food sources of carbohydrates can be ingested during physical exercise. However, extra attention to gastrointestinal problems must be paid. Intestinal training will possibly be essential to avoid disorders. Intramuscular and hepatic glycogen stores must also be considered, a factor that appears to mediate the ergogenicity of carbohydrates ingested during physical exercise. The intake of slow digestibility and slow absorption carbohydrates during physical exercise does not have robust scientific support for prescription. Level of evidence: moderate; recommendation strength: strong.***

## Carbohydrates after physical exercise

Following physical exercise, carbohydrate intake aims to recover depleted glycogen stores[157, 158]. Despite the ability to recover glycogen (1 to 2 mmol/kg of muscle/h) from glucose produced by gluconeogenesis, in the presence of exogenous carbohydrates, the resynthesis rate is much higher (5 to 10 mmol/kg of muscle/h)[157-159]. Skeletal muscle is conditioned to recover glycogen stores primarily by activating AMP-activated protein kinase (AMPK) and glucose transporter type 4 (GLUT4), by increasing insulin sensitivity and activating the enzyme glycogen synthase[157, 158, 160]. The recovery of muscle glycogen is challenging in situations where two physical exercise sessions occur between 12 and 15 hours. Therefore, evaluating the organization of training is essential. Some athletes may perform more than one workout per day and physical exercise practitioners may exercise in the morning and afternoon to modify body composition or for leisure.

A recent SRMA evaluated the effect of carbohydrate intake on glycogenesis[161]. The average value of carbohydrate intake was 1.02 g/kg/h (0.5 to 1.5 g/kg/h). The average recovery time was 2.9 hours (1 to 5 hours). Compared to the control group, carbohydrate intake had a positive effect on glycogen synthesis (23.5 mmol/kg/h; 95% CI: 19 – 27.9 mmol/kg/h). Ingesting carbohydrates more frequently was positively associated with faster glycogen recovery ($R^2 = 0.44$; $p = 0.027$). Interestingly, post-exercise glycogen storage (≤ 150 mmol/kg vs. > 150 mmol/kg) was not associated with the speed of glycogen recovery. Furthermore, the authors evaluated the effect of combining proteins and carbohydrates to recover glycogen, and no positive effects were found (ES: 0.4; 95% CI: -2.7 – 3.4). However, based on other studies, it is suggested that when carbohydrate intake is < 0.8 g/kg or between 0.8-0.9 g/kg, the addition of protein (0.3 to 0. 4 g/kg) optimizes glycogenesis[162, 163].

After physical exercise, depending on the training context, offering carbohydrates that promote a rapid glycemic response can optimize glycogen recovery[157, 162]. In cases where eating a meal takes a long time, for example, for logistical reasons (e.g., the distance between the training location and the athlete's home), facilitating carbohydrate intake with easily accessible drinks or foods (e.g., fruits, carbohydrate supplements, coconut water) favors the rapid recovery of muscle glycogen. On the other hand, when physical exercise sessions are separated by a sufficient interval (around 20 to 24 hours), there is no need to accelerate the recovery of glycogen stores significantly if the next training session does not induce significant glycogen depletion. More recently, Naderi et al.[164] suggested several practices related to carbohydrate intake before, during, and after physical exercise, highlighting the importance of nutrition. However, for people with high energy demands, carbohydrate supplementation facilitates dietary management.

Finally, ingestion of 1 to 1.2 g/kg/h is suggested to optimize the recovery of glycogen stores[157,161], and fractionation up to the 4-hour post-physical exercise can optimize glycogenesis. Furthermore, ingesting drinks and foods that are easily digestible can be an important strategy for rapid recovery. It is essential to consider that EA must be adequate for optimal recovery of muscle glycogen. All carbohydrate recommendations are presented in **Chart 7.**

**Chart 7.** Carbohydrate recommendations for athletes and exercisers

| **Carbohydrate recommendation** | **Information about physical exercise** | **Observations, effects and references** |
|---|---|---|
| 3-5 g/kg/day | Low intensity exercises | Adequate recovery of muscle glycogen over time, respecting the volume and intensity of physical exercise. Consider demands related to changes in body composition and health status. Finally, respect sociocultural aspects to choose the best carbohydrate food source for each individual[55, 124, 132]. |
| 5-7 g/kg/day | Moderate intensity exercise (~ 1 h/day) | |
| 6-10 g/kg/day | Endurance Exercises (~ 1 – 3h per day) – moderate to high intensity | |
| 8-12 g/kg/day | High training duration > 4-5 h per day – moderate to high intensity | |
| **Moments close to physical exercise** | | |
| **Carbohydrate loading** | 8-12 g/kg/day 24 – 48 before the competition | Consider gastrointestinal tolerance when increasing carbohydrate intake (e.g., the day before a race) because it may be an unusual amount for the athlete.<br>Consider the relevance of this recommendation, which will probably be higher in long-duration exercise (> 90 min).<br>Clinical studies evaluating this type of strategy are scarce; its use is based on the concept that increasing glycogen stores before a competition is essential for physical exercise that may be limited by muscle glycogen. |
| **Before physical exercise** | 1 – 4 g/kg of body mass, 1 – 4 h before starting physical exercise<br><br>Evaluate practical aspects and potential gastrointestinal issues | Endurance physical exercise<br>ES: 0.2; 95% CI: 0 – 0.30<br>Short-term physical exercise (< 60 min)<br>ES: 0.0; 95% CI: -0.3 – 0.2<br>Long-term physical exercise (≥ 60 min)<br>ES: 0.3; 95% CI: 0.1 – 0.5<br><br>Strength physical exercise<br>Mean ES: 0.61; 95% CI: 0.11 – 1.11<br>Subgroup analysis<br>> 45 min ES: 1.02; 95% CI: 0.07 – 1.97<br>< 45 min ES: 0.23; 95% CI: -0.21 – 0.67<br>Fasting > 8 h ES: 0.39; 95% CI: 0.06 – 0.72<br>Fasting < 8 h ES: 0.76; 95% CI: -0.19 – 1.71 |

| | | |
|---|---|---|
| **Effect of carbohydrate type before physical exercise** | Slow glycemic response vs. fast glycemic response | Time trial exercises<br>ES: -0.18; 95% CI: -0.58 – 0.22 (no effect)<br>Submaximal exercises + time trial tests<br>ES: -0.17; 95% CI: -0.55 – 0.22 (no effect)<br>Exercises until voluntary exhaustion<br>ES: -0.36; 95% CI: -0.93 – 0.22 (no effect) |
| **During physical exercise** | | |
| **Mouthwash** | | Mean power: ES: 0.25; 95% CI: 0.04–0.46<br>Physical exercise time: ES: -0.13; 95% CI: -0.36–0.10<br>Maltodextrin: ES: 0.15; 95% CI: 0.04–0.27<br>10 s rinsing: ES: 0.22; 95% CI: 0.05–0.39 |
| **Consumption during physical exercise** | Considering all volumes (time) of physical exercise. | Mean ES 0.43; 95% CI: 0.35–0.51[152]<br>ES cycling: 0.47; 95% CI: 0.38 – 0.57<br>ES running: 0.35; 95% CI: 0.17 – 0.52<br>The effect of carbohydrate intake during physical exercise is positive and may vary between different types of sports and by level of physical fitness; however, it remains stable regardless of age or biological sex. |
| **During physical exercise (varying volumes of physical exercise)** | < 1 h | ES: 0,19; 95% IC: -0,16 – 0,55<br>Non-significant effect for short workouts, regardless of intensity. |
| | 1 – 2h | ES: 0.41; 95% CI: 0.27 – 0.55 |
| | 2 – 4 h | ES: 0.51; 95% CI: 0.40 – 0.62 |
| **High amounts of carbohydrates during physical exercise** | 80 – 100 g/h | ES: 0.82; 95% CI: 0.31 – 1.34 |
| | > 100 g/h | ES: 0.17; 95% CI: -0.23 – 0.57 |
| **After physical exercise** | Time to next exercise session < 8h | Carbohydrate consumption should occur immediately after the end of physical exercise to optimize the recovery of glycogen stores.<br>1.0 -1.2 g/kg/h (23.5 mmol/kg/h; 95% CI: 19 – 27.9 mmol/kg/h)<br>More frequent consumption can optimize glycogen recovery. |
| | Between 0 – 4 h | Carbohydrate amounts between 1 and 1.2 g/kg/h, ingested in a fractional manner, contribute to the rapid recovery of muscle glycogen. Working with beverages that offer glucose polymers with a rapid glycemic response and meals that are easy to empty can facilitate the total intake of carbohydrates and, consequently, glycogen recovery. |

| | | |
|---|---|---|
| | For muscle glycogen recovery in 24h | To recover muscle glycogen in 24 h, the energy demand imposed by physical exercise must be considered, as well as the intensity that regulates glycogen depletion and glucose oxidation.<br>Moderate-intensity exercise: 5 to 7 g/kg/24h<br>High-intensity exercise: 6 to 10 g/kg/24h<br>Extreme-intensity exercise: 8 to 12 g/kg/24h<br>During longer recovery periods (> 6 h), when the athlete is able to ingest energy and carbohydrates adequately, the food organization must be conducted in partnership with the athlete, with the aim of facilitating carbohydrate ingestion. Furthermore, in these circumstances, there is no relevance in the fractionation or the form of presentation (liquid or solid) as long as the total amount is reached. |
| | Combine proteins and carbohydrates after physical exercise to recover muscle glycogen. | Adding protein to a carbohydrate-containing drink/meal does not optimize muscle glycogenesis (ES: 0.4; 95% CI: -2.7–3.4), unless carbohydrate intake is below the recommendation (<0.8 or between 0.8 and 0.9 g/kg); in this case, adding protein (0.3 to 0.4 g/kg) may promote positive effects on glycogenesis.<br>It is a common practice (culturally) to ingest different macronutrients throughout meals, especially after physical exercise. However, attention should be paid when the athlete mistakenly gives preference to protein over carbohydrates, which may result in inadequate recovery of muscle glycogen. |

**Legend:** Effect sizes (ES) described in standardized mean difference (SMD); g: grams; kg: kilograms; h: hour; ~: approximately; >: larger; <: smaller; CI: Confidence Interval.
**Source**: Adapted from Thomas et al.[5]

***This Guideline recommends the intake of carbohydrates with a rapid glycemic response after physical exercise, especially when the recovery time between training sessions is short (e.g., < 8 h). However, if the time between training sessions is longer (e.g., only the next day), there is no need to accelerate the recovery of muscle glycogen immediately after physical exercise. In these cases, adequate distribution of carbohydrates in meals throughout the day, respecting energy demand and carbohydrate recommendations, is sufficient to guarantee the recovery of muscle glycogen. Finally, we recommend that professionals consider each individual's eating habits, considering the sociocultural aspects of each athlete. Level of evidence: high; recommendation strength: strong.***

## Carbohydrate restriction

Carbohydrates are the primary energy source for skeletal muscles during physical exercise. The carbohydrate stores of the body are limited to approximately 400 g while triacylglycerol stores are virtually unlimited[165,166]. Triacylglycerols can provide a significant amount of energy during exercise, with their contribution peaking at an intensity corresponding to around 65% of $\dot{V}O_{2max}$. However, at higher intensities (around 80 to 100% of $\dot{V}O_2$max), glucose becomes the primary energy substrate for energy production[122].

From a physiological point of view, the role of triacylglycerols in the practice of physical exercise is indispensable, not only because their stores are substantially greater than that of muscle glycogen but also because they are a way of preserving glycogen stores, especially for long-term physical exercises performed by trained individuals[166, 167]. In a recent SRMA and meta-regression, it was observed that the rate of use of intramuscular triacylglycerols was similar (-23.7%; 95% CI: -28.7 – -18.7%) regardless of the state (fed or fasted)[168].

Despite the importance of carbohydrates, strategies that encourage the reduction of this macronutrient along with an increase in lipid intake have started to gain space both in the scientific community and society[169, 170]. It is believed that "programming" skeletal muscles to use more free fatty acids during physical exercise improves physical performance. The hypothesis is related to the greater capacity to use the "almost unlimited" triacylglycerols stores, reducing the use of glucose as an energy substrate (glycogen) and the exogenous need for carbohydrates[169]. In a recent review, researchers highlighted the ability of skeletal muscle to optimize the oxidation capacity of fatty acids (approximately 1.5 g/min) in response to a low-carbohydrate diet, in different relative intensities ranging from about 45 to 70% of maximum aerobic capacity[171].

Overall, the interventions of the studies included in this document were primarily short-term, ranging from a few days to 12 weeks; the longest study only considered self-reported adherence to food, which introduces a potential bias). Additionally, the studies were mostly parallel and presented small sample sizes[126,127,172-207]. The carbohydrate intake in these studies was less than 5% or between 5 and 10% of total energy needs. Strategies such as "sleep low", "train twice a day", "train on an empty stomach", or "avoid carbohydrates during or after physical exercise" were used to manipulate carbohydrate availability and could potentially influence the type of energy substrate used by skeletal muscles.

While randomization and blinding are fundamental in verifying the effects of interventions, nutrition-related studies present their limitations. Studies with the objective of testing isolated nutrients include a harmless placebo group in their design. However, nutrient/food/feeding studies face challenges[1,208]. For instance, some studies allow participants to choose their groups based on their familiarity with the diet, which can impact expectations due to previous experiences[1,208]. Beliefs and sensory aspects of food can also lead to erroneous estimates. Finally, studies with dietary interventions are generally not double-blind, as participants are aware of what they are ingesting, which poses a methodological limitation when evaluating the effect of carbohydrate restriction on physical performance[1,208].

In a recent SRMA, the factors randomization, allocation, and blinding reached a 20, 40, and 30% high risk of bias for studies that tested the effect of the ketogenic diet on physical performance parameters assessed in endurance exercises, respectively[209]. Another SRMA, which evaluated the effect of carbohydrate periodization (based on the Physiotherapy Evidence Database PEDro scale), showed scores between 5 and 7 points[210].

Depending on the mechanism of action, the most used tests are endurance physical exercises, predominantly 5, 10, and 20 km runs. Some studies evaluated the time to exhaustion and Wingate test, but others did not report how physical performance was evaluated. In the study conducted by Gejl and Nybo[210], the authors found that studies that tested carbohydrate periodization did not find a positive effect on physical performance compared to chronic and high carbohydrate intake (ES: 0.17; 95% CI -0.15 – 0.49). In a recent SRMA published by Cao et al.[209], the authors did not find a positive effect of a ketogenic diet on $\dot{V}O_{2max}$ (ES: -0.06; 95% CI -0.36 – 0.25), time to exhaustion (ES: -0.13; 95% CI -0.66 – 0.40), and perceived exertion (ES: 0.14; 95% CI -0.58 – 0.86). Despite the proposed mechanisms, the effect of partial or total carbohydrate restriction on performance is not favorable. In addition, some studies have reported physical performance impairment[127, 211].

Considering strength exercises, carbohydrate intake plays an essential role in physical performance, even though the ingestion of very high amounts is not necessary. Also, there is no evidence to support the restriction of carbohydrates to optimize strength exercise performance (e.g., bodybuilders, powerlifters, and Olympic weightlifters)[212]. Similar results were found for the concurrent exercise[213]. Ketogenic diet may not affect or reduce FFM, especially the muscle mass component. However, this factor is more dependent on the magnitude of the energy deficit and protein intake[214]. Finally, carbohydrate restriction has been associated with high-intensity physical performance impairment, lower glucose oxidation capacity and substantial increases in fat utilization associated with an increased oxygen cost, particularly in high-intensity domains (>70% $\dot{V}O_{2peak}$)[127, 171, 211].

There are some preliminary evidences suggesting that low-carbohydrate, high-fat diets may have adverse effects. These evidences include (i) increased levels of interleukin-6 and hepcidin, which can impair iron metabolism, and (ii) negative changes in the integrity of the intestinal barrier and markers of bone mass formation[215-220]. Therefore, it is important to consider these potential risks when considering a low-carbohydrate, high-fat diet for physical performance optimization.

***This Guideline does not recommend low-carb strategies, especially ketogenic diet, to optimize physical performance. Despite the mechanism of metabolic flexibility in which fatty acids could favor the preservation of glycogen stores, the available scientific evidence are not sufficient to recommend this strategy. Furthermore, there is evidence of potential adverse effects, which implies non-superiority and potential risks compared to diets with adequate amounts of carbohydrates. The level of evidence is moderate; the recommendation strength is weak.***

## Proteins

The maintenance of skeletal muscle occurs through a dynamic balance between protein synthesis and degradation (protein turnover)[221]. An increase in muscle mass will occur when muscle protein synthesis (MPS) surpasses muscle protein catabolism. Strength training[222] as well as adequate protein intake stimulate the MPS pathway[223]. In this context, among the possible dietary anabolic stimuli, adequate protein intake is an important intervention to optimize strength training adaptations, including skeletal muscle hypertrophy.

The recommended protein intake for sedentary adults is 0.8 to 1.0 g/kg/day[224, 225], while for those engaging in physical exercise, it is recommended to consume 1.2 to 2.2 g/kg/day[226-228]. It is important to note that these recommendations can be influenced by the type of training or sport (e.g., strength or endurance), training level, carbohydrate availability, and energy intake[229]. Athletes performing predominantly aerobic exercise require higher amounts of protein than sedentary individuals due to increased amino acid oxidation and greater need for muscle recovery[229]. Therefore, it is suggested that individuals engaging in aerobic exercise consume 1.2 to 1.7 g/kg/day of protein. For those primarily focused on muscle hypertrophy and therefore undergoing mainly strength training, the protein recommendation is 1.6 to 2.2 g/kg/day[228]. A high-protein diet can lead to an additional increase of 500-700 g in lean body mass in strength training practitioners[227]. Additionally, protein intake close to 2.0 g/kg/day is suggested in conditions of negative energy balance to prevent muscle mass loss[230-233].

In a study conducted by Nunes et al.[227], the authors observed that increased protein intake resulted in lean mass increase with an ES of 0.22 (95% CI: 0.14–0.30), with a moderate level of scientific evidence. Regarding the increase in lower limb muscle strength, the ES was 0.40 (95% CI: 0.09–0.34), with a low level of evidence. Similarly, the effect of protein intake on upper limb strength showed an ES of 0.18 (95% CI: 0.03–0.33), with a low level of evidence. Regarding protein quality, it is well known that several factors influence the acute stimulation of MPS, such as differences in digestibility, amount, and bioavailability of amino acids (especially leucine)[234]. Generally, proteins with higher amounts of essential amino acids, particularly leucine, seem to promote greater MPS stimulation[235-237]. For example, whey protein ingestion induces greater MPS stimulation compared to casein and soy protein[235]. This superiority in MPS stimulation is mainly due to the higher leucine content in whey protein, as leucine is crucial for stimulating a key protein in the protein synthesis pathway (mechanistic target of rapamycin - mTOR). However, since information related to MPS is derived from acute studies, it is important to evaluate the effects of protein sources on muscle mass gain in chronic studies (randomized clinical trials). A systematic review and meta-analysis demonstrated that whey protein supplementation had the same effect on lean mass and strength gains as soy protein intake in individuals undergoing strength training and consuming high-protein diet[238]. Additionally, a recent study showed that a high-protein diet (approximately 1.6 g/kg/day) exclusively based on plant proteins had the same effect on strength and muscle mass gains as a diet containing primarily animal protein sources[239]. Therefore, current evidence suggests that the protein source does not seem relevant for muscle hypertrophy in individuals consuming high-protein diets, but this remains unknown for those with suboptimal protein intake (< 1.6 g/kg/day), suggesting that the protein source may be more important only in individuals with lower protein intake. Since this conclusion is based on limited evidence, further studies are needed to confirm these hypotheses.

Besides total protein intake, there is a suggested ideal protein dose per meal. It is recommended to consume 20 to 40 g of protein per meal[240-242]; or 0.25-0.30 g of protein/kg/meal (young adults) and 0.4 g of protein/kg/meal (elderly) to maximally stimulate MPS[243]. It is important to note that these recommendations are mainly based on studies using whey protein, which is rich in leucine[243]. Therefore, when consuming protein sources with less leucine, larger protein doses per meal may be required[244]. Based on acute studies, it is suggested to consume 20 to 30 g of protein per meal throughout the day. However, despite the importance of protein dose per meal for MPS, long-term studies evaluating the effect of protein dose per meal on muscle hypertrophy are needed, as acute MPS evaluation does not seem to correlate with long-term muscle hypertrophy[245]. Yasuda et al.[246] demonstrated that individuals consuming protein evenly distributed throughout the day (approximately 30 g/meal) had the same appendicular lean mass gains as those consuming protein irregularly. One possible explanation for the lack of effect of protein distribution throughout the day on muscle hypertrophy may be derived from studies showing that muscles can utilize more than ~30 g of protein per meal to achieve a maximal protein synthesis stimulus[247, 248]. Thus, it is possible that individuals consuming the same total protein amount throughout the day, but in different distributions, may achieve similar muscle hypertrophy results over time. Therefore, the evidence on the relevance of protein distribution throughout the day for muscle mass gains is inconclusive. However, this evidence is based on a limited number of studies, and more research is needed for a reliable conclusion. To date, the most important factor appears to be the adequacy of total protein intake.

Finally, it is well known that strength training acutely increases MPS for up to 24 to 48 hours in untrained individuals[249, 250] and up to approximately 16 hours in trained individuals[249]. The increase in protein synthesis induced by strength training enhances the sensitivity of the skeletal muscle to ingested amino acids during this period[251]. Acute studies demonstrate that MPS is greater when protein is consumed immediately after exercise[252]. However, randomized, placebo-controlled clinical trials have shown that immediate post-exercise protein intake does not seem crucial for greater lean mass and strength gains, with total daily protein intake being more important[253-255].

***This Guideline recommends that protein intake should be individualized, considering that the total daily protein amount is the most important factor for muscle hypertrophy and/or recovery. Energy balance and exercise type are key determinants of daily protein requirements. Protein sources do not seem relevant for muscle hypertrophy in individuals consuming high-protein diets (1.6-2.2 g/kg); however, studies evaluating the effect of protein***

***sources on muscle hypertrophy in individuals with suboptimal intake (<1.6 g/kg/day) are lacking. Moreover, the effect of protein intake distribution throughout the day remains unclear, as few studies have investigated this intervention chronically. Despite limited data, the evidence suggesting that protein intake distribution throughout the day has significant relevance for muscle hypertrophy is inconclusive. Current evidence suggests that there is no need to consume protein immediately after exercise to promote greater muscle mass gains. Protein does not exert an ergogenic effect; thus, its intake should be oriented towards maintaining muscle mass and optimizing training-induced adaptations. Evidence level: moderate; strength of recommendation: strong.***

## Lipids

Lipid intake is essential to meet daily energy demands. Additionally, lipids are crucial components of cell membranes, and they are involved in hormone production and the absorption and transport of fat-soluble vitamins[256]. Lipids are found in foods mainly in the form of triacylglycerols (TAG), which consist of a glycerol molecule linked to three fatty acids. In terms of size, the main fatty acids range from 2 to 24 carbons (C2 to C24) and they can be classified as saturated and unsaturated, with the latter further categorized into monounsaturated and polyunsaturated fatty acids[257]. In foods, TAGs are found as medium-chain (MCT), long-chain (LCT), and very-long-chain (VLCT) triglycerides, with LCTs being the most abundant in the human diet[258].

MCTs include the fatty acids caproic (C 6:0), caprylic (C 8:0), capric (C 10:0), and cauric (C 12:0)[258]. MCTs are not commonly found in foods, with coconut oil being the main source[258]. Despite gaining popularity in recent years as an ergogenic aid for physical exercise, coconut oil intake does not influence physical performance[259]. The appeal of the rapid metabolism of MCTs in coconut oil is not scientifically supported, as its composition is primarily lauric acid (about 50%). Only 25 to 30% of lauric acid is absorbed via the portal vein, while 70% is transported as LCT, therefore by chylomicrons[260, 261]. Additionally, 25% of coconut oil consists of myristic (C 14:0) and palmitic (C 16:0) acids, both LCTs[260, 261]. Consequently, the supplement industry has focused on creating dietary supplements with high amounts of MCTs, particularly caprylic and capric acids[258]. LCTs mainly consist of myristic, palmitic, stearic (C 18:0), oleic (C 18:1), linoleic (C 18:2), and α-linolenic (C 18:3) acids, which are the most commonly found in the diet[262].

Polyunsaturated fatty acids consist of two families, ω-3 and ω-6, characterized by the position of the first double bond relative to the terminal methyl group in the fatty acid molecule. α-Linolenic acid and Linoleic acid are examples of polyunsaturated fatty acids from the ω-3 and ω-6 families, respectively. These two fatty acids are not synthesized in humans, and the lack of their intake causes deficiency symptoms, which makes them essential nutrients. Therefore, they must be consumed in the diet. α-Linolenic acid serves as a precursor for the synthesis of longer-chain, more unsaturated ω-3 fatty acids, such as Eicosapentaenoic acid (EPA; 20:5 ω-3) and Docosahexaenoic acid (DHA; 22:6 ω-3), which are present in significant amounts in fish oil. It is suggested that the primary biological function of α-Linolenic acid is to be a substrate for EPA and DHA synthesis. However, evidence indicates that the conversion of α-Linolenic acid to EPA and DHA in humans is relatively low, with the conversion to EPA estimated at 8-12% and to DHA less than 1%[263-267]. Some studies have suggested a potential effect of ω-3 on increasing muscle mass and strength[268]. It is hypothesized that ω-3 fatty acids may benefit skeletal muscle in two ways: by reducing inflammation and facilitating amino acid uptake by the muscle. Regarding the impact of inflammation on muscle mass, increased expression of pro-inflammatory cytokines is suggested to trigger proteolysis regulators, which in turn promote muscle loss[269]. Thus, since ω-3 fatty acids can reduce the production of pro-inflammatory substances, these fatty acids could benefit muscle mass by preserving this tissue[270]. In terms of amino acid uptake, it is speculated that ω-3 increases membrane fluidity and facilitates amino acid uptake through incorporation into skeletal muscle cell membranes, optimizing muscle protein synthesis[268, 271]. However, despite the proposed mechanisms, a recent SRMA found that ω-3 supplementation did not affect muscle mass increase, although a slight effect on strength gain was observed[272]. It is important to note that some studies included in the SRMA had limitations, such as lack of control over participants' physical exercise level and dietary intake, especially protein intake[268]. Thus, current evidence on the effects of ω-3 intake on muscle mass and strength is conflicting[268], and it is still unclear whether ω-3 intake can favor muscle mass gain.

In 2023, the World Health Organization (WHO) recommended that total lipid intake should be up to 30% of total energy needs[273]. For the general population, it is recommended to prefer unsaturated lipids, while saturated lipids should account for up to a maximum of 10% of total energy needs[273, 274]. The latest consensus on applied nutrition for physical exercise[5,6] suggests that lipid intake should follow public health guidelines, from any person who exercises to elite athletes, considering cultural and regional aspects, even though recommendations regarding the amount and type of lipid are quite similar[275, 276]. Regarding the type of lipid, it is recommended to follow the proportions of saturated, monounsaturated, and polyunsaturated fatty acids according to recommendations for metabolic and cardiovascular health[275, 276]. Thus, in addition to physical exercise, it is crucial to consider the health status of the exercise practitioner or elite athlete. Although lipid intake is not extensively explored in athletes with cardiovascular diseases, the identification and discussion of these diseases in athletes are increasing[277-279]. In Brazil, the Position on Fat Consumption and Cardiovascular Health is used to guide lipid recommendations for people with or without cardiovascular or metabolic diseases[276].

Given the importance of fatty acids for ATP production during physical exercise, some studies have aimed to evaluate the effect of lipid intake before physical exercise on the physical performance of adults, hypothesizing that increased fatty acid oxidation reduces the rate of muscle glycogen utilization and consequently improves performance, particularly in prolonged physical exercises[280-282]. However, several studies have shown that increasing lipid intake before physical exercise, regardless of the molecule size (e.g., MCT or LCT), did not have a glycogen-sparing effect nor improved physical performance[283-286]. Although some studies suggest a potential ergogenic effect from lipid-rich meals before physical exercise[287, 288], the sample sizes were small and the results need to be replicated in future studies.

As mentioned earlier, MCTs stand out as a pre-exercise resource to optimize physical performance. A recent systematic review published by Chapman-Lopez and Koh[289] revealed that studies investigating the effect of MCT supplementation on physical performance mostly had low risk of bias. The tested doses ranged from 25 to 85 g, with the main placebo (comparison group) being carbohydrate or LCT solutions. The primary administration time was 1 hour before or during physical exercise. The main outcomes evaluated were time to exhaustion, time trial, speed, and work rate, which showed inconclusive results. Regarding studies that assessed glucose and fatty acid oxidation, no significant changes were observed. It is worth noting that several studies reported adverse effects such as gastrointestinal discomfort, diarrhea, and vomiting[290-295].

While acute lipid intake before physical exercise does not promote positive effects on physical performance, it is important to consider the daily intake recommendation of this macronutrient in athletes and sportspeople, which is no less than 25% to 30% of the total energy needs[296]. Therefore, in low energy availability (LEA) conditions, it is essential to assess the need to adjust lipid intake to prevent hormonal, immunological, and metabolic disorders associated with low lipid intake[296-299].

***This Guideline does not recommend acute lipid intake (e.g., MCT or LCT) before physical exercise. Evidence level: high. Strength of recommendation: strong. However, considering the importance of lipids for vital functions, the average intake should range from 25% to 30% of total caloric intake, not falling below 20% of total energy needs. Evidence level: high; strength of recommendation: strong.***

## Micronutrients

In the previous sections, we discussed the role of macronutrients in physical performance. However, vitamins also play crucial roles in human metabolism, directly affecting athletic performance. Notably, it is understood that the demand for micronutrients is higher for elite athletes. This increased need is due to greater excretion through sweat, urine, and feces, higher turnover, reduced absorption, and increased requirements for training-induced adaptations[300].

However, the increased demand does not necessarily justify the use of dietary supplements. The "food-first" approach is often recommended, which is defined as "whenever possible, nutrients should be obtained from foods and beverages rather than dietary supplements"[301]. This is supported by Larson-Meyer et al.[35], who described that dietary supplements cannot compensate for poor food choices and an unbalanced diet. Additionally, Close et al.[301] introduced the concept of "food first, but not always", emphasizing the need to assess nutrient needs according to training periods, with dietary supplements potentially being necessary to correct nutritional inadequacies. Therefore, considering the inability of the body to produce vitamins and minerals endogenously, external intake is required during specific periods[302].

## Vitamins

Vitamins are micronutrients found in various types of foods and they are categorized according to their solubility. Water-soluble vitamins include vitamin C and the B-complex vitamins (e.g., thiamine, riboflavin, niacin, pantothenic acid, pyridoxine, biotin, folate, and cobalamin), while fat-soluble vitamins include vitamins A, D, E, and K[303]. To ensure adequate micronutrient intake from exogenous sources, athletes are highly encouraged to eat a diet rich in vegetables (e.g., fruits, vegetables, and greens)[303]. However, in cases of inadequate energy intake, poor diet quality, and reduced intestinal absorption, the use of micronutrient supplements may be a viable strategy to meet minimum daily requirements[302]. Therefore, the decision to supplement micronutrients should consider their availability through diet and the individual's micronutrient status.

While the importance of obtaining minimum micronutrient levels for vital functions is understood, especially concerning antioxidant vitamins, there are also discussions about the need for vitamin supplementation to optimize physical performance or muscle recovery in athletes. Physical exercise increases the production of substances such as reactive oxygen species (ROS), which can cause muscle damage and impair recovery between training sessions[304]. This guideline discusses the main vitamins relevant to the exercise context.

### Vitamin C and E

The effects of vitamin C and E supplementation on exercise-induced adaptations have been extensively studied. It is believed that high doses of these vitamins (e.g., ≥ 1000 mg of vitamin C or ≥ 400 IU of vitamin E) can reduce mitochondrial biogenesis and skeletal muscle protein synthesis, thereby diminishing the positive adaptations associated with physical exercise[305, 306]. This antagonistic effect is attributed to the role of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in activating critical intracellular signaling pathways for skeletal muscle adaptation[305].

However, a recent SRMA of nine studies found that vitamin C and E supplementation (500 to 1000 mg of vitamin C and 400 to 900 IU of vitamin E for 8 to 24 weeks) did not attenuate maximal aerobic capacity (ES: -0.14; 95% CI: -0.43 – 0.15) or endurance exercise performance (ES: -0.01; 95% CI: -0.38 – 0.36)[307]. Seven of the studies included presented low risk of bias. Additionally, regarding strength training adaptations (e.g., lean body mass and muscle strength), nine studies were evaluated. Vitamin C (1000 mg) and E (400 IU) supplementation for 4 weeks to 6 months did not alter lean body mass (ES: -0.07; 95% CI: -0.36 – 0.23) or muscle strength (ES: -0.15; 95% CI: -0.16 – 0.46)[307]. These findings suggest neutral effects of these vitamins on training adaptations. More recently, Rogers, Lawlor, and Moeller[308] corroborated these findings, showing no positive effects of vitamin C on exercise-related outcomes (e.g., muscle damage, pain perception, physical performance, and training adaptations)[308]. Given the lack of enhancement in physical performance, vitamin C and E supplementation for athletes should be discouraged if the goal is to optimize physical performance.

On the other hand, vitamin C has been studied for other outcomes (e.g., markers of muscle recovery and opportunistic infections). An SRMA of 18 randomized clinical trials with 313 participants (13% athletes; 33% physically active individuals; 54% healthy adults) found that oral vitamin C supplementation (400 to 3000 mg; 1–28 days) reduced lipid peroxidation (measured by malondialdehyde [MDA] and thiobarbituric acid reactive substances [TBARs]) immediately after exercise (ES: -0.488; 95% CI: -0.888 – -0.088), 1 hour post-exercise (ES: -0.521; 95% CI: -0.911 – -0.131), and between 1 and 2 hours post-exercise (ES: -0.449; 95% CI: -0.772 – -0.126)[309]. Other exercise-related parameters also decreased in response to vitamin C supplementation, such as IL-6 (TE: -0.764; 95% CI: -1.279 – -0.248). However, the level of evidence of these studies ranged from very low to moderate[309]. Another SRMA found similar parameters with combined vitamin C and E supplementation. The findings, at least in part, support the small or neutral effect of these antioxidant vitamins on exercise-related parameters[310]. Moreover, as surrogate outcomes, regardless of these changes and the level of evidence, the clinical relevance of these modifications for athletes remains unclear. This discussion was highlighted in a Cochrane review on the subject[311].

## Vitamin D

Vitamin D exists in two forms: ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3), obtained through dietary sources or supplements (about 10%) and sun exposure (about 90%). Both are converted to 25-hydroxyvitamin D (25-OH-D) in the liver, the form commonly measured in blood samples. In the kidneys, 25-OH-D is converted into its active form, calcitriol ($1,25(OH)_2D$)[312, 313]. Vitamin D plays crucial roles in bone metabolism, as well as in the immune, cardiovascular, reproductive, and endocrine systems, all muscle types, brain, skin, and liver[314]. The prevalence of vitamin D deficiency is relatively high and varies among athletes due to various factors, including training location, clothing worn during training, skin color, and country of origin[315, 316]. Recently, an SRMA identified deficiency prevalence of 30% (95% CI: 22–39%) in adult athletes[317].

For athletes, serum vitamin D levels ≥ 30 ng/ml (≥75 nmol/l) are recommended; however, studies have detected lower blood levels ranging from 21 to 29 ng/ml (52.5 to 72.5 nmol/l), classified as insufficiency, or ≤ 20 ng/ml (≤50 nmol/l) classified as deficiency, especially in sports and/or locations with low sun exposure[318-322]. Vitamin D insufficiency/deficiency negatively affects cardiorespiratory performance and muscle function in physically active individuals[323-328]. In athletes, lower vitamin D levels may increase the risk of injuries, bone fractures, and upper respiratory tract infections[320, 321, 329-331].

In a study conducted by Davey et al.[330] with 1,082 military personnel, 78 individuals (7.2%) experienced 92 stress fractures. Military personnel with baseline serum 25(OH)D concentrations below 20 ng/mL (< 50 nmol/L) were more likely to suffer stress fractures than recruits with serum 25(OH)D concentrations above this limit (OR: 1.6; 95% CI: 1–2.6). Millward et al.[332] partially corroborated these findings, observing that athletes with low 25(OH)D levels who did not reach ≥ 40 ng/mL had a 12% higher rate of stress fractures (95% CI: 6–19%) compared to those with low vitamin D levels but increased to ≥ 40 ng/mL.

Another study with 1,644 military personnel found that individuals with sufficient vitamin D levels had a 40% lower risk of upper respiratory tract infections during 12 weeks of training compared to those whose baseline serum 25(OH)D levels were below 20 ng/mL (< 50 nmol/L)[321]. Vitamin D supplementation can correct serum vitamin D levels[313, 314, 321]. An SRMA conducted by Farrokhyar et al.[333] revealed that the effect of vitamin D supplementation on serum 25(OH)D levels in athletes with levels below 30 ng/mL showed a linear response concerning dose and duration. Athletes who supplemented ≤ 2000 IU/day for 12 weeks increased vitamin D levels by 7.11 ng/mL (95% CI: 1.64–12.58) but it did not reach sufficient levels (30 ng/mL). With doses close to 3000 IU/day for 12 weeks, the average increase was 15.23 ng/mL (95% CI: 10.71–19.74), and with 5000 IU/day for 12 weeks, the increase was 27.8 ng/mL (95% CI: 16.91–38.83 ng/mL). Even athletes with sufficient vitamin D levels, when supplemented (3800 to 4000 IU/day) for 24 weeks, showed an improvement in vitamin D levels (13.89 ng/mL; 95% CI: 0.37–27.41), despite greater variability in the effect. Despite methodological flaws in the SRMA studies, the results consistently support the positive effect of vitamin D supplementation on optimizing serum 25(OH)D levels. Therefore, doses between 2000 and 4000 IU/day are expected to optimize 25(OH)D levels after 12 to 24 weeks of supplementation.

Despite potential positive effects of adequate serum 25(OH)D levels maintenance on reducing the risk of bone fractures and upper respiratory tract infections, as well as the positive effects of vitamin D supplementation on serum levels correction, there is still insufficient and robust evidence confirming that supplementation improves performance or training adaptations. Furthermore, there is no consensus on appropriate supplementation doses based on age, type of sport, or training load[333].

While some studies of low to moderate quality have shown performance improvements[313, 321, 334-336], others have not confirmed these findings[314, 337-339]. Given the high degree of heterogeneity in methods, populations, types, duration, and intensities of training, as well as physical conditioning, age, and biological sex, it is still uncertain whether vitamin D supplementation positively impacts physical performance.

In the SRMA conducted by Farrokhyar et al.[333], athletes from various sports were evaluated. Vitamin D doses ranged from 400 IU for 52 weeks to 18750 IU in 8 days. The study evaluated 531 athletes, 388 with insufficient vitamin D levels and 143 with sufficient levels. Performance parameters such as vertical jump (0.92 cm; 95% CI: -0.55–2.44; 4 studies; n = 98; 2850 to 5714 IU; 12 weeks), 1RM bench press strength (2.05 kg; 95% CI: -5.5–9.6; 3 studies; n = 70; 2850 to 5714 IU; 12 weeks), or 10 to 30 m sprint test (0.04 s; 95% CI: -0.04–0.12; 2 studies; n = 44; 2850 to 5714 IU; 12 weeks) were not optimized in response to vitamin D supplementation. Only handgrip strength (with low external and ecological validity) was improved (2.56 kg; 95% CI: 1.00–4.13; 3 studies; n = 110; 2000 to 3800 IU; 12 weeks).

More recently, another SRMA examined the effect of vitamin D supplementation on maximum strength and power in athletes[340]. Out of the twelve included studies, only 2 had low risk of bias. The authors did not observe positive effects of vitamin D supplementation on physical performance, further highlighting that the level of evidence remains low. However, it is clear that vitamin D supplementation has positive effects in correcting insufficient levels[340].

***This Guideline recommends monitoring vitamin levels, particularly vitamin D. Additionally, it is recommended to correct vitamin deficiencies through diet and, if necessary, through supplements with high bioavailability. Regarding vitamin D, sun exposure is essential. Therefore, identifying the training environment and clothing worn by the athlete can help correct serum levels of this vitamin. The use of dietary surveys combined with serum vitamin measurements can provide a better understanding of the availability of these nutrients. For monitoring and correcting deficiencies: Level of evidence: moderate; Strength of recommendation: strong. However, the effect of vitamin supplementation on physical performance is uncertain; therefore, for this outcome: Level of evidence: moderate; Strength of recommendation: weak.***

## Minerals

Similarly to vitamins, minerals play a role in various regulatory pathways, and they have essential effects on physical exercise. This guideline discusses the main minerals studied in the context of physical exercise.

### Calcium

Calcium is involved in numerous processes in the human body, such as muscle contraction, hormone secretion, enzyme activation, and nerve impulse conduction[341]. Additionally, along with vitamin D, calcium is crucial for maintaining bone mineral density (BMD)[342]. Approximately 99% of the calcium in the human body is stored in the bones as hydroxyapatite and in the blood it can be found in three forms: ionized (free), protein-bound, and complexed (chelated). Protein-bound calcium accounts for 40% of blood calcium and it is not utilized by peripheral tissues, while chelated calcium (e.g., calcium phosphate, calcium carbonate, and calcium oxalate) corresponds to about 9% of blood calcium. Finally, 51% of blood calcium is in the free (ionized) form[341].

Athletes experiencing energy deficit may have insufficient calcium levels, especially those with low intake of dairy or other calcium-rich foods[342]. Furthermore, athletes with lactose intolerance or those following a vegetarian diet may be more susceptible to calcium deficiency. In 2011, the Institute of Medicine (IOM) established the daily calcium requirements by age group. For adults over 19 years old, the recommended daily intake is 1000 mg[343]. However, based on the available literature it is unclear if the same recommendation can be applied to athletes, especially since ionized calcium levels decrease during physical exercise, leading to an increase in parathyroid hormone (PTH) released by the parathyroid glands[344].

Lower calcium intake from the diet results in increased PTH, in osteoclast activity, and consequently in bone catabolism, which can increase the risk of bone fractures and training time reduction and significantly impact physical performance and participation in important competitions in a negative manner[342]. The effect of physical exercise on BMD varies. Previous studies suggest that some sports (e.g., long-distance running, cycling, swimming, certain types of dancing, and horse riding) may favor higher risk of bone problems[345-349]. On the other hand, basketball seems to promote higher BMD compared to other sports[350]. Therefore, evaluating the type of sport for calcium management appears to be crucial. Although preliminary, some studies have showed interesting results, such as smaller changes in ionized calcium, PTH, and bone degradation markers when athletes were supplemented with calcium (e.g., 1000 to 1350 mg) before a physical exercise session[344, 351-353]. In this context, an increase in calcium intake for athletes (e.g., 1500 mg) has been suggested and it is recommended that it be divided into smaller doses (e.g., 500 mg) to optimize bioavailability[354].

In summary, there is no evidence that acute calcium supplementation before physical exercise can impact physical performance. However, athletes should be encouraged to obtain this micronutrient from a balanced and varied diet. Supplementation may be necessary to maintain adequate calcium levels depending on the sport and dietary patterns, primarily due to potential risks associated with bone mass.

## Iron

Iron is an essential nutrient for many body functions and it is crucial for those who engage in physical exercise. Iron is fundamental to the heme prosthetic group linked to hemoglobin in red blood cells. Their main function is to transport oxygen to the cells. The iron stock in hemoglobin is approximately 1.5 g in women and 2.0 g in men[355]. Iron deficiency is associated with, but not limited to, fatigue and exercise intolerance. Iron deficiency can occur with or without anemia, with the main cause of iron deficiency being non-anemic iron deficiency[356]. Anemia is defined when hemoglobin levels are < 13 g/dL for men and < 12 g/dL for non-pregnant women. Additionally, the World Health Organization defines mild anemia when hemoglobin levels are between 11 and 13 g/dL[356].

Individuals with lower iron intake due to dietary patterns or reduced absorptive capacity as a consequence of gastrointestinal diseases, as well as those with higher demands or losses (e.g., athletes), may be susceptible to iron deficiency. Iron absorption varies depending on the food source; while iron absorption from animal-based foods ranges between 15% and 40%, absorption from plant-based foods ranges between 1% and 20%[357, 358]. The lower absorption of iron from plant-based foods is partly due to the presence of phytates and polyphenols. On the other hand, vitamin C enhances the bioavailability of non-heme iron. Thus, the food matrix and meal composition play an important role in iron bioavailability[357].

In athletes, hemolysis, sweating, and gastrointestinal bleeding can be significant causes of iron deficiency. In women, iron deficiency can be even more frequent, particularly due to menstruation, which leads to iron loss[359, 360]. Iron deficiency appears to range from 3% to 11% in male athletes and 15% to 35% in female athletes[361-363]. Additionally, low energy availability especially associated with carbohydrate restriction can be a risk factor for iron deficiency[217].

Given the role of iron in oxygen transport to cells and the need for oxygen in ATP production, endurance athletes would be most affected by iron deficiency. Therefore, monitoring this parameter is crucial[364]. Although ferritin is the blood parameter used to establish iron deficiency, it is possible that, in some conditions, iron insufficiency exist regardless of ferritin levels[356]. Ferritin levels < 30 ug/L have high sensitivity (92%) and specificity (98%) for determining iron insufficiency, with or without anemia. However, values vary according to laboratories. Despite ferritin being an important marker, assessing transferrin saturation is important. Saturation levels between 16% and 20% or less may indicate lower iron availability for adequate erythropoiesis[356]. In athletes, non-anemic iron deficiency has been considered when ferritin levels reach < 20 ug/L and transferrin saturation < 16%[365]. However, values for ferritin vary between < 12 and < 40 ug/L[366].

Currently, the RDA for iron is 8 mg/day for men and 18 mg/day for women[367]. Individuals with non-anemic iron deficiency may experience early symptoms of fatigue and exercise intolerance. Moreover, physically active individuals may benefit from iron supplementation under these conditions. A systematic review and meta-analysis found that iron treatment (oral supplementation n = 15; intramuscular injection n = 2) in an adult population (mean age 22.3 years) with non-anemic iron deficiency (ferritin ≤ 35 ug/L and hemoglobin > 12 g/dL) had a positive effect on various parameters associated with iron metabolism[368]. For example, its effect on ferritin levels (ES: 1.088; 95% CI: 0.914 – 1.263), iron levels (ES: 1.004;

95% CI: 0.828 – 1.181), and iron saturation (ES: 0.741; 95% CI: 0.564 – 0.919) were considered robust, while its effect on hemoglobin levels was considered moderate (ES: 0.695; 95% CI: 0.533 – 0.836). Similarly, the effect on $\dot{V}O_{2max}$ was considered moderate (ES: 0.610; 95% CI: 0.399 to 0.821). The primary studies of this review predominantly involved women (n = 363). The tested oral iron dosage ranged from 10 to 425 mg, 1 to 3 times per day, for 6 to 15 weeks[368].

More recently, Houston et al.[369] evaluated the effect of iron supplementation (e.g., oral n = 14; intravenous n = 3; intramuscular n = 1) on physical performance parameters of physically active women and elite athletes. Among the studies that evaluated the effect of oral supplementation, 13 assessed the effect of ferrous sulfate (16 to 200 mg; 28 to 112 days) and only one study evaluated the effect of ferrous fumarate (120 mg; 90 days). Two studies assessed performance through 15 km time-trial protocols and observed no positive effect on physical performance (ES: -0.09; 95% CI: -0.53 – 0.35). Four studies evaluated physical performance through an incremental cardiopulmonary exercise test to volitional exhaustion and found no positive effect on physical performance (ES: 0.25; 95% CI: -0.22 – 0.73). Finally, in nine studies, $\dot{V}O_{2max}$ also did not change (ES: 0.11; 95% CI: -0.15 – 0.37) after iron supplementation. Based on the above results, iron supplementation seems effective in correcting potential deficiencies, especially in female athletes. While an adequate diet is crucial, monitoring iron stores is fundamental. However, iron supplementation did not have a positive effect on physical performance. Yet, the data come from studies with methodological limitations.

## Magnesium

Magnesium plays countless roles in the human body, being the fourth most abundant mineral and the second intracellular divalent cation[370]. Less than 1% of magnesium is found in the blood, while 60% to 65% are found in bones and approximately 34% to 39% in other tissues and organs[370]. Magnesium participates in at least 300 enzymatic reactions, including protein synthesis, energy production, and cell growth, among others[370]. It can be obtained through oral intake, intravenous or intramuscular administration[371]. Magnesium deficiency occurs in conditions of lower dietary intake, reduced renal absorption, or impaired gastrointestinal absorption. Approximately 30% to 40% of the ingested magnesium is absorbed and some factors act as moderators of the absorption process. For example, the amount of phytate, oxalate, calcium, phosphorus, iron, copper, manganese, and zinc and intestinal diseases reduce absorption[372]. Magnesium can be widely found in green leafy vegetables such as spinach, legumes, nuts, seeds, and whole grains[372].

Considering its role in energy production and muscle functionality, it has been studied as a potential ergogenic aid[373]. The RDA for magnesium ranges from 400 to 420 mg for men and 310 to 320 mg for women aged 14 to 70 years. Blood levels of magnesium represent magnesium status in the body; however, this parameter seems to be not sensitive enough, especially due to differences between blood and tissue changes[373]. Thus, the primary way to assess magnesium deficiency is through a loading test, which involves checking magnesium retention after administration. Urine samples are collected between 4 and 24 hours, and if retention is greater than or equal to 80%, magnesium deficiency is suggested[373].

Magnesium deficiency among athletes appears to vary, mainly because studies use different methods to check magnesium status, with dietary intake assessment being the most common. Furthermore, the effect of magnesium supplementation on physical performance is unclear, as studies present various methodological limitations[374-379].

## Zinc

Zinc is considered a trace mineral, widely found in the body (2 to 3 g) and involved in several important biological processes, including cell division and growth, carbohydrate metabolism, insulin action, taste, and smell[380]. Moreover, zinc is crucial for the functioning of immune system cells[381]. The RDA for zinc is 11 mg/day for males aged 14 and over, 9 mg/day for females aged 14 to 18, and 8 mg/day for females aged 19 and over[382].

Expected zinc levels in adults range from 80 to 120 mcg/dL, with zinc deficiency defined as levels below 70 and 74 mcg/dL for females and males, respectively. Serum zinc measurement may not accurately reflect the actual status of this mineral, particularly as it is affected by various factors[383]. Physical exercise increases zinc consumption through diet and the activity of the enzyme superoxide dismutase, which has zinc as a cofactor, suggesting a higher demand for zinc due to adaptations from training[384]. However, current evidence does not suggest zinc supplementation as an ergogenic aid for athletes, despite potential antioxidant effects[385, 386].

***This Guideline recommends that the levels of these minerals should be monitored through diet and the analysis of risk factors (e.g., dietary patterns, antinutritional factors, type of sport, biological sex, etc.), especially in situations of high energy demand due to physical exercise. However, the effect of supplementation, particularly when the outcome is physical performance, is trivial or small. Thus, for monitoring: level of evidence: moderate; strength of recommendation: strong. In contrast, for improving physical performance: level of evidence: low; strength of recommendation: weak.***

## Hydration

Water is the primary chemical constituent of the adult body (approximately 60%) and it plays an active role in various vital metabolic processes[387]. The availability of water is crucial for the proper functioning of the body, especially under stressful conditions such as physical exercise, particularly when it is performed in hot environments[388]. The importance of hydration for physical exercise has been extensively discussed in recent applied nutrition position statements related to exercise[5].

The daily fluctuation in water content is approximately 0.2 to 0.7% of body mass. Athlete dehydration refers to the reduction or loss of body water volume that is not adequately compensated during physical exercise, resulting from sweat production and/or insufficient fluid intake for replenishment and it is established when there is a total body water decrease of 3-4% (>2% of total body mass)[389, 390]. Dehydration can be presented with different characteristics, such as (i) hypotonic dehydration, (ii) isotonic dehydration, and (iii) hypertonic dehydration, the most frequently observed type during physical exercise. Hypertonic dehydration involves a greater proportion of water loss compared to electrolytes loss, which happens during significant sweating losses experienced by athletes during strenuous activities in thermally stressful environments[391].

As previously mentioned, the effects of dehydration on physical performance typically begin with body mass loss of 2%. Therefore, for a 70 kg athlete, this is equivalent to 1.4 kg[388, 389], which is usually associated with the onset of thirst[392]. Symptoms of hypertonic dehydration range from mild (such as thirst, discomfort, and loss of appetite) to moderate and severe, including reduced blood volume, increased perceived exertion, nausea, decreased concentration capacity, impaired thermoregulation, and, in extreme cases involving losses greater than 6% of body mass, vertigo, weakness, reduced breathing capacity, muscle spasms, and delirium, which can potentially lead to renal impairment[393-396]. In extreme cases with significant water losses of approximately 8%, blood viscosity increases, which can trigger cardiac arrest and potentially lead to "sudden death"[393-396].

The symptoms of dehydration arise from physiological alterations, such as a decrease in plasma volume, leading to cardiovascular changes (e.g., increased submaximal heart rate, decreased cardiac output, reduced stroke volume, decreased blood pressure, and increased plasma osmolality)[397]. Additionally, there is a reduction in cutaneous blood flow, which hinders thermoregulation by decreasing sweat production, reducing heat loss through evaporation, and consequently raising body temperature[388, 389, 398]. Furthermore, a relative reduction in visceral blood flow, especially to the liver, negatively impacts the blood-buffering process carried out by this organ[387]. Dehydration also leads to reduced muscular blood flow, impairing oxygen delivery to skeletal muscles during physical exercise, which negatively affects aerobic capacity[399]. Deshayes et al.[399] observed in a SRMA that a reduction of 3.6 ± 1.0% in body mass negatively affected physical performance. Dehydration during predominantly aerobic exercise contributed to a 2.4 ± 0.8% reduction in physical performance (95% CI: 0.8 – 4%). As such, dehydration is expected to reduce the total exercise time, especially in prolonged activities, due to a significant increase in core body temperature, which raises the risk of hyperthermia, increases perceived exertion, and leads to gastrointestinal disturbances[396, 400]. Dehydration can also cause electrolyte imbalances, which may be one of the factors responsible for muscle cramps, impairing athletic performance[388].

More recently, the deterioration of cognitive aspects associated with dehydration has also been identified as an important factor in decreased physical performance[396, 401]. Finally, athletes who voluntarily reduce body water before a competition (e.g., in combat sports and bodybuilding) may increase their risk of dehydration-related disorders)[402, 403].

## Control of Water Loss Magnitude

To maintain hydration homeostasis, it is essential for athletes to establish control procedures. There are several simple, low-cost, and highly accurate methods for assessing the occurrence and degree of dehydration during physical exercise, such as: (i) monitoring body mass fluctuations to identify water loss during exercise sessions, training, or competitions; (ii) tracking urine volume, color, and density throughout the day; and (iii) recognizing the sensation of thirst[394, 404]. Other alternative strategies are also effective including (i) plasma osmolality assessment and (ii) hematocrit examination, but at the same time they are more costly and complex[394, 404].

Assessing dehydration is crucial for aiding in the planning of hydration and fluid replacement strategies. Dehydration can be measured by weighing the body with minimal clothing on a calibrated scale immediately before an exercise session. During exercise, water intake should be monitored and recorded, as well as urine volume if urination occurs during the session. After the session, final weighing with minimal clothing should be conducted again to determine water loss. For every kilogram of weight lost, it is estimated that 1 L of water has been lost through evaporation and sweating[405]. Water loss during physical exercise varies widely; while some athletes may lose about 1 L of water, others may lose ≥ 3 L, depending on the environmental conditions or the individual factors during exercise[388].

## Development of Hydration Strategies in Sports

Developing a hydration strategy requires evaluating the environment in which the exercise will take place. Several factors guide the development of this strategy, such as environmental conditions and the associated level of thermal stress, time of the day, sport regulations, clothing, duration and intensity of the exercise, nutritional habits, fluid availability logistics, acclimatization, age, sex, among others. Notably, four key periods should be considered in the development of the strategy: (i) before; (ii) during; (iii) after exercise; and (iv) throughout the day[406]. Strategies must respect the specific characteristics of each exercise and sport. Furthermore, beverages may contain carbohydrates only, carbohydrates and electrolytes, or just electrolytes. These different combinations can influence gastric emptying, the hydration capacity of the beverage, thermoregulation, and, consequently, physical performance. These aspects are important because the impact of the beverage on physical performance depends on gastric emptying, fluid absorption in the intestine, fluid retention capacity, and renal excretion[406].

**Hydration Strategies Before Exercise**

Ideally, athletes should achieve a state of euhydration before beginning any exercise session or competition. Previous studies suggest that it is common for athletes to start physical exercise already partially dehydrated, which increases the risk of dehydration-related problems during exercise[402, 407]. Various parameters related to physical performance (e.g., $\dot{V}O_{2max}$, lactate threshold) worsen when an individual does not begin exercise in a euhydrated state. Sufficient fluid intake in the hours leading up to exercise is recommended, tailored to individual needs, and should be enough to eliminate any sensation of thirst at the start of the exercise. Additionally, maintaining clear urine color, regular urination throughout the day, and keeping body mass within a maximum variation limit (< 1%) is advised. The available literature since the publication of ACSM[5] emphasizes the importance of maintaining adequate hydration status for exercise, primarily due to the negative effects of fluid loss[400]. Therefore, it is suggested that 5 to 10 mL of water/kg of body mass be consumed 2 to 4 hours before the start of exercise[5].

It is important to highlight that the guideline strongly opposes the practice of voluntary dehydration before exercise, commonly adopted by fighters, which involves the use of diuretics, laxatives, induced vomiting, elimination of fluid intake throughout the day, physical exercise with plastic clothing, or environments like saunas[402, 403]. These practices may be harmful to physical integrity, as acute water losses exceeding 8% of body mass may result in a condition that elevates the risk of "sudden death". The attempt to achieve hyperhydration solely with water before exercise is also inadequate, as the renal system will naturally detect an increase in body fluid volume, leading to increased urine production. Thus, the intake of sodium or glycerol has been studied to optimize the state of hyperhydration before exercise and its impact on athletic performance. Hyperhydration appears to optimize physical performance in exercises to voluntary exhaustion (14.3 to 26.2%), with lower effect for time-trial exercises (11.4%)[408, 409]. However, the level of evidence for these strategies is still low and more studies are needed to establish a more robust recommendation for sodium and glycerol intake (see the section on electrolytes and glycerol).

**Hydration Strategies During Exercise**

One of the limiting factors of hydration during exercise is the rate and capacity of gastric emptying, which is typically around 1 L of fluid per hour of exercise[388]. When sweat production exceeds the rate of 1 L/h, providing fluids at the same rate may induce gastric discomfort. On the other hand, voluntary intake of rehydration drinks may not be sufficient, which requires the establishment of an individualized fluid intake strategy[410]. In this case, amounts between 100 and 200 mL of beverage every 15 minutes of exercise or 2 to 3 mL of fluids/kg of body mass are recommended[5, 388, 400, 411].

Throughout the activity, the total exercise duration will be a determining factor for the preferential intake of water or isotonic beverages. It is noted that the intake of isotonic beverages is recommended to minimize the risk of hyponatremia (< 135 mmol/L) during prolonged physical exercises (> 60 minutes)[387].

**Electrolytes**

Regarding electrolytes, sodium stands out, primarily because it is the main cation in extracellular fluids (135–145 mmol/L) and participates in various reactions in the body. Although studies are inconclusive, sodium and other electrolytes appear to be associated with the occurrence of exercise-induced cramps. However, the available scientific evidence does not support this relationship[412]. A reduction in sodium levels leads to hyponatremia (< 135 mmol/L). This phenomenon seems to be more common in highly voluminous exercises lasting 4–6 hours or longer. Additionally, the consumption of hypotonic drinks, mainly pure water (osmolality ~30 mOsm/kg), during exercise may contribute to exercise-associated hyponatremia[413, 414]. The presence of sodium in the formulation of an isotonic drink is essential, primarily to (i) improve the drink's palatability; (ii) prevent hyponatremia; (iii) enhance the intestinal glucose absorption rate through the co-transport mechanism[415]. Sodium consumption before exercise, around 3–4 g/L, usually offered in salt capsules, has been proposed to increase water retention during exercise and prevent hyponatremia. However, the scientific data supporting this recommendation are inconclusive and more studies are needed. In one study that compared sodium consumption (120 mmol/L or 180 mmol/L), no differences were observed in urinary or plasma volume, suggesting that values > 120 mmol/L may not provide additive effects on plasma volume[416]. Furthermore, it is possible that beverages with high sodium content may cause gastrointestinal issues[417]. Despite these considerations, beverages with high sodium content (> 50 mmol/L) have low palatability, making ad libitum consumption during exercise challenging. Therefore, the presentation form (dilution in the drink or capsules) directly impacts sodium acceptance and consumption before and during exercise.

The sodium concentration in an isotonic drink should range between 50 and 70 mg/100 mL (500–700 mg/L), but it is also important to assess sweat rate (> 1.2 L/h), subjective perception of "salty" sweat, and exercise volume (> 2 hours) to tailor the sodium recommendation individually[418]. Despite the importance of sodium, hyperhydration, notably with pure water due to its hypotonic nature, is believed to be the primary factor related to hyponatremia[419].

Other electrolytes commonly found in commercial isotonic drinks include potassium and magnesium, mainly to ensure a proper drink osmolality. However, the loss of these minerals in sweat is extremely low and likely has little effect on plasma osmolality. According to the Sociedad Española de Medicina del Deporte[415], potassium and magnesium concentrations in isotonic drinks should range between 11.7 and 58.5 mg/100 mL and between 0.24 and 1.8 mg/100 mL, respectively. It should be noted that some sports drinks marketed in Brazil have high potassium concentrations. When combined with other associated factors, these high concentrations could pose a danger to the athlete, therefore they are not recommended.

The appropriate plasma osmolality is around 280 mOsm/L, so the recommended osmolality for beverages should be between 275–300 mOsm/kg to be considered isotonic[400, 420]. Interestingly, hypotonic beverages may support better central hydration status (greater capacity to attenuate exercise-induced plasma volume decline) during prolonged exercise compared to isotonic or hypertonic beverages containing carbohydrates, electrolyte-containing drinks without carbohydrates, or water, as recently described in a meta-analysis[391]. However, as mentioned earlier, concern for hyponatremia should guide the best combination of water and electrolytes and, if necessary, carbohydrates, for optimal physical performance. Finally, there is no consensus on the ideal temperature for ingesting liquids, but it is recommended not to exceed 10°C to 22°C to accelerate gastric emptying and stimulate voluntary intake[388, 421].

## Post-Exercise Fluid Replacement

The goal of post-exercise fluid replacement is to restore hydration homeostasis, compensate for fluid and electrolyte losses, and facilitate the recovery process of muscle glycogen and muscle protein synthesis[415]. For these purposes, the volume of liquids to be ingested and their composition will depend on the loss of fluids and electrolytes. In terms of volume, the recommendation is to replace 1 to 1.5 mL (100 to 150%) for every 1 mL of fluid lost[422].

***This Guideline recommends that athletes and high-performance sportspeople be monitored regarding their hydration status, especially if training occurs in hot environments. Adequate intake of water and sports drinks before, during, and after exercise promotes positive health effects and physical performance. However, it is necessary to appropriately consider the beverage osmolality. Isotonic drinks are more suitable for providing carbohydrates and electrolytes, especially sodium. Additionally, hypotonic drinks can optimize hydration during exercise, despite the risk of hyponatremia. Evidence level: high; strength of recommendation: strong.***

## Dietary Supplements

The use of dietary supplements is widely discussed by scientists and professionals in the sports science field. Prevalence data suggest that approximately 60% (95% CI: 55–64%) of elite athletes use dietary supplements, with isolated vitamin and mineral supplements (50%; 95% CI: 43–57%) or multivitamin and mineral complexes being the most frequent (34%; 95% CI: 30–40%). Furthermore, elite athletes seem to use them more frequently compared to non-athletes[423].

Concerns and considerations regarding supplement use stem from factors such as: (i) product quality; (ii) the risk of unintentional doping due to cross-contamination; (iii) intolerance and adverse effects on human health; (iv) the actual benefits according to the desired outcomes[18, 424, 425]. Additionally, despite the fact that some substances have biological effects that may optimize physical performance, it is important to consider frequent questions about the discrepancy between observed effects in the laboratory, the ability to apply them in real-life settings, and the difficulty in distinguishing placebo effects from expected biological effects[18, 426]. Lastly, other elements that need to be studied in depth are the interaction between substances, repeated use of substances at shorter intervals (e.g., hours), individual response to supplementation, variations in effect depending on the athlete's fitness level, and genetic variations[18].

Some evidence-based supplements such as caffeine and sodium bicarbonate can trigger gastrointestinal symptoms that can impair performance or interfere with training in some situations. Athletes who decide to use these supplements may need to “train the gut”, which means implementing mitigation strategies to minimize GI-related adverse effects and maximize these supplements ergogenic properties[18].

In 2018, the International Olympic Committee published its posit ion on dietary supplements in sports[26]. In line with this publication, dietary supplements considered ineffective for optimizing physical performance will not be discussed in this guideline. Therefore, only dietary supplements with concrete and well-supported biological effects will be presented, including: (i) caffeine, (ii) creatine, (iii) sodium bicarbonate, (iv) beta-alanine, (v) nitrate, and (vi) glycerol.

### Caffeine

Caffeine supplementation is well-known for its ability to enhance performance across a wide range of exercise durations and intensities[427]. Meta-analytical evidence suggests that caffeine can improve performance in aerobic endurance exercises [428-431], strength exercises [428, 432, 433], and anaerobic power[428, 432, 434], as well as in sport-specific tests [435]. The ES appears to vary from small to moderate (0.22 – 0.61), depending on the type of physical exercise, with the greatest effects observed in aerobic endurance exercises and the smallest in maximal strength and power exercises[427]. In the umbrella review published by Grgic et al.[427], the authors evaluated 11 reviews comprising 21 meta-analyses, with levels of evidence ranging from very low (n = 3) to low (n = 7) and moderate (n = 11). In summary, for the outcome of speed (maximum speed in physical performance tests lasting 45 seconds to 8 minutes with fixed duration or distance) assessed in running, cycling, or rowing tests, the ES was 0.41 (95% CI: 0.15 – 0.68); for endurance exercise with duration ≥10 minutes, the ES was 0.51 (95% CI: 0.41 – 0.62); while comparisons evaluating the combination of carbohydrates and caffeine vs. carbohydrates alone showed an ES of 0.26 (95% CI: 0.15 – 0.38). For high-intensity interval exercise, the ES was 0.16 (95% CI: 0.01 – 0.31), and for time trials it was 0.40 (95% CI: 0.11 – 0.70). In the Wingate test (30 seconds), peak power and average power presented ES of 0.27 (95% CI: 0.08 – 0.47) and 0.18 (95% CI: 0.05 – 0.31), respectively. ES for strength in the 1RM test was 0.20 (95% CI: 0.03 – 0.36), for vertical jump 0.17 (95% CI: 0.00 – 0.34), and for muscular endurance 0.38 (95% CI: 0.29 – 0.48)[427].

The ergogenic effects of caffeine are primarily attributed to its ability to act as a non-selective antagonist of adenosine receptors in the central nervous system, increasing arousal, behavioral activation, and vigilance, while it also reduces perceptions of effort and pain during physical exercise[436].

Despite the ability of caffeine to optimize physical performance, there appears to be some variability in individual responses to its supplementation[437, 438], which can either enhance or impair its beneficial effects. Factors suggested as moderators of the effect of caffeine include habitual caffeine intake[439] and genetics[437], although it is unclear to what extent these factors may influence the effects of this supplement. While some studies suggest no influence of habitual caffeine intake on its supplementation effect[440, 441], most indicate that the effect of supplementation occurs regardless of it[440, 442-447]. Indeed, meta-analytic data suggest that habitual caffeine intake does not affect physical performance in response to isolated caffeine supplementation at recommended doses and that abstaining from caffeine before competitions is not necessary to optimize physical performance[428]. Therefore, based on the available data, there is no need for concern or specific adjustments regarding caffeine supplementation for those who consume caffeine-containing foods or beverages.

In addition to habitual consumption, polymorphisms in the CYP1A2 (caffeine metabolism) and ADORA2A (wakefulness-promoting effect of caffeine) genes have been suggested as modifiers of the effects of caffeine[437, 448]. However, data are contradictory across studies and there is no robust scientific evidence supporting the genotypic factor as essential for the ergogenic effect of caffeine. For example, some studies suggest that genetic polymorphisms may influence ergogenic response to caffeine on physical performance[448-450]; however, other studies show conflicting results[451-454].

Regarding the CYP1A2 gene polymorphism, one meta-analysis suggests that individuals with AA (fast metabolizers) and AC (intermediate metabolizers) genotypes exhibit improved physical performance with caffeine supplementation, while those with the CC genotype (slow metabolizers) experience worsened physical performance. However, conflicts of interest and the low quality of the studies may have influenced these findings[451-454]. Therefore, the majority of the studies in the literature does not demonstrate an influence of genetic polymorphisms on the ergogenic effect of caffeine[448], not supporting recommendations for caffeine supplementation based on genotype.

Caffeine can enhance physical performance when supplemented in the forms of capsules, gum, solutions, gels, coffee, and energy drinks[455, 456]; however, most studies have used capsules as the administration method, which facilitates dosage accuracy, making this the preferred supplementation form. The "optimal" caffeine dose appears to range from 3 to 6 mg/kg of body mass[26], although lower doses (e.g., 1 and 2 mg/kg) have also been shown to effectively improve physical performance[457, 458]. Doses exceeding 6 mg/kg do not seem to provide additional benefits and they may increase the risk of side effects such as anxiety, irritability, nausea, and tremors[459]. The "ideal" timing for caffeine intake seems to depend on the absorption rate of the chosen administration form. Capsules should be taken around 60 minutes pre-exercise[26] and gum should be chewed approximately 15 minutes before the test[460]. However, repeated caffeine supplementation in lower doses may be effective for long-duration aerobic endurance exercises[461].

Approaches for preventing caffeine-induced gastrointestinal disturbances include using low-to-moderate doses (<500 mg) and avoiding/minimizing exacerbating factors (stress, anxiety, other stimulants, fasting).

***This Guideline recommends the isolated use of caffeine as an ergogenic aid at appropriate doses. The recommendation should primarily consider individual characteristics. To avoid gastrointestinal issues, it is also recommended to "train the gut" with supplementation before. Level of evidence: high; strength of recommendation: strong.***

## Creatine

Creatine was discovered in 1832 and it is characterized as a nitrogenous amine[462]. Creatine is endogenously formed from the amino acids glycine, arginine, and methionine, primarily through reactions involving the liver and kidneys[463]. Additionally, creatine can be obtained through diet (e.g., animal-derived foods such as beef and fish). In an omnivorous diet, the intake of creatine is expected to be approximately 1 to 1.5 g/day, although it depends on the variability of the foods consumed[463, 464]. Its primary reservoir is the skeletal muscle, which stores about 95% of the creatine of the body[464]. Since saturation is not achieved exclusively through diet or endogenous production regardless of dietary patterns (e.g., omnivores vs. vegetarians), supplementation is necessary for those who wish to maximize creatine stores[465].

During supplementation, endogenous creatine production decreases but returns to normal levels after discontinuation[466, 463.] Over the last few decades, creatine has been identified as one of the most important ergogenic supplements[467], mainly due to its ability to bind to inorganic phosphate and form phosphocreatine/creatine phosphate, thereby optimizing the ATP resynthesis capacity of the ATP-CP system during high-intensity, short-duration physical exercises.

Two protocols are commonly used to increase creatine stores in skeletal muscle. The loading protocol involves supplementing creatine (approximately 20 g/day) for a period of 5 to 7 days, with doses of about 5 g taken four times a day[468, 469]. The relevance of the loading protocol is debated for physically active individuals or even high-performance athletes who do not compete within a week (approximately 7 days). If there is no need to rapidly maximize creatine stores in skeletal muscle, the maintenance protocol is sufficient to maximize muscle creatine content, although it requires more time. The maintenance protocol, in turn, involves supplementing creatine in single doses of 0.03 to 0.05 g/kg for a period of at least 28 days. Both loading and maintenance protocols achieve similar levels of creatine in muscle tissue[468, 469]. Following these periods (e.g., 5 to 7 days or 4 weeks), a 20 to 40% increase in total creatine stores (the sum of free and phosphorylated creatine) and phosphocreatine is expected[465, 468].

Regardless of the protocol, carbohydrate intake associated with creatine supplementation optimizes creatine uptake by skeletal muscle, an effect dependent on insulin[470]. Therefore, organizing creatine intake with carbohydrate-rich foods and supplements may improve creatine retention in skeletal muscle. Creatine monohydrate is considered the primary form of creatine, mainly due to its high bioavailability, stability, low cost, and the substantial body of scientific evidence supporting its efficacy and safety[465, 471, 472].

The effects of creatine supplementation have been evaluated in numerous clinical trials over the past decades. Consequently, different systematic reviews and meta-analyses have been published considering various outcomes related to physical performance[473-478]. For example, the effect of creatine supplementation on lower limb muscle strength was evaluated using the squat and leg press exercises, with ES of 0.33 (95% CI: 0.047–0.62) and 0.29 (95% CI: 0.098–0.49), respectively[478]. Additionally, the effect of creatine was evaluated on upper limb muscle strength using chest exercises performed with a barbell (e.g., bench press) or with dumbbells. The ES were 0.26 (95% CI: 0.13–0.39) and 0.67 (95% CI: 0.14–1.2), respectively[477].

For repeated sprints, the effect of creatine supplementation was evaluated in various ways. For peak power, creatine had no positive effect compared to the placebo group (ES: 0.41; 95% CI: -0.08–0.90). However, positive effects were observed for mean power (ES: 0.61; 95% CI: 0.23–1.00). In subgroup analysis, the effect was observed in sprints performed on a bicycle (ES: 0.82; 95% CI: 0.19–1.45), whereas no positive effect was observed in running sprints (ES: 0.49; 95% CI: -0.01–0.99). Nevertheless, for the latter, the p-value was 0.06 with low heterogeneity (33%), suggesting, beyond the statistical analysis, a potential clinically relevant effect for high-performance athletes, as minimal differences can be decisive in competition[476].

In soccer players, the effect was evaluated for predominantly aerobic and anaerobic performance tests. In the anaerobic Wingate test, a large effect size of creatine was observed compared to placebo (ES: 2.26; 95% CI: 1.40–3.11)[464]. In contrast, when analyzing $\dot{V}O_{2max}$ as an outcome, no ergogenic effect of creatine supplementation was observed (ES: -0.20; 95% CI: -0.39– -0.001)[474]. Similarly, another SRMA showed that creatine supplementation did not improve performance in endurance-type physical exercises (ES: -0.07; 95% CI: -0.32–0.18)[473].

Creatine supplementation is endorsed by the IOC[467] and the ACSM[5]. Additionally, the International Association of Athletics Federations (World Athletics), in its 2019 consensus, also endorsed the use of creatine as an ergogenic aid[479]. Finally, the safety of creatine supplementation has been tested Based on the available evidence, its use is safe at recommended doses, particularly for healthy adults, athletes, or high-performance athletes[472].

***This Guideline recommends creatine supplementation in adequate amounts to increase muscle strength and physical performance in high-intensity, short-duration exercises. Level of evidence: high; Strength of recommendation: strong. On the other hand, creatine supplementation for endurance-type exercises (for short periods) is not recommended: Level of evidence: moderate; Strength of recommendation: weak.***

## Buffers

The human body has various acid-buffering systems ($H^+$ ions), which include intracellular and blood chemical buffers, dynamic buffering, and the actions of the respiratory and renal systems that maintain intra- and extracellular pH within physiological ranges[480]. These systems are well-regulated and highly efficient under normal physiological conditions[481, 482]. During high-intensity physical exercise, there is intense production of $H^+$ ions, exceeding the capacity of the buffering systems to neutralize them, leading to accumulation of $H^+$ ions, drop in intramuscular pH, and acidosis[483, 484]. Muscular acidosis can affect physical performance through several mechanisms, such as (i) inhibition of key glycolytic pathway enzymes[485]; (ii) competition between $H^+$ ions and $Ca^{2+}$ ions for binding sites in the muscle contractile apparatus[486]; (iii) reduction in the rate of ATP production. All these mechanisms impair muscle contraction, reducing the cross-bridge formation speed and the force production by skeletal muscle, leading to muscle fatigue[487, 488]. Therefore, increasing the concentration of intra- or extracellular buffers in the body through nutritional strategies such as beta-alanine supplementation and/or sodium bicarbonate supplementation becomes potentially ergogenic and will be discussed below.

## Beta-Alanine

Beta-alanine is a non-proteinogenic amino acid produced in small quantities by the liver during uracil degradation metabolism. It is essential for the synthesis of carnosine in skeletal muscle and other tissues[489]. Carnosine is a dipeptide formed by a beta-alanine linked to L-histidine and its synthesis is catalyzed by the enzyme carnosine synthase. Carnosine is abundantly found in human skeletal muscle (approximately 10 to 40 mmol/kg of dry muscle)[490, 491], where it plays significant physiological roles, such as maintaining acid-base homeostasis[489, 492, 493], regulating $Ca^{2+}$ handling and sensitivity during skeletal muscle contraction[494, 495], and scavenging toxic products of lipid peroxidation[496].

The low availability of beta-alanine is the limiting factor for intramuscular carnosine synthesis. Therefore, increasing beta-alanine intake becomes the most effective way to enhance muscle carnosine synthesis[497]. Although beta-alanine is naturally present in animal-derived foods (e.g., meat, fish, and poultry), the daily intake in an omnivorous diet is only about 500 mg[498, 499]. While this amount is sufficient for omnivores to have about twice the muscle carnosine content as vegetarians[500], the amount of beta-alanine obtained through diet is far below the doses necessary for muscle carnosine to reach near saturation levels (approximately 3.0 to 6.0 g/day)[490]. For this reason, beta-alanine supplementation is recommended, which can increase muscle carnosine content by approximately 40 to 100%[490, 501], depending on the dose and duration of supplementation administered[490, 502].

Beta-alanine supplementation should be performed chronically, daily, for periods typically ranging from 4 to 12 weeks, although longer periods may be used if necessary. Doses range from 3.2 to 6.4 g/day and should be split throughout the day, with individual doses of 0.8 to 1.6 g taken at minimum intervals of 3 to 4 hours[503], with or without meals[504]. Splitting the supplementation is important to avoid paresthesia, an acute side effect characteristic of high doses of beta-alanine ingestion, and to reduce losses of beta-alanine through urine and muscle oxidative metabolism. It is important to note that paresthesia is associated with plasma peaks of beta-alanine and both its occurrence, and the intensity of symptoms depend on the plasma concentration of beta-alanine resulting from the dose ingested. Symptoms typically begin around 20 minutes after ingestion and may persist for up to about 1-hour post-ingestion[503,504].

Beta-alanine can be consumed in powder form (either diluted in water or encapsulated) or in special slow-release coated tablets or pills. The use of special tablets aims to delay intestinal absorption, creating a more prolonged plasma beta-alanine increase profile with smaller peaks, reducing the incidence or intensity of paresthesia and allowing better utilization of beta-alanine for carnosine synthesis[505]. If consumed in powder form, it is recommended to use single doses below 800 mg, as they generally do not result in paresthesia. Higher doses up to 1600 mg will likely cause moderate paresthesia and doses above 1600 mg may cause intense paresthesia. If consumed in coated tablets, it is recommended to use doses below 1600 mg[503-505].

A solid body of evidence[503, 506] shows that beta-alanine supplementation has positive, though small (0.2 to 3.0%), but statistically significant effects on performance in high-intensity physical exercises, whether continuous or intermittent, lasting approximately 30 seconds to 10 minutes, as these are exercises in which there is intense muscle acidosis[503]. Given the need for chronic beta-alanine supplementation to adequately increase intramuscular carnosine, athletes along with their technical team should consider the routine, adherence possibility, and investment required in the product before starting supplementation.

## Sodium Bicarbonate

The bicarbonate ion ($HCO_3^-$) is found in high concentrations in the bloodstream (approximately 25 mmol/L)[507-509], making it the main extracellular buffer in the human body[510]. As mentioned earlier, during high-intensity physical exercise, there is an accumulation of $H^+$ ions in skeletal muscle[483, 484], and some of these ions are transported out of the muscle and buffered in the interstitium or blood. Bicarbonate ions neutralize $H^+$ ions in the bloodstream, leading to the formation of carbonic acid ($H_2CO_3$) and later water and $CO_2$, which is exhaled by the lungs. This mechanism is essential to maintaining blood pH within physiological ranges[481, 482, 508].

The purpose of sodium bicarbonate supplementation is to increase blood bicarbonate concentration, thereby increasing the extracellular buffer reserve. For an ergogenic effect, blood bicarbonate increases must exceed a certain threshold. The exact threshold is still a matter of debate. It lacks direct evidence and likely varies among individuals. However, it is assumed that increases must be at least 4 to 6 mmol/L above baseline values[511]. Therefore, appropriate doses of sodium bicarbonate should be administered.

Supplementation can be performed acutely or chronically[481, 511]. For acute supplementation, a dose of 0.3 g/kg body mass is considered optimal, as it will likely result in blood bicarbonate increases above ergogenic thresholds, sustaining these increases for about 4 hours post-ingestion[507]. Doses of 0.2 g/kg body mass can also elevate blood bicarbonate above ergogenic thresholds but for substantially shorter periods[481, 507, 511]. Doses above 0.3 g/kg body mass are not recommended for acute supplementation, as they do not offer greater performance benefits and increase the incidence and severity of adverse effects[511]. Acute sodium bicarbonate supplementation should be performed 60 to 180 minutes before physical exercise and/or competition[511].

Chronic supplementation can be performed 3 to 7 days before physical exercise and/or competition[511]. Daily doses of 0.4 g/kg to 0.5 g/kg body mass should be administered during this period. However, the daily dose should be split into smaller doses throughout the day (0.1 g/kg to 0.2 g/kg per dose)[481, 511]. The advantages of chronic supplementation protocols include reduced gastrointestinal side effects associated with acute sodium bicarbonate ingestion[511] and ergogenic effects that can be maintained for longer periods, with reports indicating an ergogenic window of up to 48 hours after supplementation ends[508]. However, attention should be paid to the high sodium content and its potentially deleterious health effects associated with high doses of sodium bicarbonate, especially when consumed chronically.

The most common side effects of sodium bicarbonate supplementation include nausea, vomiting, abdominal pain and bloating, flatulence, and diarrhea, which can vary in incidence and intensity among individuals. Taking smaller doses with a carbohydrate-rich meal are strategies that can minimize the likelihood or severity of side effects[481, 511, 512]. New strategies to mitigate side effects include splitting the 0.3 g/kg dose into smaller doses of 0.1 to 0.15 g/kg, using smaller doses (0.2 g/kg) combined with individualized timing to peak (that requires prior determination of the time to peak blood bicarbonate for each individual, which is not feasible outside research settings), and using gastro-resistant capsules.

Despite the side effects and adverse effects of sodium bicarbonate supplementation, its ergogenic efficacy is well-established in the literature[507, 508, 513-515]. Sodium bicarbonate supplementation shows small (approximately 2%) but significant effects on continuous or intermittent high-intensity physical exercises lasting between 30 seconds and 12 minutes, such as combat sports and some cycling, running, rowing, and swimming modalities in men[481, 509] and women[513]. Therefore, sodium bicarbonate supplementation can be used as a strategy to delay fatigue during high intensity exercise[26]; however, it is important to consider individual variability for side effects related to supplementation, as they can negatively affect performance during physical exercise.

***This Guideline recommends the use of beta-alanine and sodium bicarbonate to improve performance in high-intensity physical exercises where acid-base imbalance due to $H^+$ ion accumulation is the key factor for peripheral fatigue. However, the cost-benefit should be evaluated, as well as the adverse effects generated, especially by sodium bicarbonate. The amounts found in "pre-workout" supplements are insufficient; thus, use should be***

***individually planned for each substance. Evidence level: high; recommendation strength: strong.***

## Nitrate

Nitric oxide (NO) is a gaseous molecule that plays a key role in several regulatory mechanisms essential to human life, such as vasodilation, mitochondrial respiration, glucose and calcium homeostasis, muscle contractility, and more[516]. Due to its short half-life in the human body, NO production occurs continuously via two distinct but complementary pathways. The first pathway occurs under aerobic conditions and involves the conversion of L-arginine to NO by the enzyme nitric oxide synthase (NOS)[517]. The second pathwaybecomes more active under anaerobic conditions and involves the progressive reduction of nitrate and nitrite to NO[518]. Vegetables rich in nitrate, such as lettuce, arugula, spinach, and beetroot contribute to NO production when NOS activity is limited. Nitrate metabolism primarily involves the mouth and gastrointestinal tract and it can influence metabolic aspects related to physical exercise[519].

Since the initial findings that nitrate supplementation in the form of sodium nitrate or beetroot juice can increase plasma nitrite concentration and reduce the oxygen cost of submaximal exercise by approximately 5%[520, 521], many studies have investigated its effects. Currently, several SRMA suggest that nitrate can benefit physical performance[522-529]. Evidence indicates that nitrate can improve performance in activities such as cycling (4, 10, and 16.1 km)[520, 530], middle-distance running (1500 m)[531], long-distance running (3000 m)[532], rowing (2000 m)[533], canoeing (500 m)[534], and team sports[535].

Over the past decade, several high-quality SRMAs have been published. The SRMA conducted by Hoon et al.[529] a moderate effect on time-to-exhaustion exercises (ES = 0.79; 95% CI: 0.23 – 1.35) was reported, despite great imprecision. In 2017, the SRMA conducted by McMahon et al.[528] a small to moderate effect on time-to-exhaustion exercises (ES = 0.33; 95% CI: 0.15 – 0.50) was identified. More recently, Senefeld et al.[523] observed a small effect of nitrate on physical performance (ES = 0.174; 95% CI: 0.120 – 0.229).

Despite the strong evidence for the ergogenic effect of nitrate[522, 523], several factors seem to influence the efficacy of its supplementation[522, 523]. Recent meta-analytic data suggest that nitrate is most effective in exercises lasting between 2 and 10 minutes[522], although there is evidence that it benefits the performance of continuous exercises lasting up to about 30 minutes[520], with no effects when the volume exceeds 30 minutes[536-539]. Regarding aerobic capacity, individuals with a maximum $\dot{V}O_2$ of ≥ 65 ml/kg/min (e.g., elite endurance athletes) do not seem to benefit significantly from nitrate supplementation, although some studies suggest that around 20-25% of highly trained endurance athletes ($\dot{V}O_{2max}$ ≥ 65 ml/kg/min) may benefit from this supplement. As for biological sex, while there is strong evidence that nitrate supplementation can improve male performance, most studies investigating the ergogenic effect of nitrate in women has not shown positive effects[522, 523]. However, it is possible that the lack of effect in most studies on the female population is due to suboptimal methodological aspects in these studies rather than sex differences per se[522]. Regarding supplementation strategy, recent SRMA[522, 523] suggest that nitrate can improve performance when supplemented acutely or chronically at doses > 5 mmol (> 310 mg), with the last dose ingested ≥ 120 minutes before exercise. A recent Delphi consensus published by Shannon et al.[540] suggests that consuming 8-16 mmol acutely or 4-16 mmol chronically 2-4 hours before exercise seems to produce positive effects on physical performance.

Finally, concerning intervening factors, the use of antibacterial mouthwash may reduce the diversity of bacteria in the oral cavity, impairing the reduction of nitrate to nitrite[541] and possibly attenuating its potential ergogenic effect[522]. Besides its ergogenic potential, nitrate intake may lower systemic blood pressure[542] and improve vascular function in individuals with hypertension[543], as well as increase physical tolerance in people living with chronic obstructive pulmonary disease[544], peripheral arterial disease[545], and heart failure with preserved ejection fraction[546]. Moreover, there is a consensus that acute supplementation of up to 16 mmol of nitrate is not toxic and does not have harmful effects on health[540]. Although there is a consensus on the absence of toxicity from chronic nitrate supplementation, more studies are needed to determine its safety, though harmful health effects are unlikely if plant-based foods are the source of nitrate[540].

***This Guideline recommends nitrate supplementation primarily from beetroot juice. However, it is important to note that performance improvements seem to occur mainly in trained individuals but not in elite athletes. Evaluating gastrointestinal tolerance and the kinetics of nitrate conversion to nitric oxide is essential for individual supplementation adjustments. Level of evidence: moderate; strength of recommendation: strong.***

## Glycerol

Glycerol (1, 2, 3-propanetriol) is a three-carbon alcohol naturally found in the human body, produced and distributed in various cells in low quantities (< 0.1 mmol/L)[547]. In healthy individuals, blood glycerol concentrations range from 0.05 to 0.3 mmol/L. Glycerol is part of the triacylglycerol structure and it can also be an intermediate in metabolic pathways such as glycolysis, gluconeogenesis, and fatty acid synthesis[547]. In eutrophic individuals, 38% of glycerol is converted to glucose in the liver and less than 10% of the $CO_2$ produced comes from glycerol oxidation, suggesting its minor role as an energy substrate[547].

Glycerol can also be obtained as a dietary supplement, and it is easily found in pharmacies under the name glycerin. The exogenous use of glycerol aims to increase water retention for sports performed in high-temperature conditions. Glycerol absorption in the intestine occurs simultaneously with increased water absorption by osmosis. Thus, in its free form, it is considered an osmotically active substance and, therefore, an agent capable of increasing the cellular hydration status[548, 549]. Additionally, glycerol reduces urine production because it is reabsorbed in the proximal and distal tubules of the nephrons, which increases the osmolarity of the interstitial fluid around the epithelial cells, creating a favorable gradient for fluid reabsorption. Therefore, glycerol contributes to increasing the osmotic pressure of body fluids, resulting in transient intracellular water retention[548, 549].

Given that dehydration can negatively affect physical performance, glycerol supplementation emerges as a potential ergogenic aid, especially in long-duration physical activities where dehydration during exercise leads to decreased physical performance[408]. Previous research has shown that a 2% reduction in body mass negatively affects physical performance[550]. The continuous loss of body water in the form of sweat can result in an increase in core temperature and heart rate, as well as a progressive reduction in pulmonary and systemic pressures and an overall decline in cardiac output as stroke volume decreases beyond the compensatory limit, reducing exercise tolerance[551].

It is believed that hyperhydration before physical exercise may provide thermoregulatory advantages during exercise, especially in high temperature conditions[408]. Hyperhydration reduces core temperature and increases sweat production, optimizing body cooling[408]. Glycerol was once banned for use in physical exercise due to its ability to mask doping substances in the bloodstream[552]. However, in 2018, it was formally removed from the World Anti-Doping Agency (WADA) prohibited list.

After 12 hours of fasting, glycerol ingestion (1.2 g/kg) increased blood levels from 0.05 mmol/L to 19.3 ± 3.6 mmol/L in approximately 90 minutes[553]. The most frequently studied amount of glycerol ranges between 1.2 and 1.4 g/kg of body mass with 20 to 26 mL of water per kg of body mass, 30 to 180 minutes before physical exercise[554]. Its use can also be applied during physical exercise (0.125 g/kg) with 5 mL of water/kg[554]. Finally, after exercise, it is suggested to ingest approximately 1 g of glycerol/kg of body mass with 1.5 L of water or according to the amount of water lost during exercise (about 150%)[554].

Studies evaluating the effect of glycerol supplementation on physical performance often involve small samples and present varied results. The main source of variation in results appears to be the environment. Notably, studies conducted in hot conditions showed more positive results. Conversely, in environments with suitable temperatures for sports practice, the effects of glycerol on physical performance seem to be small. A SRMA published in 2007 establishes that the effects of glycerol on physical performance need further exploration[555]. In this paper, the authors suggest a 2.62% improvement (95% CI: 0.07 – 5.17%) in physical performance (ES: 0.35; 95% CI: 0.14 – 0.56)[555].

***This Guideline recognizes that despite the potential positive effects, further well-designed studies are needed to understand the real impact of glycerol on physical performance. Moreover, various adverse effects have been reported over the years, such as headaches, gastrointestinal discomfort, and nausea. Level of evidence: moderate; strength of recommendation: weak.***

**Bioactive Compounds**

Bioactive compounds are organic substances extracted from natural sources that are present in both plant and animal-based foods. The mechanisms of action of bioactive compounds primarily involve reducing inflammatory processes and oxidative stress[556]. The effects of polyphenols on various parameters related to physical exercise have been investigated in recent years, but despite potential positive effects on markers of exercise-induced muscle damage, the level of evidence is low[557, 558]. This is due to the numerous methodological flaws observed in the studies, leading to a high risk of bias in the estimates. The SRMA conducted by Carey et al.[559] demonstrated that the intake of polyphenolic flavonoids improved muscle recovery (7.14%; 95% CI: 5.5 – 8.78) and reduced muscle soreness (-4.12%; 95% CI: -5.82, -2.41). Additionally, the use of herbal supplements is quite common. However, the level of evidence for outcomes such as physical performance is low. Narrative reviews that detail mechanisms of action suggest different biochemical pathways that are modulated and could partially explain the potential positive effect[560].

***This Guideline does not encourage the intake of bioactive compounds through dietary supplements to optimize physical performance. Adequate intake of a diverse range of foods, especially fruits, vegetables, and greens, should be part of the dietary routine for athletes and sportspeople to ensure sufficient amounts of these compounds. Level of evidence: low; strength of recommendation: weak.***

**Doping**

It is understood that the effect of dietary supplements on physical performance or body composition is modest, which may drive the use of substances with greater potential ergogenic effects. Consequently, it has become increasingly common for athletes to seek dietary supplements that can maximize physical performance, regardless of the degree of scientific evidence and strength of recommendation[561]. However, many of these dietary supplements, particularly those containing multiple substances, may include compounds considered prohibited and classified as doping by WADA[424, 425].

Furthermore, it is believed that individuals who use dietary supplements are more susceptible to using substances considered as doping. For example, Hurst et al.[562] recently found that doping was 2.74 times more prevalent (95% CI: 2.10 – 3.57) among supplement users compared to non-users. While the doping frequency was 14.7% among dietary supplement users, it was 6.7% among those who did not use supplements[562].

Dietary supplements contain various undeclared substances, including: (i) testosterone and derivatives; (ii) 1,3-dimethylamylamine (1,3-DMAA); (iii) higenamine; (iv) sibutramine, andarine, ostarine, synephrine, yohimbine, ephedrine, and (v) diuretics[563-565]. Other substances may also be present in dietary supplements, varying by the country of origin and their potential effects on the body[564]. In Brazil, stimulants and anabolic agents are more commonly found in dietary supplements[566].

Moreover, in Brazil, doping control tests are conducted by the Brazilian Doping Control Authority (ABCD), which summons athletes for specific exams to obtain biological material for investigation. In this context, the methods for evaluating substances considered as doping have been increasingly optimized and applied, particularly in major competitions like the Olympics[567-569.]

One of the justifications for defending athletes with positive doping tests has been the use of contaminated dietary supplements, known as unintentional doping[564]. Although dietary supplements originally do not contain any substances classified as doping (e.g., caffeine, creatine, beta-alanine, sodium bicarbonate, nitrate, and glycerol), several studies have shown that dietary supplements contain undeclared substances that are considered prohibited and, therefore, doping[564]. It is believed that some dietary supplements contain hormones or other substances classified as prohibited[564].

A recent study published by Alaedini et al.[570] identified the presence of testosterone in 11 samples of dietary supplements, nine of which were whey protein. Notably, testosterone or its derivatives are the most commonly found substances in dietary supplements. Similarly, a French study reported a high prevalence of 1,3-dimethylbutylamine (DMBA)[571]. Findings from a Chinese study are comparable, showing various anabolic steroids in dietary supplements[572].

Several factors need to be considered, such as the quality of information available on the Internet, conflicts of interest from prescription to scientific evidence, and the ease of acquiring dietary supplements, which often provide incomplete information about their components[573-576]. Thus, the constant analysis of products and the dissemination of results can serve as a warning system, helping athletes and other consumers make informed choices, avoiding unintentional doping, and primarily, safeguarding health.

Currently, it remains complex to distinguish dietary supplements with or without prohibited substances. Therefore, it is crucial for athletes to be cautious about the source of the product purchased, to seek brands with greater reliability, and to avoid products that may have significant media appeal or suspicious characteristics. Dietary supplements with multiple ingredients may also have a higher probability of containing undeclared substances.

***This Guideline does not recommend the use of pre-workout dietary supplements with multiple substances or dietary supplements with unclear descriptions of their components. The risk of unintentional doping is high, and therefore, the selection of dietary supplements should consider various aspects, particularly the integrity of the company producing the supplement. Notably, the use of substances listed on the WADA prohibited list is entirely inappropriate, as it exposes athletes to doping and various health problems. Level of evidence: moderate; strength of recommendation: strong.***

## Athletes in Competitions and Team Sports

As mentioned earlier, nutrition studies applied to physical exercise generally exhibit high internal validity, but they often suffer from low external and ecological validity. This limitation complicates the extrapolation of laboratory evidence to real-world settings.

Therefore, translating nutritional recommendations into practical food choices should be based on the expertise of Sports and Exercise Nutritionists and the individual needs of each athlete, considering specific habits and practices. Notably, the decision-making process regarding the choice of pre-competition menus should be grounded in an understanding of athletes' habits, individual needs, and adherence to collective dietary plans.

In this context, one of the major challenges for technical teams is guiding the dietary intake of athletes involved in team sports, especially on competition days. For instance, selecting the appropriate menu on competition days is particularly challenging when athletes are away from training centers, such as during land or air travel[479]. A recent systematic review evaluated the effect of nutritional counseling in team sports, highlighting that such interventions can enhance athletes' knowledge of dietary practices, thereby increasing their autonomy and improving food choices to optimize physical performance and recovery between training sessions or post-competition[577]. However, due to the heterogeneity among the included studies in terms of sport modality, competition level, age, and biological sex of the athletes, as well as the type of intervention (e.g., online or in-person), it is difficult to establish ideal nutritional education interventions for each analyzed variable.

Another systematic review reported findings that support nutritional counseling[578]. The authors found that nutritional counseling led to positive changes in dietary knowledge, which in turn influenced dietary intake (quality and/or adequacy), ultimately benefiting physical performance, recovery, and issues related to energy deficiency.

Increased energy intake before competition enhances physical performance and specific complaints can be avoided, particularly gastrointestinal issues, by when appropriate foods are selected[579]. Nutritional planning for team sports competitions requires attention to food safety, yet there is weak evidence supporting the inclusion of specific foods for athletic performance. We believe that this approach should be systematic and supervised by Nutritionists specializing in Sports and Exercise, maintaining muscle recovery with individualized care according to the different training phases.

## Athletes with Special Needs

Although sports for individuals with disabilities are gaining increasing attention, the literature on nutrition for this population remains scarce. Moreover, factors such as the heterogeneity of disabilities (e.g., the level of spinal cord injury, whether it is a complete or incomplete injury; type and extent of amputation; type of cerebral palsy), data collection difficulties, and most importantly the lack of methods developed specifically for this population (e.g., body composition assessment) do not yet allow for the determination of evidence levels to answer the following questions. Nonetheless, we provide some reflections based on the limited available evidence that may guide Nutritionists in their professional practice.

### Estimating Energy Needs in Para-Athletes

The determination of energy needs in para-athletes is influenced by physical, metabolic, and neurological factors[580, 581], with both BMR/RMR and the EEE, components of TDEE, potentially being affected by the diversity and specificity of disabilities.

### Spinal Cord Injury (SCI)

The alteration of neurological function leads to muscle mass atrophy below the level of injury, impacting BMR/RMR. Thus, factors such as injury level, type of injury (complete or incomplete), reduced heart rate, and maximum oxygen uptake during physical exercise must be considered[582]. RMR continues to decrease over time, mainly due to the reduction in muscle mass[582-585].

Studies indicate that individuals with SCI have lower BMR/RMR compared to those without disabilities[582, 585]. For instance, in a systematic review on nutritional aspects in adults with chronic SCI[585], seven of the 22 studies included observed significant variability (4-15%) between values measured by indirect calorimetry and those estimated by equations.

Lean mass is higher in physically active individuals with SCI than in sedentary controls[584]. Two studies with athletes provide evidence supporting this statement, as well as evaluating the agreement of predictive equations with BMR measured by indirect calorimetry. Pelly et al.[583] compared the RMR of athletes with SCI (n=6; wheelchair tennis, wheelchair basketball, handcycling, and water skiing) with non-disabled individuals, matching them by lean mass and found no differences between groups. When RMR was adjusted for lean mass, it was higher in athletes with SCI compared to controls, even with differences in body composition between the two groups. This finding seems to be related to the injury level of the participants: if the SCI is between T1 and T10 (high paraplegia), it may influence the integrity and metabolic activity of tissues that significantly contribute to RMR. When comparing the measured RMR with the estimated RMR, they found that Cunningham's predictive model[42, 44] best estimated RMR in these athletes, with a difference of 64 kcal/day. Owen's equations[586, 587] significantly underestimated it, while Mifflin's[588], Harris-Benedict's[43], and Schofield's[589] equations overestimated it, but without statistical significance.

Broad et al.[584] identified in rugby players (n = 8) with SCI that equations using lean mass (predictive models by Chun[590], Cunningham[42, 44], Mifflin[588], Nightingale[591], and Owen[586, 587]) as a predictive variable showed strong agreement with RMR measured by indirect calorimetry, with Cunningham's model being the most accurate.

## Visual Impairment (VI)

Only one study compared the RMR measured by indirect calorimetry in track and field athletes with VI (n = 11), across different events, with predictive equations developed for non-disabled populations. The study found no significant difference between the measured RMR and the estimated RMR using the Owen[586, 587], DRI, FAO/WHO[592], and Mifflin[588] predictive models. However, the equations overestimated the RMR by 146 to 341 kcal/day[593].

## Amputation (AMP)

Energy expenditure increases with more proximal amputation[581]. In non-athletes, the RMR values assessed by indirect calorimetry were underestimated by the predictive models of Mifflin[588], Harris and Benedict[43], and Owen[586, 587] by 65%, 61%, and 74%, respectively[594]. Additionally, the energy expenditure for ambulation must be considered. In the study by Mengelkoch et al.[595], which evaluated energy expenditure during walking and running in non-athletes with transfemoral (above-knee) amputations, the $\dot{V}O_2$ during walking and running was observed to be higher by 45-78% and 29-34%, respectively, compared to non-amputees. Another study compared the energy expenditure in unilateral amputees at different levels (transfemoral, transtibial, and partial foot) using treadmill ergospirometry at four different speed combinations. In all cases, energy expenditure was lower for transtibial (below-knee) amputees and higher for transfemoral amputees[596].

In athletes, only two studies compared the measured BMR/RMR by indirect calorimetry with predictive equations for non-disabled populations. Juzwiak et al.[593] observed that all predictive equations overestimated the measured BMR (n = 11) except for the Owen predictive models. Beezhold et al.[597] found that the measured RMR (n = 1) was higher than the results obtained by the Harris and Benedict[43] and Mifflin[588] equations. There is insufficient evidence to establish the magnitude of the impact of disability on BMR/RMR in athletes with AMP. However, caution is needed, considering the type and extent of the amputation.

## Cerebral Palsy (CP)

In people with CP, inefficiency in ambulation and an inability to control movement (ataxia, spasticity, athetosis) can affect the estimation of energy needs. In non-athletes, two studies measured BMR using indirect calorimetry in adults. One study showed that the contribution of BMR to TDEE in ambulatory adults with CP is lower than in non-ambulatory individuals, at 65% and 74%, respectively. In contrast, the contribution of physical activity energy expenditure was higher in ambulatory adults (25%) than in non-ambulatory adults (16%). Moreover, adults with CP (n = 21) exhibiting athetosis had a 14% higher BMR, after adjusting for lean mass, compared to adults without disabilities[598].

In athletes, only one study on track and field athletes with CP (n = 8) across different events compared the BMR measured by indirect calorimetry with various predictive equations (Cunningham[42, 44], Owen[586, 587], Harris and Benedict[43], DRI, FAO/WHO[592], and Mifflin[588]), and all equations overestimated the RMR in these athletes by 125 to 299 kcal/day[593]. There is insufficient evidence to determine the magnitude of the impact of disability on BMR/RMR in athletes with CP.

Considering the existing evidence regarding the impact of disabilities on energy requirements and estimation methods, the use of indirect calorimetry is recommended for assessing BMR/RMR in para-athletes. Caution is advised when using predictive equations for BMR/RMR estimation in this population, as equations developed for non-athletes or non-disabled athletes may either overestimate or underestimate BMR/RMR.

## Body Composition Assessment in Para-athletes

Numerous methods are widely used to estimate body composition in athletes without disabilities. However, these methods have limitations, some degree of measurement error, and lack validation for estimating body composition in para-athletes, especially those with spinal cord injury (SCI), the focus of most studies. This is because the assumptions of these methods may not be met due to the specific characteristics of each disability, leading to difficulties in interpretation[599].

## Spinal Cord Injury (SCI)

In a SRMA published by Raguidin et al.[600], which included 11 studies on body composition assessment in adults with SCI at different injury levels, assessed by DXA, it was observed that individuals with tetraplegia have a higher body fat percentage and lower lean mass compared to those with paraplegia. Moreover, factors such as muscle spasticity, hormonal alterations, particularly testosterone, growth hormone, and insulin-like growth factors, and the level of injury are related to the specificities of SCI and can influence body composition, mainly the ability to maintain lean mass, muscle strength, and accumulate total and regional fat.

In athletes with SCI, two studies (n = 16 and n = 30, respectively)[601, 602] compared different methods of body composition assessment (air displacement plethysmography [BODPOD®], skinfold thickness [SF], BIA, and DXA) at different injury levels, and found that all methods underestimated body fat percentage compared to DXA.

Three studies evaluated which predictive equation would be most suitable for this population. Bulbulian et al.[603] suggested that predictive equations are not valid for estimating body density in athletes with paraplegia (n = 22), overestimating body density and underestimating relative fat. Goosey-Tolfrey et al.[601] showed that predictive equations might underestimate body fat percentage by 8% to 14% compared to DXA, a finding also observed by Willems et al.[604]. Additionally, Goosey-Tolfrey et al.[601] developed two predictive equations with a high correlation to DXA, using 7 SF measurements and calf circumference ($R^2 = 0.84$). Sutton et al.[605] identified that waist circumference had a strong relationship with body fat percentage measured by DXA and observed that the difference between body fat percentage values obtained by DXA and those estimated by equations in women with SCI increased with higher body fat percentages.

### *Cerebral Palsy (CP)*

In non-athlete adults, a systematic review published by Hombergen et al.[606] assessed the impact of CP on physical fitness, including three studies among the nine included in the review that investigated body composition using predictive equations based on skinfold measurements. Conflicting results were found when compared to results obtained in adults without disabilities. In a study with adults with CP, Hildreth et al.[607] compared body composition estimates using BIA, DXA, and skinfold thickness (Jackson and Pollock predictive equation[608, 609]) with measurements obtained using DLW. They observed that the BIA and skinfold methods overestimated or underestimated body fat percentage, respectively, while DXA showed adequate agreement with DLW.

In athletes, only two studies analyzed body composition results using different methods. Ruciman et al.[610] evaluated body composition using DXA in six athletes with hemiplegic CP, sprinters from classes T37 and T38 (high classes), and observed no difference in bone mineral density and fat mass between the affected and unaffected sides. However, lean mass was higher on the unaffected side than on the affected side, despite similar fat mass between both sides. Sarabia et al.[611] evaluated the body composition of 102 seven-a-side soccer players with different types of CP (spastic diplegia, athetosis/ataxia, spastic hemiplegia, and minimal impairment) and compared them with non-disabled players using three-component body composition models: fat mass, lean mass, and bone mass. All CP groups showed differences in skinfold thickness on both sides of the body compared to the control group. Additionally, the spastic hemiplegia group showed differences between the affected and unaffected sides of the body in all measured variables, except for trunk skinfold thickness, demonstrating the importance of measuring both sides (affected and unaffected) in athletes with CP.

### Amputation (AMP)

Assessing body composition in people with AMP is challenging due to the lack of standardized methods for this population. It is also crucial to consider the location, level, and extent of the amputation[612]. In athletes, a study by Cavedon et al.[613] with men (n = 42) distributed into two groups, above-knee and below-knee amputations, in different sports, investigated the impact of amputation on body composition using DXA. They observed that the level of amputation impacts body composition. For example, men with above-knee AMP had lower total body mass, lean mass, and bone mineral content. However, no differences were observed in body fat percentage and the fat mass/lean mass ratio in both groups. This shows that the level of amputation does not seem to influence fat accumulation associated with reduced lean mass. In another study[612] with male athletes (n = 29) with unilateral lower limb amputation, body composition measured by DXA was compared with values estimated by nine equations developed for athletes and nine for non-athletes. The authors observed that the results obtained with the application of the Durnin and Womersly[614] predictive model were similar to the body fat percentage measured by DXA; however, with a bias of 8.71%. The Forsyth and Sinning[615] equation overestimated the body fat percentage measured by DXA. All other applied predictive equations underestimated body fat percentage compared to DXA. It was observed that as the body fat percentage measured by DXA increased, so did the underestimation/overestimation of body fat percentage estimated by the equations. Additionally, the authors proposed two equations with good predictive performance, one using four skinfold measurements ($R^2 = 0.89$) and the other using the sum of nine skinfold measurements ($R^2 = 0.93$).

Considering the existing evidence on the impact of disability on body composition in para-athletes using different methods, DXA appears to be the most accurate method. However, caution is necessary in some situations, such as the presence of spasticity, the use of pacemakers, or the presence of metal rods or pins that may prevent or induce errors in measurement.

**Low Energy Availability (LEA) in the Para-athlete Population**

There is limited evidence on the risk of LEA among para-athletes with various disabilities, particularly women, with the majority of studies focusing on individuals with SCI. The heterogeneity of this population complicates accurate assessment of LEA, making it difficult to determine which symptoms are related to LEA and which ones are associated with impairment caused by the disability[581, 616, 617]. Moreover, the lack of specific cut-off points for the para-athlete population makes precise evaluation challenging, as cut-offs developed for the non-disabled population are often used[580, 617].

Only one study has evaluated LEA in athletes with different disabilities. Joaquim et al.[618] assessed EA in male and female sprinters with VI (n = 10), AMP (n = 3), and CP (n = 4) using four-day photographic records to assess energy intake, body composition via skinfold thickness, and actigraphy to evaluate EEE. The EA for athletes with VI, AMP, and CP was 36, 37, and 38 kcal/kg of FFM/day, respectively. No athlete exhibited LEA for more than two days.

**Spinal Cord Injury (SCI)**

In athletes, only two studies have evaluated EA, both finding a high prevalence of LEA. Egger et al.[619] assessed males (n = 8) and females (n = 6) with SCI over seven consecutive days using food diaries to evaluate energy intake, while EEE was calculated based on published data on EEE in wheelchair athletes. They found that 93% of the athletes consumed less than 45 kcal/kg of FFM/day. Upon analyzing all days, LEA was observed in 73% of the women and 30% of the men. Hertig-Godeschalk et al.[620] assessed males (n = 6) and females (n = 8) with SCI, using three-day food diaries to evaluate energy intake and calculated EEE according to published data on wheelchair athletes. They found that women had lower EA compared to male athletes, with LEA observed on 58% of the days for women and 34% of the days for men.

***This Guideline recommends that evaluations related to body composition and energy expenditure of athletes with special needs should consider each individual's condition. Important aspects such as LEA should also be monitored, and, if necessary, tools need to be developed. Evidence level: low; recommendation strength: strong. The advancement of Paralympic sport requires studies capable of understanding the nuances of this population, specifically the effects of dietary and nutritional interventions to optimize physical performance.***

**Conclusion**

In conclusion, this guideline recommends quantifying total energy expenditure for dietary planning and using 24-hour recall combined with dietary history to assess the food intake of athletes. Additionally, it recommends screening for REDs by calculating EA and using validated questionnaires, and if available, verifying the primary and secondary criteria established by the International Olympic Committee to maximize screening and risk stratification. Regarding macronutrient management, the guideline recommends carbohydrate intake before prolonged exercise to improve physical performance, while no positive effects are evidenced for short-duration exercise. It also recommends carbohydrate intake during prolonged exercise to enhance physical performance, especially easily digestible and absorbable carbohydrates (e.g., maltodextrin, sucrose, glucose + fructose). For the post-exercise period, this guideline recommends the intake of high glycemic index carbohydrates, especially when recovery time between training sessions is short (e.g., < 8 hours). However, if the time between training sessions is longer (e.g., only the next day), there is no need to accelerate muscle glycogen recovery immediately after exercise. On the other hand, this guideline does not recommend low-carbohydrate strategies to optimize physical performance, nor the acute intake of lipids (e.g., MCT or LCT) before exercise. It recommends that protein intake should be individually adjusted, considering that total daily protein intake is the most important factor for muscle hypertrophy and/or muscle recovery. However, protein does not have an ergogenic effect; therefore, its intake should be aimed at maintaining muscle mass and optimizing training-induced adaptations. Regarding vitamins and minerals, this guideline recommends correcting deficiencies through diet and, if necessary, through supplements with high bioavailability. It also recommends that high-performance athletes be monitored for hydration status, especially if training takes place in hot environments, and hydration adjustments should be based on fluid loss during exercise. Furthermore, this guideline recommends the appropriate use of isolated ergogenic aids such as caffeine, creatine, sodium bicarbonate, beta-alanine, and nitrate. However, despite the potential positive effects, further well-designed studies are needed to improve understanding on the true effect of glycerol on physical performance. Additionally, this guideline does not recommend the use of multi-ingredient pre-workout supplements or supplements with uncertain compound descriptions. The risk of unintentional doping is high; therefore, the selection of supplements should consider various aspects, especially the credibility of the supplement manufacturer. Finally, this guideline recommends that for athletes with special needs, evaluations related to body composition and energy expenditure should be conducted considering each condition and important aspects such as LEA and REDs should also be monitored.

## Conflicts of Interest

MVLSQ, MCCT, EPO, MD, GDP, ARZB, ABB, RRM, RAC, MLA, GFB, SL, KGMS, CMM, LSG, CRJ, DPJ, DCG, JCBM, CFB, AC, HSS, RFC, MNP, CFPC, MS, FLL, VM, EARS, LPN, CGM, FPN, MTM, BS, MMR, RCB, SMLR, TRS reported no conflicts of interest. GGA has had publication costs of articles and congress travel expenses paid by Natural Alternatives International Inc., a company that produces beta-alanine. GGA has also participated as a collaborator researcher in a study on beta-alanine supplementation funded by Natural Alternatives International Inc.

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