Nutritional Supplements & Food in Sport:
Compared effect of ingesting a commercial or homemade recovery beverage with similar nutritional content after exhaustive eccentric exercise on muscle damage, functional recovery, soreness markers, inflammation, oxidative stress, and metabolic parameters

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Keywords: SPORTS NUTRITION, ATHLETES, NUTRITIONAL INTAKE, CARBOHYDRATES, PROTEINS.
I dedicate my thesis to my parents. They have the ability to always see the best in me. They encourage me to be the best version of myself, giving me the love, the inspiration, the strength, the tools, the opportunities, the courage, and the stability I need to go further.
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Abstract

**Purposes:** The purposes of this dissertation were (a) to characterize the nutritional supplements (NS) usage among high-performing athletes, (b) to compare the nutritional intake and nutritional adequacy from food between users and non-users, (c) to understand the determinants of NS usage, and (d) to compare the effect of a commercial (CS) and homemade (MS) recovery beverage, with similar nutritional content, ingested after exercise-induced muscle damage (EIMD).

**Methods:** Considering NS usage (a, b, c), 304 high-level Portuguese athletes from 13 sports completed a (i) NS usage questionnaire, assessing information also on sociodemographic, health-related, and sport characteristics, and (ii) a semi-quantitative food-frequency questionnaire, regarding the previous 12 months. The Estimated Average Requirement (EAR) cut-point method was used to calculate prevalence of micronutrient inadequacy (PMI). To study the impact of CS and MS on EIMD recovery (d), 13 national-level athletes performed 2 trials of an exhaustion protocol comprising a minimum of 300 concentric/eccentric knee extension/flexion repetitions, in a crossover counterbalanced and randomized experimental study. During the 2 h after the protocol, participants ingested 0.8 g carbohydrate·kg⁻¹·h⁻¹, 0.26 g protein·kg⁻¹·h⁻¹ and 60.8 ± 1.0 mg vitamin C, in form of CS or MS (skimmed milk, strawberries and banana). Functional tests were performed before, and immediately after, 24 h, and 48 h after exercise. Soreness scores and blood samples were taken at the same moments and 2 h after exercise.

**Results:** In the final sample (n=292, 68% male, 20.4 ± 4.9 y), 66% of athletes reported NS usage. Multivitamins/minerals were the most frequently consumed supplement, the main cited reason to consume NS was to accelerate recovery, and physicians were the preferable source of information. Non-scientifically supported choices revealed a lack of information and appropriate nutritional strategies (e.g. use of glutamine and magnesium). Due to miss-reporting and incomplete information, the sample was afterwards reduced to 241 athletes (66% males, 13–37 y). NS users reported a generally higher nutritional intake
from food than non-users, even after adjustment for confounders, and a lower PMI for some micronutrients. After adjustment, supplement usage was associated with being ≥18 y, performing individual sports and >2 h gym/week, with a higher intake of meat, eggs, and yogurt, and a lower intake of processed meat, vegetable oils, margarine, chips, and fast food. Regarding the study to compared the effect of CS and MS, the final sample comprised 10 athletes (21.7 ± 3.4 yrs, 73.3 ± 4.5 kg) – 3 were excluded since they were already in a muscle damage process at the beginning of one of the trials [creatine kinase (CK) >1083 U·L⁻¹]. We found that CS and MS led to similar recovery patterns from EIMD regarding muscle damage (CK, myoglobin, lactate dehydrogenase, aldolase, aspartate aminotransferase, alanine aminotransferase), functional recovery (eccentric peak torque of the quadriceps, countermovement jump height), soreness markers (general muscle soreness, soreness from palpation), inflammation (white blood cells, neutrophils, monocytes, basophils, eosinophils, lymphocytes, interleukin-6, tumour necrosis factor-α, C-reactive protein), oxidative stress (total antioxidant status, protein carbonyls, uric acid, glutathione reductase), and metabolic parameters (glucose, total proteins, non-esterified fatty acids, β-hydroxybutyrate, triglycerides, total cholesterol, low-density lipoprotein, high-density lipoprotein, creatinine, urea, alkaline phosphatase, cardiac-specific troponin I).

Conclusions: We conclude that (i) NS consumption among Portuguese athletes was widespread, (ii) athletes consuming supplements were those with a better nutritional intake, (iii) NS users were different from non-users on sociodemographic and sporting characteristics, (iv) food choices of NS users seemed to be both health- and sport-driven and (v) both beverages, independently of their commercial or homemade nature, led to similar recovery patterns from EIMD.

Keywords: SPORTS NUTRITION, ATHLETES, NUTRITIONAL INTAKE, CARBOHYDRATES, PROTEINS.
Resumo

Objetivos: Os objetos desta dissertação foram (a) caracterizar o uso de suplementos nutricionais (NS) em atletas portugueses de alto nível, (b) comparar a ingestão nutricional e a adequação nutricional derivadas exclusivamente de alimentos entre utilizadores e não utilizadores de NS, (c) compreender os determinantes do uso de NS e (d) comparar o efeito da ingestão de uma bebida de recuperação comercial (CS) com um batido caseiro (MS), com conteúdo nutricional semelhante, ingeridos após dano muscular induzido pelo exercício (EIMD).

Métodos: Relativamente ao uso de NS (a, b, c), 304 atletas portugueses de alto nível de 13 modalidades completaram (i) um questionário sobre o uso de suplementos, avaliado também informação sociodemográfica, dados relacionados com a saúde e características desportivas e (ii) um questionário de frequência alimentar semiquantitativo, relativos aos 12 meses transatos. O método de ponto de corte pelas necessidades médias estimadas (EAR) foi utilizado para calcular a prevalência de inadequação de micronutrientos (PMI). Para estudar o impacto do CS e MS na recuperação do EIMD (d) 13 atletas de nível nacional realizaram 2 ensaios que incluíam um protocolo até à exaustão de, pelo menos, 300 repetições concêntricas/excêntricas dos extensores/flexores do joelho, através de um estudo crossover contrabalancado e randomizado. Durante as 2h após o protocolo, os participantes ingeriram 0,8g hidratos de carbono·kg\(^{-1}\)·h\(^{-1}\), 0,26g proteína·kg\(^{-1}\)·h\(^{-1}\) e 60,8 ± 1,0mg vitamina C na forma de CS ou MS (leite magro, morango e banana). Os testes funcionais foram realizados antes, imediatamente após, 24h e 48h após o exercício. A avaliação do desconforto muscular e as amostras sanguíneas foram recolhidas aos mesmos momentos e 2h após o exercício.

Resultados: Na amostra final (n=292, 68% homens, 20,4 ± 4,9 anos), 66% dos atletas reportaram terem consumido NS. Os multivitamínicos/multiminerais foram os suplementos mais frequentemente consumidos, a razão mais citada para usar NS foi acelerar a recuperação, e o médico foi a fonte de informação preferencial. Devido a miss-reporting e informação incompleta, a amostra foi
posteriormente reduzida a 241 atletas (66% homens, 13–37 anos). Utilizadores de NS reportaram, em geral, uma maior ingestão nutricional derivada dos alimentos, mesmo após ajuste para confundidores, e uma PMI mais baixa para alguns micronutrientes. Após ajuste, a utilização de suplementos foi associada a ter ≥18 anos, praticar uma modalidade individual e >2h ginásio/semana, a maior ingestão de carne, ovos e iogurte e menor ingestão de carne processada, óleos vegetais, margarina, batatas fritas e *fast food*. Relativamente ao estudo para comparar o efeito do CS e do MS, a amostra final foi constituída por 10 atletas (21,7 ± 3,4 anos; 73,3 ± 4,5 kg) – 3 foram excluídos por estarem em processo de dano muscular no início de um dos ensaios [cínase da creatina (CK) >1083 U·L⁻¹]. Padrões de recuperação similares foram obtidos por CS e MS em parâmetros de dano muscular (CK, mioglobina, desidrogenase do lactato, aldolase, aspartato aminotransferase, alanina aminotransferase), recuperação funcional (*peak torque* excêntrico dos quadríceps, altura do *countermovement jump*), desconforto muscular (geral e à apalpação), inflamação (leucócitos, neutrófilos, monócitos, basófilos, eosinófilos, linfócitos, interleucina-6, fator de necrose tumoral-α, proteína C reativa), *stress* oxidativo (estado antioxidante total, proteínas carboniladas, ácido úrico, redutase da glutationa), e metabólicos (glicose, proteínas totais, ácidos gordos não esterificados, β-hidroxitítrato, triglicerídeos, colesterol total, lipoproteína de baixa densidade, lipoproteína de alta densidade, creatinina, ureia, fosfatase alcalina, troponina i cardio-específica).

Conclusões: Nós concluímos que (i) o consumo de NS entre os atletas portugueses era generalizado, (ii) os atletas que consumiam NS eram os que tinham uma melhor ingestão nutricional, (iii) os utilizadores eram diferentes dos não utilizadores em características sociodemográficas e desportivas, (iv) as escolhas alimentares dos utilizadores pareciam ser orientadas para a saúde e para o desporto e (v) ambas as bebidas, independentemente da sua natureza comercial ou caseira, levaram a padrões de recuperação de EIMD similares.

**Palavras-chave:** NUTRIÇÃO NO DESPORTO, ATLETAS, INGESTÃO NUTRICIONAL, HIDRATOS DE CARBONO, PROTEÍNAS.
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AA</td>
<td>Arachidonic acid</td>
</tr>
<tr>
<td>ASAE</td>
<td>Autoridade de Segurança Alimentar e Económica</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine aminotransferase</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate aminotransferase</td>
</tr>
<tr>
<td>BCAA</td>
<td>Branch-chain amino acids</td>
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<tr>
<td>%BF</td>
<td>Body fat percentage</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>BMR</td>
<td>Basal metabolic rate</td>
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<tr>
<td>CFSAN</td>
<td>Center for Food Safety and Applied Nutrition</td>
</tr>
<tr>
<td>CHO</td>
<td>Carbohydrates</td>
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<tr>
<td>CI</td>
<td>Confidence intervals</td>
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<tr>
<td>CK</td>
<td>Creatine kinase</td>
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<tr>
<td>CLA</td>
<td>Conjugated linoleic acid</td>
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<tr>
<td>CMJ</td>
<td>Countermovement jump</td>
</tr>
<tr>
<td>CO(_2)</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>CoQ(_{10})</td>
<td>Co-enzyme Q</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CS</td>
<td>Commercial supplement</td>
</tr>
<tr>
<td>cTnI</td>
<td>Cardiac-specific troponin I</td>
</tr>
<tr>
<td>DGAV</td>
<td>Direção-Geral de Alimentação e Veterinária</td>
</tr>
<tr>
<td>DHA</td>
<td>Docosahexaenoic acid</td>
</tr>
<tr>
<td>DHLA</td>
<td>Dihydrolipoic acid</td>
</tr>
<tr>
<td>DOMS</td>
<td>Delayed onset muscle soreness</td>
</tr>
<tr>
<td>DRI</td>
<td>Dietary Reference intakes</td>
</tr>
<tr>
<td>DSHEA</td>
<td>The Dietary Supplement Health and Education Act</td>
</tr>
<tr>
<td>EAA</td>
<td>Essential amino acids</td>
</tr>
<tr>
<td>EAR</td>
<td>Estimated Average Requirement</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
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<tr>
<td>EGCG</td>
<td>Epigallocatechin gallate</td>
</tr>
<tr>
<td>EI</td>
<td>Energy intake</td>
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<tr>
<td>EIMD</td>
<td>Exercise-induced muscle damage</td>
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<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<tr>
<td>EPA</td>
<td>Eicosapentaenoic acid</td>
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<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
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<tr>
<td>FFQ</td>
<td>Food-frequency questionnaire</td>
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<tr>
<td>GI</td>
<td>Glycemic index</td>
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<tr>
<td>GLUT-4</td>
<td>Glucose transporter-4</td>
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<tr>
<td>GPx</td>
<td>Glutathione peroxidase</td>
</tr>
<tr>
<td>GR</td>
<td>Glutathione reductase</td>
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<tr>
<td>GSH</td>
<td>Reduced glutathione</td>
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<tr>
<td>GSSG</td>
<td>Oxidized glutathione</td>
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<tr>
<td>HDL</td>
<td>High-density lipoprotein cholesterol</td>
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<tr>
<td>HMB</td>
<td>Hydroxymethylbutyrate</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Interleukin-1 beta</td>
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<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
</tr>
<tr>
<td>ISAK</td>
<td>International Society for the Advancement of Kinanthropometry</td>
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<tr>
<td>LA</td>
<td>α-lipoic acid</td>
</tr>
<tr>
<td>LDH</td>
<td>Lactate dehydrogenase</td>
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<tr>
<td>LDL</td>
<td>Low-density lipoprotein cholesterol</td>
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<tr>
<td>LPO</td>
<td>Lipid peroxide</td>
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<tr>
<td>LTs</td>
<td>Leukotrienes</td>
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<tr>
<td>Mb</td>
<td>Myoglobin</td>
</tr>
<tr>
<td>MS</td>
<td>Homemade milkshake</td>
</tr>
<tr>
<td>MPS</td>
<td>Muscle protein synthesis</td>
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<tr>
<td>MPB</td>
<td>Muscle protein breakdown</td>
</tr>
<tr>
<td>NADPH</td>
<td>Nicotinamide adenine dinucleotide phosphate</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>NEFA</td>
<td>Non-esterified fatty acids</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NS</td>
<td>Nutritional supplement(s)</td>
</tr>
<tr>
<td>$O_2^-$</td>
<td>Superoxide ion</td>
</tr>
<tr>
<td>OH$^+$</td>
<td>Hydroxyl radical</td>
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<tr>
<td>OR</td>
<td>Odds ratios</td>
</tr>
<tr>
<td>PAL</td>
<td>Physical activity level</td>
</tr>
<tr>
<td>PC</td>
<td>Protein carbonyl</td>
</tr>
<tr>
<td>PG</td>
<td>Prostaglandin</td>
</tr>
<tr>
<td>PMI</td>
<td>Prevalence of micronutrient inadequacy</td>
</tr>
<tr>
<td>PTq</td>
<td>Peak torque of the quadriceps</td>
</tr>
<tr>
<td>PUFAs</td>
<td>Polyunsaturated fatty acids</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
</tr>
<tr>
<td>TAS</td>
<td>Total antioxidant status</td>
</tr>
<tr>
<td>TBARS</td>
<td>Thiobarbituric acid reactive species</td>
</tr>
<tr>
<td>TEV</td>
<td>Total energy value</td>
</tr>
<tr>
<td>TNF-$\alpha$</td>
<td>Tumour necrosis factor-alpha</td>
</tr>
<tr>
<td>UNESCO</td>
<td>United Nations Educational, Scientific and Cultural Organization</td>
</tr>
<tr>
<td>US</td>
<td>United States</td>
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<tr>
<td>VAS</td>
<td>Visual analogue scale</td>
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<tr>
<td>VO$_2$max</td>
<td>Maximal oxygen consumption</td>
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<tr>
<td>WBC</td>
<td>White blood cells</td>
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CHAPTER I

1. INTRODUCTION

2. AIMS
1. Introduction

The manipulation of athletes’ nutritional practices with the specific propose of sports performance enhancement has been used since ancient times (Grivetti & Applegate, 1997). The continuous advances in science made possible the huge development that sports nutrition undertook in the last decades (Maughan & Burke, 2011). These progresses have also been an opportunity for the supplements industry, which started to produce and advertise an increasing number of products specifically targeted to exercising individuals (Molinero & Márquez, 2009). Nowadays, supplementation is an accepted and widespread practice among athletes, supported and facilitated by the existing large variety of brands and products, and by the aggressive marketing of the manufactures (Molinero & Márquez, 2009).

There is no clear definition for what constitute a nutritional supplement (NS). The terms NS, dietary supplement, ergogenic aid, and sports supplements have been indiscriminately used by authors to refer the same substances (Braun et al., 2009). For the purpose of this dissertation, we considered the definition suggested by Petróčzi and collaborators (Petróčzi et al., 2007), where NS are products taken orally with the aim to supplement the diet with vitamins, minerals and/or other substances. Supplements may contain vitamins, minerals, herbs, amino acids, and/or a concentrate, metabolite, constituent, extract, or a combination of any of these. This definition goes in line with the one from the United States (US) Food and Drug Administration (FDA) (Food and Drug Administration, 1994), which considers an NS a product (other than tobacco) with the intention to supplement the diet that bears or contains one or more of the following substances: a vitamin, a mineral, an herb or other botanical, an amino acid, a dietary substance for use by man to supplement the diet by increasing the total dietary intake, or a concentrate, metabolite, constituent, extract, or combination of these ingredients. In Europe (European Commission, 2002), including in Portugal (“Decreto-Lei n.º 136/2003 de 28 de Junho”, 2003), NS are designated as foodstuffs intended “to supplement the
normal diet and which are concentrated sources of nutrients (vitamins and minerals) or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form". Additionally, substances used by athletes may also belong to the group of foodstuffs intended for particular nutritional uses (European Commission, 2009; Ministério da Agricultura do Desenvolvimento Rural e das Pescas, 2010), particularly under the category of foods intended to meet the expenditure of intense muscular effort, especially for sportsmen/sportswomen. However, no specific rules have been set so far regarding foods and supplements for sport people (European Commission). Nevertheless, article 13 of Regulation 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control (European Commission, 2013) stipulates that “by 20 July 2015, the Commission shall (...) present to the European Parliament and to the Council a report on the necessity, if any, of provisions for food intended for sportspeople”. This report may be accompanied by an appropriate legislative proposal, if deemed necessary.

The available evidence suggests that the prevalence of NS usage among athletes is higher compared to general population (Braun et al., 2009). Moreover, the use of supplements within sports context is increasing over time (Huang et al., 2006), and the combination of substances is a common practice among athletes (Suzic Lazic et al., 2011; Tscholl et al., 2010). Evidence also suggests that NS usage is related to some factors as age (Erdman et al., 2007), sport (Huang et al., 2006; Suzic Lazic et al., 2011), and level of competition (Erdman et al., 2006; Giannopoulou et al., 2013). Gender, in its turn, does not seem to influence the prevalence of NS usage (Dascombe et al., 2010; Salgado et al., 2014); however, being female or male appears to predispose the type of chosen supplements (Erdman et al., 2007; Slater et al., 2003). Type of sport (Huang et al., 2006; Tsitsimpikou et al., 2009) is other factor that also seems to influence the used supplements. Regarding age, it may (Tscholl et al., 2010) or may not (Erdman et al., 2007) be related with the type of supplements chosen
by athletes. Sportspeople consume NS mainly driven by performance- and health-related reasons (Wiens et al., 2014). Frequently cited specific motivations include to improve recovery, improve health, enhance performance, prevent or treat illness, and compensate for a poor diet (Maughan et al., 2011). Furthermore, athletes seem to feel a certain obligation to consume NS, since they believe heavy training increases the need for NS usage (Dascombe et al., 2010), and that they need to consume NS if they want to become champions (Dietz et al., 2014). Athletes seek advice on NS from different figures. Coaches, physicians, and nutritionists, in particular, are often identified as the main sources of information (Giannopoulou et al., 2013), but family and teammates also have an important role as advisors (Lun et al., 2012). To date, there was no study on the characterization of NS consumption in Portugal.

Studies with non-athletic population have repeatedly concluded that NS consumers tend to have healthier lifestyle and food choices than non-consumers (Beitz et al., 2004; McNaughton et al., 2005; Reinert et al., 2007). It has been suggested that, in general population, people taking supplements may be the least likely to need them (McNaughton et al., 2005). In the sports field, one study (Beshgetoor & Nichols, 2003) reported that supplemented female athletes had higher intakes of some micronutrients. However, the dietary information included both food and NS, making impossible to ascertain the provenience (food or supplement) of the nutrient. Although a healthier dietary behaviour by athletes consuming NS seems also to occur (Beshgetoor & Nichols, 2003), more studies are necessary to confirm this relationship.

Despite the large prevalence of use, athletes do not always fully understand the possible risks that may arise from NS consumption (Braun et al., 2009; Dascombe et al., 2010). The same supplement may be of use in some circumstances but detrimental to performance in others (Maughan et al., 2011). Of more concern is the poor quality insurance of NS. Regulation of NS varies between countries, and the increasing market of online sales allows a tremendous offer of different products, sometimes with uncertain origin.
(Maughan et al., 2011). In the US, the country that probably represents the larger NS market, The Dietary Supplement Health and Education Act of 1994 (DSHEA) (Food and Drug Administration, 1994) considers that supplements, independently of their form, belong to a special category under the general umbrella of foods, not drugs. This means that NS are not under the same regulations nor are subject to the strait control that is applied to the pharmaceutical industry. Indeed, the oversight of these products is the responsibility of FDA’s Center for Food Safety and Applied Nutrition (CFSAN). In Europe, NS are also regulated as foods (European Food Safety Authority, 2013). Particularly in Portugal, the Direção-Geral de Alimentação e Veterinária (DGAV) is responsible for regulation and control of both food supplements and foodstuffs for particular nutritional uses (Direção Geral de Alimentação e Veterinária, 2014). Additionally, the Autoridade de Segurança Alimentar e Económica (ASAE) is the authority for supervision and protection of the legislation compliance in this matter (Autoridade de Segurança Alimentar e Económica, 2014).

The poor quality of NS is a concern for sports community. There are reports of contamination with impurities, namely lead, broken glass, and animal faeces, due to poor quality control during manufacture or storage (Maughan et al., 2011). Also, reports of undeclared allergens or of microbiological contamination are, unfortunately, frequent (Maughan, 2013). This scenario, in addition to demonstrate lack of good manufacturing practice, may cause acute health consequences and jeopardize a crucial period of training or competition (Maughan et al., 2011). But more worrisome is the possible contamination of NS with prohibitive substances in sports (Geyer et al., 2008). In some cases, the amount of the banned substance present in the supplement may represent a health hazard for all consumers; in others, the concentration may be too small to cause any health or performance effect, but is sufficiently high to cause a failed doping test (Geyer et al., 2011). Although there are some products that deliberately contain substances prohibited by the World Anti-Doping Agency (Judkins & Prock, 2013), others – 15% (Geyer et al., 2004) to 25% (Burke et al.,
2009) – are contaminated with them, i.e. these substances are not declared in the label. Some of these cases may result from inadvertent cross-contamination due to poor quality control, but others, with higher amounts of the contaminant, seem to involve deliberate adulteration (Maughan, 2013). Therefore, there is a high and real risk for athletes consuming contaminated supplements to inadvertently fail a doping test (Geyer et al., 2011). Since the contamination of supplements became a serious issue, reputable supplement companies have been taking several measures to prevent adulteration (Judkins & Prock, 2013). These include product and manufacturing audits to attest the quality control along the production process, and the testing of products for trace amounts of prohibitive substances by specialized sports anti-doping laboratories (Judkins & Prock, 2013).

The new World Anti-Doping Code (World Anti-Doping Agency, 2015) establishes that the presence of a prohibited substance or its metabolites or markers in an athlete’s sample is punished with 4 years of ineligibility (Article 10.2.1). In the cases the detected prohibited substance came from a contaminated product, and depending on the degree of fault, the period of ineligibility may be reduced. This reduction is based on no significant fault or negligence, and may range from a reprimand and no period of ineligibility, up to 2 years of ineligibility (Article 10.5.1.2). However, the 2015 Code explicitly refers that a reduction of the period of ineligibility, based on no significant fault or negligence, for a positive test resulting from a mislabelled or contaminated NS would not apply, since athletes have been warned against the possibility of supplements contamination. The sanctions for athlete support personnel that had advised the use a supplement that was found to be contaminated are less defined. The 2015 Code (World Anti-Doping Agency, 2015) establishes it is a responsibility of the International Olympic Committee (Article 20.1.7), International Paralympic Committee (20.2.7), International Federations (Article 20.3.10), National Olympic Committees and National Paralympic Committees (Article 20.4.10), National Anti-Doping Organizations (Article 20.5.7), and Major Event Organizations (Article 20.6.5), within their jurisdiction, to investigate
whether athlete support personnel or other persons may have been involved in each case of doping. International Federations (Article 20.3.10) and National Anti-Doping Organizations (Article 20.5.7) have additionally to ensure proper enforcement of consequences.

The United Nations Educational, Scientific and Cultural Organization (UNESCO) in its International Convention against Doping in Sport (United Nations Educational Scientific and Cultural Organization, 2005) declares that “states Parties shall themselves take measures or encourage sports organizations and anti-doping organizations to adopt measures, including sanctions or penalties, aimed at athlete support personnel who commit an anti-doping rule violation or other offence connected with doping in sport”. Portugal is one of the States Parties that approved and adopted the aforementioned Convention (Ministério dos Negócios Estrangeiros, 2007). In the Lei n.º 38/2012 de 28 de Agosto (Assembleia da República, 2012; Ministro Adjunto e dos Assuntos Parlamentares, 2013) that approves the anti-doping law in sport, adopting internally the rules established in the World Anti-Doping Code, it is stated at n.º 1 of artigo 45.º “who administer the athlete (...) any substance (…), or who assists, encourages, help, allowing the concealment, or any other type of complicity involving an anti-doping rule violation shall be punished with imprisonment from 6 months to 3 years, unless there is an authorization for therapeutic use”. Moreover, by n.º 4 of artigo 64.º of the same law (Assembleia da República, 2012), for the athlete support personnel that practice the criminal offenses aforementioned referred, a suspension of the sport activity for 4 to 25 years shall be applied for the first offense.

Taking all these arguments into account, the investigation of alternatives that are safer and, at least, similarly effective is of extremely importance. Those would protect both athletes and athlete support personnel. Besides the doping risk of supplements containing banned substances, the potential health risks of these contaminating compounds should not be neglected (Geyer et al., 2008). For example, hepatic and renal consequences have been described due to the
consume of NS containing undeclared anabolic steroids (Krishnan et al., 2009). Therefore, a safer option would be advantageous also in terms of public health.

The American Dietetic Association, Dietitians of Canada, and American College of Sports Medicine joint position statement (Rodriguez et al., 2009) considered creatine, caffeine, sports drinks, gels, bars, sodium bicarbonate, protein, and amino acid as supplements that perform as claimed. More recently, L-arginine, beta-alanine, nitrate, and the already mentioned creatine, caffeine, and alkalinizing agents (sodium bicarbonate and sodium citrate) were considered NS with good evidence for improvements in exercise performance (Maughan et al., 2011). In this particular paper (Maughan et al., 2011), the authors excluded sports drinks, energy bars, gels, and other sports foods from the definition of NS. Considering that some of the abovementioned supplements are naturally present in food, food might be considered as a possible alternative to supplements. In some cases, such as creatine, fairly high intakes of specific foods would be necessary to achieve the ergogenic doses (Tarnopolsky, 2010), making this alternative less viable. But for others, namely proteins and carbohydrates (CHO), the ergogenic doses can be more easily achieved using food.

Mixed protein sources as meat, fish, eggs, and dairy products may be just as effective as essential amino acids alone, with the advantages of cost and palatability (Maughan & Burke, 2011). Regarding CHO, both solid foods and liquid forms appear to be equally effective in providing substrate for muscle glycogen synthesis (Keizer et al., 1987; Reed et al., 1989). Moreover, food contains a range of other nutrients that may contribute to the athlete’s individual nutritional goals (Maughan, 2013). The use of food with the intent to substitute supplements, and as a practical approach in the context of sports nutrition has been recently suggested (Maughan & Burke, 2011; Reid, 2013). However, using food with these proposes may not be easy for athletes who are not familiar with food composition. In this way, supplements might be more practical to use than food, since nutritional composition per portion is usually described
on the label. Still, it is important to point out that the poor quality assurance of supplements may compromise the faithfulness of the label information (Maughan, 2013).

Protein supplements are between the most used supplements (Lun et al., 2012; Maughan, 2013; Tsitsimpikou et al., 2009), and are considered potential sources for illegal substances, more specifically for anabolic androgenic steroids (Geyer et al., 2004; Rodriguez et al., 2009). Athletes performing resistance training are among those who most likely use protein supplements (Maughan, 2013) and consume higher doses of protein (Tipton, 2011); therefore, they might be at a greater risk to inadvertently fail a doping test. Important to notice are the facts that athletes’ protein ingestion tends to be higher than recommended (Tipton, 2011), supplementation significantly increases this intake (Lun et al., 2009), and food alone is sufficient to meet recommendations for dietary protein (Lun et al., 2009). Furthermore, protein intake is recommended for exercise recovery (Beelen et al., 2010; Van Loon, 2013), and recovery enhancement is one of the main reasons to use supplements (Sato et al., 2012). Indeed, after exercise, optimal nutritional intake is determinant to replenish endogenous substrate stores and to facilitate muscle-damage repair and reconditioning.

Muscle damage occurs predominantly after an exhaustive or unaccustomed intense exercise, and is more pronounced when eccentric contractions are involved (Clarkson & Hubal, 2002). When the muscle lengthens during contraction, greater stress and strain are placed upon the involved structures, inducing injury at a greater severity compared to concentric and isometric muscle actions (Clarkson & Hubal, 2002). In practical terms, the main undesirable consequences are weakness and pain (Allen, 2001), that can negatively influence the adherence to an exercise-training program. Although the mechanisms and their order of occurrence are not entirely establish yet, exercise-induced muscle damage (EIMD) is believed to comprise both mechanical and metabolic pathways, leading to structural damage of skeletal
muscle cells (Howatson & van Someren, 2008). This seems to result from the combination of several events, namely sarcomere disruption due to the high mechanical tension on the myofibril, impaired excitation-contraction coupling related to altered intracellular calcium homeostasis, inflammation, and oxidative stress (Friden & Lieber, 1992; Howatson & van Someren, 2008; Proske & Morgan, 2001). Nutritional strategies have been proposed to reduce the negative impact associated with EIMD, namely the use of CHO, proteins, and antioxidants (Howatson & van Someren, 2008). This topic will be further developed in the study IV of this dissertation.

Food sources rich in the above-mentioned nutrients may be interesting for post-exercise recovery. Milk is a recognize food source of proteins with high-biological value. Milk contains both casein and whey proteins, resulting in sustained elevations of blood amino acid concentrations (Bos et al., 2003; Roy, 2008). Whey protein is a soluble protein that is rapidly digested and absorbed after ingestion, leading to a faster and more transient rise in plasma amino acid concentration compared to casein (Van Loon, 2013). Casein, on the other hand, tends to clot in the stomach after ingestion, delaying digestion and absorption, which results in a slower release of amino acids to the blood (Van Loon, 2013). Additionally, milk proteins contain considerable amounts of leucine (with the whey fraction being richer in this amino acid) which highly influence protein synthesis (Phillips et al., 2009). Fruit is an excellent source of fructose and glucose, which is not present in the natural form of protein rich foods, and is naturally rich in minerals and vitamins. Berries are known to be a particularly rich fruit source of antioxidants (Proteggente et al., 2002; Wang et al., 1996). Strawberry, in particular, is considered a functional food with multiple health benefits, including antioxidant and anti-inflammatory effects (Basu et al., 2014). The antioxidant properties have been mostly attributed to its content in ascorbic acid and polyphenols, particularly ellagitannins and anthocyanins (Aaby et al., 2007). The anti-inflammatory properties of strawberries have been linked to their high content in flavonoids, specially anthocyanins (Giampieri et al., 2014). Banana is a natural rich source of CHO and, as strawberries, have a
glucose:fructose rate near 1:1 (0.96:1 for banana and 0.87:1 strawberries) (Unwin et al., 1991). By using a mixture of CHO from banana and strawberries, it is possible to obtain a rich antioxidant solution with a ratio of glucose:fructose near 1:1, which leads to one of the highest exogenous CHO absorption rate (Jeukendrup, 2010). Thus, the combination of skimmed milk with berries and banana might be used as a practical homemade strategy to recover from exercise.

Food seems, therefore, a possible choice for the recovery meal. Recently, some investigators have focused their attention on studying the effect of food on recovery in comparison to placebo (Bowtell et al., 2011; Cockburn et al., 2013; McLeay et al., 2012), with positive and promising outcomes. However, the majority of studies in this field of research still compares the effect of NS with placebo. Due to this, sports community seem to perceive that an efficient recovery can only be achieved by using supplements. Otherwise, improve recovery would not probably be one of the most cited reasons to consume supplements and, ultimately, the prevalence of nutritional supplements usage would not be increasing over time (Huang et al., 2006), despite the concerns mentioned above. In a real-life situation, athletes need to choose between a supplement and food, not between a supplement and placebo, or food and placebo. The choice between a supplement and food reflects a real-world problematic and, thus, would be particularly pertinent for field application. To date, the compared recovery effects of food with an equivalent nutritional supplement have been neglected even though its importance has been denoted (Cockburn et al., 2008). The information that would arise from this comparison would help both professionals and athletes to make informed and scientifically driven choices regarding the nature of the recovery meal.
2. Aims

The specific aims of this dissertation were:

1. To determine the prevalence of NS usage, the type of supplements used, the reasons for usage, and the source of advice among high-performing athletes (study I);

2. To compare the nutritional intake and nutritional adequacy from food between NS users and non-users (study II);

3. To understand the determinants of NS usage (study III);

4. To compare the effect of ingesting a commercial or homemade recovery beverage with similar nutritional content after EIMD on muscle damage, functional recovery, soreness markers (study V), inflammation, oxidative stress (study VI), and metabolic parameters (study VII).
References


CHAPTER II

3. STUDIES
3. Studies

3.1 Study I

3.2 Study II

3.3 Study III

3.4 Study IV

3.5 Study V
3.6 Study VI

3.7 Study VII
3.1 Study I
Nutritional Supplements Usage by Portuguese Athletes

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Abstract: In this study, we determined the prevalence of nutritional supplements (NS) usage, the type of supplements used, the reasons for usage, and the source of nutritional advice among Portuguese athletes. Two hundred ninety-two athletes (68% male, 12–37 years old) from 13 national sports federations completed a questionnaire that sought information on socio-demographics, sports data, and NS usage. Most athletes (66%) consumed NS, with a median consumption of 4 supplements per athlete. The most popular supplements included multivitamins/minerals (67%), sport drinks (62%), and magnesium (53%). Significant differences for the type of NS consumed were found between gender and age groups and the number of weekly training hours. Most athletes used NS to accelerate recovery (63%), improve sports performance (62%), and have more energy/reduce fatigue (60%). Athletes sought advice on supplementation mainly from physicians (56%) and coaches (46%). Age and gender were found to influence reasons for use and the source of information. Reasons for NS usage were supported scientifically in some cases (e.g., muscle gain upon protein supplementation), but others did not have a scientific basis (e.g., use of glutamine and magnesium). Given the high percentage of NS users, there is an urgent need to provide athletes with education and access to scientific and unbiased information, so that athletes can make assertive and rational choices about the utilization of these products.

Key words: vitamins and minerals, sport drinks, performance, recovery, fatigue, physicians, coaches

Introduction

As training programs become ever more demanding, athletes rely increasingly on nutrition to get an advantage over the competition [1]. It is known that the use of nutritional supplements (NS) among sportsmen and sportswomen is more widespread than in the general population, and that elite athletes tend to use these substances more than the non-elite do [2]. However, good evidence of efficacy and safety exists for only a limited number of these substances [3]. To worsen the scenario, as many as 25% of supplements are contaminated with prohibited compounds [4]. Therefore, besides posing a potential risk to health, the use of NS can lead to positive results in a doping test.

There is not a single definition of what constitutes an NS [3], but it is generally accepted that an NS is a product taken orally with the intention to supplement the diet by increasing the total intake of vitamins, minerals, or other nutritional substances. In this context, supplements may contain vitamins, minerals, herbs, amino acids and/or a concentrate,
To better inform athletes about the risks and benefits of oral supplementation, however, it is crucial to identify the factors determining the rational practice of NS use. In Portugal—the setting of this study—information on the use of NS among athletes is scarce, contrary to the situation in other countries [6–9]. Therefore, the objectives of this research were to: determine the prevalence of NS usage among Portuguese athletes, describe the type of supplements used, the main reasons for using them, and the source of advice for their usage according to age, gender, and sport. It was also a purpose for this study to access athletes’ understanding of the benefits of NS and to investigate the presence or absence of informed choices. Moreover, previous research has identified gender differences, but less focus has been placed on the differences in NS use between types of sport, age, and training frequency. Therefore, it was also an aim of this study to describe the types of relationships that have been neglected in previous studies.

Material and Methods

Sample

Three hundred four athletes representing the Portuguese national teams of 13 national sports federations (cycling, athletics, triathlon, gymnastics, rugby, basketball, volleyball, judo, swimming, baseball, handball, boxing, and fencing) volunteered to participate in this study by filling out a questionnaire about the use of NS. The sports were conveniently selected for this study. Twelve athletes were excluded from the study due to incomplete questionnaires.

Informed consent was obtained from all athletes. Additionally, for those under 18 years old, formal authorization from their guardians was required. The study was approved by the Scientific Council of the Faculty of Nutrition and Food Sciences at the University of Porto and by each of the 13 national sports federations.

Questionnaire

Thirty-one questions were developed by a group of experienced nutritionists in order to characterize the prevalence of NS usage, the main reasons for consumption, and the advisors that athletes relied on for information for the 12 months prior to questionnaire administration. This questionnaire also assessed information on socio-demographic and sports data. A pilot study with 11 athletes was conducted to pre-test the questionnaire for clarity and relevance.

The questionnaire was filled out in the presence of a qualified and trained nutritionist or was sent in to the sports federation (boxing and fencing) throughout the year of 2008; the two methods yielded similar results regarding NS usage (p = 0.334).

We used a broad definition of NS that included all types of supplements, namely ergogenic aids, sports foods, and dietary/nutritional supplements. In order to help athletes remember which type of supplements they had taken over the previous year, 30 closed-ended options were provided: multivitamins/minerals; antioxidants; β-carotene; vitamins E, B1, C, B6, and B12, calcium, iron, and magnesium; proteins; branch-chain amino acids (BCAAs); glutamine; arginine; other amino acids; β-alanine; sport drinks; gels; other carbohydrate supplements (for example, bars); creatine; herbs or plants; testosterone/Tribulus terrestris; omega-3; conjugated linoleic acid (CLA); hydroxymethylbutyrate (HMB); glucosamine; ginseng; caffeine; and l-carnitine. Reasons for NS usage were chosen from a 13-item list and included: stay healthy; increase strength; increase speed; increase endurance; accelerate recovery; increase focus; improve sports performance; have more energy/reduce fatigue; prevent/treat diseases or injuries; correct dietary flaws; gain muscle mass; decrease stress; and lose weight. Advisor type was chosen from an 8-item list: physician; coach; family; nutritionist; friends, him/herself; other athletes; and media. In all 3 topics, an additional open-ended question was included to allow answers other than those provided in the given list.

The lists of options considered in this study were based on other studies [6, 8, 10, 11] and on the authors’ anecdotal experience with Portuguese athletes.

Data Analysis

For statistical analyses, the 10 most taken supplements, the 6 most chosen reasons for usage, and the 6 most reported sources of information were used. Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 18. Descriptive data were reported as percentages, as the mean ± standard deviation when data were normally distributed (height, weight, body mass index, and age), or as the median when not (international performances, hours of training per week, and number of any of these [5].
of NS used). Chi-square test for categorical variables and Mann-Whitney test and Spearman correlation for non-parametric data were also used.

Phi (φ) coefficients were calculated to describe the relationships between reasons for NS usage and type of NS (Table III), and between type of advisor and type of NS (Table IV) [12]. To evaluate normality, the Kolmogorov–Smirnov test was performed. Values of p < 0.05 were considered statistically significant.

### Results

#### Sample Characterization

The final sample (n = 292, 68% males, 12–37 years of age) comprised athletes belonging to the following Portuguese national teams: volleyball (22.6%), swimming (12.3%), triathlon (10.6%), cycling (9.6%), judo (8.2%), athletics (6.2%), baseball (5.5%), handball (5.5%), rugby (5.5%), gymnastics (5.1%), basketball (4.1%), fencing (3.4%), and boxing (1.4%). The sample characterization is given in Table I. Table II shows the gender and age distribution by sport.

#### Supplement Intake

The majority of athletes (66%) reported using at least one NS. In total, 997 supplements were reported for 192 athletes, with a median consumption of 4 supplements per athlete (range, 1–16).

Differences in the median number of NS were found according to age groups (<18 years = 2, ≥18 years = 5; p < 0.001), gender (male = 5, female = 4; p = 0.039), and sport (p < 0.001; Figure 1). A positive and significant correlation between the number of NS and weekly hours of training (ρ = 0.465; p < 0.001) was also found.

No statistically significant correlation was found between the number of NS used and the number of international performances.

### Table I: Selected socio-demographic and athletic data of athletes. Data are presented as percentages (%), the mean ± standard deviation or the median (minimum–maximum value).

<table>
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<th>Characteristics</th>
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<tr>
<td>Height (cm)</td>
<td>178.9 ± 10.8</td>
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<td>Weight (kg)</td>
<td>72.6 ± 13.5</td>
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<td>Body mass index (kg/m²)</td>
<td>22.5 ± 2.5</td>
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<tr>
<td>Male</td>
<td>68%</td>
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<td>Female</td>
<td>32%</td>
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<td>Age (years)</td>
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<tr>
<td>&lt;18 years</td>
<td>20.4 ± 4.9</td>
<td>31%</td>
<td></td>
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<tr>
<td>≥18 years</td>
<td></td>
<td>69%</td>
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<tr>
<td>Years of education</td>
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<td>≤9</td>
<td>15.0%</td>
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<tr>
<td>10–12</td>
<td>42.5%</td>
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<tr>
<td>≥13</td>
<td>42.5%</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>International performances</td>
<td>6 (0–200)</td>
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<tr>
<td>Hours of training per week (h)</td>
<td>13 (4–33)</td>
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### Table II: Relative percentages (number) of athletes per sport by gender and age.

<table>
<thead>
<tr>
<th>Sport</th>
<th>Gender</th>
<th>Age</th>
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<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Volleyball</td>
<td>52% (34)</td>
<td>48% (32)</td>
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<tr>
<td>Swimming</td>
<td>61% (22)</td>
<td>39% (14)</td>
</tr>
<tr>
<td>Triathlon</td>
<td>68% (21)</td>
<td>32% (10)</td>
</tr>
<tr>
<td>Cycling</td>
<td>89% (25)</td>
<td>11% (3)</td>
</tr>
<tr>
<td>Judo</td>
<td>79% (19)</td>
<td>21% (5)</td>
</tr>
<tr>
<td>Athletics</td>
<td>56% (10)</td>
<td>44% (8)</td>
</tr>
<tr>
<td>Baseball</td>
<td>100% (16)</td>
<td>0%</td>
</tr>
<tr>
<td>Handball</td>
<td>100% (16)</td>
<td>0%</td>
</tr>
<tr>
<td>Rugby</td>
<td>0%</td>
<td>100% (16)</td>
</tr>
<tr>
<td>Gymnastics</td>
<td>73% (11)</td>
<td>27% (4)</td>
</tr>
<tr>
<td>Basketball</td>
<td>100% (12)</td>
<td>0%</td>
</tr>
<tr>
<td>Fencing</td>
<td>100% (10)</td>
<td>0%</td>
</tr>
<tr>
<td>Boxing</td>
<td>100% (4)</td>
<td>0%</td>
</tr>
</tbody>
</table>

Type of Supplements

The most widely used supplement was multivitamins/minerals (67%), followed by sport drinks (62%), and magnesium (53%). The 10 most commonly used supplements are shown in Figure 2.

Regarding gender, males used more protein (56% vs. 33%, \( p = 0.003 \)), vitamin C (29% vs. 13%, \( p = 0.011 \)), and iron (31% vs. 13%, \( p = 0.007 \)) supplements than females did.

Age-based differences were found between the consumption of multivitamins/minerals (<18 years=54%, ≥18 years = 71%, \( p = 0.030 \)), protein (<18 years=28%, ≥18 years = 56%, \( p = 0.001 \)), antioxidants (<18 years=0%, ≥18 years = 21%, \( p < 0.001 \)), creatine (<18 years=0%, ≥18 years = 22%, \( p < 0.001 \)), vitamin C (<18 years=9%, ≥18 years = 29%, \( p = 0.005 \)), and iron (<18 years=14%, ≥18 years = 29%, \( p = 0.034 \)).

No association was found between the type of supplements consumed and sport. However, differences were found in weekly hours of training for the use of proteins (users=19 h, non-users = 13 h; \( p < 0.001 \)), sport drinks (users=18 h, non-users = 12 h; \( p < 0.001 \)), sport gels (users=20 h, non-users = 13 h; \( p < 0.001 \)), glutamine (users=20 h, non-users = 13 h; \( p < 0.001 \)), creatine (users=12 h, non-users = 16 h; \( p = 0.012 \)), vitamin C (users=20 h; non-users = 14 h; \( p < 0.001 \)), and iron (users=20 h; non-users = 13 h; \( p < 0.001 \)).

Reasons for Usage

The most cited reasons for the usage of NS (Figure 3) were to accelerate recovery (63%), improve sports performance (62%), and have more energy/reduce fatigue (60%).
To increase strength (27% vs. 11%; p = 0.009), increase endurance (25% vs. 7%; p = 0.003), accelerate recovery (67% vs. 51%; p = 0.025), and gain muscle mass (22% vs. 7%; p = 0.010) were referred to as reasons more often by males than by their female counterparts.

Age-based differences were also found for reasons such as increasing strength (<18 years = 2%, ≥18 years = 28%; p < 0.001), accelerating recovery (<18 years = 31%, ≥18 years = 72%; p = 0.029), and gaining muscle mass (<18 years = 7%, ≥18 years = 21%; p = 0.027).

No association was found between type of sport and the reasons for consuming NS.

In order to assess the athletes’ understanding of the benefits of consuming NS and their informed use, associations between supplements and reasons for their use were made. Twenty-nine associations were found (Table III). Based on the scientific information available to date, it was not surprising to see the use of multivitamins/minerals (p = 0.017; φ = 0.167, p = 0.024), vitamin C (p = 0.009; φ = 0.184, p = 0.011), and iron (p = 0.002; φ = 0.222, p = 0.002) for staying healthy; sport drinks for accelerating recovery (p < 0.001; φ = 0.325, p < 0.001) and having more energy/reduce fatigue (p = 0.031; φ = 0.147, p = 0.043); and proteins for increasing strength (p < 0.001; φ = 0.26, p < 0.001) and gaining muscle mass (p = 0.002; φ = 0.22, p = 0.002). However, there were also some rational but non-scientific based associations as the use of multivitamin/mineral for giving more energy/reducing fatigue (p = 0.003; φ = 0.214, p = 0.003); glutamine for staying healthy (p = 0.033; φ = 0.145, p = 0.046), accelerating recovery (p < 0.001; φ = 0.370, p < 0.001), and preventing/treating diseases or injuries (p = 0.010; φ = 0.183, p = 0.012); and magnesium for accelerating recovery (p = 0.011; φ = 0.177, p = 0.015). There were some casuistic relationships between sport gels and the willingness for staying healthy (p < 0.001) and preventing/treating diseases or injuries (p = 0.004).
Table III: Associations between the 8 most chosen reasons for nutritional supplements usage and the 10 most taken nutritional supplements.

<table>
<thead>
<tr>
<th>Reason</th>
<th>Multivitamins/minerals</th>
<th>Protein</th>
<th>Sport drinks</th>
<th>Sport gels</th>
<th>Antioxidants</th>
<th>Glutamine</th>
<th>Creatine</th>
<th>Magnesium</th>
<th>Vitamin C</th>
<th>Iron</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stay healthy</td>
<td>YY: 75%</td>
<td>NY: 60%</td>
<td>YY: 41%</td>
<td>YY: 38%</td>
<td>YY: 9%</td>
<td>YY: 33%</td>
<td>YY: 37%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p = 0.017</td>
<td></td>
<td>p &lt; 0.001</td>
<td>p = 0.003</td>
<td>p &lt; 0.001</td>
<td></td>
<td>p = 0.09</td>
<td>p = 0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(φ = 0.167;</td>
<td></td>
<td>(φ = 0.307;</td>
<td>(φ = 0.145;</td>
<td>(φ = 0.199;</td>
<td></td>
<td>(φ = 0.184;</td>
<td>(φ = 0.222;</td>
<td>(φ = 0.011)</td>
<td>(φ = 0.002)</td>
</tr>
<tr>
<td></td>
<td>p = 0.024)</td>
<td></td>
<td>p &lt; 0.001)</td>
<td>p = 0.046)</td>
<td>p = 0.06)</td>
<td></td>
<td>p = 0.011</td>
<td>(φ = 0.222;</td>
<td>(φ = 0.011)</td>
<td>(φ = 0.002)</td>
</tr>
<tr>
<td>Increase strength</td>
<td>YY: 74%</td>
<td>NY: 43%</td>
<td>YY: 56%</td>
<td>YY: 41%</td>
<td>YY: 38%</td>
<td>YY: 9%</td>
<td>YY: 33%</td>
<td>YY: 37%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p = 0.001</td>
<td></td>
<td>p = 0.001</td>
<td>p = 0.001</td>
<td>p = 0.001</td>
<td></td>
<td>p = 0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(φ = 0.264;</td>
<td></td>
<td>(φ = 0.549;</td>
<td>(φ = 0.010)</td>
<td>(φ = 0.014)</td>
<td>(φ = 0.001)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.001)</td>
<td></td>
<td>p &lt; 0.001)</td>
<td>p &lt; 0.001)</td>
<td>p &lt; 0.001)</td>
<td></td>
<td>p &lt; 0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase endurance</td>
<td>YY: 76%</td>
<td>NY: 42%</td>
<td>YY: 32%</td>
<td>YY: 40%</td>
<td>YY: 47%</td>
<td>YYYY: 40%</td>
<td>YYYY: 47%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p = 0.037</td>
<td></td>
<td>p = 0.012</td>
<td>p = 0.001</td>
<td>p = 0.001</td>
<td></td>
<td>p = 0.015</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>(φ = 0.14;</td>
<td></td>
<td>(φ = 0.188;</td>
<td>(φ = 0.010)</td>
<td>(φ = 0.178)</td>
<td>(φ = 0.247;</td>
<td>(φ = 0.014)</td>
<td>(φ = 0.001)</td>
<td>(φ = 0.001)</td>
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</tr>
<tr>
<td></td>
<td>p = 0.051)</td>
<td></td>
<td>p = 0.010)</td>
<td>p = 0.014)</td>
<td>p = 0.001)</td>
<td></td>
<td>p = 0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accelerate recovery</td>
<td>YY: 63%</td>
<td>NY: 42%</td>
<td>YY: 32%</td>
<td>YY: 40%</td>
<td>YY: 47%</td>
<td>YYYY: 40%</td>
<td>YYYY: 47%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p = 0.001</td>
<td></td>
<td>p = 0.001</td>
<td>p = 0.002</td>
<td>p = 0.001</td>
<td></td>
<td>p = 0.011</td>
<td>p = 0.048</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(φ = 0.357;</td>
<td></td>
<td>(φ = 0.370;</td>
<td>(φ = 0.04)</td>
<td>(φ = 0.04)</td>
<td></td>
<td>p = 0.009</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p &lt; 0.001)</td>
<td></td>
<td>p &lt; 0.001)</td>
<td>p &lt; 0.001)</td>
<td>p &lt; 0.001)</td>
<td></td>
<td>p = 0.069</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improve sports performance</td>
<td>YY: 75%</td>
<td>NY: 54%</td>
<td>YY: 68%</td>
<td>YY: 44%</td>
<td>YY: 38%</td>
<td>YY: 44%</td>
<td>YYYY: 40%</td>
<td>YYYY: 40%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p = 0.003</td>
<td></td>
<td>p = 0.031</td>
<td>p = 0.002</td>
<td>p = 0.001</td>
<td></td>
<td>p = 0.015</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(φ = 0.214;</td>
<td></td>
<td>(φ = 0.147;</td>
<td>(φ = 0.183;</td>
<td>(φ = 0.205;</td>
<td></td>
<td>p = 0.005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p = 0.003)</td>
<td></td>
<td>p = 0.043)</td>
<td>p = 0.012)</td>
<td>p = 0.005)</td>
<td></td>
<td>p = 0.005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevent/treat diseases or</td>
<td>YY: 75%</td>
<td>NY: 61%</td>
<td>YY: 44%</td>
<td>YY: 40%</td>
<td>YY: 44%</td>
<td>YYYY: 40%</td>
<td>YYYY: 40%</td>
<td>YYYY: 40%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>injuries</td>
<td>p = 0.007</td>
<td></td>
<td>p = 0.003</td>
<td>p = 0.004</td>
<td>p = 0.003</td>
<td></td>
<td>p = 0.015</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(φ = 0.188;</td>
<td></td>
<td>(φ = 0.212;</td>
<td>(φ = 0.183;</td>
<td>(φ = 0.205;</td>
<td></td>
<td>p = 0.005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p = 0.010)</td>
<td></td>
<td>(φ = 0.133;</td>
<td>(φ = 0.214;</td>
<td>(φ = 0.205;</td>
<td></td>
<td>p = 0.005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gain muscle mass</td>
<td>YY: 74%</td>
<td>NY: 45%</td>
<td>YY: 53%</td>
<td>YY: 44%</td>
<td>YY: 44%</td>
<td>YYYY: 40%</td>
<td>YYYY: 40%</td>
<td>YYYY: 40%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p = 0.002</td>
<td></td>
<td>p = 0.001</td>
<td>p = 0.003</td>
<td>p = 0.002</td>
<td></td>
<td>p = 0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(φ = 0.22;</td>
<td></td>
<td>(φ = 0.483;</td>
<td>(φ = 0.010)</td>
<td>(φ = 0.002)</td>
<td></td>
<td>p = 0.001</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>p = 0.002)</td>
<td></td>
<td>p = 0.002)</td>
<td>p = 0.002)</td>
<td>p = 0.001)</td>
<td></td>
<td>p = 0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

YY: percentage of athletes that chose both variables; NY: percentage of athletes that used the nutritional supplement but did not point that reason. The significant p-values obtained by chi-square test, and the phi (φ) coefficients (and their corresponding p-values) are presented. Empty cells represent non-significant or non-valid chi-square tests.
Table IV: Associations between the 6 most chosen nutritional supplements advisor and the 10 most taken nutritional supplements.

<table>
<thead>
<tr>
<th>Advisor</th>
<th>Multivitamins/ minerals</th>
<th>Protein</th>
<th>Sport drinks</th>
<th>Sport gels</th>
<th>Antioxidants</th>
<th>Glutamine</th>
<th>Creatine</th>
<th>Magnesium</th>
<th>Vitamin C</th>
<th>Iron</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physician</td>
<td>YY: 73 %</td>
<td>NY: 59 %</td>
<td>p = 0.028</td>
<td>(φ = 0.150; p = 0.038)</td>
<td>YY: 29 %</td>
<td>NY: 18 %</td>
<td>p = 0.049</td>
<td>(φ = 0.132; p = 0.069)</td>
<td>YY: 33 %</td>
<td>NY: 17 %</td>
</tr>
<tr>
<td>Coach</td>
<td>YY: 38 %</td>
<td>NY: 21 %</td>
<td>p = 0.001</td>
<td>p &lt; 0.001</td>
<td>YY: 11 %</td>
<td>NY: 22 %</td>
<td>p = 0.003</td>
<td>p = 0.005</td>
<td>YY: 34 %</td>
<td>NY: 19 %</td>
</tr>
<tr>
<td>Family</td>
<td>YY: 81 %</td>
<td>NY: 62 %</td>
<td>p = 0.016</td>
<td>(φ = 0.165; p = 0.023)</td>
<td>YY: 11 %</td>
<td>NY: 22 %</td>
<td>p = 0.044</td>
<td>p = 0.006</td>
<td>YY: 33 %</td>
<td>NY: 17 %</td>
</tr>
<tr>
<td>Nutritionist</td>
<td>YY: 73 %</td>
<td>NY: 59 %</td>
<td>p = 0.028</td>
<td>(φ = 0.150; p = 0.038)</td>
<td>YY: 29 %</td>
<td>NY: 18 %</td>
<td>p = 0.049</td>
<td>(φ = 0.132; p = 0.069)</td>
<td>YY: 33 %</td>
<td>NY: 17 %</td>
</tr>
</tbody>
</table>

YY: percentage of athletes that chose both variables; NY: percentage of athletes that used the nutritional supplement but did not indicate that advisor. The significant p-values obtained by chi-square test, and the phi (φ) coefficients (and their corresponding p-values) are presented. Empty cells represent non-significant or non-valid chi-square tests.
Source of Information

Physicians (56%) and coaches (46%) were the main sources of information and advice (Figure 4).

Gender-based differences were found for family advice (males = 7%; females = 22%; p = 0.007).

Age significantly influenced the search for advice from coaches (< 18 years = 60%, ≥ 18 years = 42%, p = 0.029) and family (< 18 years = 36%, ≥ 18 years = 32%, p < 0.001).

No statistical differences were found between sports and sources of information.

Table IV shows the relationships between the source of information and the type of NS used. When the source of information was the physician (p = 0.028; φ = 0.150, p = 0.038) or the athlete him/herself (p = 0.016; φ = 0.165, p = 0.023), the use of vitamins and minerals was more prevalent. On the other hand, supplements to improve training performance were used more often when the coach was the advisor (p < 0.05).

Discussion

Number and Type of NS Intake

To our knowledge, this is the first study to report NS usage in Portuguese national team athletes. Intake of dietary supplements was widespread among our sample population, with 66% of the responders having consumed one or more type of NS over the year prior to data collection. This high percentage of usage was also reported in other countries [9, 13].

Regarding these numbers, we believe that key dietary issues and other major determinants of performance improvement and health maintenance might be receiving much less consideration than they merit [9]. Therefore, it is of high importance that athletes be advised by qualified health professionals, in particular specialists in food and sports nutrition, who can tailor diet and NS as per the athletes’ specific needs.

It has been previously reported that a combination of NS is not unusual [10, 14]; similarly in this study, we found that the median level of consumption was 4 supplements per athlete. In accordance with other studies [9, 13], our data showed that the number of supplements taken was higher in older (over-18) athletes. Supplements such as multivitamins/minerals, protein, antioxidants, creatine, vitamin C, and iron were significantly more consumed by athletes aged 18 or older than by under-18 athletes.

We also found that males took more supplements than females, and that the number of supplements increased with increasing weekly training hours. Cycling was the sport where NS consumption was highest (10 supplements per athlete) and fencing the sport with the lowest consumption level (2 supplements per athlete). Not surprisingly, cycling is known for being one of the sports with the highest prevalence of NS use [15].

In agreement with other studies [13, 14], the most taken supplement was multivitamins/minerals. Exercise may indeed increase the requirements for certain vitamins and minerals, many of which are involved in energy production and muscle synthesis and repair, but vitamin and mineral supplements are not needed if adequate energy to maintain body weight is drawn from a rich diet [16]. However, athletes who follow low-energy, low-micronutrient-dense, or unbalanced diets may require these types of supplements [16].

A similar tendency was found for magnesium, also highly consumed by German athletes [13]. Although even marginal magnesium deficiency impairs exercise performance, magnesium supplementation of physically active individuals with good magnesium status does not enhance physical performance [17]. Therefore, increased dietary intake of magnesium from food or supplements should only be an option when a deficiency in this mineral is proven.

In accordance with published data [8, 13, 18], sport drinks were among the most taken NS. These findings are in agreement with the guidelines that encourage athletes to consume sports beverages in order to maximize exercise performance [19].

In the current study, male athletes were more likely to use protein preparations than female athletes, as also seen in other studies [6, 8, 13]. The same effect was observed for iron intake, contrary to what was found by Petroczi and collaborators [7], and to the fact that females are more prone to developing iron deficiency compared to males [20]. For vitamin C, the results of this study were similar to those of another [7] where the proportion of male users was higher than the proportion of female users. The higher use of both iron and vitamin C by male athletes in this study may be explained by the fact that vitamin C positively affects iron metabolism [21], which is why these two micronutrients are often prescribed simultaneously.

The number of weekly training hours was positively related with the use of 6 supplements (proteins, sport drinks, sport gels, glutamine, vitamin C, and iron) except for creatine, which showed an inverse association. Creatine is classically related to power, strength, and explosive force. Therefore, this result is in congruence...
with the fewer training hours of a typical strength athlete, who is a usual consumer of creatine.

**Reasons for Usage**

In the present study, the primary reasons for taking supplements were to accelerate recovery, improve sports performance, and have more energy/reduce fatigue—all of which are performance-related. These findings contrast with other studies [11, 13, 22, 23] that report mainly health-related reasons. Moreover, in studies where performance-related reasons were most cited [9, 10], no specific reference to recovery is made. In the present study, not only was accelerated recovery the most important motivation, but also the reason for consuming more different types of supplements (Table III). An explanation for this might be that only high-level athletes participated in this study; these athletes are exposed to high training volumes, power, and intensities, and fast recovery is their major concern.

From a general perspective, multivitamins/minerals, vitamin C and iron were consumed primarily for health-related issues, whereas sport drinks were taken to guarantee endurance, energy, and recovery, and proteins and creatine to promote muscle mass and recovery. Other studies have also found an association between multivitamins/minerals and energy gain [11, 24, 25], and proteins with muscle build-up [18]. Whether these relationships were due to the athletes’ knowledge about NS efficacy and potency was not assessed. Nonetheless, there seems to exist—at least in some cases—a rational choice for the supplement, albeit not supported by scientific information. That is, it seems that athletes look for information about supplements, yet perhaps not in scientific resources; e.g., peer-reviewed journals. For example, glutamine was taken to improve health and to accelerate recovery, but these effects have not been scientifically proven [26, 27]. Notwithstanding the lack of scientific proof, they are frequently suggested in glutamine supplement labels. Another example is that of multivitamins/minerals, magnesium, vitamin C, and iron, which were being taken for health and/or performance reasons. However, their use is only rational if athletes are at risk for poor micronutrient status [16].

Reasons for taking NS were related to gender and to age. As in our study, Tian et al. [18] found that males use supplements mainly for performance and strength enhancement. However, the sport did not influence the reasons for taking these substances; i.e., the 8 most chosen reasons were similar regardless of the type of sport. This phenomenon might be due to the fact that all sports have some goals in common.

**Source of Information**

In this study, the physician was the most common source of information, followed by the coach. Although the same results were obtained by de Silva et al. [9], in most studies the primary source of information is the coach or the family [6, 11, 23, 24, 28]. Therefore, it appears that Portuguese athletes perceive supplements as an issue that deserves medical attention. However, the percentage of athletes seeking the advice of nutritionists was low. This can be explained—at least partially—by the fact that in Portugal, few nutritionists are working directly with athletes.

On the other hand, as mentioned above, NS advice is often sought from coaches; this may be due to the close relationship between coaches and athletes favoring trust. Therefore, coaches must be aware of the benefits and risks of NS in order to provide good advice and recommendations. However, under-18 athletes obtained information on NS not only from their coaches but also from family members, suggesting that families should receive education on NS and, if necessary, be prepared to ask for medical/nutritional advice.

In this study, advice from physicians was sought in the case of health supplements, and that of coaches in the case of performance and ergogenic supplements. Therefore, a complementary approach might be necessary for the athlete’s best interest.

A limitation of this study was that it was not possible to quantify the frequency and the duration of NS intake. The study of these variables in future studies may shed new light on sports supplementation patterns. Moreover, more specific studies about this topic are needed in order to investigate the consumption of NS within each specialty, namely in athletics (long- and middle-distance running, sprints, hurdles, jumps, throws, combined events, and race-walking), swimming (competitive swimming, synchronized swimming, and open-water swimming), cycling (road cycling, off-road cycling, and BMX riding), and also within weight classes in the case of sports with weight categories, such as boxing. Another limitation was the difference in the number of athletes per sport, which might have biased the comparisons between sports in favor of the most represented sports.

In conclusion, this study demonstrated that the prevalence of NS usage is widespread among Portuguese athletes. Non-scientifically supported choices revealed a lack of information and appropriate nu-
tritional strategies, which are essential for the safe use of NS. It is important that athletes choose NS with caution and be assisted by a professional health care provider specializing in sports nutrition. Given the high percentage of NS users, there is an urgent need to provide athletes with education and access to scientific and unbiased information, in order to make right and wise choices about the use, or not, of these products. Not only athletes, but also their families and all professionals working with them, must ensure the adequacy of NS for each individual sport and athlete. It is also important for athletes to understand that under a balanced diet, gains from the majority of NS are minimal or non-existent; in fact, their inadequate consumption could result in inadvertent doping.

Acknowledgements

The authors would like to thank all the athletes and the 13 federations that participated in this study. Mónica Sousa acknowledges the Fundação para a Ciência e a Tecnologia (FCT) regarding the grant SFRH/BD/75276/2010. Vítor Hugo Teixeira and Pedro Moreira acknowledge the FCT for the funded project PEst-OE/SAU/UI0617/2011.

References


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3.2 Study II
Nutritional supplements usage in high-performance athletes is related with lower nutritional inadequacy from food

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ABSTRACT

The use of nutritional supplements (NS) among athletes is widespread. However, little is known about the relationship between nutritional adequacy and NS usage. The aims of this study were to evaluate the NS usage and to compare the nutritional intake from food and prevalence of micronutrient inadequacy (PMI) between NS users and non-users. Portuguese athletes from 13 sports completed a NS usage questionnaire and a semi-quantitative food-frequency questionnaire assessing information over the previous 12 months. The Estimated Average Requirement (EAR) cut-point method was used to calculate PMI. General linear models were used to compare nutritional intake and NS usage. Chi-squared tests and logistic regression were performed to study, respectively, relationships and associations between PMI and NS usage. From the 244 athletes (66% males, 13–37 y), 64% reported NS usage. After adjustment, NS users showed a higher intake from food ($P < 0.05$), for at least one gender, for energy and for 7 of the 17 studied nutrients. The highest PMI were seen for vitamins D and E, calcium, folate, and magnesium. After adjustment, NS users, irrespective of gender, reported lower PMI for calcium (OR 0.28, 95% CI 0.12, 0.65) and female users for magnesium (OR 0.06, 95% CI 0.00, 0.98). Athletes using NS reported a higher nutritional intake from food, and a lower PMI for some nutrients. Perhaps, those who were taking NS were probably the ones who would least benefit from it.

Keywords: Proteins, carbohydrates, vitamins, minerals, sport.
INTRODUCTION

Athletic performance can be enhanced by an adequate and individually-adapted dietary intake. Sports nutrition guidelines suggest that protein intake should be between 1.2–1.7 g·kg\(^{-1}·d\)\(^{-1}\) (Rodriguez et al., 2009), carbohydrates (CHO) ingestion may range from 3–12 g·kg\(^{-1}·d\)\(^{-1}\), depending on the duration and type of exercise (Burke et al., 2011), and fat should contribute to 20–35% of total energy value (TEV) (Rodriguez et al., 2009). Recently, these guidelines – developed for adults – were also considered adequate for adolescents (Desbrow et al., 2014). Moreover, athletes should reach, at least, the Dietary Reference intakes (DRI) for all micronutrients (Rodriguez et al., 2009).

Regardless of the growing body of scientific evidence concerning the sports nutrition impact on performance, and the supposedly easier access to reliable information, athletes are still reporting slightly unbalanced diets. Generally, protein intake tends to be higher than recommended (Erdman et al., 2013) while, that of carbohydrates is sometimes below the recommended range (Reed et al., 2014). The adequate consumption of some micronutrients is also a source of concern, with some studies (Nogueira & Da Costa, 2004; Papadopoulou et al., 2012) showing intakes under the DRI.

The wide usage of nutritional supplements (NS) by athletic populations is largely recognized (Sousa et al., 2013). Though, in the sports field, it has not been appropriately demonstrated if supplementation is advantageous and rationale for those who are taking it. Some studies (Petróczki et al., 2007; Sousa et al., 2013) had already shown that the reasons to use certain types of NS, namely multivitamins/minerals and individual micronutrients such as vitamin C and iron, are not always science-based. Moreover, the use of these vitamin/mineral supplements will only result in a performance enhancement if it corrects a nutritionally unbalanced diet (Rodriguez et al., 2009). Additionally, epidemiological studies have been shown that NS users tend to have better health-related behaviours, namely a healthier nutritional intake (McNaughton et
Therefore, those taking NS are potentially the ones who need them less. However, little is known regarding the nutritional adequacy of athletes using NS.

Therefore, the aims of this study were (i) to assess the NS usage among high-performance athletes, (ii) to evaluate nutritional inadequacy considering the micronutrients from food, (iii) to compare nutritional intake, and (iv) prevalence of micronutrient inadequacy (PMI) between NS users and non-users. For this purpose, we used the NS definition suggested by Petróczi and collaborators (Petróczi et al., 2007) which considers that NS are products taken orally with the aim to supplement the diet with vitamins, minerals and/or other substances. Supplements may contain vitamins, minerals, herbs, amino acids and/or a concentrate, metabolite, constituent, extract, or a combination of any of these.

METHODS

Participants and study design

Three hundred and four athletes representing the Portuguese national teams in 13 sports (cycling, athletics, triathlon, gymnastic, rugby, basketball, volleyball, judo, swimming, baseball, handball, boxing and fencing) volunteered to participate in this study. The sports were conveniently selected for the study. Informed consent was obtained from all athletes. Additionally, formal authorisation from the guardians was required for those <18 years old. The study was approved by the Scientific Council of the Faculty of Nutrition and Food Sciences of the University of Porto, and by each of the 13 national sports federations.

The participants filled out 2 self-administered questionnaires: one about NS usage and a semi-quantitative food-frequency questionnaire (FFQ). Both
questionnaires assessed information over the previous 12 months. The questionnaires were completed in the presence of a qualified and trained nutritionist or sent in to the respective sport federation (boxing and fencing) throughout the year of 2008; the two methods yielded similar results regarding NS usage ($P = 0.369$) and energy intake ($P = 0.897$).

**Nutritional Intake**

Dietary intake was obtained by a self-administered, semi-quantitative FFQ, validated for the Portuguese adult population (Lopes et al., 2007). The FFQ is an 86-item questionnaire that includes food groups and beverage categories, and a frequency section with nine possible responses, ranging from ‘never or less than 1 time per month’ to ‘6 or more times per day’. The food intake was calculated by weighting one of the nine possibilities of frequency of consumption by the weight of the standard portion size of the food-item. A seasonal variation factor was considered for foods in which production and consumption were not regular over the year. Energy and nutrient intake with more sport relevance (proteins, carbohydrates, lipids, vitamins A, C, E, D, B6 and B12, thiamine, riboflavin, folate, magnesium, zinc, calcium, selenium, and iron), without including the NS contribution, were estimated using the software Food Processor SQL® (ESHA Research Inc., Salem, OR, USA) added with Portuguese foods and recipes.

To identify under- and over-reporting, the ratio energy intake (EI) to basal metabolic rate (BMR) was used (Livingstone & Black, 2003). EI was obtained from data analyses whereas BMR was estimated using Schofield equations (Schofield et al., 1985). The under-reporting cut-off was set at 0.9, as it was used in another study for a similar purpose (Farajian et al., 2004), and the one for over-reporting at 4.0, which corresponds to the physical activity level (PAL) upper limit for professional endurance athletes (Westerterp, 2013).
The PMI was determined by the Estimated Average Requirement (EAR) cut-point method (Murphy et al., 2002), calculating the proportion of individuals whose intake was below the EAR from the Food and Nutrition Board of the Institute of Medicine, for the respective gender and age group [to consult EAR values please see (Institute of Medicine, 2011)]. PMI for iron was not calculated since this method should not be used when requirements are not normally distributed (Murphy et al., 2002). Age groups were defined according to the Food and Nutrition Board of the Institute of Medicine categories.

**Nutritional supplements usage and other information**

A broad definition of NS was used, which included all types of supplements, namely ergogenic aids, sports food, and dietary/nutritional supplements. Thirty closed-ended options for NS were provided with an additional open-ended question.

This questionnaire also assessed information on weight, height, age, gender, years of education (year of attendance or concluded years if the athlete was not currently studying), type of sport, hours of training, and number of international performances, as described in detail elsewhere (Sousa et al., 2013).

**Statistical analysis**

Descriptive data were reported as proportions (%), mean ± standard deviation when data were normally distributed [height, weight, body mass index (BMI)], or as median (interquartile range) when not (age, number of international performances, hours of training, and energy and nutrients intake).
The Kolmogorov-Smirnov test was used to evaluate normality. Student’s T-test for normally distributed variables, Mann–Whitney U test for non-parametric data, and chi-squared test for categorical variables were used to compare groups. For the chi-squared tests with statistically significant results, phi coefficients were also calculated to describe the relationships between the variables.

In order to compare nutritional intake between NS users and non-users, nutritional variables were adjusted for total energy intake, using the nutrient residual model (Willett et al., 1997). In this model, energy-adjusted nutrients intake is computed as the residuals from the regression analysis, with total energy intake as the independent variable and absolute intakes as the dependent variable. Afterwards, univariate general linear models non-adjusted and adjusted for confounders were performed. Non-normal distributed variables (total energy intake, absolute nutrients intake, and hours of training) were logarithmically transformed to attain normal distribution for the purpose of residual models and univariate general linear models.

The relationships between PMI and NS usage were performed using chi-squared tests. For those with statistically significant relationships, phi coefficients were calculated and logistic regression was subsequently performed, with and without confounders’ adjustment. Odds ratios (OR), and 95% confidence intervals (CI), were calculated by reference with the micronutrient intake ≥EAR.

Considering the biological plausibility related to dietary intake, age, BMI, education, and total energy intake were considered as confounders. For this purpose, age was categorized as ≤18 and ≥19 years old to attain similar number of athletes per group, and respect the DRI age group cut-offs. Moreover, regarding the statistical significance differences between users and non-users by gender for sport and hours of training (Table 1), these variables were also included as confounders.
All statistical procedures were completed using Statistical Package for Social Sciences® version 20 (SPSS Inc., Chicago, IL, USA). The level of significance was set at $P < 0.05$.

RESULTS

From the 304 athletes, 44 were excluded due to incomplete information, and 19 due to miss-reporting (16 for under-reporting and 3 for over-reporting). Therefore, the final sample comprised 241 athletes (66% males, 13–37 y, 71.5 ± 13.0 kg, 178 ± 11 cm). Similar NS prevalence of use ($P = 0.370$) and energy intake ($P = 0.842$) were reported by athletes regardless the method of data collection. An overview of basic characteristics of supplement users and non-users, by gender, is given in Table 1.

The majority of athletes (64%), reported to have used NS in the previous 12 months. The top 8 reported supplements were multivitamins/minerals (71%), sport drinks (59%), magnesium (58%), protein (47%), glutamine (28%), vitamin C (28%), iron (24%), and sport gels (21%).

A significant difference of consumption between users and non-users from food intake was found for 8 of the 17 studied nutritional parameters (Tables 2 and 3). In all situations, users showed a higher intake of the respective nutrient than non-users. Regarding the crude values, differences were found for proteins in males, and for proteins, CHO, vitamin D and B12, and selenium in females. After adjustment for energy intake, age, education, sport, hours of training, and BMI, male users showed a higher ingestion of CHO, riboflavin, folate, and calcium, and female users of energy, proteins, vitamin D, and selenium.
Table 1: Characteristics of nutritional supplements user and non-user athletes according to gender.

Data are presented as the mean ± standard deviation, the median (interquartile range) or n (%).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Users (n=108)</td>
<td>Non-users (n=52)</td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
<td>181 ± 9</td>
<td>186 ± 10</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>74.3 ± 12.0</td>
<td>81.6 ± 10.0</td>
</tr>
<tr>
<td><strong>Body mass index (kg·m⁻²)</strong></td>
<td>22.6 ± 2.3</td>
<td>23.5 ± 1.8</td>
</tr>
<tr>
<td><strong>Age (years old)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 – 13</td>
<td>21 (5)</td>
<td>19 (6)</td>
</tr>
<tr>
<td>14 – 18</td>
<td>21 (20%)</td>
<td>20 (38%)</td>
</tr>
<tr>
<td>19 – 30</td>
<td>78 (72%)</td>
<td>31 (60%)</td>
</tr>
<tr>
<td>31 - 50</td>
<td>9 (8%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td><strong>Education (years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 9</td>
<td>13 (13%)</td>
<td>6 (12%)</td>
</tr>
<tr>
<td>10 – 12</td>
<td>36 (37%)</td>
<td>20 (41%)</td>
</tr>
<tr>
<td>≥ 13</td>
<td>49 (50%)</td>
<td>23 (47%)</td>
</tr>
<tr>
<td><strong>Sports</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Team</td>
<td>31 (29%)</td>
<td>38 (73%)</td>
</tr>
<tr>
<td><em>Baseball</em></td>
<td>5 (5%)</td>
<td>8 (16%)</td>
</tr>
<tr>
<td>Handball</td>
<td>9 (8%)</td>
<td>5 (10%)</td>
</tr>
<tr>
<td><em>Rugby</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Basketball</td>
<td>7 (6%)</td>
<td>4 (7%)</td>
</tr>
<tr>
<td>Volleyball</td>
<td>10 (9%)</td>
<td>21 (40%)</td>
</tr>
</tbody>
</table>
**Table 1 (continued)** Characteristics of nutritional supplements user and non-user athletes according to gender.

Data are presented as the mean ± standard deviation, the median (interquartile range) or n (%).

<table>
<thead>
<tr>
<th></th>
<th>Males Users (n=108)</th>
<th>Non-users (n=52)</th>
<th>P</th>
<th>Females Users (n=45)</th>
<th>Non-user (n=36)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Individual</td>
<td>77 (71%)</td>
<td>14 (27%)</td>
<td></td>
<td>30 (67%)</td>
<td>4 (11%)</td>
<td></td>
</tr>
<tr>
<td>Judo</td>
<td>11 (10%)</td>
<td>4 (7%)</td>
<td></td>
<td>4 (9%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Fencing</td>
<td>3 (3%)</td>
<td>5 (10%)</td>
<td></td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Boxing</td>
<td>3 (3%)</td>
<td>0</td>
<td></td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Cycling</td>
<td>26 (24%)</td>
<td>0</td>
<td></td>
<td>2 (4%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Triathlon</td>
<td>6 (6%)</td>
<td>0</td>
<td></td>
<td>5 (11%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Athletics</td>
<td>9 (8%)</td>
<td>1 (2%)</td>
<td></td>
<td>7 (16%)</td>
<td>1 (3%)</td>
<td></td>
</tr>
<tr>
<td>Swimming</td>
<td>18 (17%)</td>
<td>2 (4%)</td>
<td></td>
<td>12 (27%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Gymnastics</td>
<td>1 (1%)</td>
<td>2 (4%)</td>
<td></td>
<td>0</td>
<td>3 (8%)</td>
<td></td>
</tr>
<tr>
<td>International</td>
<td>9 (28)</td>
<td>7 (27)</td>
<td>0.390‡</td>
<td>4 (15)</td>
<td>0 (10)</td>
<td>0.066‡</td>
</tr>
<tr>
<td>performances</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hours of training</td>
<td>18 (8)</td>
<td>11 (9)</td>
<td>&lt;0.001‡</td>
<td>13 (7)</td>
<td>8 (4)</td>
<td>&lt;0.001‡</td>
</tr>
<tr>
<td>(h·week⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† T-test
‡ Mann–Whitney U test
§ Chi-squared test. For the statistically significant P, the phi (φ) coefficients (and their corresponding P) are presented
* P < 0.05
Table 2: Comparison of energy, and macronutrients intake from food between nutritional supplements users and non-users according to gender\(^1\). Crude and adjusted p-values are presented. Data are presented as the median (interquartile range).

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th></th>
<th>Females</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Users (n=108)</td>
<td>Non-users (n=52)</td>
<td>Crude P(^\dagger)</td>
<td>Adjusted P(^\ddagger)</td>
</tr>
<tr>
<td>Energy (kcal·d(^{-1}))</td>
<td>2798 (1344)</td>
<td>2756 (996)</td>
<td>0.898</td>
<td>0.815(^\ddagger)</td>
</tr>
<tr>
<td>Proteins (g·kg(^{-1})·d(^{-1}))</td>
<td>1.8 (0.8)</td>
<td>1.3 (0.6)</td>
<td>0.011*</td>
<td>0.067(^\ddagger)</td>
</tr>
<tr>
<td>Proteins (g·d(^{-1}))</td>
<td>126 (50)</td>
<td>116 (45)</td>
<td>0.315</td>
<td>0.236</td>
</tr>
<tr>
<td>Carbohydrates (g·kg(^{-1})·d(^{-1}))</td>
<td>4.8 (2.8)</td>
<td>4.2 (1.9)</td>
<td>0.170</td>
<td>0.040(^\ddagger)*</td>
</tr>
<tr>
<td>Carbohydrates (g·d(^{-1}))</td>
<td>351 (163)</td>
<td>337 (143)</td>
<td>0.938</td>
<td>0.260</td>
</tr>
<tr>
<td>Lipids (g·d(^{-1}))</td>
<td>94 (49)</td>
<td>96 (46)</td>
<td>0.511</td>
<td>0.077</td>
</tr>
</tbody>
</table>

\(^1\) Energy and nutrients are presented as unadjusted variables
\(^\dagger\) General linear model
\(^\ddagger\) General linear model adjusted for energy (\(^\ddagger\) except energy), age, education, sport, hours of training, and BMI (\(^\ddagger\) except BMI)

\(*\ P < 0.05\)
Table 3 Comparison of micronutrients intake from food between nutritional supplements users and non-users according to gender.\(^1\)

Crude and adjusted p-values are presented. Data are presented as the median (interquartile range).

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th></th>
<th>Females</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Users (n=108)</td>
<td>Non-users (n=52)</td>
<td>Crude (P^†)</td>
<td>Adjusted (P^‡)</td>
</tr>
<tr>
<td>Vitamin A ((\mu g\cdot d^{-1}))</td>
<td>2549 (1708)</td>
<td>2543 (1986)</td>
<td>0.609</td>
<td>0.083</td>
</tr>
<tr>
<td>Vitamin C (mg·d(^{-1}))</td>
<td>164 (139)</td>
<td>163 (113)</td>
<td>0.179</td>
<td>0.053</td>
</tr>
<tr>
<td>Vitamin E (mg·d(^{-1}))</td>
<td>10.7 (7.1)</td>
<td>11.1 (4.8)</td>
<td>0.494</td>
<td>0.479</td>
</tr>
<tr>
<td>Vitamin D ((\mu g\cdot d^{-1}))</td>
<td>5.5 (3.5)</td>
<td>5.0 (3.4)</td>
<td>0.460</td>
<td>0.768</td>
</tr>
<tr>
<td>Thiamine (mg·d(^{-1}))</td>
<td>2.3 (1.1)</td>
<td>2.2 (1.1)</td>
<td>0.975</td>
<td>0.181</td>
</tr>
<tr>
<td>Riboflavin (mg·d(^{-1}))</td>
<td>3.3 (1.8)</td>
<td>2.9 (1.3)</td>
<td>0.509</td>
<td>0.026*</td>
</tr>
<tr>
<td>Vitamin B6 (mg·d(^{-1}))</td>
<td>3.3 (1.5)</td>
<td>2.8 (1.3)</td>
<td>0.308</td>
<td>0.205</td>
</tr>
<tr>
<td>Vitamin B12 ((\mu g\cdot d^{-1}))</td>
<td>13.4 (8.3)</td>
<td>11.5 (9.2)</td>
<td>0.818</td>
<td>0.109</td>
</tr>
<tr>
<td>Folate ((\mu g\cdot d^{-1}))</td>
<td>510 (299)</td>
<td>461 (237)</td>
<td>0.334</td>
<td>0.032*</td>
</tr>
<tr>
<td>Magnesium (mg·d(^{-1}))</td>
<td>453 (220)</td>
<td>417 (164)</td>
<td>0.532</td>
<td>0.160</td>
</tr>
<tr>
<td>Zinc (mg·d(^{-1}))</td>
<td>17.0 (7.7)</td>
<td>15.7 (6.6)</td>
<td>0.584</td>
<td>0.293</td>
</tr>
<tr>
<td>Calcium (mg·d(^{-1}))</td>
<td>1388 (845)</td>
<td>1149 (738)</td>
<td>0.090</td>
<td>0.019*</td>
</tr>
<tr>
<td>Iron (mg·d(^{-1}))</td>
<td>22.4 (10.6)</td>
<td>21.0 (13.7)</td>
<td>0.853</td>
<td>0.225</td>
</tr>
<tr>
<td>Selenium ((\mu g\cdot d^{-1}))</td>
<td>130 (60)</td>
<td>114 (73)</td>
<td>0.622</td>
<td>0.122</td>
</tr>
</tbody>
</table>

\(^1\) Energy and nutrients are presented as unadjusted variables

\(^†\) General linear model

\(^‡\) General linear model adjusted for energy, age, education, sport, hours of training, and BMI

\(* P < 0.05\)

\(* P < 0.05\)
Table 4 Comparison of prevalence of micronutrient inadequacy between nutritional supplements users and non-users according to gender. Data are presented as the n (%).

<table>
<thead>
<tr>
<th>Males PMI</th>
<th>Females PMI</th>
<th>PMI</th>
<th>Total PMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Users (n=108)</td>
<td>Non-users (n=52)</td>
<td>P†</td>
<td>Users (n=153)</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>2 (2%)</td>
<td>0</td>
<td>N.V.</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>6 (6%)</td>
<td>4 (8%)</td>
<td>N.V.</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>62 (57%)</td>
<td>30 (58%)</td>
<td>0.555</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>102 (94%)</td>
<td>46 (89%)</td>
<td>N.V.</td>
</tr>
<tr>
<td>Thiamine</td>
<td>0</td>
<td>0</td>
<td>N.V.</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>0</td>
<td>0</td>
<td>N.V.</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>0</td>
<td>0</td>
<td>N.V.</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>0</td>
<td>0</td>
<td>N.V.</td>
</tr>
<tr>
<td>Folate</td>
<td>21 (19%)</td>
<td>13 (25%)</td>
<td>0.272</td>
</tr>
<tr>
<td>Magnesium</td>
<td>25 (23%)</td>
<td>12 (23%)</td>
<td>0.580</td>
</tr>
<tr>
<td>Zinc</td>
<td>4 (4%)</td>
<td>1 (2%)</td>
<td>N.V.</td>
</tr>
<tr>
<td>Calcium</td>
<td>15 (14%)</td>
<td>15 (29%)</td>
<td>0.022*</td>
</tr>
<tr>
<td>Selenium</td>
<td>1 (1%)</td>
<td>0</td>
<td>N.V.</td>
</tr>
</tbody>
</table>

PMI, prevalence of micronutrient inadequacy; N.V., non-valid chi-squared test

† Chi-squared test. For the statistically significant P, the phi (φ) coefficients (and their corresponding P) are presented

* P < 0.05
A huge percentage of the sample (92%) showed an inadequate intake for vitamin D, and more than half of the sample (58%) for vitamin E. A high PMI level was also found for folate, magnesium, and calcium, as shown in Table 4. The PMI for the other nutrients was low or non-existent. Female users reported a significantly lower PMI for magnesium and calcium, and male users for calcium. Regardless of gender, NS users showed to have a lower PMI for folate and calcium.

Additionally, female non-users were significantly more likely to present magnesium (crude OR 0.12, 95% CI 0.03, 0.60) and calcium (crude OR 0.35, 95% CI 0.13, 0.94) inadequacy than users. When adjusted for energy intake, BMI, education, sport, and hours of training, the PMI for calcium (OR 0.18, 95% CI 0.28, 1.11), but not for magnesium (OR 0.06, 95% CI 0.00, 0.98), ceased to be associated with supplement usage. Male users were less likely to have an inadequate calcium intake (crude OR 0.40, 95% CI 0.18, 0.90; adjusted OR 0.29, 95% CI 0.10, 0.82). Furthermore, NS users (irrespective of gender) were less likely to have an inadequate calcium intake (crude OR 0.36, 95% CI 0.19, 0.67; adjusted OR 0.28, 95% CI 0.12, 0.65). No association was observed for folate (crude OR 0.56, 95% CI 0.30, 1.05; adjusted OR 0.69, 95% CI 0.30, 1.60).

DISCUSSION

One of the main findings of this study was that elite Portuguese athletes who consumed NS reported a better nutritional intake from food than non-users. Moreover, the athletes who did not use NS reported a higher PMI for several nutrients. Additionally, taking NS showed to be associated, independently of confounders, with lower odds of inadequate intake from food sources of calcium, irrespectively of gender, and of magnesium, for female athletes.
The tendency for healthier food choices and better dietary intakes for several nutrients by NS users compared to non-users have been systematically described in non-athletic populations (Bailey et al., 2011; Beitz et al., 2004; McNaughton et al., 2005). In the sports field, there is one study (Beshgetoor & Nichols, 2003) with female master (≥35 y) cyclists and runners which also showed that supplemented athletes reported higher intakes of some micronutrients. However, the dietary information included both food and NS making it difficult to compare with the present data. Nevertheless, if one takes into account that health and performance issues were the most chosen reasons to justify NS consumption among Portuguese athletes (Sousa et al., 2013), it seems that there is a propensity, also among athletes, for NS use by those who are more self-concerned.

When comparing the ingestion of macronutrients from food between NS users and non-users, the higher weight-adjusted protein intake found in both male and female users for crude values, and in females for adjusted ones also, is of special interest. Moreover, the users groups’ intake was above the upper recommendation limit, whereas the intake of the non-users groups was within the recommended range [1.2–1.7 g·kg⁻¹·d⁻¹ (Rodriguez et al., 2009)]. With regard to CHO intake, male users shown to have higher weight-adjusted ingestion after confounders’ adjustment compared to non-users, with both groups showing median ingestions under the minimum recommended for the studied sample: 5 g·kg⁻¹·d⁻¹ (Burke et al., 2011). Female users also reported higher CHO intake (crude values), but median ingestion for both groups was slightly above 5 g CHO·kg⁻¹·d⁻¹. Taking into account that the increased intake of proteins and CHO through the use of NS was already seen in high-performance athletes from 38 different modalities (Lun et al., 2009), and that protein supplements, sport drinks, and sport gels, were amongst the most consumed NS, it is reasonable to expect that the combined intake of food and supplements would result in an higher median-group intakes for NS users. This is especially relevant for proteins since a higher ingestion would lead to a greater deviation from the recommended range. Higher protein intakes than the recommendation
are frequently seen among athletes (Erdman et al., 2013; Tipton, 2011). Over the years, the negative impact of high protein intake on bone mass and renal function in healthy individuals has been demystified (Bonjour, 2005; Institute of Medicine, 2005). The excess of protein seems to lead to urea production, resulting in a higher need of water, and oxidation of the carbon skeletons (Phillips, 2012). Concerning the athletic performance, protein intakes higher than recommendations may have a negative impact if the extra protein is achieved at the expense of CHO (Phillips, 2012). Regarding our data, this seems to be the case since the median of CHO intake for NS users was below the lower limit for males and marginally above for females, and the lipid consumption was within the recommendation values (30.8 ± 6.3 % TEV and 31.7 ± 5.4 % TEV for female and male users, respectively). Therefore, these athletes might benefit from specific nutritional guidance in order to increase CHO ingestion, and implement strategies to adjust protein intake.

Although macronutrient ingestion was near the recommended values, probably reflecting an adequate energy intake, a high PMI was reported for several micronutrients. Intakes below the DRI for folate (Beshgetoor & Nichols, 2003; Gibson et al., 2011; Iglesias-Gutiérrez et al., 2005), vitamin E (Beshgetoor & Nichols, 2003; Gibson et al., 2011; Iglesias-Gutiérrez et al., 2005), calcium (Farajian et al., 2004; Gibson et al., 2011; Nogueira & Da Costa, 2004), and magnesium (Farajian et al., 2004; Iglesias-Gutiérrez et al., 2005; Nogueira & Da Costa, 2004), were also found in other athletic groups, namely triathletes (Nogueira & Da Costa, 2004), female master (≥35 y) cyclists and runners (Beshgetoor & Nichols, 2003), swimmers and water polo players (Farajian et al., 2004), female junior soccer players (Gibson et al., 2011), and adolescent soccer players (Iglesias-Gutiérrez et al., 2005). Moreover, compared to non-users, supplement users (irrespective of gender) had 3.6 times lower odds to have inadequate calcium intake, and female users had 16.7 times lower odds to have inadequate magnesium intake, with confounders’ adjustment in both situations. Concerning vitamin D, its insufficient ingestion within athletic populations has been a hot topic in the sports nutrition field, due to its possible
negative impact on bone health, immune function, inflammatory modulation, and also on muscle function and performance (Larson-Meyer & Willis, 2010; Powers et al., 2011). Regarding our participants, they also seem to show the same tendency concerning vitamin D insufficient intake. Notwithstanding the huge prevalence of inadequate intake, vitamin D supplement was not between the most taken supplements in our study and in others (De Silva et al., 2010; Tian et al., 2009). Nevertheless, beyond food and supplementation, vitamin D can also be obtained by endogenous synthesis in the skins’ dermis after ultraviolet B radiation activation (Owens et al., 2014). Surprisingly, deficient intakes of the mentioned micronutrients are still a reality, albeit their insufficient supply can lead to performance impairments (Rodriguez et al., 2009). This may be a result of an ineffective transmission of knowledge from nutritionists and other health professionals to athletes, resistance to change to a healthier dietary behaviour, and/or a lack of care regarding these particular nutrients. Macronutrients, especially proteins, are frequently the primarily nutritional concern for athletes, which might lead to forgetfulness of CHO and micronutrients.

Some limitations regarding the NS questionnaire have already been discussed elsewhere (Sousa et al., 2013). Considering that our aim was to compare users with non-users, both punctual and habitual users were considered as NS users which may constitute a limitation. The time extent of data gathering also did not permit to assess biochemical parameters, namely vitamin D status. Future studies may opt to evaluate shorter periods of time in order to be feasible to collect information regarding nutritional status, and more specific data regarding NS usage and nutritional intake. Concerning the used FFQ, although it has an acceptable validity (Lopes, 2000; Moreira et al., 2003; Pinto et al., 2010), the interpretation of results should be done with caution. Even though FFQs are often used to estimate absolute intakes, results should be cautiously evaluated since multiple food records are a preferred method for more precise estimates (Shim et al., 2014). Nevertheless, this study allowed the collection of information regarding long-term NS usage and the athletes’ dietary
intake during the same period of time. Studies performing a simultaneous approach are scarce, and the information that arose from these combined questionnaires is of great and emerging interest. Moreover, this study was performed with a considerable high number of high-level athletes from several different sports, which gave the opportunity to add some novel information about this particular group of sportsmen and sportswomen.

In conclusion, high-performance athletes that used NS reported a generally higher nutritional intake from food and a lower PMI for some micronutrients. Considering the dietary results comparing NS users with non-users, professionals working with athletes should review and question NS use. Perhaps, the athletes who are using or want to use NS, might be the ones who are already more concerned about nutrition-related aspects and with least need to supplement their diet with macro- and/or micronutrients. On this, athletes seem to follow the tendency of general population (Kirk et al., 1999; McNaughton et al., 2005). Moreover, one should bear in mind that supplements cost money and may result in inadvertent doping (Geyer et al., 2011). Therefore, attention and care should be given to the type of NS that an athlete is taking, in order to prevent unnecessary supplementation. Moreover, this study shows that athletes using NS might benefit from general dietary adjustment, since the amount of ingested proteins seemed higher than recommendations whereas the intake of CHO was near the lower borderline. Furthermore, and given the high PMI for several micronutrients, these athletes would probably benefit not only from a quantitative nutritional approach, but also from a qualitative one focusing on nutritionally-dense foods.

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REFERENCES


3.3 Study III
Nutritional supplement-usage associated characteristics of high-performing athletes

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ABSTRACT

**Purpose:** To analyze whether sociodemographic and sporting characteristics, health-behaviors, and food intake were associated with nutritional supplements (NS) usage among high-performance athletes.

**Methods:** Portuguese athletes from 13 sports completed (i) a NS usage questionnaire, assessing information on sociodemographic (gender, age, height, weight, athlete’s and parental education level), health-related (smoking, daily time of sleeping, walking and siting), and sporting (type, number of international performances, weekly hours of training and of gym) characteristics, and (ii) a semi-quantitative food-frequency questionnaire (86 items), regarding the previous 12 months. Variables were recoded into two categories. Logistic regression was performed to study associations between all variables and NS usage.

**Results:** From the 241 athletes (66% males, 13–37 y), 64% reported NS use. After adjustment, supplement usage was associated with being ≥18 y (odds ratio [OR] 2.57, 95%; confidence interval [CI] 1.17–5.65), performing individual sports (OR 5.45, 95%; CI 2.49–11.93), and ≥2 h gym/week (OR 2.42, 95%; CI 1.15–5.11). Regardless of confounders, supplement users reported to have a higher consumption of meat (OR 2.83, 95%; CI 1.36–5.90), eggs (OR 2.53, 95%; CI 1.07–5.96), and yogurt (OR 2.24, 95%; CI 1.08–4.62), and a lower intake of processed meat (OR 0.32, 95%; CI 0.15–0.72), vegetable oils (OR 0.35, 95%; CI 0.17–0.74), margarine (OR 0.37, 95%; CI 0.18–0.76), chips (OR 0.22, 95%; CI 0.10–0.48), and fast food (OR 0.42, 95%; CI 0.19–0.91).

**Conclusions:** Athletes using NS had different characteristics from non-users, and seem to have healthier and more sports-directed food choices.

**Keywords:** Food, age, sport, health.
INTRODUCTION

The consumption of nutritional supplements (NS) is highly prevalent among athletes, with some studies reporting up to 90% of users (De Silva et al., 2010). Although a virtually uncountable number of supplements exist, only a few are supported by scientific evidence for health or performance positive effect (Maughan et al., 2011). Yet, the use of NS among athletes is widespread, and appears to be increasing with time (Huang et al., 2006). Not surprisingly, some studies (Petróczi et al., 2007; Sousa et al., 2013) have shown that athletes' underlying reasons to consume NS are not always congruent with the supplement's rationale. Moreover, in one study (Dascombe et al., 2010) the majority of the supplemented athletes did not know their supplements' active ingredient, side effects, or mechanism of action, denoting a probable lack of informed choices on NS. Moreover, the safety of supplement use in terms of sport practice, due to a possible failed doping test, is a current matter of concern (Geyer et al., 2008).

Some athletes are probably more prone to use supplement than others, as this also seems to occur in general population (Reinert et al., 2007). In studies involving athletic populations, some characteristics have been related to NS usage, namely the type of sport (Froiland et al., 2004), age (Erdman et al., 2007), and level of competition (Erdman et al., 2006). Moreover, healthier behaviours (McNaughton et al., 2005) and healthier food choices (Beitz et al., 2004) have been positively associated with NS consumers in general populations, making them the ones who probably least need them. However, athletes are a particular population, where performance, and not necessarily health, is the goal. Although athletes are known to use supplements more than general population (Sobal & Marquart, 1994), little is known concerning health-related behaviours and food choices of those using NS.

Taking all this into account, it is important to understand the characteristics associated with the NS-user athlete, in order to better educate,
guide, and prevent the misuse and abuse of these substances. Therefore, the main aim of the present study was to analyze sociodemographic and sporting characteristics, health-behaviors, and food consumption of high-performance athletes, in order to investigate if and how these factors were associated with NS usage.

METHODS

Participants and study design

Three hundred and four athletes representing the Portuguese national teams in 13 sports – volleyball (22.0%), swimming (12.8%), triathlon (10.2%), cycling (9.5%), judo (7.9%), athletics (6.6%), handball (5.6%), baseball (5.3%), rugby (5.3%), gymnastic (5.3%), basketball (4.3%), fencing (3.9%), and boxing (1.3%) – volunteered to participate in this study. Informed consent was obtained from all athletes, and formal authorisation from the guardians was required for those <18 years old. The study was approved by the Scientific Council of the Faculty of Nutrition and Food Sciences of the University of Porto, and by each national sports federation. Participants filled out 2 questionnaires: one about NS usage and a semi-quantitative food-frequency questionnaire (FFQ). Both questionnaires assessed information over the previous 12 months. The questionnaires were completed in the presence of a qualified and trained nutritionist or sent in to the respective sport federation (boxing and fencing) throughout the year of 2008; the two methods yielded similar results regarding NS usage ($P = 0.369$) and energy intake ($P = 0.897$).

Dietary Intake

Dietary intake was measured via a semi-quantitative FFQ validated for the Portuguese adults (Lopes et al., 2007) and also used on adolescents (Abreu...
et al., 2014). The FFQ was designed according to Willett (Willett, 1998) and adapted to include typical Portuguese food items. The questionnaire comprises 86 food items or beverage categories, and a frequency section with 9 possible responses, ranging from ‘never or less than 1 time per month’ to ‘6 or more times per day’. Any food not listed in the questionnaire could be added by participants in a free-response section. Food intake was calculated with consideration of portion and the respondents’ ratings of frequency and seasonality of each item. Energy intake, without including the NS contribution, was estimated using the software Food Processor SQL® (ESHA Research Inc., Salem, OR, USA) added with Portuguese foods and recipes.

To identify miss-reporting, the ratio energy intake (EI) to basal metabolic rate (BMR) was used (Livingstone & Black, 2003). EI was obtained from food data analysis whereas BMR was estimated using Schofield equations (Schofield et al., 1985). The under-reporting cut-off for this study was set at 0.9, similarly to other study with athletes and with an identical purpose (Farajian et al., 2004). Ratios >4.0 were considered as over-reporting, since this value corresponds to the physical activity level (PAL) upper limit for professional endurance athletes (Westerterp, 2013).

Participants were divided into 2 categories according to the amount of each food group consumed: one corresponding to an intake lower than or equal to the median amount of the total sample (low intake), and the other corresponding to an intake higher than the median amount of the total sample (high intake). For the following 51 foods, beverages, or groups of foods, qui-squared test were performed: vegetables, vegetable soup, pulses, fresh fruit, canned fruit, olives, nuts, fish (fat fish, low-fat fish, canned fish, mollusc, seafood, shellfish) fat fish, low-fat fish, canned fish, mollusc, sea food and shellfish, meat (beef, lamb, pork, offal, poultry, rabbit), low-fat meat (poultry, rabbit), red meat (beef, lamb, pork), offal, processed meats (sausages, ham, smoked ham, chorizo), eggs, olive oil, vegetable oils, butter, margarine, milk (fat milk, semi-skimmed milk, skimmed milk), fat milk, semi-skimmed milk, skimmed
milk, yogurt (all types), cheese (all types), milk-based pudding (custard, vanilla), ice cream, starches (pasta, potato, chips, rice), pasta, potato other than fried, chips, rice, bread (white and dark), white bread, dark bread, ready-to-eat cereals, sugar sweetened beverages, coffee, tea, alcoholic beverages, fast food (pizza, hamburgers, snack fried foods), pastry (cakes, sugar biscuits/wafers, chocolate candy, marmalade/jam, sugar), cakes and sugar biscuits/wafers, chocolate candy, marmalade/jam, sugar, and crackers/cookies (approximately 20% of sugar or less). Only statistically significant test were reported (Table 2).

**Nutritional supplements usage and other information**

A broad definition of NS was used, which included all types of supplements, namely ergogenic aids, sports food, and dietary/nutritional supplements, as fully described elsewhere (Sousa et al., 2013). Other assessed information included weight, height, age, gender, years of education of the athlete (year of attendance or concluded years if the athlete was not currently studying) and of his/her parents, type of sport, weekly hours of training and of gym, number of international performances, hours of sleep per day, and daily walking and sitting time (time spending watching TV, playing video games, on computer, reading, driving, eating, on class) during most days of the week.

For the purpose of statistical analyses, all variables were recoded into two categories. Weekly hours of training and of gym, number of international performances, and daily sitting time, were recoded using the same methodology described above for food items. For body mass index (BMI) the same procedure was used but groups were defined by the mean. Age was divided into adolescents (<18 y) and adults (≥18 y), education by national mandatory school education (9 years of schooling), sports into team (rugby, basketball, volleyball, baseball, handball) and individual (cycling, athletics, triathlon, gymnastic, judo, swimming, boxing, fencing), sleeping duration into <8 h/day and ≥8h/day, walking time by World Health Organization recommendations (World Health
Organization, 2010): (i) <60 min for adolescents and <30 min for adults, or (ii) ≥60 min for adolescents and ≥30 min for adults, and smoking habits by smokers and non-smokers.

**Statistical analysis**

Descriptive data were reported as proportions (%), and as mean ± standard deviation when data were normally distributed (height, weight). Kolmogorov-Smirnov test was used to evaluate normality. Chi-squared tests were used to investigate the relationships between supplement usage and sociodemographic factors, health-related behaviours, sporting characteristics, and food consumption. For those with statistically significant relationships, logistic regression was subsequently performed without and with confounders’ adjustment. Gender, age, BMI, father and mother's education, type of sport, weekly hour of training and of gym, for being statistically different between NS users and non-users (Table 1), and energy were considered as confounders. Odds ratios (OR) and 95% confidence intervals (CI) were calculated by reference with the first (lower) category of each variable. All statistical procedures were completed using Statistical Package for Social Sciences® version 20 (SPSS Inc., Chicago, IL, USA). The level of significance was set at $P < 0.05$.

**RESULTS**

From the 304 athletes, 44 were excluded due to incomplete information, and 19 due to miss-reporting. Therefore, the final sample comprised 241 athletes (66% males, 13–37 y, 71.5 ± 13.0 kg, 178 ± 11 cm).

The majority of athletes (64%, n = 153) reported to have used NS in the previous 12 months. Overall, a greater percentage of male than female athletes
Study III

(P = 0.047) reported NS usage, as shown in Table 1. Moreover, adult athletes (P < 0.001) and those with lower BMI (P = 0.013), also reported a higher NS usage. Although athletes' years of education were not related with NS usage (P = 0.168), parents' education was, with less than 9 years of education being related to supplement usage (P < 0.01). Regarding sporting characteristics, to perform an individual modality (P < 0.001), to train more than 12.5 h/week (P < 0.001), and to attend the gym more than 2 h/week (P = 0.010) were related with NS usage. Concerning health-related behaviours, there were no statistically significant differences (P > 0.05) between NS users and non-users for sleeping duration, walking, and sitting daily time, and smoking. Older athletes, athletes involved in individual modalities, and those performing >2h gym/week had, respectively, 2.6, 5.4, and 2.4 times higher odds to be a NS user than younger athletes and those performing team sports and ≤2h gym/week, after adjustment for the other variables with statistically significant chi-squared test in Table 1. No other factors were independently associated with supplement usage by athletes.

Twenty-one food groups and beverages categories were found to be related with supplement usage (Table 2). Athletes with lower daily intake of olives, vegetable oils, margarine, and chips were significantly more likely to be a NS user than athletes consuming higher portions of these foods. On the other hand, athletes with higher intake of nuts, pulses, fish, canned fish, meat, low-fat meat, red meat, eggs, skimmed milk, yogurt, pasta, coffee, tea, and marmalade/jam were significantly more likely to be a NS user than athletes consuming lower portions of these foods. Moreover, athletes with higher intake of meat, eggs, and yogurt had, respectively, 2.8, 2.5 and 2.2 times higher odds to be a NS user than athletes consuming lower quantities of these foods, independently of energy, gender, age, BMI, father's and mother's education, sport, and weekly hours of training and of gym. Contrariwise, after adjustment, athletes with lower intake of processed meat, vegetable oils, margarine, chips, and fast food, were 3.1, 2.8, 2.7, 4.5, and 2.4 lower odds to be a NS user than athletes that reported a higher intake of these foods.
Table 1 Associations between nutritional supplements usage and sociodemographic characteristics, sporting factors, and health-related behaviours.

Data are presented as percentage (n).

<table>
<thead>
<tr>
<th></th>
<th>Non-users (n=88)</th>
<th>Users (n=153)</th>
<th></th>
<th>Crude OR (95% CI)</th>
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<th>Adjusted OR (95% CI)</th>
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<tbody>
<tr>
<td></td>
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<td></td>
<td>P</td>
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<td>P</td>
<td></td>
<td>P</td>
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<tr>
<td>Gender</td>
<td></td>
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<tr>
<td>Female</td>
<td>40.9% (36)</td>
<td>29.4% (45)</td>
<td>0.047</td>
<td>Ref</td>
<td>0.070</td>
<td>Ref</td>
<td>0.958</td>
</tr>
<tr>
<td>Male</td>
<td>59.1% (52)</td>
<td>70.6% (108)</td>
<td></td>
<td>1.66 (0.96–2.88)</td>
<td></td>
<td>0.98 (0.41–2.34)</td>
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<tr>
<td>Age (years old)</td>
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<tr>
<td>&lt;18</td>
<td>47.7% (42)</td>
<td>19.0% (29)</td>
<td>&lt;0.001</td>
<td>Ref</td>
<td>&lt;0.001</td>
<td>Ref</td>
<td>0.019</td>
</tr>
<tr>
<td>≥18</td>
<td>52.3% (46)</td>
<td>81.0% (124)</td>
<td></td>
<td>3.90 (2.18–6.99)</td>
<td></td>
<td>2.57 (1.17–5.65)</td>
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<tr>
<td>BMI (kg/m²)</td>
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<tr>
<td>≤22.3</td>
<td>46.0% (40)</td>
<td>61.8% (94)</td>
<td>0.013</td>
<td>Ref</td>
<td>0.018</td>
<td>Ref</td>
<td>0.155</td>
</tr>
<tr>
<td>&gt;22.3</td>
<td>54.0% (47)</td>
<td>38.2% (58)</td>
<td></td>
<td>0.53 (0.31–0.90)</td>
<td></td>
<td>0.55 (0.24–1.26)</td>
<td></td>
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<tr>
<td>Education (years)</td>
<td></td>
<td></td>
<td>0.168</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤9</td>
<td>12.0% (19)</td>
<td>17.9% (25)</td>
<td></td>
<td>Ref</td>
<td>N.P.</td>
<td>N.P.</td>
<td>N.P.</td>
</tr>
<tr>
<td>&gt;9</td>
<td>88.0% (10)</td>
<td>82.1% (115)</td>
<td></td>
<td></td>
<td>N.P.</td>
<td>N.P.</td>
<td>N.P.</td>
</tr>
<tr>
<td>Father’s Education (years)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>≤9</td>
<td>26.4% (23)</td>
<td>43.7% (66)</td>
<td>0.006</td>
<td>Ref</td>
<td>0.009</td>
<td>Ref</td>
<td>0.504</td>
</tr>
<tr>
<td>&gt;9</td>
<td>73.6% (64)</td>
<td>56.3% (85)</td>
<td></td>
<td>0.46 (0.26–0.82)</td>
<td></td>
<td>0.69 (0.24–2.04)</td>
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<tr>
<td>Mother’s Education (years)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>≤9</td>
<td>21.4% (18)</td>
<td>44.7% (68)</td>
<td>&lt;0.001</td>
<td>Ref</td>
<td>&lt;0.001</td>
<td>Ref</td>
<td>0.198</td>
</tr>
<tr>
<td>&gt;9</td>
<td>78.6% (66)</td>
<td>55.3% (84)</td>
<td></td>
<td>0.34 (0.18–0.62)</td>
<td></td>
<td>0.49 (0.16–1.46)</td>
<td></td>
</tr>
<tr>
<td>Sport</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Team⁴</td>
<td>20.5% (18)</td>
<td>69.9% (107)</td>
<td>&lt;0.001</td>
<td>Ref</td>
<td>&lt;0.001</td>
<td>Ref</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Individual⁵</td>
<td>79.5% (70)</td>
<td>30.1% (46)</td>
<td></td>
<td>9.05 (4.85–16.86)</td>
<td></td>
<td>5.45 (2.49–11.93)</td>
<td></td>
</tr>
<tr>
<td>International performances (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤6</td>
<td>56.2% (45)</td>
<td>49.3% (68)</td>
<td></td>
<td>N.P.</td>
<td>N.P.</td>
<td>N.P.</td>
<td>N.P.</td>
</tr>
<tr>
<td>&gt;6</td>
<td>43.8% (35)</td>
<td>50.7% (70)</td>
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<td></td>
<td>N.P.</td>
<td>N.P.</td>
<td>N.P.</td>
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**Table 1 (continued)**  Associations between nutritional supplements usage and sociodemographic characteristics, sporting factors, and health-related behaviours.

Data are presented as percentage (n).

<table>
<thead>
<tr>
<th></th>
<th>Non-users (n=88)</th>
<th>Users (n=153)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Crude OR (95% CI)</td>
</tr>
<tr>
<td><strong>Training (h/week)</strong></td>
<td></td>
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</tr>
<tr>
<td>≤12.5</td>
<td>69.0% (60)</td>
<td>39.1% (59)</td>
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<tr>
<td>&gt;12.5</td>
<td>31.0% (27)</td>
<td>60.9% (92)</td>
</tr>
<tr>
<td><strong>Gym (h/week)</strong></td>
<td></td>
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<tr>
<td>≤2</td>
<td>70.9% (61)</td>
<td>54.7% (82)</td>
</tr>
<tr>
<td>&gt;2</td>
<td>29.1% (25)</td>
<td>45.3% (68)</td>
</tr>
<tr>
<td><strong>Sleeping (h/day)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤8</td>
<td>42.0% (37)</td>
<td>31.6% (48)</td>
</tr>
<tr>
<td>≥8</td>
<td>58.0% (51)</td>
<td>68.4% (104)</td>
</tr>
<tr>
<td><strong>Walking (min/day)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adolescents: &lt;60, adults &lt;30</td>
<td>55.0% (49)</td>
<td>52.0% (78)</td>
</tr>
<tr>
<td>Adolescents: ≥60, adults ≥30</td>
<td>43.0% (37)</td>
<td>48.0% (72)</td>
</tr>
<tr>
<td><strong>Sitting (h/day)</strong></td>
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<tr>
<td>≤8</td>
<td>44.2% (38)</td>
<td>55.8% (48)</td>
</tr>
<tr>
<td>&gt;8</td>
<td>54.7% (82)</td>
<td>45.3% (68)</td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
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</tr>
<tr>
<td>No</td>
<td>95.4% (83)</td>
<td>98.7% (151)</td>
</tr>
<tr>
<td>Yes</td>
<td>4.6% (4)</td>
<td>1.3% (2)</td>
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</table>

OR, odds ratio; CI, confidence interval; Ref, reference group; BMI, body mass index; N.P., test not performed; N.V., test not valid
1 Chi-squared test
2 Logistic regression
3 Logistic regression adjusted for all other variables in this table with statistical significant chi-squared test, and energy intake
4 Volleyball, rugby, handball, baseball, basketball
5 Swimming, cycling, judo, athletics, triathlon, fencing, gymnastic, boxing
Table 2 Associations between nutritional supplements usage and daily food intake.
Data are presented as percentage (n).

<table>
<thead>
<tr>
<th></th>
<th>Non-users (n=88)</th>
<th>Users (n=153)</th>
<th>P</th>
<th>Crude OR (95% CI)</th>
<th>P</th>
<th>Adjusted OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Olives</strong></td>
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</tr>
<tr>
<td>Low intake</td>
<td>43.2% (38)</td>
<td>58.8% (90)</td>
<td>0.014</td>
<td></td>
<td>0.020</td>
<td></td>
<td>0.268</td>
</tr>
<tr>
<td>High intake</td>
<td>56.8% (50)</td>
<td>41.2% (63)</td>
<td></td>
<td>Ref</td>
<td>0.53 (0.31–0.90)</td>
<td>0.68 (0.34–1.35)</td>
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</tr>
<tr>
<td><strong>Nuts</strong></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Low intake</td>
<td>60.2% (53)</td>
<td>40.5% (62)</td>
<td>0.002</td>
<td>2.23 (1.30–3.80)</td>
<td>1.46 (0.70–2.05)</td>
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<tr>
<td>High intake</td>
<td>39.8% (35)</td>
<td>59.5% (91)</td>
<td></td>
<td>Ref</td>
<td>0.68 (0.34–1.35)</td>
<td>1.46 (0.70–3.05)</td>
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</tr>
<tr>
<td><strong>Pulses</strong></td>
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</tr>
<tr>
<td>Low intake</td>
<td>43.2% (57)</td>
<td>28.4% (31)</td>
<td>0.013</td>
<td>1.91 (1.11–3.28)</td>
<td>0.268</td>
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<tr>
<td>High intake</td>
<td>56.8% (75)</td>
<td>71.6% (78)</td>
<td></td>
<td>Ref</td>
<td>0.68 (0.34–1.35)</td>
<td>1.90 (0.92–3.92)</td>
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<tr>
<td><strong>Fish</strong></td>
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</tr>
<tr>
<td>Low intake</td>
<td>62.5% (55)</td>
<td>43.1% (66)</td>
<td>0.003</td>
<td>2.20 (1.28–3.76)</td>
<td>0.268</td>
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<tr>
<td>High intake</td>
<td>37.5% (33)</td>
<td>56.9% (87)</td>
<td></td>
<td>Ref</td>
<td>0.68 (0.34–1.35)</td>
<td>1.88 (0.90–3.91)</td>
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<tr>
<td><strong>Canned fish</strong></td>
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<tr>
<td>Low intake</td>
<td>69.3% (61)</td>
<td>52.3% (80)</td>
<td>0.007</td>
<td>2.06 (1.19–3.58)</td>
<td>0.010</td>
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<td>0.070</td>
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<tr>
<td>High intake</td>
<td>30.7% (27)</td>
<td>47.7% (73)</td>
<td></td>
<td>Ref</td>
<td>0.68 (0.34–1.35)</td>
<td>1.94 (0.95–3.99)</td>
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</tr>
<tr>
<td><strong>Meat</strong></td>
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<tr>
<td>Low intake</td>
<td>63.6% (56)</td>
<td>45.1% (69)</td>
<td>0.004</td>
<td>2.13 (1.24–3.65)</td>
<td>0.010</td>
<td></td>
<td>0.006</td>
</tr>
<tr>
<td>High intake</td>
<td>36.4% (32)</td>
<td>54.9% (32)</td>
<td></td>
<td>Ref</td>
<td>0.68 (0.34–1.35)</td>
<td>2.83 (1.36–5.90)</td>
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<tr>
<td><strong>Low-fat meat</strong></td>
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<td>Low intake</td>
<td>60.2% (53)</td>
<td>45.1% (69)</td>
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<td>1.84 (1.08–3.14)</td>
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<td>39.8% (35)</td>
<td>54.9% (84)</td>
<td></td>
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<td>0.68 (0.34–1.35)</td>
<td>1.09 (0.54–2.18)</td>
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<tr>
<td><strong>Red meat</strong></td>
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<tr>
<td>Low intake</td>
<td>62.5% (55)</td>
<td>49.0% (75)</td>
<td>0.029</td>
<td>1.73 (1.02–2.96)</td>
<td>0.044</td>
<td></td>
<td>0.208</td>
</tr>
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<td>High intake</td>
<td>37.5% (33)</td>
<td>51.0% (78)</td>
<td></td>
<td>Ref</td>
<td>0.68 (0.34–1.35)</td>
<td>1.60 (0.77–3.30)</td>
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</tr>
<tr>
<td><strong>Processed meat</strong></td>
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</tr>
<tr>
<td>Low intake</td>
<td>43.2% (38)</td>
<td>56.2% (86)</td>
<td>0.035</td>
<td>0.59 (0.35–1.01)</td>
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<td>56.8% (50)</td>
<td>43.8% (67)</td>
<td></td>
<td>Ref</td>
<td>0.68 (0.34–1.35)</td>
<td>0.32 (0.15–0.72)</td>
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</tr>
</tbody>
</table>
Table 2 (continued) Associations between nutritional supplements usage and daily food intake.
Data are presented as percentage (n).

<table>
<thead>
<tr>
<th>Food</th>
<th>Non-users (n=88)</th>
<th>Users (n=153)</th>
<th>( P^i )</th>
<th>Crude OR (95% CI)</th>
<th>( P )</th>
<th>Adjusted OR (95% CI)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs</td>
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</tr>
<tr>
<td>Low intake</td>
<td>77.3% (68)</td>
<td>56.9% (87)</td>
<td>0.001</td>
<td>Ref</td>
<td>0.002</td>
<td>Ref</td>
<td>0.034</td>
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<tr>
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<td></td>
<td>2.58 (1.43–4.66)</td>
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<td>2.53 (1.07–5.96)</td>
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<tr>
<td>Vegetable oils</td>
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<td>0.019</td>
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<td>0.028</td>
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<td>0.006</td>
</tr>
<tr>
<td>Low intake</td>
<td>52.3% (46)</td>
<td>66.7% (102)</td>
<td></td>
<td>Ref</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>High intake</td>
<td>47.7% (42)</td>
<td>33.3% (51)</td>
<td></td>
<td>0.55 (0.32–0.94)</td>
<td></td>
<td>0.35 (0.17–0.74)</td>
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</tr>
<tr>
<td>Margarine</td>
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<td>0.010</td>
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<td>0.007</td>
</tr>
<tr>
<td>Low intake</td>
<td>37.5% (33)</td>
<td>54.9% (84)</td>
<td></td>
<td>Ref</td>
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<tr>
<td>High intake</td>
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<td>45.1% (69)</td>
<td></td>
<td>0.49 (0.29–0.84)</td>
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<td>0.37 (0.18–0.76)</td>
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<tr>
<td>Skimmed milk</td>
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<td>0.560</td>
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<td>Low intake</td>
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<td>66.7% (102)</td>
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<tr>
<td>High intake</td>
<td>20.5% (18)</td>
<td>33.3% (51)</td>
<td></td>
<td>1.94 (1.05–3.61)</td>
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<td>1.27 (0.57–2.82)</td>
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<td>Yogurt</td>
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<tr>
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<td>47.7% (73)</td>
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<td>52.3% (80)</td>
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<td>2.12 (1.23–3.65)</td>
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<td>2.24 (1.08–4.62)</td>
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<tr>
<td>High intake</td>
<td>35.2% (31)</td>
<td>54.9% (84)</td>
<td></td>
<td>2.24 (1.30–3.85)</td>
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<td>1.39 (0.68–2.82)</td>
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<td>Potato (other than fried)</td>
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<tr>
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<td>50% (44)</td>
<td>62.7% (96)</td>
<td></td>
<td>Ref</td>
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</tr>
<tr>
<td>High intake</td>
<td>50% (44)</td>
<td>37.3% (57)</td>
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<td>0.59 (0.35–1.01)</td>
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<td>0.64 (0.32–1.27)</td>
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<td>Chips</td>
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<td>&lt;0.001</td>
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<td>&lt;0.001</td>
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<tr>
<td>Low intake</td>
<td>30.7% (27)</td>
<td>62.1% (95)</td>
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<td>Ref</td>
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<tr>
<td>High intake</td>
<td>69.3% (61)</td>
<td>37.9% (58)</td>
<td></td>
<td>0.27 (0.16–0.47)</td>
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<td>0.22 (0.10–0.48)</td>
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<tr>
<td>Coffee</td>
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<td>51.0% (78)</td>
<td></td>
<td>Ref</td>
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<tr>
<td>High intake</td>
<td>34.1% (30)</td>
<td>49.0% (75)</td>
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<td>1.86 (1.08–3.20)</td>
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<td>1.37 (0.64–2.94)</td>
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</table>
Table 2 (continued) Associations between nutritional supplements use and daily food intake.

Data are presented as percentage (n).

<table>
<thead>
<tr>
<th></th>
<th>Non-users (n=88)</th>
<th>Users (n=153)</th>
<th>( p^1 )</th>
<th>Crude OR (95% CI)(^2 )</th>
<th>( P )</th>
<th>Adjusted OR (95% CI)(^3 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tea</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low intake</td>
<td>61.4% (54)</td>
<td>47.7% (73)</td>
<td>0.028</td>
<td>Ref</td>
<td>0.042</td>
<td>Ref</td>
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<tr>
<td>High intake</td>
<td>38.6% (34)</td>
<td>52.3% (80)</td>
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<td>1.74 (1.02–2.97)</td>
<td>0.028</td>
<td>0.77 (0.37–1.59)</td>
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<tr>
<td><strong>Fast food</strong></td>
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<tr>
<td>Low intake</td>
<td>46.6% (41)</td>
<td>58.8% (90)</td>
<td>0.044</td>
<td>Ref</td>
<td>0.067</td>
<td>Ref</td>
<td>0.028</td>
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<tr>
<td>High intake</td>
<td>53.4% (47)</td>
<td>41.2% (63)</td>
<td></td>
<td>0.61 (0.36–1.04)</td>
<td>0.067</td>
<td>0.42 (0.19–0.91)</td>
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<tr>
<td><strong>Marmalade/jam</strong></td>
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</tr>
<tr>
<td>Low intake</td>
<td>67.0% (59)</td>
<td>51.6% (79)</td>
<td>0.014</td>
<td>Ref</td>
<td>0.021</td>
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<td>High intake</td>
<td>33.0% (29)</td>
<td>48.4% (74)</td>
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<td>1.91 (1.10–3.29)</td>
<td>0.29</td>
<td>1.29 (0.61–2.71)</td>
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</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval; Ref, reference group

\(^1\)Chi-squared test
\(^2\)Logistic regression
\(^3\)Logistic regression adjusted for energy intake, gender, age, BMI, father’s and mother’s education, sport, hours of training per week, and hours of gym per week

Includes fat fish, low-fat fish, canned fish, mollusc, seafood, and shellfish

Includes beef, lamb, pork, offal, poultry, and rabbit

Includes poultry, and rabbit

Includes beef, lamb, and pork

Includes sausages, ham, smoked ham, and chorizo

Includes pizza, hamburgers, and snack fried foods
DISCUSSION

The collected data demonstrated that some sociodemographic and sporting characteristics, namely gender, age, BMI, parents’ education, type of sport, hours of training and gym/week, and a range of foods, seem to be related to NS consumption. The use of NS is highly prevalent among athletes (Maughan et al., 2011) and, taking into account our results, Portuguese athletes are not an exception. Athletes seem to believe that the use of these substances is almost mandatory for achieving maximal performance. The multi-billion-dollar industry of supplements has been fuelled this conviction with aggressive advertisement campaigns on their products promising performance enhancing, frequently without specific scientific evidence (Bishop, 2010). In fact, media has been recently considered a predictor of NS use in adolescent boys (Frison et al., 2013). Curiously, Dietz and collaborators (Dietz et al., 2014) recently concluded that young athletes with the aim to enhance performance in order to become an Olympic or World Champion had 3.7 times more odds to be a NS user.

Our results seems to evidence that athletes with more sports experience (more training load, and older) were associated with NS usage. Accordingly, a group of 72 Austrian athletes from different sports (kayaking, field hockey, rowing, waterpolo, swimming, athletics, and netball) believed that heavy training increases the need for supplement usage (Dascombe et al., 2010). Additionally, in our study, those performing >2 h gym/week and with 18 years or older were more likely to be a NS user independently of confounders. Age (Erdman et al., 2007) have been related with NS usage, with the oldest athletes reporting higher supplementation prevalence than the youngsters. Reasons that might explain this difference include: (i) more and easier access and more exposure to supplements in older ages, (ii) young athletes’ dietary intake is usually more controlled by parents and, therefore, youth athletes might have less freedom to engage in NS usage, and (iii) older athletes might be more pressured by their peers to consume NS (McDowall, 2007). It is inquisitive to notice that 40.8% of
the adolescents’ athletes in our study reported to have taken at least one NS during the year before, although the usage of NS for the propose of sports enhancement is discouraged (Desbrow et al., 2014). However, the recommendation of no NS consumption does not apply to sports foods and drinks, and the definition of nutritional supplement used in this study covers all types of supplements.

In agreement with Giannopoulou and colleagues (2013), type of sport also seems to affect NS usage, with a greater prevalence of supplementation between those performing individual modalities. Importantly, this association was maintained after adjustment for gender, age, BMI, parents’ education, sport, hours of training per week, and hours of gym per week. This might be explained by underlying personality differences between individual and team sport athletes. Allen and collaborators (Allen et al., 2011) used a five-factor model of personality dimension to compare personality profiles of athletes involved in individual vs. team sports. They concluded that athletes participating in individual sports were less extraverted (i.e. were more introverted), had less neuroticism (i.e. were more emotionally stable), more openness (were more open to new experiences), and more conscientiousness (i.e. were more organized, punctual, hardworking, and careful).

In the present study, similarly to what was found in other studies with athletes (Giannopoulou et al., 2013; Scofield & Unruh, 2006), NS usage was related with being male. Industry marketing of sports supplements might be a liable party since it is primarily aimed at males (Evans Jr et al., 2012). Yet, ORs shows no association between gender and NS usage without or with confounders’ adjustment. This last result, obtained from a more robust statistical analysis than chi-squared test, seems more concordant with the trend showed across literature: gender does not seem to be related to NS intake among athletes (Dascombe et al., 2010; Erdman et al., 2007; Nieper, 2005; Sundgot-Borgen et al., 2003). Contrarily, in general population, women seem more likely to use NS than men (Lyle et al., 1998; McNaughton et al., 2005; Reinert et al.,
Nevertheless, more research is needed to establish whether gender differences on supplementation prevalence does exists in athletic population.

Curiously, parental level of education was negatively associated with supplement usage, i.e., more years of education was associated with non-use without confounders’ adjustment. In one study with American children and adolescents (Evans Jr et al., 2012) the opposite was verified: the higher the mothers’ education, the higher the odds for NS consumption, while fathers’ level of education was not associated with NS usage. It is possible that this result is geographically dependent. In our study and in another (Dietz et al., 2014), athletes’ education was not associated with NS usage. It seems that parents’ educational level has a higher impact on NS consumption of their descendants than that of athletes’.

Among general population, supplement usage has been described as an “overall approach to living healthy” (Dickinson et al., 2014). Our data, together with results of others (Dietz et al., 2014), supports the idea that athletes have a similar attitude towards supplementation. For instance, we found that athletes with lower BMI values were more likely to be NS users, not independently of confounders. In other studies (Mullie et al., 2011; Reinert et al., 2007), lower BMI levels were also associated with NS intake; this was considered by authors as a healthy lifestyle characteristic. Moreover, in our study, NS usage was associated with a higher intake of foods perceived as healthier, such as nuts, pulses, fish, low-fat meat, skimmed-milk, yogurt (even after adjustment), and tea. Additionally, consuming NS showed to be associated with a lower intake of fat-rich foods such as olives, vegetable oils, margarine, chips (the last 3 even after adjustment), processed meat, and fast food (the last 2 only after adjustment). Overall, supplement users seemed to have more favourable diets than non-supplement users. The dietary differences between users and non-users in the current study are consistent with those of other studies in general population. For example, supplement users were more likely to be higher consumers of food considered to be healthy as dairy products (Beitz et al.,
2004; Lyle et al., 1998; McNaughton et al., 2005; Reinert et al., 2007), fish (Beitz et al., 2004; Reinert et al., 2007), tea (Beitz et al., 2004), fruit (Beitz et al., 2004; Frank et al., 2000; Harrison et al., 2004; McNaughton et al., 2005), and vegetables (Frank et al., 2000; Harrison et al., 2004), among other foods.

It is curious that the higher intake of very good food sources of protein (fish, meat, eggs, yogurt, and skimmed milk) were associated with supplementation among athletes. Greater meat ingestion was still associated with NS usage independently of adjustment variables, contrarily to the results of a study (Reinert et al., 2007) in general population. The higher likelihood of having a higher meat intake by supplement users, in the present study, might be explained by the importance that protein has among athletes. Meat-rich diets, with a performance enhancement propose, extend as far back as ancient Greece (Grandjean, 1997). Also interesting to notice is the lower consumption of vegetable oils and margarine by NS-user athletes, even independently of confounders. In studies among general population, the intake of vegetable fats tends to be higher among NS users (Beitz et al., 2004; McNaughton et al., 2005). This result has been considered as a healthier behaviour, since supplement users seem to pretermut saturated fats in favour of vegetable ones (Reinert et al., 2007). In the specific case of athletes, the intake of fat is frequently kept to the least possible in order to maintain/achieve an ideal physic (Manore, 2013). Additionally, higher intakes of empirical sports-related foods as pasta, marmalade/jam, and coffee were also associated with supplement usage. Therefore, athletes using supplements seem to be those who not only had healthier choices, but also sport-conscientious decisions regarding food intake. In fact, performance related explanations and “stay healthy” were the primary reasons for Portuguese athletes to justify supplement usage (Sousa et al., 2013).

Concluding, our results indicate that athletes using NS were different from non-users on a variety of sociodemographic and sporting characteristics. Moreover, food choices of NS users seem to be both health- and sport-driven.
These results are aligned with those from general population, where NS users have presumably healthier diet characteristics. Information such as gender, age, BMI, parental education, type of sport, training load, and food intake should be taken into account as it provides an important insight on the athlete’s dietary supplementation profile. These characteristics may help sports and health professionals to identify an alleged or future NS user, enabling the development of a timely and self-directed supplement scheme based on scientific evidence.

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REFERENCES


3.4 Study IV
Dietary strategies to recover from exercise-induced muscle damage

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Abstract
Exhaustive or unaccustomed intense exercise can cause exercise-induced muscle damage (EIMD) and its undesirable consequences may decrease the ability to exercise and to adhere to a training programme. This review briefly summarises the muscle damage process, focusing predominantly on oxidative stress and inflammation as contributing factors, and describes how nutrition may be positively used to recover from EIMD. The combined intake of carbohydrates and proteins and the use of antioxidants and/or anti-inflammatory nutrients within physiological ranges are interventions that may assist the recovery process. Although the works studying food instead of nutritional supplements are very scarce, their results seem to indicate that food might be a favourable option as a recovery strategy. To date, the only tested foods were milk, berries, and pomegranate with promising results. Other potential solutions are foods rich in protein, carbohydrates, antioxidants and/or anti-inflammatory nutrients.

Keywords
Food, inflammation, nutrients, oxidative stress

Introduction
Exhaustive or unaccustomed intense exercise can cause muscle damage, which results in muscle soreness, temporary decrease in muscle force, oedema, inflammation and an increase of intramuscular proteins in blood (Howatson & Van Someren, 2008; Smith et al., 2008). It has been described that the combination of a novel type of exercise with eccentric contractions leads to the occurrence of a higher degree of damage (Howatson & Van Someren, 2008), and its severity is influenced by the type, intensity and duration of training (Schoenfeld, 2012). Although the energy cost is lower for eccentric contractions compared with concentric ones, for the same power output, the former can cause a large degree of muscle damage (Evans, 2000; Newham et al., 1983). Eccentric contractions have also been considered more damaging than isometric ones (Clarkson & Hubal, 2002). It is believed that this is due to the increased generation of tension as muscle lengthens, resulting in a higher load distributed amongst the same number of fibres that causes a higher load per fibre ratio (Clarkson & Hubal, 2002; Enoka, 1996).

One of the most undesirable consequences of exercise-induced muscle damage (EIMD), especially in practical athletic terms, is its negative impact on muscle function, namely the decrease in muscle force-generating capacity, which is seen particularly after exercises involving eccentric contractions (Cheung et al., 2003; McGinley et al., 2009). Injury-induced strength loss, due to eccentric contractions, starts immediately after the end of the exercise and, depending on the severity of damage, it may persist from several days (McGinley et al., 2009) to 5–6 weeks (Howell et al., 1993). Muscle strength may decline up to 40%–50% after the exercise (Howell et al., 1993; Ingalls et al., 1998), leading to a large deleterious impact on athletic performance. On the other hand, pain, tenderness, swelling and stiffness typically appear only within the first 24–48 h after eccentric exercise, being its duration also related to the extent of the damage (Allen, 2001; McGinley et al., 2009). Given the delayed nature of these symptoms, they are altogether often called “delayed onset muscle soreness” (DOMS) (Allen, 2001). Although EIMD can have detrimental effects, it has also been proposed that the associated inflammation and increased protein turnover are essential for hypertrophic adaptation (Evans & Cannon, 1991). Schoenfeld (2012), in his recent review, concluded that there is theoretical rationale supporting that EIMD may enhance the accretion of muscle proteins, although it seems that muscle growth can also occur in the relative absence of muscle damage. Furthermore, there may be a threshold beyond which damage does not have further effect on hypertrophy (Kosmulski et al., 2000), and that excessive damage, particularly due to its induced force loss, can impair athletes’ ability to train, which would consequently have an detrimental impact on muscle growth (Schoenfeld, 2012).

Nevertheless, due to its consequences, EIMD can hinder the adherence to an exercise training programme (Howatson & Van Someren, 2008). So, the study of interventions that may help to reduce the negative impact of EIMD, in order to accelerate the recovery process, may play a significant role for the sports population. The most common strategies used to prevent and treat EIMD are nutritional, pharmacological, stretching, massage, electrical therapy, cryotherapy and exercise (Howatson &
Van Soneren, 2008). Regarding the nutritional approach, the existing review literature is almost null and not focused on practical recommendations.

Thus, the aims of this review are to briefly summarize the muscle damage process, focusing on oxidative stress and inflammation, and to describe how nutrition may be positively used to help recovering from EIMD. Although nutrition is believed to provide prophylactic and therapeutic effect in reducing EIMD (Howatson & Van Soneren, 2008), this review will focus especially on its therapeutic effects, particularly during the recovery period. For this review, databases PubMed and Scopus were used and searches were performed up to March 2013. Combinations of the following keywords were used as search terms: "muscle damage", "recovery", "oxidative stress", "inflammation", "exercise", "food", "antioxidants", "proteins", "carbohydrates" and "omega-3 fatty acids". References of retrieved articles were used whenever they were considered relevant. Additionally, the book Nutrition (Insel et al., 2007) was used to search nutritional contents of food.

Muscle damage

Although the exact mechanisms responsible for muscle damage remain unclear, it is believed that both mechanical and metabolic pathways are involved (Torres et al., 2012) and that the magnitude of damage is influenced by the mode, intensity and duration of exercise (Bowtell et al., 2011). A damage model, divided into two general phases, has been proposed: (i) a primary damage that occurs during the exercise, involving mechanical and metabolic alterations (Bebeling & Clarkson, 1989; Tee et al., 2007), and (ii) a secondary damage associated with the inflammatory response (Howatson & Van Soneren, 2008). EIMD involves, therefore, a complex interaction of events (Tromborg et al., 2011), which seems to include sarcoplasmic disruption due to the high mechanical tension on the myofibril (Prowse & Morgan, 2001), impured excitation–contraction coupling related to altered intracellular calcium homeostasis (Warren et al., 2001), oxidative stress (Pawer, 1999) and inflammation (Peake et al., 2005). These events will lead to structural damage of the skeletal muscle cells and degradation of cell membrane, resulting in fibre necrosis and, ultimately, in fibre remodeling (Howatson & Van Soneren, 2008). The possible sources of oxidants and the inflammatory process associated with EIMD will be further developed in the next sections.

Oxidative stress

It is largely accepted that exercise can create an imbalance between oxidant and antioxidant levels, known as oxidative stress (Leeuwenburgh & Heinecke, 2001). This phenomenon is caused by the production of reactive oxygen species (ROS) both acutely, i.e. during exercise, and throughout the long-term response to the EIMD (Powers & Jackson, 2008). ROS play an important role as mediators of EIMD (Pinaud et al., 2006; Sacheck & Blumberg, 2001). Although it is widely recognized that ROS lead to the increment of markers of lipid, protein and DNA oxidation, ROS actions regarding EIMD seem to be associated with the oxidation of critical redox-sensitive sites within skeletal muscle – see Powers & Jackson (2008) for comprehensive review. Beyond their negative impact, ROS can also lead to positive outcomes, as contraction-induced adaptive responses of muscle fibres and regulation of gene expression (Powers & Jackson, 2008; Powers et al., 2011b).

Several mechanisms have been proposed as potentially liable parties of ROS increment during exercise. The electron transport associated with the mitochondrial respiratory chain has been considered one of the major intracellular source of ROS during exercise (Di Meco & Venditti, 2001). It is known that around 0.15% of the consumed oxygen is not completely reduced to water in the respiratory chain (St-Pierre et al., 2002), being converted into superoxide ion (O$_2^-$) (Boveris et al., 1972) primarily in complexes I and III of the mitochondrial electron transport chain (Pinaud et al., 2006). Another alternative cause for ROS production could be ischemia-reperfusion (Pinaud et al., 2006).

During exhaustive exercise, working muscles are the primary tissue to be supplied with blood, while other tissues may undergo partial ischemia due to the reduced blood flow (Vollaard et al., 2005). Additionally, during exercise performed at intensities at or above maximal oxygen consumption (VO$_2$max), muscle fibres may experience hypoxia since oxygen supply is beneath the energy demand (Packer, 1997). The ischemia conditions trigger the conversion of xanthine dehydrogenase to xanthine oxidase, which upon reoxygenation of the hypoxic tissue produces O$_2^-$ (Gomes et al., 2012). However, this mechanism has been shown to happen only in few studies (Gomes et al., 2012). Other processes that may be involved in ROS production during exercise include: (i) autooxidation of haem proteins, namely haemoglobin and myoglobin (Mb) (Pinaud et al., 2006), (ii) the activity of the enzyme nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase) (Powers & Jackson, 2008), (iii) the phospholipase A$_2$-dependent processes (Powers & Jackson, 2008; Powers et al., 2011b), and (iv) nitric oxide (NO) synthase (Powers & Jackson, 2008). Additional exercise-related changes that might be involved in ROS production are: (i) the increase in catecholamines which can lead to ROS release during their metabolic inactivation (Clarkson & Thompson, 2000), (ii) the production of lactic acid that is able to convert O$_2^-$ into hydroxyl radical (OH) (Clarkson & Thompson, 2000), (iii) the rise in muscle temperature and (iv) the increase in carbon dioxide (CO$_2$) (Arbogast & Reid, 2004).

Although ROS may mediate cell damage, exercise-induced cell damage can also stimulate ROS production, since during the inflammatory response to EIMD the infiltrated leukocytes (neutrophils and macrophages) can release ROS (Leeuwenburgh & Heinecke, 2001). Their oxidative burst involves the production of O$_2^-$ that can be rapidly removed by reaction with other free radicals or due to conversion to hydrogen peroxide (H$_2$O$_2$) by superoxide dismutase (SOD) (Hampton et al., 1998). Furthermore, neutrophils can convert H$_2$O$_2$ into hydroxyl acid, a highly potent oxidant, via myeloperoxidase (Vollaard et al., 2005). Myeloperoxidase, an enzyme expressed primarily by neutrophils, has been shown to increase following exercise, and may remain elevated for days (Childs et al., 2001). Additionally, it was demonstrated that the level of myeloperoxidase activity per neutrophil is increased by exercise (Suzuki et al., 1996). Moreover, activated macrophages are a rich source of nitric oxide (NO) and they can lyse muscle cells in vitro through nitric-oxide-dependent mechanisms (Nguyen & Tidball, 2003). In addition, the presence of muscle cells seems to induce a higher NO production by macrophages and their cytolytic capacity appears to be increased by the presence of neutrophils (Nguyen & Tidball, 2003). Similar to neutrophils, macrophages are also thought to play a major role in promoting muscle damage after muscle injury (Tidball, 2005). However, recent findings demonstrated that macrophages recruited by damaged skeletal cells exhibit a phagocytic and pro-inflammatory profile, which is rapidly converted to an anti-inflammatory phenotype (Arnold et al., 2007). This phenotype is associated with myogenensis and muscle growth (Arnold et al., 2007), suggesting that macrophages do not contribute to secondary damage. Other aspect of the inflammatory phenomenon resultant from EIMD will be developed in the subsequent section.
Inflammation

Intense physical exercise, especially eccentric exercise, triggers a rapid and sequential invasion of muscle by inflammatory cells which can persist for days to weeks (Leeuwenburgh & Heinecke, 2001; Tidball, 2005). White blood cells (WBC) are the major cellular mediators of inflammation (Cannon & St. Pierre, 1998) and their increased concentrations after EIMD are believed to be mainly due to the rise of neutrophils and monocytes/macrophages (Evans, 2000; Mota et al., 2009; Sacheck & Blumberg, 2001; Uuso & Clarkson, 2003). Lymphocytes are also recruited during strenuous exercise but their count declines immediately after the end of the exercise (Pender sen & Toft, 2000). The inflammatory process is believed to be mediated (i) by neuroendocrinological factors, such as adrenaline, noradrenaline, growth hormone and cortisol (Pender sen & Toft, 2000) and (ii) by cytokines, namely pro-inflammatory factors such as tumor necrosis factor-α (TNF-α) and interleukin-1 beta (IL-1β), and the inflammation-responsive cytokine IL-6 (Pender sen & Hoffman-Goetz, 2000).

Although it is generally accepted that cytokines have a central role in the inflammatory process, the exact mechanism of action of each one remains unclear (Smith et al., 2008) (for detailed review see Cannon & St Pierre (1998)). Endothelial cells also play a significant role in regulating the inflammatory response, namely (i) by expressing leukocyte’s adhesion molecules, which are determinant for the influx of neutrophils and monocytes, (ii) possibly by producing NO, which is vasodilator, and (iii) by secreting several cytokines as IL-1α and -1β, IL-6 and IL-8 (Cannon & St. Pierre, 1998). The local inflammatory process is afterwards accompanied by a systemic response known as acute-phase response, similar to what happens in an infection (Evans, 2000; Pender sen & Hoffman-Goetz, 2000). Eccentric exercise results in a greater rise of neutrophil counts compared with concentric exercise, where both circulating and skeletal muscle neutrophils increase (Evans, 2000). Exercise causes demargination of neutrophils, increasing the circulating populations (Tidball, 2005). Within 1 h of increased muscle loading, neutrophils invasion begins and their concentrations can be elevated for periods as long as 5 days (Fielding et al., 1993). The mobilization of neutrophils seems to depend on exercise intensity and to be mediated by the secretion of stress hormones, such as catecholamines, cortisol and growth hormone (Pender sen & Toft, 2000; Sacheck & Blumberg, 2001). Their function after infiltration in the damaged muscle is to phagocytose the necrotic myofibers and help to degrade cellular debris (Cannon & St. Pierre, 1998), by releasing proteases and oxygen radicals, as explained in the previous section, which can damage muscle even further and also other healthy surrounding tissues (Evans & Cannon, 1991; Leeuwenburgh & Heinecke, 2001; Tidball, 2005). This process is known as secondary damage (Smith et al., 2008). In fact, evidence has shown that administration of an antibody that blocks the respiratory burst and degranulation of neutrophils prior to a single eccentric contraction, led to a significant decrease in muscle damage (Brockton et al., 2003). Moreover, neutrophils are thought to be one of the most important players in the secondary damage since they are the immune cells that predominate in the injured tissue at the time when secondary damage occurs (Smith et al., 2008). Neutrophils may also magnify the inflammatory process via the release of inflammatory cytokines as IL-6 (Smith et al., 2008), IL-1β and TNF-α (Cannon & St. Pierre, 1998).

The inflammatory cell pattern changes within the first 24 h, with the number of neutrophils starting to decrease and the macrophages count beginning to increase (Smith et al., 2008). Thus, macrophages are evident in the damaged muscle around 1 day after exercise and their counts may remain high up to 7–14 days post-exercise (Round et al., 1987). There is a poor understanding of macrophages’ functions during exercise-induced inflammation and their role in muscle damage is complex, since they secrete various growth factors, cytokines and free radicals, and act as antigen-presenting cells, regulating the cellular immune response (Tidball, 2005). Some of the cytokines released by macrophages include IL-1β, TNF-α and IL-6, which magnify the inflammatory process and coordinate the various elements of the systemic acute-phase response (Sachek & Blumberg, 2001; Smith et al., 2008). As neutrophils, they also have the ability to phagocytose damaged tissue and both seem to play a key role in muscle repair and remodelling (Tidball, 2005). The discussion of the mechanisms by which these inflammatory cells contribute to muscle repair and remodelling is beyond the scope of this review; for that, other review papers (Smith et al., 2008; Tidball, 2005) are suggested.

Nutritional strategies

Due to the heavy sport’s schedule of athletes, training or competing more than once within a single day is oftentimes their routine. Therefore, maximising and accelerating the recovery processes is crucial to potentiate their performance (Betta & Williams, 2010). Some interventions have been proposed to reduce the negative effects associated with EIMD, like nutrition, pharmacological strategies, electrical and manual therapies, cryotherapy and active exercise (Howatson & Van Someren, 2008; Torres et al., 2012). With training programmes becoming more demanding any possible help should be considered, and nutrition is an area that obviously can make a difference (Maughan et al., 2004). Given the fact that feeding is a mandatory physiological demand, it is of countless interest to potentiate the athletes’ food intake in order to maximise their training programme. Recovering faster and more efficiently will allow athletes to train more and to respond to training more positively, leading to the expected performance improvements. It has been widely stated that during post-exercise recovery, optimal nutritional intake is essential to facilitate muscle repair and regeneration (BeeLEN et al., 2010). Some nutrition interventions have been considered capable of assisting recovery after EIMD.

Proteins

Proteins alone

Few studies have been conducted that have examined the role of protein supplementation in preventing or alleviating symptoms associated with EIMD (Howatson et al., 2012; Jackman et al., 2010; Matsumoto et al., 2009; Nosaka et al., 2006; Shimomura et al., 2006), with the majority of them using branched chain amino acids (BCAA). It has been concluded that the ingestion of amino acids seem to be able to reduce muscle damage – measured by creatine kinase (CK) (Howatson et al., 2012; Matsumoto et al., 2009; Nosaka et al., 2006), aldolase (Nosaka et al., 2006), Mb (Nosaka et al., 2006), lactate dehydrogenase (LDH) (Matsumoto et al., 2008), granulocyte elastase (Matsumoto et al., 2009) or muscle soreness (Howatson et al., 2012; Jackman et al., 2010; Matsumoto et al., 2009; Nosaka et al., 2006; Shimomura et al., 2006) – decrease sensation of fatigue (Matsumoto et al., 2009; Shimomura et al., 2006), and accelerate the functional recovery process (Howatson et al., 2012). However, in one study (Jackman et al., 2010), no differences were found for CK, Mb, IL-6, maximal isometric strength and low-frequency fatigue compared with placebo.

It is widely accepted that a positive muscle protein balance is necessary to facilitate the muscle repair and adaptation from EIMD (Hawley et al., 2006). Regarding muscle protein synthesis
(MPS) and net protein accretion, it has been concluded that they can be promoted by an early post-exercise protein consumption (Phillips, 2011). For optimal stimulation of muscle protein synthesis, recent data suggest an intake of 20–25 g protein following resistance exercise (Moore et al., 2009; Phillips, 2011). Essential amino acids (EAAs) seem to be the primarily responsible for the stimulation of muscle protein synthesis (Volpi et al., 2003) and breakdown of muscle (Hawkinson & Van Someren, 2008), suggesting that the combination of these two macronutrients can be a valuable strategy. However, some studies (Breen et al., 2010; Green et al., 2008; White et al., 2008; Wojcik et al., 2001) do not support these findings. The possible reasons for these discrepancies are (i) the inherent inter-individual variability for indirect systemic markers of muscle damage, namely CK (Bets & Williams, 2010), which was the only blood parameter used to assess muscle damage in the four studies that did not find positive results, and (ii) the different exercise protocols applied.

CHO ingestion after exercise has been shown to improve net protein balance by attenuating the exercise-induced increment in muscle protein breakdown, which has been attributed to a rise in plasma insulin (Beelen et al., 2010; Burlakova et al., 2004). However, even a single dose of protein was administrated, the co-ingestion of CHO and proteins did not seem to further improve protein synthesis and protein breakdown (Koopman et al., 2007; Staples et al., 2011). Still, it is important to note that the amount of CHO used in those studies was considerably low: 0.15 or 0.6 g/kg (Koopman et al., 2007) and 30 g (Staples et al., 2011). Although conflicting data still exist regarding whether or not the ingestion of CHO plus proteins has an undoubtedly advantage versus protein alone, it seems clear that it is not a disadvantage combining these two macronutrients during the recovery time. Additionally, the palatability of a CHO-protein solution has usually a better acceptance than one with proteins only. Moreover, low glycogen levels have been shown to possibly have a negative impact on MPS (Churchley et al., 2007; Cereer et al., 2005; Wojtaszewski et al., 2003) and to promote muscle protein breakdown (Lemon & Mullin, 1990). It is important to note that a high volume of resistance exercise can lead to a decrease in muscle glycogen stores (MacDougall et al., 1999; Robergs et al., 1991) and that muscle glycogen re-synthesis is impaired by EIMD (Costill et al., 1990; O’Reilly et al., 1987; Seifert et al., 2005). It is known that CHO feeding during the recovery period can stimulate greater rates of muscle glycogen re-synthesis than when no CHO are ingested at all (Betsa & Williams, 2010). Furthermore, it has already been shown that a high CHO intake (8.5 g CHO/kg/d) after an eccentric exercise leads to a higher increase in intramuscular CHO storage compared to a lower amount (4.25 g CHO/kg/d) (Costill et al., 1990). Moreover, a recent review paper (Beelen et al., 2010) suggested that the co-ingestion of 0.2–0.4 g/kg protein with 0.8 g/kg CHO (compared to the recommended amount of 1.2 g/kg CHO alone), in addition to provide proteins that are essential to stimulate MPS, seems to result in optimal muscle glycogen-repletion rate (Beelen et al., 2010; Van Loon et al., 1999). This phenomenon may be due to the synergistic influence of CHO and protein on insulin secretion (Van Loon et al., 2000).

Therefore, and regarding the existing evidence, it seems that ingesting 0.8–1.2 g CHO/kg/d and 0.2–0.4 g protein/kg, preferably in the early recovery period, with a minimum content of 20 g high-quality protein, may enhance the recovery after EIMD. Some discussions have been raised recently regarding the importance of nutrient timing consumption (Aragon & Schoenfeld, 2013) and if CHO and proteins really need to be consumed as soon as possible after the exercise. Even though it is not yet certain that a real advantage for the early feeding after exercise exists, certainly a quick nutrient delivery after a demanding effort will not be a disadvantage. Furthermore, from a practical point of view, the promotion of an early nutritional recovery strategy, preferably carried out within the sports context, may enhance the adherence to that strategy and ensures a correct and proportional feeding.

Antioxidant supplementation

Whether or not athletes benefit from the use of antioxidant supplements remains a hot topic and it is still controversial. Powers and collaborators, in a recent review about this topic (Powers et al., 2011a), highlighted the arguments that have been used for and against antioxidant supplementation. Briefly, the most commonly used arguments to support antioxidant supplementation are: (i) the fact that exercising leads to an increase in ROS production and that increased levels of antioxidants could counteract the ROS, preventing or reducing damage and, therefore, muscle pain (Urso & Clarkson, 2003), (ii) that some antioxidants shown to improve endurance performance (Kelly et al., 2009) and to delay fatigue, and (iii) that some athletes may not achieve the nutritional recommendations for antioxidant intake just with food (Machefer et al., 2007; Palazzetti et al., 2004; Rankinen et al., 1998). On the other hand, some arguments have been used against antioxidant supplementation, namely: (i) the fact that regular exercise leads to an increase in enzymatic and non-enzymatic antioxidants in muscle fibres (Powers et al., 2011b), (ii) that antioxidant supplementation may impair muscle function or delay some adaptations induced by exercise (Coombes et al., 2001; Tesaiera et al., 2009), by interfering with cell signalling functions of ROS, affecting muscular performance (McGinley et al., 2009), (iii) that antioxidant supplementation does not seem to lead to better outcomes, compared with placebo, regarding muscle function, inflammation (Beaton et al., 2002) and redox status (Theodorou et al., 2011) after eccentric exercise; (iv) that antioxidant supplementation may contribute to increase muscle damage and oxidative stress (Childs et al., 2001), and (v) that some studies do not support the concept that antioxidant supplementation is beneficial to human health (Bylakovic et al., 2007) and doubts have been placed about the long-term effects of antioxidant supplementation in high doses (McGinley et al., 2009). Moreover, it has been reported that the protective effect of a diet, with natural sources of antioxidants, is not equivalent to the protective effect of supplementation (Halliwell, 2000). Given these facts, it is currently suggested (Peternel & Coombes, 2003).
2011; Powers et al., 2011a) that due to the limited evidence to recommend antioxidant supplements, athletes should rather focus on consuming a well-balanced and energetically adequate diet, which can provide antioxidant-rich foods.

**Antioxidant and/or anti-inflammatory nutrients in food**

Keeping in mind that high doses of antioxidants seem to have detrimental consequences, the use of antioxidant-rich foods seems to be the best option. These foods can provide an amount of antioxidant within the physiological range, while nutritional supplements usually provide supra-physiological doses. Therefore, this review will only focus on the key antioxidants found in food and a brief explanation of their main mechanisms will be given below. Some of these compounds also seem to have an anti-inflammatory action, which may have further benefits on the recovery from EIMD. Given that, most studies regarding muscle damage used nutritional supplements rather than food, reference will be made not only to the studies that used nutrient-dense foods but also to those that utilized the isolate substances in humans. It is important to mention that the level of evidence regarding the cause and health effect relationship, for the majority of these substances, is not yet sufficient for the European Food Safety Authority (EFSA) to consider making health claims. Therefore, more research in this area, especially clinical trials with human beings, will help to understand the possible relationship between the intake of these substances and their possible effects.

**Vitamin C and/or vitamin E**

Vitamin C, or ascorbic acid, is a potent water soluble vitamin, present in the cytosolic compartment of the cells (Evans, 2000). It is found mainly in citrus fruits, with sweet peppers, strawberries, cruciferous and leafy vegetables, being also good sources (Gerber, 2003). This vitamin exerts its functions by scavenging ROS and reactive nitrogen species, as well as regenerating other antioxidant molecules from their radical species, namely vitamin E, β-carotene and glutathione (Carr & Peri, 1999).

Vitamin E is the most important lipid-soluble antioxidant vitamin, and it is virtually found in all cell membranes (Evans, 2000). Its main sources are vegetable oils, especially sunflower, safflower and nuts (Gerber, 2003). It exists in eight different isomers: α, β, γ and δ-tocopherol and α, β, γ and δ-tocotrienol (Gülçin, 2012), being the α-tocopherol the most important biologically active form (McGinley et al., 2009).

Vitamin E is known for its ability for stopping the progression of the lipid peroxidation chain reaction and also for acting as a scavenger of superoxide, hydroxyl and lipid peroxyl radicals (McGinley et al., 2009).

An exhaustive review (McGinley et al., 2009) about the effects of supplementation with these two antioxidants, alone or combined, concluded that there is little evidence to support its protection against muscle damage, although there is evidence showing that both can reduce indices of oxidative stress. Moreover, the typical large supplementation dosages can even have a detrimental effect on the adaptive and recovery processes since it may interfere with the signalling functions of ROS (McGinley et al., 2009). Studies after that review continue to show contradictory results, (i) some showing positive outcomes of supplementation concerning muscle soreness (Silva et al., 2010), muscle damage (measured trough LDH (Silva et al., 2010) and CK (Nakhostin-Roohi et al., 2008)), oxidative status (Nakhostin-Roohi et al., 2008; Silva et al., 2010) and inflammation (Silva et al., 2010), (ii) and others showing no effect on muscle damage using CK (Theodorou et al., 2011), muscle soreness (Theodorou et al., 2011), muscle function by isometric peak torque (Theodorou et al., 2011), inflammation (Nakhostin-Roohi et al., 2008; Silva et al., 2010), and oxidative status (Theodorou et al., 2011).

**Polyphenols**

Polyphenols are the biggest group of phytochemicals and are known for being strong antioxidants (Tsao, 2010). Flavonoids are the largest group of polyphenolic compounds with more than 4000 identified varieties distributed among fruits, vegetables, green tea and wine (Cabrera et al., 2006; Zadra et al., 2010; Marzocchella et al., 2011). It has been suggested by a large number of publications that these compounds have immunomodulatory, antioxidant and anti-inflammatory properties (González-Gallego et al., 2010; Marzocchella et al., 2011). Flavonoids seem to exert their antioxidant activity by scavenging ROS (García-Lafuente et al., 2009) and by impairing ROS production by inhibiting NADPH oxidase, xanthine oxidase and superoxide dismutase (Cotelle, 2001). In addition, they also have the ability to inhibit lipid peroxidation, chelating redox active metals, activating antioxidant enzymes and reducing α-tocopherol radicals (Heim et al., 2002). Some mechanisms have been proposed in order to explain the anti-inflammatory effects of flavonoids, as the reduction of the activities of the arachidonic acid metabolizing enzymes A2 (phospholipase A2), inhibition of NO synthase, inhibition of pro-inflammatory molecules (IL-1β, IL-2, IL-6, TNF-α, among others), and modulation of pro-inflammatory gene expression (Marzocchella et al., 2011).

The flavonoid quercetin has been used in some studies (Nieman et al., 2007a,b; O’Fallon et al., 2012) in the context of EIMD. Apart from a diminished post-exercise expression of MyD88, MyD88, IL-8 and IL-10 mRNA in one of the studies (Nieman et al., 2007b), quercetin, contrary to the expected, failed to positively influence muscle strength, muscle damage, inflammation and plasma cytokine, and hormone levels. Another flavonoid, the epigallocatechin gallate (EGCG) that is a catechin found in high concentrations in green tea, was also investigated along with muscle damage (Kerkvick et al., 2010). Its supplementation resulted in reduced muscle soreness compared to placebo. No differences were seen for the other tested parameters (peak torque production, LDH, CK, serum cortisol, neutrophil counts, neutrophil:lymphocyte ratio and markers of apoptosis). Regarding food, cherries and berries, known to be rich in various polyphenol compounds especially in anthocyanins (another class of flavonoids) and the flavonol quercetin (McCune et al., 2011; Szajek & Borowinska, 2008), have been used as treatment in studies related to EIMD (Bowtell et al., 2011; Connolly et al., 2006; Howatson et al., 2010; McLeay et al., 2012). Impressively, all studies showed positive results for muscle force recovery; regarding markers of muscle damage, most of the studies did not found any differences between the tested groups and the results for oxidative stress and inflammation markers varied among the studies (Table 1). Pomegranate and the respective extract are especially rich in the polyphenols ellagitannins (Medjakovic & Jungbauer, 2013), and have also been used in research concerning muscle damage. In a study (Tromboli et al., 2010), pomegranate extract showed to reduce muscle force loss but had no impact on CK, MB, IL-6 and CRP. The same research group conducted another study (Tromboli et al., 2011) with pomegranate juice in which they concluded that the supplementation attenuated the force loss and reduced soreness of the elbow flexor muscles; however, no differences from placebo were found for the knee extensor muscles. The general positive results in these studies using food as the supplementation, strengthens the possible positive effect
Table 1. Studies that used food as the strategy to recover from EIMD in humans.

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Dose</th>
<th>Duration</th>
<th>Exercise protocol</th>
<th>Muscle damage</th>
<th>Oxidative stress</th>
<th>Inflammation marker</th>
<th>Force/Performance recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sousa et al., 2008</td>
<td>14 males college students</td>
<td>2 x 355 mL cherry juice a</td>
<td>3 d pre-ex</td>
<td>2 x 20 single-knee ECC flexion</td>
<td>Muscle soreness</td>
<td>CK, DOMS, Mb</td>
<td>Isometric</td>
<td>↑</td>
</tr>
<tr>
<td>Cockburn et al., 2008</td>
<td>24 active males</td>
<td>500 mL semi-skimmed milk b</td>
<td>Immediately and 2 h after ex</td>
<td>6 x 10 ECC-CON</td>
<td>CK, DOMS, Mb</td>
<td>PT, Non-dominant leg</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Poole et al., 2009</td>
<td>10 males regional-level cyclists and triathletes</td>
<td>Low-fat chocolate milk to achieve 1.0 g/kg c</td>
<td>Immediately and 2 h after ex</td>
<td>6 x (5 min cycling at 60% VO2 max + 3 x 10 s Wingate sprints)</td>
<td>CK, Muscle soreness</td>
<td>Cycling at 85% VO2 max to exhaustion</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Bowtell et al., 2011</td>
<td>10 well-trained males</td>
<td>2 x 30 mL cherry juice d</td>
<td>7 d pre-ex</td>
<td>10 x 10 single-knee extension 80% IROM</td>
<td>CK, Norepinephrine</td>
<td>PPT</td>
<td>MVC</td>
<td>↑</td>
</tr>
<tr>
<td>Rowson et al., 2012</td>
<td>20 (13 male) recreational marathon runners</td>
<td>2 x 217 mL cherry juice e</td>
<td>5 d pre-ex</td>
<td>Marathon run</td>
<td>CK, LDH, DOMS, CRP, MVIC</td>
<td>↑</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>Tweedie et al., 2013</td>
<td>17 trained males</td>
<td>2 x 250 mL pomegranate juice f</td>
<td>7 d pre-ex</td>
<td>3 x 20 single-knee ECC flexion 0.7 rad s⁻¹ + 6 x 10 single-knee extension 110% IROM</td>
<td>Muscle soreness</td>
<td>Elbow flexors</td>
<td>Isometric</td>
<td>↑</td>
</tr>
<tr>
<td>McLarty et al., 2015</td>
<td>10 healthy females</td>
<td>1 blueberry smoothie: 200 g blueberries, 1 banana, 200 mL apple juice g</td>
<td>5 and 10 h pre-ex (immediately, 12 and 36h after ex)</td>
<td>3 x 100 single-knee ECC flexion 30° s⁻¹</td>
<td>CK, Muscle soreness</td>
<td>PC, IL-6</td>
<td>Eccentric</td>
<td>↑</td>
</tr>
<tr>
<td>Cockburn et al., 2013</td>
<td>14 healthy males</td>
<td>500 mL semi-skimmed milk b</td>
<td>Immediately after ex</td>
<td>6 x 10 ECC-CON knee flexion 1.05 rad s⁻¹</td>
<td>Passive DOMS, Active DOMS, CK, Mb</td>
<td>CMJ, Reactive strength index</td>
<td>↑</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: Pre-ex, pre-exercise; ECC, eccentric; ex, exercise; ECC-CON, eccentric-concentric; CK, creatine kinase; DOMS, delayed onset muscle soreness; Mb, myoglobin; PT, peak torque; VO2 max, maximal oxygen uptake; RM, repetition maximum; PPT, pressure pain threshold; TAS, total antioxidant status; PC, protein carbonyls; hCRP, high-sensitivity C-reactive protein; MVC, maximum voluntary contraction; LDH, lactate dehydrogenase; TRARS, thiobarbituric acid reactive species; CRP, C-reactive protein; IL, interleukin; MVIC, maximum voluntary isometric contraction; ROS-GC, radical oxygen species-generating capacity; PRAP, ferric reducing anti-oxidant power; CMJ, countermovement jump; LIST, Loughborough Intermittent Shuttle Test.

†, significant decrease; ‡, significant increase; =, no significant change.

*Comparison with a placebo/control group.
The study has four groups: milk, milk-based carbohydrate-protein supplement, sports drink and water (control). The results are expressed for milk versus control.
of physiological ranges of antioxidants over the typically used supra-physiological doses.

Carotenoids

Carotenoids are present in plants, algae and microorganisms, and they are divided into two classes: carotenes and xanthophylls (Riccioni, 2009). The major dietary sources of carotenoids are fruits and vegetables (Sembra et al., 2007), in which they are the principal pigments responsible for their colour (Gülcin, 2012). The most abundant carotenes in the diet are the β-carotene and lycopene while lutein, β-cryptoxanthin, zeaxanthin and astaxanthin, are the most common xanthophylls (Riccioni, 2009). Carotenoids are efficient antioxidants and comprise an important component of the antioxidant defence system in humans by protecting against oxidative stress, scavenging singlet molecular oxygen and free radicals, and inhibiting lipid peroxidation (Sembra et al., 2007).

To date, there is only one study in humans (Djordjevic et al., 2012) using only carotenoids (astaxanthin) as the anti-oxidant supplementation treatment. This study showed a positive effect of the supplementation on CK and on the total antioxidant status (TAS), but not on SOD.

α-Lipoic acid

Dietary α-lipoic acid (LA) can be obtained from both animal and plant sources, but is primarily found in animal-derived foods, namely red meat, liver, heart and kidney (Gorča et al., 2011). Humans can also obtain LA by de novo synthesis from fatty acids and cysteine (Biewenga et al., 1997). The reduced form of LA is known as dihydrolipoic acid (DHLA) and it is this form that predominantly interacts with ROS, although the oxidized form of LA can also inactivate free radicals (Packer et al., 2001). Furthermore, both forms may exhibit antioxidant activity by metal chelating (Packer et al., 2001). DHLA also has the capacity to reduce the oxidized forms of several important antioxidants, such as vitamin C and E, co-enzyme Q10, and glutathione (Bilaka & Whonde, 2005; Gorča et al., 2011; Kozlov et al., 1999). For these reasons, the LA/DHLA redox couple is now being recognized as one of the most powerful biologic antioxidant systems (Gorča et al., 2011).

Regarding LA, one study (Zemborn-Lačny et al., 2009b) showed no differences on muscle damage markers CK and LDH between treatments. LA supplementation, on the other hand, influenced the levels of glutathione (GSH), glutathione reducetase (GR) and glutathione peroxidase (GPx), after exercise; although the levels of total thiol, TBARS and protein carbonyl (PC), were positively changed throughout the trial compared with the control group, no different kinetics were seen between conditions with exercise. Moreover, no changes were seen after LA supplementation on the exercise parameters measured by the isokinetic device, namely peak torque, time to reach peak torque, total work, average power and maximal average peak torque. In another study (Zemborn-Lačny et al., 2009a) by the same research group, similar outcomes were found: no differences in CK levels compared to control but positive results regarding TAS, total thiol, TBARS, PC and uric acid. In a third study (Pogarty et al., 2013), there was also an increase in blood total antioxidant capacity as a result of LA supplementation while DNA damage, lipid peroxidation and hydrogen peroxide, increased following exercise only in the non-supplemented group.

Co-enzyme Q10

Co-enzyme Q, also called ubiquinones, is a natural lipophilic compound found in every living cell (Pravst et al., 2010). Co-enzyme Q10 (CoQ10) is the most abundant form in humans and most animals (Pravst et al., 2010). In addition to endogenous synthesis, food is also a source of CoQ10, with meat, fish, nuts and certain vegetable oils being the richest nutritional sources (Pravst et al., 2010). However, its dietary intake is limited to only a few percentage (Benstinger et al., 2010). CoQ10 is a key component of the mitochondrial respiratory chain but it is also known for its antioxidant properties (Littarru & Tiano, 2010). Mostly in its reduced form, CoQ10 is an effective antioxidant with capacity to protect against lipid peroxidation, DNA and protein oxidation, and to regenerate vitamin C and E as well (Pinaud et al., 2006; Pravst et al., 2010).

In one study (Díaz-Castro et al., 2012), the CoQ10 supplementation decreased oxidative markers, increased antioxidant markers and also had a positive effect on inflammatory markers. However, no markers of muscle damage or muscle performance were measured. In another study (Ostman et al., 2012), the supplementation with CoQ10 had no mediators, and in oxidative stress and CK compared with placebo. In a third one (Kon et al., 2008), supplementation with CoQ10 decreased CK, Mb and lipid peroxide (LPO), and had no influence on changes in neutrophil counts after exercise. In a last one (Malm et al., 1996), anaerobic work capacity was impaired and CK levels were increased at various points in the CoQ1 group whereas during placebo trial there were no changes. It is worth mentioning that the American College of Sports Medicine (Rodriguez et al., 2009) classified CoQ10 as an ergogenic aid that does not perform as claimed.

n-3 Polyunsaturated fatty acids

The n-3 polyunsaturated fatty acids (PUFAs), namely the long-chain n-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are extensively associated with anti-inflammatory and immunomodulatory properties (Galli & Calder, 2009). Rich sources of PUFAs include fatty fish, such as salmon, tuna and mackerel, fish oil and nuts (Insel et al., 2007; Ros & Mataix, 2006).

Typically, the phospholipids of immune cells contain proportionally more arachidonic acid (AA), an n-6 PUFA, than other long-chain fatty acids, including n-3 PUFAs (Calder et al., 1994; Kew et al., 2004). Therefore, AA is usually the major substrate for eicosanoids synthesis, which is a key mediator and regulator of inflammation (Calder, 2009). The AA-derived eicosanoids, namely prostaaglandin (PG) E2 and 4-series of leukotrienes (LTs), are generally assumed to be pro-inflammatory, although it has been recently discovered that PGE2 can also have anti-inflammatory actions (Calder, 2009). Nevertheless, it has been shown that the EPA and DHA content of the immune cells membrane can be altered through oral administration of these fatty acids (Calder, 2007). This manipulation results in a decreased production of AA-derived eicosanoids, a rise in alternative substances, such as PGE2 (less potent then PGE2) and resolvins (potent anti-inflammatory mediators), and also an altered gene expression by a direct effect of n-3 fatty acids on signalling pathways (Calder, 2009). Through these mechanisms, n-3 PUFAs intake seems to have the ability to affect phagocytosis, T-cell signalling and antigen presentation capability (Calder, 2007) and to lead to a decrease in cytokines and ROS production, and in the expression of adhesion molecules (Calder, 2006).

For these reasons, n-3 PUFAs may be of a useful nutritional help to modulate the exercise-induced inflammation and immune dysfunction resultant from EIMD. A recent review about n-3 fatty acids and physical performance (Mickleborough, 2013) concluded that supplementation with n-3 PUFAs may help to alleviate DOMS resulting from muscle damage, possibly due to the ability
of these fatty acids to increase blood flow. However, the author concluded that the evidence from human data is inconclusive to show a beneficial effect of n-3 PUFA in attenuating the inflammatory and immunomodulatory response to exercise.

Can food be an adequate alternative to supplements?

Most work, in the area of nutritional recovery from exercise, focus on the use of nutritional supplements rather than on foods. The few studies done using food — milk, cherries, blueberry or pomegranate — to recover from EIMD are described in Table 1. Although the number of studies is scarce and they used different methodologies, their results seem to indicate that food might be a favourable option as a recovery strategy. Moreover, given the issues related to potential contamination resulting in inadvertent doping (Burke et al., 2009), it is a safer option for athletes if they rely on food rather than on nutritional supplements.

Milk

Cow’s milk and its derivatives represent a very good source of proteins, lipids, amino acids, vitamins and minerals (Roy, 2008). Milk has several characteristics that make it an interesting recovery drink. One of its advantages is that it contains both casein and whey proteins in a ratio of approximately 3:1, which results in sustained elevations of blood amino acid concentrations (Bos et al., 2003; Roy, 2008). Therefore, milk has both fast dietary proteins (whey) that stimulate protein synthesis, and slowly absorbed ones (casein) which suppress muscle protein breakdown (Boirie et al., 1997; Dangin et al., 2003). Another advantage is that whey proteins contain a large proportion of BCAA, which are important in muscle metabolism and protein synthesis (Roy, 2008).

Milk was used in three (Cockburn et al., 2008, 2013; Pritchett et al., 2009) of the eight studies mentioned in Table 1. Essentially, the positive results were found regarding force performance recovery: one study (Cockburn et al., 2008) found a positive effect of semi-skimmed milk on force recovery and total work for the dominant leg, and the second one (Cockburn et al., 2013) found a positive effect of the same type of milk on limiting increases in sprint time and agility. However, the third study (Pritchett et al., 2009), found no differences on the measured recovery parameters between low-fat chocolate milk and a carbohydrate replacement beverage, despite the previously published data suggesting it has an effective recovery aid (Karp et al., 2006). The three studies found no differences between the milk group and the respective comparison group for the muscle damaged markers, namely CK (Cockburn et al., 2008, 2013; Pritchett et al., 2009), DOMS (Cockburn et al., 2008, 2013; Pritchett et al., 2009) and Mb (Cockburn et al., 2008, 2013).

Cherries

Cherries are known to have a high content in numerous phytochemicals possessing antioxidant properties, with anthocyanins and quercetin playing a special role (McCune et al., 2011). These fruits also contain vitamins C and E and some carotenoids, especially β-carotene (Ferretti et al., 2010). In addition to the antioxidants effects, the consumption of cherries has also been associated with anti-inflammatory effects, namely through inhibition of the activity of the cyclooxygenase II (Ferretti et al., 2010; Seeram et al., 2001), and with pain inhibition (in animal studies) (Tall et al., 2004).

Three (Bowtell et al., 2011; Connolly et al., 2006; Howatson et al., 2010) of the eight studies using food as the intervention to attenuate the consequences associated with muscle damage, used cherries as the treatment. The results seen in these studies (Table 1) are, as a whole, positive and promising. For all the three studies, the cherry supplementation enhanced the performance recovery. One of the studies (Connolly et al., 2006) showed a decrease in DOMS, while the other two did not find any difference in the muscle damage markers. Howatson & collaborators (2010) found a positive outcome for inflammation (CRP, IL-6 and uric acid) and oxidative stress (TAS and TBARS) markers except PC, whereas Bovell et al. (2011) only found a positive effect for PC (and not for TAS or high-sensitivity CRP).

Berries

Berries, specifically blueberries, are fruits particularly rich in antioxidants (Ehlenfeldt & Prior, 2001; Protegente et al., 2002; Wang et al., 1998). It is believed that the phenolic compounds — including phenolic acids, tannins, namely ellagitannins and flavonoids as anthocyanins, flavonols and flavanols — are mainly responsible for their antioxidant properties (Bass et al., 2010; Szadzki & Borow ska, 2008). Other substances, as β-carotene and other carotenoids and ascorbic acid, may also contribute to these properties but in smaller proportions (Szadzki & Borowska, 2008). Similar to cherries, berries also seem to have anti-inflammatory properties. They have been shown to reduced TNF-α induced up-regulation of inflammatory mediators in human microvascular endothelial cells (Youdim et al., 2002), to attenuate inflammatory gene expression in mice (DeFuria et al., 2009), and also to positively influence the NO metabolism (Bass et al., 2010; Pergola et al., 2006). The only study (McLeay et al., 2012) in the context of EIMD that used blueberries as treatment showed positive results regarding oxidative stress and force recovery, but not for the muscle damaged parameters (CK and muscle soreness).

Pomegranate

Pomegranate was also studied (Trombold et al., 2011) with interesting and positive results. In this study, the ingestion of pomegranate juice was associated with the attenuation of weakness and reduction of soreness in the elbow flexor muscles. Pomegranate is considered a potent and unique polyphenol-rich food, containing mainly ellagitannins and their derived metabolites, which can protect against most types of free radical oxidants (Visioli et al., 2011).

To date, pomegranate’s capacity to inhibit oxidative processes, and to accelerate the breakdown and the removal of oxidized lipids, has still been more studied in the health field, namely regarding atherosclerosis development and its consequent cardiovascular events (Aviram et al., 2000, 2004; Visioli et al., 2011).

Other potential solutions

Taking into account the evidence discussed throughout this review, there are some other foods that, although not studied yet, might also have the potential to be considered as an effective solution for EIMD recovery. Therefore, the following examples were chosen due to their nutritional characteristics and may be considered in future investigations.

Meat and fish, although may not be considered as conventional as liquid options, can also be a valuable alternative not only due to their content in proteins with high-biological value (Guigoz, 2011), but also because they are one of the richest sources of some compounds mentioned above, namely LA, CoQ10 and PUFA. Moreover, fatty fishes as salmon, tuna and mackerel may also be a good choice due to their high amounts in n-3 PUFA (Insel et al., 2007). Beef has already shown to be capable of stimulating MPS from young to old persons (Robinson et al., 2013; Symons...
Conclusions

Due to the fact that EIMD can impair athletes’ ability to train and perform properly, developing strategies that mitigate and accelerate the recovery process after muscle damage are of huge importance for the athletic population. Accelerating this process will result in shorter recovery periods that will allow athletes to return sooner to their normal training routine.

Although there are few studies that relate food and recovery from EIMD, the results available seem promising. Moreover, it is important to bear in mind the current issues related to the potential contamination of the nutritional supplements that athletes often use as a recovery strategy. Therefore, considering food as a potential means to recover from muscle damage becomes even more important, since it is not contaminated with prohibitive compounds.

Some foods enclose potential to be considered an effective recovery option, especially if combined to ensure the delivery of protein, carbohydrates, antioxidants and anti-inflammatory nutrients. Beyond milk, cherries, blueberries and pomegranates, that were already successfully tested regarding EIMD, other foods are considered to be possible solutions to help in the recovery process from muscle damage. These foods include protein sources as milk, meat, fish, eggs and soy, carbohydrate-rich foods, for instance bread, pasta, rice, potatoes, beans and fruit, and foods with a high content in antioxidant and/or anti-inflammatory nutrients, such as other berries, tea and nuts.

It is clear, therefore, that more studies in this specific field are needed. It is fundamental to have scientific evidence about which types or combination of foods can improve the recovery from EIMD.

Declaration of interest

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References


et al., 2007); however, to date, no studies regarding muscle damage have been conducted.

Egg may also be a valid option since it has a biologic value of 100, meaning that all the absorbed egg protein is retain by the body (Insel et al., 2007). Egg protein was already used to study MPS (Moore et al., 2009); however, to date, no study has used eggs to investigate recovery from EIMD.

In contrast to the other plant foods, the protein isolated from soya beans provides a complete, high-quality protein equal to the animal protein (Young, 1991). Soya protein is considered a fast protein since it is digested rapidly, leading to a large but transient rise in aminoaemia (Wilkinson et al., 2007). Yet, compared to fluid milk, it seems to lead to a less acute rise in muscle protein synthesis (Wilkinson et al., 2007). This may be due to the fact that the leucinemia is greater and more prolonged with milk consumption than with soya, probably reflecting the higher leucine content of whey proteins (Phillips, 2011). Nevertheless, soya beverages can be used, for example, when there is a contraindication for milk consumption, such as cow’s milk-protein allergy or lactose intolerance.

Typical sources of CHO include bread, pasta, rice, potatoes, beans and fruit. Athletes may choose the type of CHO-rich food to consume according to the glycemic index (GI), the individual goals of each athlete and the timing of ingestion (Mondaresz & Arcelli, 2009). Fruit, in addition to have a high content in minerals, vitamins and antioxidants, is also a rich source of CHO, namely fructose and glucose. It has been shown (Jeukendrup, 2010) that when a combination of several CHO, specially glucose and fructose, is used instead of just one, the CHO absorption could be increased. This phenomenon is due to the utilization of different intestinal transporters for absorption (Jeukendrup, 2010). Particularly, the mixture glucose–fructose, namely the one with a 1:1 ratio, seems to produce one of the highest exogenous carbohydrate absorption rates (Jeukendrup, 2010). Therefore, ingesting a mixture of glucose and fructose seems to provide an optimal balance of dietary CHO for both muscle (Walls et al., 2008) and liver (Casey et al., 2000) glycogen re-synthesis.

Regarding the antioxidant potential of fruits, other berries may also be of interest for future studies, e.g. strawberries, raspberries and blackberries.

Tea is also known for its antioxidant content. Tea is originated from the leaves of Camellia sinensis L. and, according to the fermentation process, one can obtain green (not fermented), oolong (partially fermented) and black tea (fermented) (Lin et al., 2003). Green tea is considered an important dietary source of polyphenols, particularly flavonoids (Cabrera et al., 2006), being catechins the main flavonoid present (McKay & Blumberg, 2002). Catechins – especially EGCG – which are found in higher amounts in green tea than in black or oolong, are considered to have strong antioxidant potential, extensively demonstrated by in vitro and animal studies (Cabrera et al., 2006), and anti-inflammatory properties (Cabrera et al., 2006). Therefore, as the human clinical evidence is still scarce, future studies are needed in order to define the magnitude of the possible benefits and to establish, if it is the case, safe ranges of consumptions related to its benefits (Chacko et al., 2010).

Along with their high content in vitamins and minerals, unsaturated fatty acids and fibre, nuts enclose several phytochemicals that have been shown to possess a range of bioactive actions, including antioxidant and anti-inflammatory properties (Bolling et al., 2010; Chen & Blumberg, 2008). In fact, the consumption of nuts has been inversely associated with biomarkers of inflammation (Jiang et al., 2006). Regarding the antioxidant properties, it has been attributed mostly to their phenolic compounds, but a limited number of studies are available (Chen & Blumberg, 2008).
Study IV


Dietary strategies to recover from exercise-induced muscle damage


Study IV

Dietary strategies to recover from exercise-induced muscle damage


3.5 Study V
Similar effect of commercial and homemade recovery beverages on muscle damage after exhaustive eccentric exercise

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ABSTRACT

Background: Nutritional strategies to recover from exercise are fundamental for athletes. Studies typically investigate supplements or food as the recovery meal, and the comparison between both has been neglected. Therefore, this study aimed to compare the effect of 2 recovery beverages [commercial (CS) vs. homemade (MS)] with similar nutritional content on markers of muscle damage.

Methods: Ten male athletes (21.7 ± 3.4 yrs, 73.3 ± 4.5 kg) performed 2 trials of an eccentric exhaustion protocol. During the 2 h after the protocol, participants ingested 0.8 g carbohydrate·kg⁻¹·h⁻¹ and 0.26 g protein·kg⁻¹·h⁻¹ in form of CS or MS (skimmed milk, strawberries and banana). Functional tests were performed before (M1) and immediately (M2), 24 h (M4), and 48 h (M5) after exercise. Soreness scores and blood samples were additionally taken 2 h after exercise (M3).

Results: There was a time main-effect (P < 0.05), consistent with the occurrence of muscle damage, for protein kinase (M4>M1–M5; M5>M1), myoglobin (M3>M1–M5; M2>M1, M4–M5), aldolase (M4>M1–M2; M5>M1–M3), aspartate (M4>M1–M3; M5>M1) and alanine (ALT; M5>M1–M3) aminotransferases, eccentric peak torque of the quadriceps (M2<M1–M5; M4<M1), countermovement jump height (M2<M1–M5), and soreness (general: M1<M2–M5; palpation: M1<M2, M4–M5; M3<M5). Aldolase and ALT presented a beverage main-effect (P < 0.05), but significance was lost when evaluated as changes from pre-exercise values. No significant time × beverage interaction-effects were detected (P > 0.05). No significant differences were found for lactate dehydrogenase.

Conclusion: Both beverages, independently of their commercial or homemade nature, led to similar recovery patterns.

Keywords: Food, nutritional supplements, soreness, muscle function, practice.
INTRODUCTION

The development of nutritional strategies to recover from exercise has been a priority in the sports field over the last years. Due to this interest, numerous studies have been developed, mainly comparing nutritional supplements with placebo. Consequently, athletes seem to perceive that an efficient recovery can only be achieved by using supplements. Accordingly, the most referred reason to use supplements is to accelerate recovery (Sousa et al., 2013). Recently, some investigators have focused their attention on studying the effect of food on recovery in comparison to placebo (Bowtell et al., 2011; Cockburn et al., 2013; McLeay et al., 2012), with positive and promising outcomes. However, although food seems effective, this is not yet realized by sports community given that prevalence of nutritional supplements usage is increasing over time (Huang et al., 2006).

The possible contamination of supplements with prohibitive substances, possibly resulting in inadvertent doping and health issues (Geyer et al., 2008), is a serious issue to consider. An alternative that is safer and, at least, with the same effectiveness would be of great interest for athletes and sports professionals, and would also be a better option in terms of public health. Based on evidence (Sousa et al., 2014), food seems to be a valid option for exercise recovery, with the advantage of not having the contamination risk associated with supplements. In a real-life situation, athletes need to choose conscientiously between a supplement and food, not between a supplement/food and placebo. Unfortunately, the compared recovery effects of food with an equivalent nutritional supplement have been neglected even though its importance has been denoted (Cockburn et al., 2008).

A growing body of evidence shows post-exercise combined intake of protein with carbohydrates (CHO) may attenuate the negative impact of exercise-induced muscle damage (EIMD) (Cockburn et al., 2008; Luden et al., 2007; Mitchell, 2013). A recent systematic review (Sousa et al., 2014) has
suggested the intake of 0.8–1.2 g CHO·kg\(^{-1}\)·h\(^{-1}\) and 0.2–0.4 g protein·kg\(^{-1}\)·h\(^{-1}\) soon after the damaging exercise to enhance the recovery process. The protein content would account for the positive muscle protein balance that is necessary for muscle repair and adaptation (Hawley et al., 2006). On the other hand, CHO would: (i) assist the insulin rise, attenuating muscle protein breakdown, (ii) promote muscle glycogen re-synthesis (Jentjens & Jeukendrup, 2003) – low muscle glycogen levels may have a negative impact on muscle protein synthesis (Creer et al., 2005), and promote muscle protein breakdown (Lemon & Mullin, 1980) – and (iii) enrich the beverage palatability.

We hypothesized that a supplement and a homemade meal, with similar nutritional content, would lead to similar recovery outcomes from EIMD. Therefore, the aim of the present study was to compare the effects of ingesting a commercial CHO and protein supplement (CS) or a homemade milkshake with skimmed milk, strawberries and banana (MS), with similar CHO and protein content, on muscle damage, functional recovery, and muscle soreness after an exhausting eccentric protocol. This study reflects a real-world problematic and, thus, will be particularly pertinent for field application. The generated information will help both professionals and athletes to make informed and scientifically driven choices regarding the nature of the recovery meal.

**METHODS**

**Participants**

Thirteen adult male national-level track-and-field athletes (8 jumpers, 2 throwers and 3 sprinters) volunteered to participate. The study was approved by the Ethics Commission of the University of Porto, and conducted in compliance with the World Medical Association’s Declaration of Helsinki (2008). The trial is registered at ClinicalTrials.gov (NCT01555775). All participants were informed verbally and in writing regarding the experimental procedures before giving their
written informed consent. Exclusion criteria included previous acute knee/ankle injuries, lactose intolerance, and allergy to cow's-milk protein and/or strawberries.

**Experimental design**

Participants completed 2 trials separated by at least 2 weeks, in a crossover design (Figure 1). On each occasion, they arrived at the laboratory after an overnight fast (≥10 h) and rested in a seated position while a blood sample was taken from an antecubital vein (M1). Participants were then asked to score general muscle soreness and muscle soreness from palpation on a 100 mm-length visual analogue scale (VAS) (Bijur et al., 2001). Soreness from palpation was measured by hand-pressure on the front mid-thigh; measurements were made by the same investigator for each subject on each occasion. In both soreness determinations subjects marked a point along a 100 mm line from “no pain” to “maximal pain” that represented the perceived soreness in the quadriceps muscle group. The participants then completed a 5-min warm-up on a cycle ergometer at 70–100 rpm. After that, countermovement jump (CMJ) height was determined, followed by the determination of the eccentric peak torque of the quadriceps (PTq). Afterwards, the exhaustion protocol was conducted. Different lower limbs were tested for each trial in a random way to minimize the repeated bout effect (Howatson & van Someren, 2007). After exhaustion (M2), PTq determination was repeated along with a second blood sample, CMJ, and soreness scores. During the first 2 h after the end of the exhaustion protocol the participants drank the CS or MS. Beverages were randomized and the treatment was blinded to the participants. Two hours after the end of the exhaustion protocol (M3), the soreness determinations were repeated, and a third blood sample was collected. The VAS was also used at this time point to assess the beverage flavour acceptability. Participants returned to the laboratory 24 h (M4) and 48 h (M5) after the end of the protocol, in a fasted state (≥10 h). On each occasion, a resting blood sample was
obtained, and general muscle soreness and muscle soreness from palpation were reassessed. Participants then repeated the warm-up, CMJ, and PTq determination.

**Figure 1** Experimental protocol design.

PTq, Eccentric peak torque of the quadriceps; MS, homemade milkshake; CS, commercial supplement.

—► Blood sampling and visual analogue scale

—► Countermovement jump

**Exhaustion protocol and peak torque**

To induce exhaustion, a concentric/eccentric knee extension/flexion protocol was conducted at the first day of each trial. Participants were correctly placed in the test chair of the isokinetic dynamometer (Biodex® system IV, Biodex Medical Systems Inc., Shirley, NY, USA) and the range of motion set at 60° [from 50° to 110° knee flexion (0° = full knee extension)]. The participants initially performed 8 sub-maximal repetitions at a constant angular velocity of 60°·s⁻¹ to be familiar with the movement. After resting for 60 s, they completed 3 maximal repetitions to determine the subject’s maximal PTq (the best of the 3).
After this, the exhaustion protocol was conducted, which consisted of 3 bouts of 100 repetitions, with a 200-s rest time between sets. In the third set, subjects performed 100 + n repetitions until exhaustion. Participants were considered exhausted when the torque of 3 consecutive repetitions fell below 25% of the initial PTq value (Rozzi et al., 1999). Participants received verbal encouragement to perform maximally.

**Jumps**

Athletes started the CMJ test in the upright position, equal weight-bearing, with feet at hip width and holding their hands on the iliac crest. Participants bent their knees to 90° of flexion and in one continuous movement, without stopping at the lowest position, started their upward motion to jump as high as possible on an Ergojump® (Digitime 1000, Digitest, Finland). The best from 2 attempts was used for the analysis.

**Beverages composition**

Firstly, the amount of the CS in powder form (Supreme Gainers®, GoldNutrition, Econutraceuticos, Alcabideche, Portugal) was determined to guarantee 0.8 g CHO·kg\(^{-1}\)·h\(^{-1}\) and 0.26 g protein·kg\(^{-1}\)·h\(^{-1}\). Then, a milkshake was formulated, with specific amounts of foods, to achieve a similar amount of CHO and protein. The Portuguese Food Composition Table (Martins et al., 2007) was used to get the nutritional content of foods. The MS included 100 g of strawberry, and variable amounts of banana (170 ± 99 g) and skimmed milk (443 ± 114 g) to reach the CHO and protein targets. Water (Caldas de Penacova®, Água das Caldas de Penacova, Penacova, Portugal) and vitamin C (Cebiolon®, Merck, Merck and Company, Inc., Whitehouse Station, NJ, USA) were added to the CS to match the MS content (620 ± 35 g and 60.8 ± 1.0 mg, respectively). The final beverages composition is displayed on Table 1.
beverages were individually made for each participant and placed in black opaque containers. The total volume supplied during each hour was separated into two equal-volume drinks. Each participant received his respective container in every 30 min during the 2 h after the exhaustion protocol. Both beverages had strawberry flavour.

**Table 1** Beverages comparison (delivered per hour) for mean subject weight of 73.3 kg.

<table>
<thead>
<tr>
<th></th>
<th>Homemade milkshake (MS)</th>
<th>Commercial supplement (CS)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy (kcal)</strong></td>
<td>327</td>
<td>328</td>
</tr>
<tr>
<td><strong>Protein (g)</strong></td>
<td>18.9</td>
<td>18.8</td>
</tr>
<tr>
<td><strong>Carbohydrate (g)</strong></td>
<td>58.6</td>
<td>58.6</td>
</tr>
<tr>
<td><strong>Fat (g)</strong></td>
<td>1.9</td>
<td>2.0</td>
</tr>
<tr>
<td><strong>Vitamin C (mg)</strong></td>
<td>60.8</td>
<td>61.5</td>
</tr>
<tr>
<td><strong>α-tocopherol (mg)</strong></td>
<td>0.6</td>
<td>8.0</td>
</tr>
</tbody>
</table>

**Dietary intake and physical activity**

To help ensure similar metabolic conditions between trials, participants were instructed by a trained nutritionist to fulfil a food record and a physical activity record for the 2 days prior and during the 3 days of each trial. Athletes were asked not to make drastic changes in their diet and to avoid strenuous exercise during the study period. Dietary records’ information was transformed into energy and nutrients using Food Processor SQL® (ESHA Research Inc., Salem, OR, USA). For the physical activity record, athletes were asked to record the main physical activity for each 15 min. The athletes’ daily energy expenditure was calculated using metabolic equivalent values for each task and intensity level, after adjustment for the estimate resting metabolic rate predicted from the Harris-Benedict equation (Ainsworth et al., 2011).
**Body composition**

Body weight, height, and body fat percentage (%BF) were assessed in the week before the first trial. Weight was recorded to the nearest 0.1 kg using In-Body® 230 (Biospace, Korea) and height was measured to the nearest 0.5 cm with a Jofre® stadiometer (Braga, Portugal). Triceps, abdominal, and front thigh skinfold thickness was measured twice, at the nearest 0.1 mm, using a Cescorf® calliper (Porto Alegre, Brazil). Mean values were used to estimate %BF by Evans equation (Evans et al., 2005). All measures were taken according to the International Society for the Advancement of Kinanthropometry (ISAK).

**Biochemical analysis**

On each of the blood extraction time points, two venous blood samples were collected from each athlete, in a seated position. One sample was collected to a tube from Venosafe® (Terumo Europe, Leuven, Belgium) containing tripotassium ethylenediaminetetraacetic acid (EDTA). A complete blood count was obtained using an automated blood counter Sysmex XE-5000® (Sysmex Europe GmbH, Norderstedt, Germany). The second sample was collected to a serum separator tube from Venosafe® containing an additive of gel and clot activator. Blood was allowed to clot for 30 min and then centrifuged at 4500 r.p.m. during 15 min. The analytes creatine kinase (CK), lactate dehydrogenase (LDH), aldolase, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured using an automated clinical chemistry analyser (Olympus AU5400®, Olympus, Hamburg, Germany). Myoglobin was measured by a chemiluminescent immunoassay using Architect i2000® automated analyser (Abbott Diagnostics, Lake Forest, IL, USA). The remaining serum was collected, aliquoted and stored at -80°C until further measurements.
Plasma volume changes were calculated according to Costill and Dill (Dill & Costill, 1974) to understand if there were significant changes between M1 and M2. Since there were no significant differences ($P \geq 0.145$) between absolute and adjusted values on M2 for haematocrit, haemoglobin, red blood cells, and white blood cells, absolute data were used for statistical analysis (Kargotich et al., 1998). To identify if any participants were already in a muscle damage process at the beginning of the trials, the upper limit of the reference interval of CK for male athletes, 1083 U·L$^{-1}$, was used as a cut-off (Mougios, 2007).

**Statistical procedures**

Descriptive data were reported as proportions (%) and means ± standard deviations for normal variables, or as medians (interquartile range), when variables were not normally distributed. The Kolmogorov-Smirnov test was used to evaluate normality. Paired-sample T-test was used to evaluate differences in (i) haemoconcentration between absolute and adjusted values (described in the previous paragraph), (ii) beverages flavour acceptability, and (iii) $n$ repetitions between trials. Non-normal distributed variables were logarithmically transformed to attain normal distribution (CK, myoglobin, AST, ALT, soreness from palpation). Linear mixed models were used to analyse the differences in the studied parameters over time and between beverages. In the model, beverage, time, time × beverage, treatment sequence, and lower-limb sequence were treated as fixed effect variables and participant as a random effect variable. Treatment and leg sequences were counter-balanced and, thereafter, randomized by random number generation before the first trial. Nutritional and physical activity data were analysed by two-way repeated-measures ANOVA to determine if these conditions were similar between trials. The Mauchly sphericity test was used to check homogeneity of covariance; violations of the assumption of sphericity were dealt using Greenhouse-Geisser adjustment. All statistical procedures were performed using the Statistical
Package for Social Sciences® version 20 (SPSS Inc., Chicago, IL, USA). The level of significance was set at $\alpha = 0.05$.

RESULTS

The final sample comprised 10 athletes (21.7 ± 3.4 yrs, 73.3 ± 4.5 kg, 178 ± 5 cm, 8.4 ± 2.1%BF) since 3 were excluded due to CK activity >1083 U·L$^{-1}$ at the beginning of one of the trials. Participants reported similar ($P > 0.05$) nutritional intake (energy, protein, CHO, fat, and vitamin C) and energy expenditure, and performed similar $n$ repetitions ($P = 0.326$) in both trials. They found the flavour of both beverages similar ($P = 0.593$).

Table 2 presents data regarding muscle damaged biomarkers over time and by beverage. For CK, the highest values were observed 24 h after exercise ($M_4$>all the other moments; $P \leq 0.01$). After 48 h, CK levels were still higher than baseline ($M_5$>$M_1$; $P = 0.021$). Myoglobin increased gradually until 2 h after exercise ($M_3$>all the remaining moments, and $M_2$>$M_1$, $M_4$–$M_5$; $P < 0.05$). AST activity remained unaltered during the first 3 time points, peaked 24 h after exercise ($M_4$>the previous moments; $P < 0.01$), and stayed higher than baseline at 48 h ($M_5$>$M_1$; $P = 0.043$). Aldolase activity increased until 24 h after exercise ($M_4$>$M_1$–$M_2$; $P < 0.01$) and remained higher than baseline until 48 h after the protocol ($M_5$>$M_1$–$M_3$; $P < 0.05$). The CS trial presented lower aldolase activity levels than the MS trial. ALT activity increased over time, reaching a higher level at 48 h ($M_5$>$M_1$–$M_3$; $P < 0.05$). A beverage main-effect was also detected, with the CS trial levels being low than the MS ones. No significant alterations were observed for LDH. Similar statistical analyses were performed considering the changes from pre-exercise values for aldolase and ALT. No statistical significant differences were found in terms of beverage (no significant beverage main-effect) for both parameters ($P > 0.264$).
Table 2 Muscle damage biomarkers over time and by beverage.

Data are presented as mean ± SD or median (interquartile range).

<table>
<thead>
<tr>
<th></th>
<th>Before exercise (M1)</th>
<th>Immediately after exercise (M2)</th>
<th>2 h after exercise (M3)</th>
<th>24 h after exercise (M4)</th>
<th>48 h after exercise (M5)</th>
<th>Total</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(U·L⁻¹; 98 obs)</td>
<td>MS</td>
<td>244 (495)</td>
<td>264 (396)</td>
<td>324 (636)</td>
<td>710 (894)</td>
<td>394 (518)</td>
<td>329 (543)</td>
</tr>
<tr>
<td></td>
<td>CS</td>
<td>181 (351)</td>
<td>226 (363)</td>
<td>311 (406)</td>
<td>586 (663)</td>
<td>403 (366)</td>
<td>275 (405)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>206 (384)</td>
<td>237 (356)</td>
<td>324 (425)</td>
<td>600 (664)*</td>
<td>403 (390)*</td>
<td>N.A.</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>MS</td>
<td>51.8 (38.5)</td>
<td>84.9 (62.7)</td>
<td>201.6 (262.9)</td>
<td>56.8 (83.9)</td>
<td>52.6 (39.0)</td>
<td>77.5 (74.3)</td>
</tr>
<tr>
<td>(ng·mL⁻¹; 98 obs)</td>
<td>CS</td>
<td>51.7 (41.5)</td>
<td>96.9 (47.7)</td>
<td>255.9 (215.2)</td>
<td>63.4 (44.5)</td>
<td>56.3 (23.3)</td>
<td>70.2 (70.4)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>51.7 (36.2)</td>
<td>85.7 (52.4)</td>
<td>209.4 (152.0)</td>
<td>56.8 (49.4)</td>
<td>56.3 (25.9)</td>
<td>N.A.</td>
</tr>
<tr>
<td>LDH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(U·L⁻¹; 98 obs)</td>
<td>MS</td>
<td>176 ± 24</td>
<td>175 ± 28</td>
<td>194 ± 33</td>
<td>181 ± 25</td>
<td>203 ± 50</td>
<td>186 ± 34</td>
</tr>
<tr>
<td></td>
<td>CS</td>
<td>169 ± 33</td>
<td>183 ± 30</td>
<td>167 ± 42</td>
<td>184 ± 22</td>
<td>183 ± 30</td>
<td>177 ± 32</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>172 ± 28</td>
<td>179 ± 28</td>
<td>180 ± 39</td>
<td>183 ± 23</td>
<td>193 ± 41</td>
<td>N.A.</td>
</tr>
<tr>
<td>Aldolase</td>
<td>MS</td>
<td>7.08 ± 5.23</td>
<td>5.38 ± 3.04</td>
<td>7.89 ± 6.18</td>
<td>10.1 ± 6.96</td>
<td>11.51 ± 7.16</td>
<td>8.42 ± 6.08</td>
</tr>
<tr>
<td>(U·L⁻¹; 98 obs)</td>
<td>CS</td>
<td>5.75 ± 3.49</td>
<td>5.61 ± 3.19</td>
<td>7.04 ± 4.19</td>
<td>9.25 ± 4.12</td>
<td>9.57 ± 4.51</td>
<td>7.44 ± 4.13</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>6.42 ± 4.38</td>
<td>5.50 ± 3.04</td>
<td>7.47 ± 5.16</td>
<td>9.65 ± 5.49*</td>
<td>10.54 ± 5.91*</td>
<td>N.A.</td>
</tr>
<tr>
<td>AST</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(U·L⁻¹; 99 obs)</td>
<td>MS</td>
<td>18 (10)</td>
<td>18 (8)</td>
<td>19 (11)</td>
<td>27.5 (17)</td>
<td>24.5 (9)</td>
<td>21 (11)</td>
</tr>
<tr>
<td></td>
<td>CS</td>
<td>17 (12)</td>
<td>20.5 (11)</td>
<td>17.5 (10)</td>
<td>23.5 (9)</td>
<td>22.5 (18)</td>
<td>20.5 (11)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>17 (10)</td>
<td>18 (8)</td>
<td>18.5 (9)</td>
<td>26 (11)*</td>
<td>24 (9)*</td>
<td>N.A.</td>
</tr>
<tr>
<td>ALT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(U·L⁻¹; 99 obs)</td>
<td>MS</td>
<td>11 (7)</td>
<td>11 (7)</td>
<td>12.5 (8)</td>
<td>12 (6)</td>
<td>13 (7)</td>
<td>12 (6)</td>
</tr>
<tr>
<td></td>
<td>CS</td>
<td>9 (5)</td>
<td>9.5 (6)</td>
<td>9.5 (6)</td>
<td>11.5 (6)</td>
<td>12 (7)</td>
<td>10 (6)</td>
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<tr>
<td></td>
<td>Total</td>
<td>9.5 (7)</td>
<td>10 (6)</td>
<td>11 (7)</td>
<td>12 (5)</td>
<td>13 (6)*</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

CK, creatine kinase; obs, observations; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; MS, homemade milkshake; CS, commercial supplement; N.A., not applicable; T, time main-effect; B, beverage main-effect; T x B, time × beverage interaction-effect.

* Significant difference from baseline (P < 0.05); † Significant differences between beverages (P < 0.05)
PTq (78 observations; Figure 2A) decreased by 98.6 ± 61.3 N·m (23.8 ± 14.2%) with the exhaustive protocol (M2<all the remaining moments; $P < 0.001$); the initial levels were restored 48 h after exercise (M4<M1; $P = 0.026$). For CMJ height (74 observations; Figure 2B), a decrement of 5.5 ± 4.6 cm (11.3 ± 9.6%) was observed immediately after exercise (M2<all the remaining moment; $P < 0.01$), but was not significantly different at 24 h. No significant differences ($P \geq 0.119$) were detected for these functional parameters regarding beverage main-effect or time × beverage interaction-effect.

**Figure 2** Changes in functional parameters over time depending on the nature of the recovery beverage.

Data are presented as mean + SD for MS and mean - SD for CS.

MS, homemade milkshake; CS, commercial supplement; PTq, eccentric peak torque of the quadriceps; CMJ, countermovement jump.

* Significant difference from baseline ($P < 0.05$); † Significant time main-effect ($P < 0.001$).
General muscle soreness (100 observations) and soreness from palpation (100 observations) also differed throughout time (Figure 3). After 48 h, both soreness scores were still higher than pre-exercise values (general: M1<M2–M5; palpation: M1<M2, M4–M5, and M3<M5; \( P < 0.05 \)). No significant \( (P \geq 0.277) \) beverage main-effect or time \( \times \) beverage interaction-effect was seen regarding soreness parameters.

**Figure 3** Changes in muscle soreness scores over time depending on the nature of the recovery beverage.
Data are presented as mean + SD for CS and mean - SD for MS (A) or median (B).

MS, homemade milkshake; CS, commercial supplement.
* Significant difference from baseline \( (P < 0.05) \); † Significant time main-effect \( (P < 0.001) \).
DISCUSSION

The aim of the present study was to evaluate if the nature (homemade vs. commercial) of the recovery beverage influenced the recovery pattern after an exhaustive exercise protocol. Our findings suggest the nutrients nature do not influence the recovery course on muscle damage, muscular function, and muscle soreness on highly trained male athletes. The time-effects observed confirm the exercise protocol successfully induced muscle damage, allowing the comparison between the beverages under conditions of dietary and physical activity control.

Previous studies have demonstrated that a CHO–P solution consumed after exercise has a positive influence on the recovery from EIMD (Cockburn et al., 2008; Cockburn et al., 2012; Cockburn et al., 2010; Luden et al., 2007; Wojcik et al., 2001). Proteins are believed to increase amino acids availability (increasing protein synthesis), whereas CHO contribute to an optimal hormonal environment due to insulin increment (decreasing protein breakdown) (Cockburn et al., 2008). The higher biomarkers’ levels after eccentric exercise are known to be a consequence of the sarcolemma damage that is proportional to the duration and intensity of the physical demand (Brancaccio et al., 2008). Changes in protein metabolism, due to the ingestion of CHO–P solutions, have been suggested to attenuate the ultrastructural damage, resulting in a better maintenance of myofibrillar, contractile protein, and sarcolemma integrity (Cockburn et al., 2008).

The majority of the analysed muscle damage biomarkers showed a kinetic consistent with EIMD (Brancaccio et al., 2010). CK levels usually peak 8 h after resistance exercise and remain markedly elevated for 24 h (Brancaccio et al., 2008). In accordance with this typical time-change, in our study the highest CK activity was observed 24 h after the end of exercise. Additionally, the response we found is in line with the participants’ level of training (Brancaccio et al., 2008), and with other works using CHO–P recovery
beverages (Green et al., 2008). Moreover, the usual kinetics only occurs if subjects rest (Brancaccio et al., 2008), confirming our participants did not engage in strenuous exercise during the study period, as requested. The myoglobin peak moment (2 h after exercise) is also in agreement with published data (Jackman et al., 2010), which showed higher levels in the first few hours after exercise both for placebo and branched-chain amino acids supplemented groups. Cockburn and collaborators (2008) compared the effect of ingesting water, CHO, milk, or CHO–P (containing ≈2.5 times more CHO than milk), after an eccentric exercise bout. Milk and CHO–P groups presented lower myoglobin concentrations (beverage-main effect), and lower CK levels at 48 h, compared to CHO group. These data, taken together with our findings, support that the nature of the CHO–P beverage (food/homemade vs. commercial) does not appear to lead to different muscle damage biomarkers' response as long as a proper nutritional load is provided. Therefore, and in agreement with van Loon (van Loon & Gibala, 2012), after exercise the focus should be placed on getting an ample amount of protein, rather than on the type and nature of the protein.

Data on LDH levels after consuming a CHO–P or a CHO solution during and after exercise are equivocal. In one study (Betts et al., 2009), LDH activity was highest immediately following exercise (intermittent shuttle-running) compared to pre-exercise levels, and remained elevated until the end of the studied period (24 h), without difference between beverages. In another study (Romano-Ely et al., 2006), where LDH activity was measured before and 72 h after riding to exhaustion, the levels of this enzyme were significantly elevated over baseline on the CHO but not in the CHO–P trial. Moreover, an eccentric exercise bout typically induces LDH increments between the third (72 h) and fifth (120 h) day after exercise (Brancaccio et al., 2010; Sietsema et al., 2010). Furthermore, LDH activity response to exercise seems to be influenced by training level, with physical training reducing post-exercise serum enzyme levels (Brancaccio et al., 2008). In our study, the fact that both of our trials contained CHO–P beverages, the participants were national-level athletes that performed an eccentric exercise bout, and blood was only collected until 48 h after
exercise, might explain why a LDH time main-effect was not detected. Nevertheless, LDH activity did not differ between the tested beverages. Regarding aldolase, its activity at 24 h and 48 h after exercise was significantly higher than pre-exercise values. In another study (Sietsema et al., 2010), where no beverages were ingested, this enzyme only differed from pre-exercise values at 72 h after the protocol.

Usually, AST and ALT levels are considered as markers of liver function, but in athletes they also reflect the release from the muscle (Nathwani et al., 2005). Although the specific time-line for these two biomarkers in the context of EIMD is not yet totally understood, it seems that the duration and type of exercise influence their responses (Brancaccio et al., 2010; Sietsema et al., 2010). Indeed, AST activity increased immediately after running a half-marathon and remained high for 24 h (Lippi et al., 2008), and a soccer match did not alter AST and ALT activities throughout the first 18 h after the game (Gravina et al., 2011). In our study, AST and ALT peaked 24 h and 48 h after eccentric exercise, respectively.

In our study, although ALT and aldolase activities differed between treatments, the beverage main-effect lost significance when evaluated as changes from pre-exercise values. Additionally, the beverage and leg sequences did not influence these outcomes ($P \geq 0.158$). This suggests the differences between beverages were probably due to marginal differences in baseline, and not from different effects from the beverages. Indeed, no significant differences were found for these 2 parameters at the beginning of both trials ($P \geq 0.373$)

Muscle force is considered a practical measure of muscle functional capacity (Jackman et al., 2010), and frequently used as a marker of functional recovery (Bowtell et al., 2011; Howatson et al., 2012; McLeay et al., 2012). The decrease of muscle force immediately after eccentric exercise, as occurred in the current study, is well documented (Allen, 2001). Consuming a CHO–P
solution after exercise has been shown to enhance the functional recovery from EIMD (Cockburn et al., 2008; Cockburn et al., 2010). Possible underlying mechanisms are the replacement of the lost amino acids during the increased protein degradation, and a positive change in the protein balance, limiting the ultrastructural damage (Cockburn et al., 2010). However, other studies (Green et al., 2008; Wojcik et al., 2001) did not attain the same results. These inconsistencies could be owing to the different exercise protocols and/or to the different methods used to assess muscle function. Nonetheless, in the present study, no differences were found between the two beverages concerning the recovery of PTq levels and CMJ performance. This suggests both beverages yielded similar recovery profiles in terms of functional recovery.

Muscle soreness usually increases during the first 24 h, and peaks at about 48 h after exercise (Cheung et al., 2003). Previous studies (Cockburn et al., 2010; Luden et al., 2007) have highlighted the positive effect of ingesting a mixture of CHO and proteins on the attenuation of muscle soreness after EIMD, probably by limiting the ultrastructural damage (Cockburn et al., 2010). In our study, muscle soreness evolved similarly between beverages, increasing immediately after exercise, and remaining high until the end of the study (48 h). The eccentric component of the exercise, known to induce injury at a greater severity compared to other muscle actions (Clarkson & Hubal, 2002), together with the intensity and duration of the protocol, might have contributed to the observed early onset of muscle soreness. Feeding has been suggested as a factor that may modulate soreness, resulting in a temporary reduction in perceived feelings of discomfort (Jackman et al., 2010). This could explain the decrement in muscle soreness from palpation observed 2 h after exercise and after the beverages intake (M3).

One possible limitation of this study is the absent of control group. Nevertheless, the aim of the present study was to compare the effect of both beverages. Several others studies (Luden et al., 2007; Wojcik et al., 2001) used the same approach when the aim was to compare beverages. Additionally,
other study (Cockburn et al., 2008) have already compared the effect of CHO–P beverages with a control group, using a similar exercise protocol. Another issue might have been the manually measurement of soreness from palpation; nevertheless, measurements were always made by the same investigator.

In conclusion, both beverages, independently of their commercial or homemade nature, led to similar recovery patterns from EIMD regarding muscle damage, functional, and soreness markers. Moreover, it is interesting to note that the participants found the beverages’ flavour similar. Taken together, these data provide important information for athletes and professionals on the sports field and has a pertinent real-world application. Compared to nutritional supplements, foods have the advantage of being cheaper and not contaminated with prohibitive substances in sport. Considering the issues related to inadvertent doping, it is a safer and probably healthier option, for athletes and for professionals working with athletes, to rely on food rather than on nutritional supplements. On the other hand, the commercial options might be more practical and are ready to be consumed. Therefore, athletes and professionals may prefer to select the nature of the recovery beverage based on other characteristics than on the recovery outcome.

ACKNOWLEDGEMENTS

We acknowledge Continente®, Sonae, Portugal for supplying the food included in the MS beverage. Mónica Sousa acknowledges the Fundação para a Ciência e a Tecnologia (FCT) and POPH/FSE regarding the grant SFRH/BD/75276/2010. CIAFEL acknowledges the FCT and POPH/FSE for the funded project PEst-OE/SAU/UI0617/2011.
REFERENCES


3.6 Study VI
Compared effect of commercial or homemade recovery beverages ingested after eccentric exercise on inflammatory response and oxidative stress

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6 Instituto de Saúde Pública (ISPUP) [Institute of Public Health], Universidade do Porto, Porto, Portugal
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ABSTRACT

Background: Exercise-induced muscle damage has inflammation and oxidative stress in its genesis. Most studies have focused on the impact of ingesting supplements to attenuate these processes. Food seems to have similar potential.

Purpose: To compare the effects of ingesting a commercial supplement (CS) or a homemade milkshake (MS), with similar nutritional content, on inflammatory response and oxidative stress after exercise.

Methods: Ten male athletes (21.7 ± 3.4 yrs, 73.3 ± 4.5 kg) performed 2 trials of an exhaustion protocol comprising a minimum of 300 concentric/eccentric knee extension/flexion repetitions. During the 2 h after the protocol, participants ingested 0.8 g CHO·kg⁻¹·h⁻¹, 0.26 g protein·kg⁻¹·h⁻¹, and 60.8 ± 1.0 mg vitamin C, in form of CS or MS (skimmed milk, strawberries, and banana). Blood samples were taken before (M1), immediately after (M2), 2h (M3), 24 h (M4), and 48 h (M5) after exercise.

Results: Significant higher counts at 2 h after exercise (M3>M1–M5; P < 0.001) were detected for leucocytes and neutrophils, whereas eosinophils and lymphocytes presented significant lower counts (M3<M1–M5; P < 0.05). Higher levels of C-reactive protein and lower levels of uric acid were found in MS trial (P < 0.001), but significances were lost when evaluated as changes from baseline. Glutathione reductase activity peaked immediately after exercise (M2>M1, M4, M5; P < 0.05). No significant differences were found for monocytes, basophils, interleukine-6, tumour necrosis factor-α, total antioxidant status, and protein carbonyls.

Conclusion: Both beverages yield similar recovery patterns on inflammatory and oxidative exercise-induced responses.

Keywords: Exercise, metabolism, sports nutrition, vitamin c, supplements.
INTRODUCTION

Athletes seem to perceive the use of supplements as mandatory to achieve maximal performance (Dascombe et al., 2010). Recovery enhancement has been repeatedly cited as a major reason to consume supplements (Sousa et al., 2013). Indeed, numerous studies have focused on the impact of ingesting supplements to recover from exercise. However, the problematic around positive doping violations and possible health consequences that can arise from contaminated supplements (Geyer et al., 2008) is of serious concern. A safer alternative, at least with similar effectiveness, preferably cheaper, and nutritionally equivalent, would be of great interest for clinical and sports practice, and also for public health. Based on the available evidence (Sousa et al., 2014), food seems to be a valid option for this purpose.

Oxidative stress and inflammation have been proposed to impair recovery after damaging exercise (Sousa et al., 2014). Exercise is well recognized to cause perturbations on the immune system; for instance, it can lead to dramatic changes in circulating leucocyte count and induce the production of cytokines (Pedersen & Hoffman-Goetz, 2000). Additionally, acute high-intensity resistance exercise may lead to oxidative stress, resulting in oxidation of multiple compounds, namely proteins (Finaud et al., 2006; Hudson et al., 2008). Besides their negative consequences, the inflammatory process and oxidative stress have central roles regarding, respectively, the healing process (Tipton, 2013) and the training-induced adaptations (S. K. Powers et al., 2011). However, avoidance of excessive inflammation (Tipton, 2013) and excessive antioxidant supplementation (S. Powers et al., 2011) have been recommended.

Some nutritional factors have been implicated in the reduction of the inflammatory and oxidative impact in response to heavy exercise, namely the ingestion of carbohydrates (CHO) (Henson et al., 2000; McAnulty et al., 2007), proteins (Kerasioti et al., 2012; Kerasioti et al., 2013) and, possibly, the intake of
antioxidant nutrients within natural amounts found in food (Sousa et al., 2014). A large body of evidence in the context of EIMD recovery has been gathering regarding nutritional supplements, but little is still know concerning the impact of natural food on inflammatory and oxidative parameters after exercise. Moreover, the compared recovery effect of food with an equivalent nutritional supplement is unknown, even though in a real-life situation, athletes have to choose a supplement or food as their recovery meal – not a supplement/food or placebo.

Therefore, the aim of the present study was to describe and compare the effects of ingesting a commercial supplement (CS) or a homemade milkshake with skimmed milk, strawberries and banana (MS), with similar CHO, protein, and vitamin C content, on markers of inflammation and oxidative stress after an eccentric protocol until exhaustion. We hypothesised that supplements and food would lead to similar recovery outcomes from EIMD.

METHODS

Participants

Thirteen adult male national-level track-and-field athletes (8 jumpers, 2 throwers and 3 sprinters) volunteered to participate in this study. The study was approved by the Ethics Commission of the University of Porto, was conducted in compliance with the World Medical Association’s Declaration of Helsinki (2008), and is registered at ClinicalTrials.gov (NCT01555775). All participants were informed verbally and in writing regarding the experimental procedures before giving their written informed consent.
Experimental design

Participants completed a single-blind, counterbalanced, randomized, crossover experimental study consisting of 2 trials separated by, at least, 2 weeks. The muscle damage was induced in the anterior musculature using an isokinetic dynamometer (Biodex® system IV, Biodex Medical Systems Inc., Shirley, NY, USA). Different lower limbs were tested for each trial in a random way to minimize the repeated bout effect (Howatson & van Someren, 2007). In each occasion, the participants arrived at the laboratory after an overnight fast (≥10 h) and rested in a seated position while a blood sample was taken from an antecubital vein (M1). Then, they warmed-up for 5-min on a cycle ergometer, with intensity ranging 70–100 rpm, were correctly positioned and strapped in the test chair of the isokinetic dynamometer, and performed 8 sub-maximal repetitions at 60°·s⁻¹ [range of motion set at 60°, from 50° to 110° knee flexion (0° = full knee extension)] to be familiar with the movement. After resting for 60 s, the participants completed 3 maximal repetitions of concentric/eccentric knee joint extension/flexion at the same constant angular velocity to determine the subject’s maximal eccentric peak torque of the quadriceps (PTq), defined as the best of the 3 repetitions. After the PTq determination, the exhaustion protocol was conducted, consisting of 3 bouts of a concentric/eccentric knee extension/flexion exercise at 60°·s⁻¹ with a 200-s rest time between sets. The first and second sets were composed by 100 repetitions and, in the third set, subjects performed 100 + n repetitions until exhaustion. Participants were considered exhausted when the torque of 3 consecutive repetitions fell below 25% of the initial PTq value (Rozzi et al., 1999). Participants received verbal encouragement to perform maximally in each occasion. After exhaustion (M2), a second blood sample was taken. During the first 2 h after the end of the exhaustion protocol the participants drank the CS or MS. Beverages were randomized and the treatment was blinded to the participants. Two hours after the end of the exhaustion protocol (M3), a third blood sample was collected. Participants returned to the laboratory 24 h (M4) and 48 h (M5) after the end of
the protocol, in a fasted state (≥10 h), to get a resting blood sample collected from an antecubital vein on each occasion.

**Beverages composition**

The two beverages had similar CHO, protein, and vitamin C content. Firstly, the amount of the commercial supplement in a powder form (Supreme Gainers®, GoldNutrition, Econutrechticos, Alcabideche, Portugal) was determined to guarantee 0.8 g CHO·kg⁻¹·h⁻¹ and 0.26 g protein·kg⁻¹·h⁻¹. Then, a milkshake was formulated, with specific amounts of foods (milk, banana, and strawberries), to achieve a similar amount of CHO and protein. The Portuguese Food Composition Table (Martins et al., 2007) was used to get the nutritional content of foods. The MS included 100 g of strawberry, and variable amounts of banana (170 ± 99 g) and skimmed milk (443 ± 114 g) to reach the CHO and protein targets. Finally, bottled water (620 ± 35 g; Caldas de Penacova®, Água das Caldas de Penacova, Penacova, Portugal) and vitamin C (60.8 ± 1.0 mg; Cebiolon®, Merck, Merck and Company, Inc., Whitehouse Station, NJ, USA) were added to the CS to match the MS content. The final beverages composition is displayed on Table 1. The beverages were individually made for each participant and placed in black opaque containers. In every 30 min, during the 2 h after the exhaustion protocol, each participant received his respective container. Both beverages had strawberry taste.

**Dietary intake and physical activity**

Participants were instructed to fulfil a food record and a physical activity record for the 2 days prior and during the 3 days of each trial. They were also asked not to make drastic changes in their diet, to avoid strenuous exercise, and to abstain from anti-inflammatory drugs during the study period. Dietary records’ information was transformed into energy and nutrients using ESHA
Food Processor SQL® (ESHA Research Inc., Salem, OR, USA). The athletes’ daily energy expenditure was calculated using metabolic equivalent values by task and intensity level, for the main physical activity in each 15 min, after adjustment for the estimate resting metabolic rate predicted from the Harris-Benedict equation (Ainsworth et al., 2011).

**Table 1** Beverages comparison (delivered per hour) for mean subject weight of 73.3 kg.

<table>
<thead>
<tr>
<th></th>
<th>Homemade milkshake (MS)</th>
<th>Commercial supplement (CS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>327</td>
<td>328</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>18.9</td>
<td>18.8</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>58.6</td>
<td>58.6</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>1.9</td>
<td>2.0</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>60.8</td>
<td>61.5</td>
</tr>
<tr>
<td>α-tocopherol (mg)</td>
<td>0.6</td>
<td>8.0</td>
</tr>
</tbody>
</table>

**Body composition**

Body weight, height, and body fat percentage (%BF) were assessed in the week before the first trial. Weight was recorded to the nearest 0.1 kg using In-Body® 230 (Biospace, Korea), and height was measured to the nearest 0.5 cm with a Jofre® stadiometer (Braga, Portugal). The %BF was calculated using Evans 3-sites equation formula (Evans et al., 2005); each skinfold thickness (triceps, abdominal and front thigh) was taken twice at the nearest 0.1 mm using a Cescorf® calliper (Porto Alegre, Brazil), and the mean value used in the equation. All measures were taken according to the International Society for the Advancement of Kinanthropometry (ISAK).
Biochemical analysis

On each of the blood extraction moments, two venous blood samples were collected from each athlete, in a seated position. One sample was collected to a tube from Venosafe® (Terumo Europe, Leuven, Belgium) containing tripotassium ethylenediaminetetraacetic acid (EDTA). A complete blood count was obtained in this tube, using an automated blood counter Sysmex XE-5000® (Sysmex Europe GmbH, Norderstedt, Germany). The remaining blood was then centrifuged at 4500 r.p.m. during 15 min and the plasma fraction was separated. Plasma interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) concentrations were measured by a quantikine high-sensitivity ELISA (R&D Systems, Inc., Minneapolis, MN, USA) at M1, M2, and M4. The second sample was collected to a serum separator tube from Venosafe® containing an additive of gel and clot activator. Blood was allowed to clot for 30 min and then centrifuged at 4500 r.p.m. during 15 min. The serum fraction was separated and the analytes creatine kinase (CK), C-reactive protein (CRP), uric acid, total antioxidant status (TAS) and glutathione reductase were measured using an Olympus AU5400 automated clinical chemistry analyser (Olympus, Hamburg, Germany). Serum protein carbonyl (PC) concentrations were measured using an enzyme-linked immunosorbent assay (ELISA) (Cell Biolabs, Inc., San Diego, CA, USA). The remaining serum and plasma were collected, aliquoted and stored at -80°C for future analysis.

Taking into account that no significant plasma volume changes were detected between absolute and adjusted values (Dill & Costill, 1974) on M2 for haematocrit ($P = 0.167$), haemoglobin ($P = 0.159$), red blood cells ($P = 0.158$), and white blood cells (WBC; $P = 0.145$), absolute data were used for statistical analysis (Kargotich et al., 1998). To identify if any participant were already in a muscle damage process at the beginning of the trials, the reference value of 1083 U·L$^{-1}$ (Mougios, 2007) for CK was used as a cut-off.
Statistical procedures

Descriptive data were reported as mean ± standard deviation for normal variables, or as median (interquartile range), when variables were not normally distributed. The Kolmogorov-Smirnov test was used to evaluate normality. Paired-sample T-test was used to evaluate differences in haemoconcentration between absolute and adjusted values, and n repetitions between trials. Non-normal distributed variables were transformed to attain normal distribution; the natural logarithm transformation was used when skewness needed to be corrected (basophils, PC) and the inversion was applied when both skewness and kurtosis required adjustment (CRP). Linear mixed models were used to analyse the differences in the studied parameters over time and between beverages. In the model, beverage, time, time × beverage, treatment sequence, and lower-limb sequence were treated as fixed effect variables and participant as a random effect variable. Treatment and leg sequences were counterbalanced and, thereafter, randomized by random number generation before the first trial. Nutritional and physical activity data were analysed by two-way repeated-measures ANOVA to determine if these conditions were similar between trials. The Mauchly sphericity test was used to check homogeneity of covariance; violations of the assumption of sphericity were dealt using Greenhouse-Geisser adjustment. All statistical procedures were performed using the Statistical Package for Social Sciences® version 20 (SPSS Inc., Chicago, IL, USA). The level of significance was set at α = 0.05.

RESULTS

The final sample comprised 10 athletes (21.7 ± 3.4 yrs, 73.3 ± 4.5 kg, 178 ± 5 cm, 8.4 ± 2.1 %BF); 3 were excluded due to CK activity >1083 U·L⁻¹ at the beginning of one of the trials. Participants reported similar (P > 0.05) nutritional intake (energy, protein, CHO, fat, and vitamin C) and energy expenditure, and performed similar n repetitions (P = 0.326) in both trials.
Inflammatory response over time and by beverage is displayed in Table 2. WBC and neutrophils differed significantly over time ($P < 0.001$), peaking 2 h after exercise. No significant beverage main-effect or time $\times$ beverage interaction-effect was found. For monocytes and basophils, no significant changes were seen. Eosinophils and lymphocytes presented a main-effect for time ($P < 0.001$), with their counts decreasing significantly 2 h after exercise. Neither a significant beverage main-effect nor a time $\times$ beverage interaction-effect was detected for these two markers. Twenty-four hours after exercise, WBC, neutrophils, eosinophils, and lymphocytes presented levels similar to baseline ($M_{1} = M_{4}$ and $M_{5}, P \geq 0.05$). No significant changes were seen for IL-6 and TNF-$\alpha$. Additionally, CRP did not statistically change over time, but a significant beverage main-effect was detected ($P < 0.001$), with the MS trial presenting higher levels than the CS one. However, this significant result disappeared when the CRP was evaluated as changes from pre-exercise values. No statistically significant time $\times$ beverage interaction-effect was found.

Considering TAS and PC, no significant changes over time, by beverage, or time $\times$ beverage interaction were detected (Table 3). Although a significant time main-effect was found for uric acid ($P = 0.031$), no pairwise comparisons were detected (Table 3). A significant beverage main-effect was also detected ($P < 0.001$), with the CS trial presenting higher levels than the MS one. The statistical significance was lost when this variable was analysed as changes from pre-exercise values. No statistically significant time $\times$ beverage interaction effect was found. Glutathione reductase activity significantly changed over time ($P < 0.001$), peaking immediately after the exhaustion protocol (Table 3). Neither a significant beverage main-effect nor a time $\times$ beverage interaction effect was detected.
Table 2 Inflammatory response over time and by beverage.
Data are presented as mean ± SD or median (interquartile range).

<table>
<thead>
<tr>
<th></th>
<th>Before exercise (M1)</th>
<th>Immediately after exercise (M2)</th>
<th>2 h after exercise (M3)</th>
<th>24 h after exercise (M4)</th>
<th>48 h after exercise (M5)</th>
<th>Total</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WBC (10^3·µL⁻¹; 100 obs)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>6.89 ± 1.25</td>
<td>7.23 ± 1.91</td>
<td>10.05 ± 2.14</td>
<td>6.79 ± 1.30</td>
<td>6.67 ± 1.83</td>
<td>7.53 ± 2.09</td>
<td>T: P &lt; 0.001</td>
</tr>
<tr>
<td>CS</td>
<td>6.86 ± 1.54</td>
<td>7.66 ± 1.62</td>
<td>9.62 ± 2.55</td>
<td>6.79 ± 2.52</td>
<td>6.54 ± 1.70</td>
<td>7.50 ± 2.26</td>
<td>B: P = 0.918</td>
</tr>
<tr>
<td>Total</td>
<td>6.88 ± 1.36</td>
<td>7.45 ± 1.74</td>
<td>9.84 ± 2.30*</td>
<td>6.79 ± 1.95</td>
<td>6.60 ± 1.72</td>
<td>N. A.</td>
<td>T × B: P = 0.921</td>
</tr>
<tr>
<td><strong>Neutrophils (10^3·µL⁻¹; 94 obs)</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>3.67 ± 0.83</td>
<td>4.00 ± 1.38</td>
<td>7.20 ± 2.45</td>
<td>3.75 ± 1.11</td>
<td>3.78 ± 1.68</td>
<td>4.38 ± 1.98</td>
<td>T: P &lt; 0.001</td>
</tr>
<tr>
<td>CS</td>
<td>3.52 ± 1.04</td>
<td>4.29 ± 1.12</td>
<td>7.10 ± 2.24</td>
<td>3.81 ± 2.27</td>
<td>3.78 ± 1.43</td>
<td>4.53 ± 2.11</td>
<td>B: P = 0.773</td>
</tr>
<tr>
<td>Total</td>
<td>3.60 ± 0.92</td>
<td>4.16 ± 1.21</td>
<td>7.14 ± 2.27*</td>
<td>3.78 ± 1.70</td>
<td>3.78 ± 1.53</td>
<td>N. A.</td>
<td>T × B: P = 0.989</td>
</tr>
<tr>
<td><strong>Monocytes (10^3·µL⁻¹; 94 obs)</strong></td>
<td></td>
<td></td>
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<tr>
<td>MS</td>
<td>0.62 ± 0.17</td>
<td>0.64 ± 0.12</td>
<td>0.67 ± 0.08</td>
<td>0.60 ± 0.12</td>
<td>0.56 ± 0.13</td>
<td>0.61 ± 0.13</td>
<td>T: P = 0.151</td>
</tr>
<tr>
<td>CS</td>
<td>0.64 ± 0.15</td>
<td>0.67 ± 0.21</td>
<td>0.65 ± 0.13</td>
<td>0.61 ± 0.14</td>
<td>0.60 ± 0.15</td>
<td>0.64 ± 0.15</td>
<td>B: P = 0.537</td>
</tr>
<tr>
<td>Total</td>
<td>0.63 ± 0.15</td>
<td>0.66 ± 0.18</td>
<td>0.66 ± 0.11</td>
<td>0.61 ± 0.12</td>
<td>0.58 ± 0.14</td>
<td>N. A.</td>
<td>T × B: P = 0.899</td>
</tr>
<tr>
<td><strong>Basophils (10^3·µL⁻¹; 94 obs)</strong></td>
<td></td>
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</tr>
<tr>
<td>MS</td>
<td>0.025 (0.03)</td>
<td>0.03 (0.03)</td>
<td>0.02 (0.01)</td>
<td>0.02 (0.02)</td>
<td>0.02 (0.02)</td>
<td>0.02 (0.02)</td>
<td>T: P = 0.382</td>
</tr>
<tr>
<td>CS</td>
<td>0.025 (0.03)</td>
<td>0.03 (0.02)</td>
<td>0.025 (0.01)</td>
<td>0.02 (0.02)</td>
<td>0.02 (0.02)</td>
<td>0.02 (0.02)</td>
<td>B: P = 0.846</td>
</tr>
<tr>
<td>Total</td>
<td>0.025 (0.03)</td>
<td>0.03 (0.03)</td>
<td>0.02 (0.01)</td>
<td>0.02 (0.02)</td>
<td>0.02 (0.02)</td>
<td>N. A.</td>
<td>T × B: P = 0.569</td>
</tr>
<tr>
<td><strong>Eosinophiles (10^3·µL⁻¹; 94 obs)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>0.20 ± 0.14</td>
<td>0.14 ± 0.11</td>
<td>0.09 ± 0.07</td>
<td>0.19 ± 0.12</td>
<td>0.18 ± 0.11</td>
<td>0.16 ± 0.12</td>
<td>T: P &lt; 0.001</td>
</tr>
<tr>
<td>CS</td>
<td>0.19 ± 0.14</td>
<td>0.17 ± 0.13</td>
<td>0.11 ± 0.07</td>
<td>0.17 ± 0.13</td>
<td>0.18 ± 0.10</td>
<td>0.16 ± 0.12</td>
<td>B: P = 0.578</td>
</tr>
<tr>
<td>Total</td>
<td>0.19 ± 0.14</td>
<td>0.16 ± 0.12</td>
<td>0.10 ± 0.07*</td>
<td>0.18 ± 0.12</td>
<td>0.18 ± 0.10</td>
<td>N. A.</td>
<td>T × B: P = 0.948</td>
</tr>
</tbody>
</table>

Total M3 > all the other moments (M1: P < 0.001; M2: P = 0.008; M4: P = 0.001; M5: P = 0.004)
Table 2 (continued) Inflammatory response over time and by beverage.

Data are presented as mean ± SD or median (interquartile range).

<table>
<thead>
<tr>
<th></th>
<th>Before exercise (M1)</th>
<th>Immediately after exercise (M2)</th>
<th>2 h after exercise (M3)</th>
<th>24 h after exercise (M4)</th>
<th>48 h after exercise (M5)</th>
<th>Total</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lymphocytes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>(10³·µL⁻¹; 94 obs)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MS</strong></td>
<td>2.37 ± 0.49</td>
<td>2.31 ± 0.77</td>
<td>1.76 ± 0.38</td>
<td>2.22 ± 0.38</td>
<td>2.12 ± 0.46</td>
<td>2.17 ± 0.53</td>
<td>T: P &lt; 0.001</td>
</tr>
<tr>
<td><strong>CS</strong></td>
<td>2.48 ± 0.67</td>
<td>2.50 ± 0.85</td>
<td>1.74 ± 0.43</td>
<td>2.03 ± 0.47</td>
<td>2.14 ± 0.39</td>
<td>2.18 ± 0.64</td>
<td>B: P = 0.800</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>2.43 ± 0.57</td>
<td>2.42 ± 0.80</td>
<td>1.75 ± 0.40*</td>
<td>2.13 ± 0.42</td>
<td>N. A.</td>
<td>2.13 ± 0.42</td>
<td>T × B: P = 0.666</td>
</tr>
<tr>
<td>Total M3&lt;all the other moments (M1, M2: P &lt; 0.001; M4: P = 0.017; M5: P = 0.037)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| **IL-6** (pg·mL⁻¹; 59 obs) |                      |                                 |                         |                          |                          |       |       |
|----------------------------|----------------------|---------------------------------|-------------------------|                          |                          |       |       |
| **MS**                     | 1.15 ± 0.59          | 1.22 ± 0.65                     | N. A.                   | 1.09 ± 0.59              | N. A.                    | 1.15 ± 0.59 | T: P = 0.425 |
| **CS**                     | 1.00 ± 0.59          | 1.23 ± 0.53                     | N. A.                   | 1.19 ± 0.39              | N. A.                    | 1.14 ± 0.51 | B: P = 0.823 |
| **Total**                  | 1.08 ± 0.58          | 1.22 ± 0.57                     | N. A.                   | 1.14 ± 0.49              | N. A.                    | N. A.   | T × B: P = 0.558 |

| **TNF-α** (pg·mL⁻¹; 59 obs) |                      |                                 |                         |                          |                          |       |       |
|-----------------------------|----------------------|---------------------------------|-------------------------|                          |                          |       |       |
| **MS**                     | 0.85 ± 0.27          | 0.89 ± 0.33                     | N. A.                   | 0.87 ± 0.24              | N. A.                    | 0.87 ± 0.27 | T: P = 0.561 |
| **CS**                     | 1.04 ± 0.34          | 1.05 ± 0.31                     | N. A.                   | 0.87 ± 0.38              | N. A.                    | 0.99 ± 0.34 | B: P = 0.166 |
| **Total**                  | 0.94 ± 0.32          | 0.98 ± 0.32                     | N. A.                   | 0.87 ± 0.31              | N. A.                    | N. A.   | T × B: P = 0.618 |

| **CPR** (mg·L⁻¹; 99 obs)   |                      |                                 |                         |                          |                          |       |       |
|---------------------------|----------------------|---------------------------------|-------------------------|                          |                          |       |       |
| **MS**                    | 0.57 (2.39)          | 0.66 (2.27)                     | 0.56 (1.86)             | 0.75 (1.57)              | 0.70 (1.13)              | 0.66 (1.51) | T: P = 0.157 |
| **CS**                    | 0.48 (0.48)          | 0.53 (0.49)                     | 0.52 (0.48)             | 0.78 (0.62)              | 0.81 (2.45)              | 0.54 (0.52) | B: P < 0.001 |
| **Total**                 | 0.53 (0.61)          | 0.55 (0.55)                     | 0.53 (0.53)             | 0.78 (0.99)              | 0.72 (0.97)              | N. A.    | T × B: P = 0.790 |

△PCR: P: T = 0.615; B = 0.941; T × B = 0.537

WBC, white blood cells; obs, observations; MS, homemade milkshake; CS, commercial supplement; N.A., not applicable; T, time main-effect; B, beverage main-effect; T × B, time × beverage interaction-effect; IL-6, interleukin-6; TNF-α, tumour necrosis factor-alpha; CRP, C-reactive protein.

* Significant difference from baseline (P < 0.05)
Table 3 Oxidative stress biomarkers over time and by beverage. Data are presented as mean ± SD or median (interquartile range).

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Before exercise (M1)</th>
<th>Immediately after exercise (M2)</th>
<th>2 h after exercise (M3)</th>
<th>24 h after exercise (M4)</th>
<th>48 h after exercise (M5)</th>
<th>Total</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAS (mmol·L⁻¹; 99 obs)</td>
<td>MS 1.70 ± 0.10</td>
<td>1.75 ± 0.10</td>
<td>1.72 ± 0.10</td>
<td>1.78 ± 0.34</td>
<td>1.62 ± 0.12</td>
<td>1.71 ± 0.18</td>
<td>T: P = 0.061</td>
</tr>
<tr>
<td></td>
<td>CS 1.70 ± 0.09</td>
<td>1.78 ± 0.09</td>
<td>1.73 ± 0.08</td>
<td>1.68 ± 0.08</td>
<td>1.68 ± 0.10</td>
<td>1.71 ± 0.09</td>
<td>B: P = 0.968</td>
</tr>
<tr>
<td></td>
<td>Total 1.70 ± 0.10</td>
<td>1.76 ± 0.09</td>
<td>1.72 ± 0.09</td>
<td>1.73 ± 0.25</td>
<td>1.65 ± 0.11</td>
<td>N. A.</td>
<td>T × B: P = 0.344</td>
</tr>
<tr>
<td>PC (nmol·mg⁻¹; 100 obs)</td>
<td>MS 8.06 (2.81)</td>
<td>8.19 (6.58)</td>
<td>9.03 (6.95)</td>
<td>8.79 (4.69)</td>
<td>9.31 (9.54)</td>
<td>8.51 (4.99)</td>
<td>T: P = 0.941</td>
</tr>
<tr>
<td></td>
<td>CS 10.31 (7.42)</td>
<td>7.98 (3.40)</td>
<td>7.17 (5.32)</td>
<td>8.78 (4.06)</td>
<td>7.54 (2.68)</td>
<td>7.83 (3.99)</td>
<td>B: P = 0.181</td>
</tr>
<tr>
<td></td>
<td>Total 8.28 (5.94)</td>
<td>7.98 (4.08)</td>
<td>8.78 (5.29)</td>
<td>8.78 (4.14)</td>
<td>7.54 (4.46)</td>
<td>N. A.</td>
<td>T × B: P = 0.280</td>
</tr>
<tr>
<td>Uric acid (mg·dL⁻¹; 99 obs)</td>
<td>MS 5.62 ± 0.77</td>
<td>6.17 ± 0.89</td>
<td>5.79 ± 0.98</td>
<td>5.47 ± 0.81</td>
<td>5.42 ± 0.63</td>
<td>5.68 ± 0.83</td>
<td>T: P = 0.031</td>
</tr>
<tr>
<td></td>
<td>CS 5.83 ± 0.80</td>
<td>6.18 ± 0.93</td>
<td>6.24 ± 0.86</td>
<td>6.01 ± 0.99</td>
<td>5.98 ± 1.48</td>
<td>6.05 ± 1.01</td>
<td>B: P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Total 5.73 ± 0.77</td>
<td>6.17 ± 0.89</td>
<td>6.02 ± 0.92</td>
<td>5.74 ± 0.92</td>
<td>5.70 ± 1.14</td>
<td>N. A.</td>
<td>T × B: P = 0.571</td>
</tr>
</tbody>
</table>

No pairwise comparisons. Δ uric acid: T: P = 0.044 (no pairwise comparisons); B = 0.093; T × B = 0.560

| Glutathione reductase (U·L⁻¹; 99 obs) | MS 52.3 ± 6.5   | 56.4 ± 8.7 | 53.3 ± 6.2 | 53.3 ± 6.3 | 49.5 ± 5.7 | 53.0 ± 6.8 | T: P < 0.001 |
|                                      | CS 51.3 ± 7.0   | 55.1 ± 8.0 | 51.7 ± 8.1 | 51.1 ± 7.20 | 50.5 ± 7.8 | 51.9 ± 7.5 | B: P = 0.150 |
| Total                                 | 51.8 ± 6.6     | 55.7 ± 8.1* | 52.5 ± 7.1 | 52.2 ± 6.7 | 50.0 ± 6.7 | N. A.     | T × B: P = 0.674 |

Total M2>M1 (P = 0.007), M4 (P = 0.022), and M5 (P < 0.001)

TAS, total antioxidant status; obs, observations; MS, homemade milkshake; CS, commercial supplement; N.A., not applicable; T, time main-effect; B, beverage main-effect; T × B, time × beverage interaction-effect; PC, protein carbonyls.

* Significant difference from baseline (P < 0.05)
DISCUSSION

The aim of the present study was to evaluate if the nature (homemade vs. commercial) of a recovery beverage influenced the recovery pattern in terms of inflammatory and oxidative stress responses after an exhaustive exercise protocol. Our findings suggest that nutrient provenance seems to be indifferent in terms of the impact on inflammatory and oxidative stress recovery on highly trained male athletes. Briefly, independently of the beverage, the exercise protocol induced a significantly increase of WBC and neutrophils, and a significantly decrease of lymphocytes and eosinophils, 2 h after exercise. Moreover, the glutathione reductase peaked immediately after exercise.

Studies (Malm et al., 1999; Pizza et al., 1995) evaluating the leukocyte and neutrophil response to eccentric exercise have shown significant increases in circulation few hours after exercise, but not at 24 h and 48 h. This rapid, but short-lived, increment in circulating neutrophils likely precedes infiltration into the damaged muscle tissue (Tiidus, 2008). Carbohydrates attenuate the immune response after exercise (Pendersen & Toft, 2000). Particularly, the ingestion of CHO before, during, and after endurance exercise (Henson et al., 2000) attenuates blood cell counts for WBC, neutrophils, and monocyte. This kinetics probably reflects a decrease in physiological stress due to increased CHO availability (Carlson et al., 2008), leading to a lessened cortisol, growth hormone (Nieman, 1998), and adrenaline response (McAnulty et al., 2007). Regarding resistance exercise, however, few studies have been conducted. In one study (Nieman et al., 2004), the rise of WBC and neutrophils, but not monocytes and lymphocytes, were attenuated with CHO ingestion, whereas other (Koch et al., 2001) reported no treatment × time interactions for any of the cells. In our study, monocyte and basophil counts did not differ through time. Although this was expected for basophils, monocytes generally have a similar response to acute resistance exercise as neutrophils, peaking up to 2 h after exercise (Freidenreich & Volek, 2012). Similarly to our findings, Niemen and collaborators (Nieman et al., 2004) observed that monocytes (measured before,
immediately after, and 1 h after exercise) did not change over time, whereas, Malm and colleagues (Malm et al., 1999) observed an elevation in circulating monocytes 6 h after eccentric exercise. In our study, the maintenance of monocytes counts might have been a consequence of CHO intake or/and the monocytes' peak occurred between the analysed time-points. The observed delayed lymphopenia and eosinopenia after resistance exercise has also been reported by others (Koch et al., 2001; Nieman et al., 1995). The decrement in these leucocytes subpopulations might be a result of the exercise-induce increase in cortisol levels (Pedersen et al., 1997). Moreover, the observed lymphopenia after exercise have also been attributed to the elimination through apoptosis, migration from circulation, or a combination of both (Pereira et al., 2012).

Our results regarding CRP, IL-6, and TNF-α suggest a lack of inflammatory systemic response, known as acute-phase immune response (Simpson et al., 2005). In our study, although an inflammatory response was observed regarding leukocytes, CRP did not statistically change over time. Other study (Bowtell et al., 2011), also using a single-leg exercise protocol, came to a similar result regarding CRP. Taken together, these data may suggest that the chosen exercise protocol does not seem sufficient to elevate CRP. CRP is a marker of systemic inflammation, and a single-leg exercise may only allow a transient cytokine release (Pedersen & Hoffman-Goetz, 2000). Moreover, in both studies participants were athletes, and biomarkers of systemic inflammation are reduced in physically active individuals (Gleeson, 2007). Other possible explanation is an attenuation of inflammation. Kerasioti and collaborators (Kerasioti et al., 2013) tested the effect of ingesting a special cake with similar CHO (0.9 g·kg⁻¹·h⁻¹) and protein (0.26 g·kg⁻¹·h⁻¹) content to our study, or a placebo cake (1.1 g CHO·kg⁻¹·h⁻¹ and 0.1g protein·kg⁻¹·h⁻¹) after 2 h of cycling at 60–65% VO₂max. The CRP increment 4 h after exercise was attenuated by 46% in the experimental trial compared to placebo. The authors proposed that the low levels of IL-6 (lower than placebo) also seen at this time point, might be responsible for the CRP drop. The lower IL-6 levels were
suggested to be due to the protein content. The whey protein present in the special cake and in both of our beverages has anti-inflammatory properties due to lactoferrin. Lactoferrin may inhibit the production of several pro-inflammatory cytokines, namely IL-6, TNF-α, and IL-1β, and/or increase anti-inflammatory cytokines, including IL-10 (Ward et al., 2005). In fact, in our study, we did not observe changes in IL-6 and TNF-α, although eccentric contractions lead to greater IL-6 increment than concentric ones (Bruunsgaard et al., 1997). Additionally, CHO ingestion influences cytokine levels, attenuating their production during and after acute exercise (Braun & Von Duvillard, 2004). Nevertheless, type, duration, and intensity of the exercise, muscle mass recruited and damaged, subjects’ training status, blood sampling time, and assays sensitivity, may influence the response pattern of cytokines, specially IL-6 (Pedersen & Hoffman-Goetz, 2000; Petersen & Pedersen, 2005; Teixeira et al., 2009).

In our study, we believe that PC did not statistically change over time because the oxidative stimulus during recovery might be reduced. The intake of antioxidant-rich foods, such as berries, has been shown to reduce EIMD (Bowtell et al., 2011; McLeay et al., 2012). However, in one study (Howatson et al., 2010), where participants performed a marathon after consuming cherry juice or placebo for 5 days, the day of the race, and for 48 h after the exercise, PC did not change over time in both groups, despite the signs of muscle damage. Additionally, whey protein, present in both of our beverages, has antioxidative capacities due to cysteine, a precursor of thiol-containing antioxidants, as reduced glutathione (GSH) and α-lipoic acid (Elia et al., 2006). Moreover, we cannot despise the fact that training is believed to increase antioxidant defences (Packer, 1997), and that our participants were national-level athletes. Therefore, it is possible that the antioxidant defence mechanisms have efficiently counteracted the exercise-induced oxidative onset. Indeed, the blood glutathione reductase, an antioxidant marker, peaked immediately after exercise. This suggests an activation of the antioxidant system (Packer, 1997) probably due to ROS production during exercise. The glutathione reductase is
the enzyme that recycles oxidized glutathione (GSSG) back to GSH, one of the major antioxidants in the body (Reid & Durham, 2002). In the presence of oxidative stress, namely after exhaustive physical exercise, the GSH:GSSG ratio decreases (Finaud et al., 2006) and the activity of glutathione reductase increases due to the physiological need to keep glutathione in the reduced form (Vollaard et al., 2005).

Even though no pairwise comparisons were detected for uric acid between time moments, there was a tendency for higher concentrations immediately after exercise, which is consistent with the literature (Howatson et al., 2010; Teixeira et al., 2013). This increased post-exercise concentration probably results from an activation of the purine catabolic pathways leading to uric acid (Sutton et al., 1980). The uric acid has been historically investigated and analysed as the principal antioxidant in human plasma (Banfi et al., 2012). Indeed, in our study there was a positive correlation \( r = 0.29, P = 0.003 \) between uric acid and TAS. Recently, another approach to the uric acid exercise-induced kinetic has arisen, suggesting that its elevations after exercise may reflect the inflammatory response to exercise (Howatson et al., 2010). In fact, we also detected a correlation between white blood cells count and uric acid \( r = 0.36, P < 0.001 \), which is stronger than the uric acid \( \times \) TAS correlation.

In our study, TAS did not change with exercise, resembling other study findings (Bowtell et al., 2011). TAS assay measures the antioxidant capacity in the hydrophilic compartment of the plasma, relying mostly on protein (28%), uric acid (19%), and ascorbic acid (3%) (Cao & Prior, 1998). Taking into account that protein (data not shown) and uric acid presented the highest values immediately after exercise, that a higher antioxidant activity seemed to occur at this time point, and that TAS did not statistically change over time, we might assume that there was a drop in ascorbic acid levels immediately after exercise, balancing TAS values (Teixeira et al., 2013). Furthermore, blood sampling timing could have influenced this result, since significant decrements were reported in TAS levels 5 min (but increments at 20 min) after cycling until exhaustion (Steinberg et al., 2006).
In summary, the observed kinetics of the analysed markers of inflammation and oxidative stress were consistent with the occurrence of muscle damage, lack of acute-phase response, and possible attenuation of oxidative stress by the ingested beverages. Since the main aim of the present study was to compare the impact of the beverages nature on the recovery of muscle damage, a control group was not added, precluding the confirmation of the hypothesis of beverage-induced oxidative stress attenuation. Regarding the differences between beverages, the MS trial showed significantly higher levels of CRP, and lower levels of uric acid. However, these effects lost statistical significance when the variables were evaluated as changes from baseline, suggesting small intra-individual differences in pre-exercise values rather than differences in beverages recovery efficiency. Indeed, no significant differences were found for these 2 parameters at the beginning of trials ($P \geq 0.254$). Therefore, we can conclude that the nature of the beverage, commercial or homemade, is indifferent in terms of the impact on inflammation and oxidative stress during recovery from EIMD. This information is of tremendous value for athletes and sports nutritionists. We demonstrated that food can be a reliable alternative to supplements in terms of exercise recovery. Therefore, professionals can suggest food as a scientifically proven alternative, with the advantages of being cheaper and safer in terms of inadvertent doping.

ACKNOWLEDGEMENTS

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REFERENCES


3.7 Study VII

Compared metabolic recovery between commercial and homemade beverages with isoglucidic and isoproteic content after eccentric exercise until exhaustion

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6 Faculdade de Ciências da Nutrição e Alimentação [Faculty of Nutrition and Food Sciences], Universidade do Porto, Porto, Portugal
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ABSTRACT

Background: Changes in metabolic parameters after exercise-induced muscle damage (EIMD) are seldom investigated, with inconsistent results reported. The combined intake of carbohydrate (CHO) and proteins have demonstrated positive impact on recovery. However, the compared effects of food with an equivalent nutritional supplement have been unmindful.

Purpose: To compare the effect of 2 isoglucidic and isoproteic recovery beverages [commercial (CS) vs. homemade (MS)] on metabolic parameters after exercise.

Methods: Ten male athletes (21.7 ± 3.4 yrs, 73.3 ± 4.5 kg) performed 2 trials of an exhaustion protocol comprising a minimum of 300 concentric/eccentric knee extension/flexion bouts. During the 2 h after the protocol, participants ingested 0.8 g CHO·kg⁻¹·h⁻¹ and 0.26 g protein·kg⁻¹·h⁻¹ in form of CS or MS (skimmed milk, strawberries, and banana). Blood samples were taken before exercise (M1), immediately after (M2), 2 h (M3), 24 h (M4), and 48 h (M5) after exercise.

Results: A main-effect for time (P < 0.05) was detected for glucose (M3<M2, M4–M5), total proteins (M2>M1–M5), non-esterified fatty acids (M2>M3–M5; M3<M1–M2, M4), β-hydroxybutyrate (M5<M1, M2), total cholesterol and low-density lipoprotein (M2>M4–M5; M1>M5), high-density lipoprotein (M2>M4–M5), and urea (no pairwise comparisons). Although beverage-main effects were observed for triglycerides, LDL, and urea, and a time × beverage effect for β-hydroxybutyrate (P < 0.05), significances were lost when evaluated as changes from baseline. No effects (P > 0.05) were detected for cardiac-specific troponin I, creatinine, and alkaline phosphatase.

Conclusion: Both beverages seemed equally efficient to recover from EIMD regarding the metabolic response.

Keywords: Food, nutritional supplements, eccentric exercise, muscle damage, metabolism.
INTRODUCTION

The study of biochemistry related to exercise-induced muscle damage (EIMD) has been more directed towards the analysis of muscle damage parameters, such as creatine kinase and myoglobin, or inflammatory markers, namely counts of white blood cells and cytokines (Clarkson & Hubal, 2002). Changes in metabolic parameters after damaging exercise, although less mentioned, have been described for decades (Kratz et al., 2002). The main concern regarding the influence of nutrition on metabolic parameters after exercise has been the study of plasma insulin responses to different combinations of carbohydrates (CHO) and proteins, although not necessarily after EIMD (Van Loon, Saris, Verhagen, et al., 2000). Recently, more focus has been placed on the variations of glucose uptake, insulin sensitivity (Tee et al., 2007), and blood lipid profile (Nikolaidis et al., 2008), but concerning the changes caused by the damaging exercise itself. In fact, little has been investigated concerning the metabolic impact of a typical EIMD recovery meal.

Nutrition can have an important role in attenuating the deleterious consequences of muscle damaging exercises (Howatson & van Someren, 2008). Particularly, the combined intake of CHO and proteins has been suggested as a possible recovery strategy to tackle the EIMD process (Sousa et al., 2014). Briefly, proteins are believed to increase amino acids availability (increasing protein synthesis), which is necessary to muscle repair and adaptation, whereas CHO may contribute to an optimal hormonal environment due to insulin increment (decreasing protein breakdown) (Cockburn et al., 2008). Additionally, CHO content would also promote muscle glycogen re-synthesis (Jentjens & Jeukendrup, 2003), improving CHO availability for the next exercise session. Moreover, CHO intake would prevent low glycogen levels that are believed to have a negative impact on muscle protein synthesis (Creer et al., 2005), and to promote muscle protein breakdown (Lemon & Mullin, 1980).
The study of nutritional interventions aimed to mitigate the muscle damage process has been a topic of interest in the past few years, but the majority of studies tended to use nutritional supplements to investigate this purpose. This may be due to the fact that supplements are easier to manipulate, and more ready to use than combinations of foods. Nevertheless, some recent works (Bowtell et al., 2011; Cockburn et al., 2013) investigated the effect of food compared to placebo in the attenuation of EIMD, globally with positive results. However, in real-life, when it comes to the recovery meal, athletes need to choose conscientiously between a supplement and food (not between a supplement/food and placebo). However, a direct comparison between isoglucidic and isoproteic strategies, only differing in the nature (commercial vs. homemade), has never been studied. Therefore, the aim of the present study was to describe and compare the effects of ingesting a commercial carbohydrate and protein supplement (CS) or a homemade milkshake with skimmed milk, strawberries and banana (MS), with similar CHO and protein content, on metabolic parameters after an eccentric protocol until exhaustion.

METHODS

Participants

Thirteen adult male national-level track-and-field athletes (8 jumpers, 2 throwers and 3 sprinters) volunteered to participate in this study. The study was approved by the Ethics Commission of the University of Porto, was conducted in compliance with the World Medical Association’s Declaration of Helsinki (2008), and is registered at ClinicalTrials.gov (NCT01555775). All participants were informed verbally and in writing regarding the experimental procedures before giving their written informed consent.
Experimental design

This study had a single-blind, randomized, crossover experimental design. Athletes completed 2 trials separated by, at least, 2 weeks. In each occasion, the participants arrived at the laboratory after an overnight fast (≥10 h) and rested in a seated position while a blood sample was taken from an antecubital vein (M1). Then, they completed a 5-min warm-up on a cycle ergometer, with intensity ranging 70–100 rpm. After that, participants were correctly positioned and strapped in the test chair of the isokinetic dynamometer (Biodex® system IV, Biodex Medical Systems Inc., Shirley, NY, USA), and the range of motion was set at 60° [from 50° to 110° knee flexion (0° = full knee extension)]. They initially performed 8 sub-maximal repetitions at 60°·s⁻¹ to be familiar with the movement and, after resting for 60 s, completed 3 maximal repetitions of concentric/eccentric knee joint extension/flexion at a constant angular velocity of 60°·s⁻¹ to determine the subject’s maximal PTq, defined as the best of the 3 repetitions. After this, an exhaustion protocol was conducted, consisting of 3 bouts of a concentric/eccentric knee extension/flexion exercise at 60°·s⁻¹ with a 200-s rest time between sets. The first and second sets were composed by 100 repetitions and subjects performed 100 + n repetitions until exhaustion in the third set. Participants were considered exhausted when the torque of 3 consecutive repetitions fell below 25% of the initial PTq value (Rozzi et al., 1999). Participants received verbal encouragement to perform maximally in each occasion. Different lower limbs were tested for each trial in a random way to minimize the repeated bout effect (Howatson & van Someren, 2007). After exhaustion (M2), a second blood sample was taken. During the first 2 h after the end of the exhaustion protocol the participants drank the CS or MS. Beverages were randomized and the treatment was blinded to the participants. Two hours after the end of the exhaustion protocol (M3), a third blood sample was collected. Participants returned to the laboratory 24 h (M4) and 48 h (M5) after the end of the protocol, in a fasted state (≥10 h), to get a resting blood sample collected from an antecubital vein on each occasion.
The two beverages had similar CHO and protein content. Firstly, the amount of the commercial supplement in a powder form (Supreme Gainers®, GoldNutrition, Econutraceuticos, Alcabideche, Portugal) was determined to guarantee 0.8 g CHO·kg⁻¹·h⁻¹ and 0.26 g protein·kg⁻¹·h⁻¹. Then, a milkshake was formulated, with specific amounts of foods, to achieve a similar amount of CHO and protein. The Portuguese Food Composition Table (Martins et al., 2007) was used to get the nutritional content of foods. The MS included 100 g of strawberry, and variable amounts of banana (170 ± 99 g) and skimmed milk (443 ± 114 g) to reach the CHO and protein targets. Finally, bottled water (Caldas de Penacova®, Água das Caldas de Penacova, Penacova, Portugal) and vitamin C (Cebiolon®, Merck, Merck and Company, Inc., Whitehouse Station, NJ, USA) were added to the CS to match the MS content (620 ± 35 g and 60.8 ± 1.0 mg, respectively). The final beverages composition is displayed on Table 1. The beverages were individually made for each participant and placed in black opaque containers. The total volume supplied during each hour was separated into two equal-volume drinks. Each participant received his respective container in every 30 min during the 2 h after the exhaustion protocol. Both beverages had strawberry flavour.

Table 1 Beverages comparison (delivered per hour) for mean subject weight of 73.3 kg.

<table>
<thead>
<tr>
<th></th>
<th>Homemade milkshake (MS)</th>
<th>Commercial supplement (CS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>327</td>
<td>328</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>18.9</td>
<td>18.8</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>58.6</td>
<td>58.6</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>1.9</td>
<td>2.0</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>60.8</td>
<td>61.5</td>
</tr>
<tr>
<td>α-tocopherol (mg)</td>
<td>0.6</td>
<td>8.0</td>
</tr>
</tbody>
</table>
Dietary intake and physical activity

Participants were instructed to fulfil a food record and a physical activity record for the 2 days prior and during the 3 days of each trial. Athletes were also asked not to make drastic changes in their diet, and to avoid strenuous exercise during the study period, in order to ensure similar metabolic conditions between trials. Dietary records’ information was transformed into energy and nutrients using Food Processor SQL® (ESHA Research Inc., Salem, OR, USA). The athletes’ daily energy expenditure was calculated using metabolic equivalent values by task and intensity level, for the main physical activity in each 15 min, after adjustment for the estimate resting metabolic rate predicted from the Harris-Benedict equation (Ainsworth et al., 2011).

Body composition

Body weight, height, and body fat percentage (%BF) were assessed in the week before the first trial. Weight was recorded to the nearest 0.1 kg using In-Body® 230 (Biospace, Korea), and height was measured to the nearest 0.5 cm with a Jofre® stadiometer (Braga, Portugal). The %BF was calculated using Evans 3-sites equation formula (Evans et al., 2005); each skinfold thickness (triceps, abdominal and front thigh) was taken twice at the nearest 0.1 mm using a Cescorf® calliper (Porto Alegre, Brazil), and the mean value used in the equation. All measures were taken according to the International Society for the Advancement of Kinanthropometry (ISAK).

Biochemical analysis

On each of the blood extraction moments, two venous blood samples were collected from each athlete, in a seated position. One sample was collected to a tube from Venosafe® (Terumo Europe, Leuven, Belgium)
containing tripotassium ethylenediaminetetraacetic acid (EDTA). A complete blood count was obtained using an automated blood counter Sysmex XE-5000® (Sysmex Europe GMBH, Norderstedt, Germany). The second sample was collected to a serum separator tube from Venosafe® containing an additive of gel and clot activator. Blood was allowed to clot for 30 min and then centrifuged at 4500 r.p.m. during 15 min. In this serum fraction, the analytes creatine kinase (CK), glucose, triglycerides, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), non-esterified fatty acids (NEFA), β-hydroxybutyrate, total proteins, urea, creatinine, and alkaline phosphatase, were measured using an automated clinical chemistry analyser (Olympus AU5400®, Olympus, Hamburg, Germany). Cardiac-specific troponin I (cTnI) was measured by a chemiluminescent immunoassays using Architect i2000® automated analyser (Abbott Diagnostics, Lake Forest, IL, USA). The remaining serum was collected, aliquoted and stored at -80°C to further measurements.

Taking into account that no significant plasma volume changes were detected between absolute and adjusted values (Dill & Costill, 1974) on M2 for haematocrit \((P = 0.167)\), haemoglobin \((P = 0.159)\), red blood cells \((P = 0.158)\), and white blood cells \((P = 0.145)\), absolute data were used for statistical analysis (Kargotich et al., 1998). To identify if any participant were already in a muscle damage process at the beginning of the trials, the reference value of 1083 U·L\(^{-1}\) (Mougios, 2007) for CK was used as a cut-off.

**Statistical procedures**

Descriptive data were reported as means ± standard deviations for normal variables, or as medians (minimum–maximum), when variables were not normally distributed. Skewedness and kurtosis were used to evaluate normality. Paired-sample T-test was used to evaluate differences in haemoconcentration between absolute and adjusted values and \(n\) repetitions between trials. Non-
normal distributed variables (β-hydroxybutyrate, cTnI) were logarithmically transformed to attain normal distribution. Linear mixed models were used to analyze the differences in the studied parameters over time and between beverages. In the model, beverage, time, time × beverage, treatment sequence, and lower-limb sequence were treated as fixed effect variables and participant as a random effect variable. Treatment and leg sequences were counter-balanced and, thereafter, randomized by random number generation before the first trial. Nutritional and physical activity data were analysed by two-way repeated-measures ANOVA to determine if these conditions were similar between trials. The Mauchly sphericity test was used to check homogeneity of covariance; violations of the assumption of sphericity were dealt using Greenhouse-Geisser adjustment. All statistical procedures were performed using the Statistical Package for Social Sciences® version 20 (SPSS Inc., Chicago, IL, USA). The level of significance was set at α = 0.05.

RESULTS

The final sample comprised 10 athletes (21.7 ± 3.4 yrs, 73.3 ± 4.5 kg, 178 ± 5 cm, 8.4 ± 2.1 %BF); 3 were excluded due to CK activity >1083 U·L⁻¹ at the beginning of one of the trials. Participants reported similar (P > 0.05) nutritional intake (energy, protein, CHO, fat, and vitamin C) and energy expenditure, and performed similar n repetitions (P = 0.326) in both trials.

Glucose (100 observations) significantly changed over time (P = 0.004), presenting the lowest values 2 h after exercise (M3<M2, M4 and M5; P < 0.05; Figure 1A). Neither a beverage main-effect (P = 0.431), nor a time × beverage interaction-effect (P = 0.443) were observed.

Total proteins (99 observations) significantly changed over time (P < 0.001), increasing acutely immediately after exercise (M2>M all the remaining
moments; \( P < 0.01 \); Figure 1B). No significant beverage main-effect \((P = 0.197)\) and time × beverage interaction-effect \((P = 0.445)\) were detected.

NEFA (100 observations) also presented a time main-effect \((P < 0.001)\) with the highest values being detected immediately after the exhaustion protocol \((M2>M3, M4 and M5; P < 0.01; \) Figure 1C), decreasing to the lowest 2 h after exercise \((M3<M1, M2 and M4; \) \( P < 0.05)\). Neither a beverage main-effect \((P = 0.718)\), nor an interaction-effect \((P = 0.971)\) were observed.

A time main-effect was observed for \(\beta\)-hydroxybutyrate (100 observations; \( P = 0.002\)), with the lowest values being detected in the last measurement, 48 h after exercise \((M5<M1 and M2; P < 0.05; \) Figure 1D). There was no significant beverage main-effect \((P = 0.463)\), but a time × beverage interaction-effect \((P = 0.038)\) was detected, with MS presenting significantly higher levels on M3 \((P = 0.049)\) and lower levels on M5 \((P = 0.041)\) compared to CS. This significant result disappeared when \(\beta\)-hydroxybutyrate was evaluated as changes from pre-exercise values \((P = 0.158)\).

For triglycerides (99 observations; Figure 2A), no time main-effect of time \((P = 0.524)\) or time × beverage interaction-effect \((P = 0.849)\) were detected, but there was a main-effect of beverage \((P = 0.003)\), with MS trial showing lower values \((75.8 \pm 25.8 \text{ mg\cdotdL}^{-1}\) than the CS one \((84.9 \pm 25.6 \text{ mg\cdotdL}^{-1}\)). No statistical significant differences were found in terms of beverage (no significant beverage main-effect, \( P = 0.671\)) when considering the changes from pre-exercise values.

Regarding the total cholesterol (99 observations), LDL (99 observations), and HDL (99 observations), all of them changed over time \((P < 0.01)\) being lower 24 h and 48 h after exercise, compared to the results obtained immediately after the exercise protocol \((M4 and M5<M2; P < 0.05; \) Figure 2B, C, D). Moreover, total cholesterol and LDL, were lower at the end of the study, compared to pre-exercise levels \((M5<M1; P < 0.05)\). A beverage main-effect
was only detected for LDL ($P = 0.033$), with MS trial showing higher values (121 ± 26 mg·dL$^{-1}$) than the CS one (117 ± 30 mg·dL$^{-1}$). However, when this variable was evaluated as changes from pre-exercise values, there was no significant beverage main-effect ($P = 0.058$). No significant time × beverage interaction-effect ($P > 0.686$) was detected for any of the three cholesterol biomarkers.

For creatinine, no main-effects or interaction-effect ($P > 0.05$) were detected (Table 2). Urea showed a time main-effect ($P = 0.040$), although without pairwise comparisons, and a beverage main-effect ($P < 0.001$), where the MS trial presented higher results than the CS one. However, both statistically significant main-effects are lost when this variable was analysed as changes from pre-exercise values ($P = 0.057$ and $P = 0.207$, respectively). For cTnI and alkaline phosphatase, no main- or interaction effects were observed ($P > 0.081$).
**Figure 1** Changes in glucose (A), total proteins (B), non-esterified fatty acids (C), and β-hydroxybutyrate (D) over time and depending on the nature of the recovery beverage.

Data are presented as mean + or - SD (A, B, C) or median (D).

MS, homemade milkshake; CS, commercial supplement; NEFA, non-esterified fatty acids; M1, before exercise; M2, immediately after exercise; M3, 2 h after exercise; M4, 24 h after exercise; M5, 48 h after exercise.

# Significant time main-effect ($P < 0.01$); * significant difference from baseline ($P < 0.05$); † significant time × beverage interaction effect ($P = 0.038$); ‡ significant difference between beverages ($P < 0.05$).
Figure 2 Changes in triglycerides (A), total cholesterol (B), low-density lipoprotein (C), and high-density lipoprotein (D) over time and depending on the nature of the recovery beverage. Data are presented as mean + or - SD.

MS, homemade milkshake; CS, commercial supplement; LDL, low-density lipoprotein; HDL, high-density lipoprotein; M1, before exercise; M2, immediately after exercise; M3, 2 h after exercise; M4, 24 h after exercise; M5, 48 h after exercise.

§ Significant beverage main-effect ($P < 0.05$); # significant time main-effect ($P < 0.01$); * significant difference from baseline ($P < 0.05$).
Table 2 Changes in kidney, heart, and liver biomarkers over time and depending on the nature of the recovery beverage. Data are presented as mean ± SD or median (minimum–maximum).

<table>
<thead>
<tr>
<th></th>
<th>Before exercise (M1)</th>
<th>Immediately after exercise (M2)</th>
<th>2 h after exercise (M3)</th>
<th>24 h after exercise (M4)</th>
<th>48 h after exercise (M5)</th>
<th>Total</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urea (mg·dL(^{-1}); 100 obs)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>34.3 ± 7.8</td>
<td>34.4 ± 7.9</td>
<td>37.6 ± 7.8</td>
<td>34.4 ± 5.7</td>
<td>34.9 ± 5.8</td>
<td>35.1 ± 6.9</td>
<td>T: P = 0.040(^\dagger)</td>
</tr>
<tr>
<td>CS</td>
<td>30.9 ± 4.7</td>
<td>31.1 ± 4.7</td>
<td>33.8 ± 4.8</td>
<td>33.5 ± 4.0</td>
<td>33.1 ± 5.0</td>
<td>32.5 ± 4.6</td>
<td>B: P &lt; 0.001</td>
</tr>
<tr>
<td><strong>Creatinine (mg·dL(^{-1}); 99 obs)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>0.94 ± 0.10</td>
<td>0.92 ± 0.11</td>
<td>0.91 ± 0.10</td>
<td>0.93 ± 0.06</td>
<td>0.93 ± 0.07</td>
<td>0.93 ± 0.09</td>
<td>T: P = 0.289</td>
</tr>
<tr>
<td>CS</td>
<td>0.97 ± 0.05</td>
<td>0.97 ± 0.10</td>
<td>0.92 ± 0.09</td>
<td>0.97 ± 0.07</td>
<td>0.92 ± 0.05</td>
<td>0.95 ± 0.07</td>
<td>B: P = 0.096</td>
</tr>
<tr>
<td><strong>cTnI (ng·mL(^{-1}); 99 obs)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>0.001 (0–0.006)</td>
<td>0 (0–0.006)</td>
<td>0 (0–0.012)</td>
<td>0 (0–0.011)</td>
<td>0 (0–0.008)</td>
<td>0 (0–0.012)</td>
<td>T: P = 0.410</td>
</tr>
<tr>
<td>CS</td>
<td>0.001 (0–0.009)</td>
<td>0 (0–0.005)</td>
<td>0.0030 (0–0.005)</td>
<td>0.001 (0–0.008)</td>
<td>0.002 (0–0.006)</td>
<td>0.002 (0–0.009)</td>
<td>B: P = 0.081</td>
</tr>
<tr>
<td><strong>Alkaline phosphatase (U·L(^{-1}); 99 obs)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>58.9 ± 14.3</td>
<td>62.7 ± 17.9</td>
<td>57.6 ± 17.0</td>
<td>59.0 ± 16.9</td>
<td>56.3 ± 15.2</td>
<td>58.8 ± 15.7</td>
<td>T: P = 0.589</td>
</tr>
<tr>
<td>CS</td>
<td>60.3 ± 15.8</td>
<td>58.7 ± 15.8</td>
<td>57.3 ± 14.0</td>
<td>58.3 ± 14.7</td>
<td>59.1 ± 14.1</td>
<td>58.7 ± 14.3</td>
<td>B: P = 0.919</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>59.6 ± 14.7</td>
<td>60.6 ± 16.5</td>
<td>57.5 ± 15.2</td>
<td>58.7 ± 15.4</td>
<td>57.7 ± 14.3</td>
<td>N. A.</td>
<td>T × B: P = 0.669</td>
</tr>
</tbody>
</table>

obs, observations; MS, homemade milkshake; CS, commercial supplement; N.A., not applicable; cTnI, cardiac-specific troponin I.\(^\dagger\)No pairwise comparisons.
DISCUSSION

The main findings of the present study were that the nature (homemade vs. commercial) of the recovery beverage seems to be indifferent in terms of the impact on metabolic recovery. Since participants reported similar intake of energy, proteins, CHO, fat, and, vitamin C and similar energy expenditure in both trials, the beverages’ effect comparison was under similar conditions of dietary intake and physical activity.

Briefly, independently of the beverage, the protocol induced a significantly decrease in blood glucose levels 2 h after exercise, in β-hydroxybutyrate at 48 h after exercise, and in total cholesterol, LDL, and HDL, 24 h and 48 h after exercise. The protocol also led to an increase in proteins and NEFA immediately after exercise, with NEFA levels dropping 2 h after exercise. Regarding differences between beverages, the MS trial showed significantly lower levels of triglycerides, and higher levels of LDL, and urea. Moreover, for β-hydroxybutyrate, MS presented significantly higher levels 2 h after exercise and lower levels 48 h after exercise. However, these effects lost significance when the variables were analysed as changes from baseline, suggesting non-significant intra-individual differences in pre-exercise values rather than differences in beverages recovery efficiency. Indeed, no statistically significant differences were found for these 4 parameters at the beginning of trials ($P > 0.05$).

The blood glucose levels drop after the supplement feeding and 2 h after the end of exercise might have occurred as a result of glucose clearance into the muscle and liver. Both muscle contraction and insulin secretion are recognized stimulators of muscle glucose uptake, in an additive way (Tee et al., 2007). Increased insulin sensitivity of skeletal muscle glucose uptake can be observed after a single exercise session, facilitating the entrance of glucose to the cells through the glucose transporter-4 (GLUT-4) (Hansen et al., 1998). Additionally, exercise training induces muscle adaptations that may contribute
to increased insulin sensitivity, such as increased GLUT-4 content, improved insulin signalling, and increased blood flow (Jentjens & Jeukendrup, 2003). Taking into consideration that our participants were highly trained, the exercise-induced adaptations in favour of glucose uptake would be expected, contributing to the fall of serum glucose levels. Furthermore, the binding of insulin to its membrane receptors also induces GLUT-4 translocation (Tee et al., 2007), and the intake of CHO exercise has been shown to further increase GLUT-4 protein expression (Kuo et al., 1999). Additionally, certain amino acids (e.g. leucine) and proteins can synergistically affect insulin release when ingested in combination with CHO (Jentjens & Jeukendrup, 2003). In fact, the ingestion of a CHO:P solution (0.8 g CHO·kg⁻¹·h⁻¹ and 0.4 g protein·kg⁻¹·h⁻¹) after exercise resulted in a higher insulin response than CHO only (0.8 g·kg⁻¹·h⁻¹) (Van Loon, Saris, Kruijshoop, et al., 2000). Furthermore, the glucose storage in the liver might also contribute to the glucose decrease seen at M3. The greater postprandial insulin levels resultant from the CHO and protein co-ingestion could stimulate the storage of the ingested CHO in the more insulin-sensitive tissues such as liver and previously exercise skeletal muscle (Beelen et al., 2010). Nevertheless, it is believed that there is a rapid phase of glycogen synthesis immediately following exercise, which generally lasts 30–60 minutes and is independent of circulating insulin levels (Jentjens & Jeukendrup, 2003). During this phase, the high rate of glucose transport into the cell is believed to be mediated by (i) the translocation of the GLUT-4 to the sarcolemma, stimulated by muscle contraction, and low glycogen levels, and (ii) a higher glycogen synthase activity, stimulated by low muscle glycogen concentration (Beelen et al., 2010). The sample collected at M3 encompasses both this initial muscle glycogen synthesis phase, and the second phase that can be influenced by insulin levels (Beelen et al., 2010). Taken together, all these mechanisms might explain the lower levels of glucose at M3.

Moreover, the high post-prandial insulin levels makes glucose the preferable substrate and suppresses lipolysis, inhibiting NEFA release from adipose tissue (Frayn, 2003). NEFA are the form to transport fat from the body
stores to its utilization sites (Frayn et al., 1997). Additionally, after a mixed meal, the esterification of fatty acid within adipose tissue is stimulated, leading to a suppression of NEFA release from adipocytes (Frayn et al., 1997). These mechanisms might explain the low NEFA levels 2 h after the exhaustion protocol and after the beverages intake. It would be interesting to have the insulin changes throughout the 48 h. Nevertheless, no significant beverage-main effect or time × beverage interaction-effect was detected for NEFA and glucose, suggesting that both beverages, independently of their commercial or homemade nature, lead to similar recovery kinetics regarding these parameters.

With the exception of triglycerides, all lipid parameters presented the highest values immediately after exercise. In the present study, the participants were not fed before and during exercise; therefore, the blood CHO and fat changes immediately after exercise reflected only the exercise demands. During prolonged and severe physical activities, both glucose and lipids are used as fuel (Beelen et al., 2010). Indeed, our finds regarding the significant increase of NEFA, total cholesterol, LDL, and HDL immediately after exercise, reflect the use of fatty acids as fuel (Waśkiewicz et al., 2012). Furthermore, 2 h after the protocol, NEFA were lower than at baseline, and at 48 h after exercise, total cholesterol, LDL, and β-hydroxybutyrate, were lower compared to baseline. Additionally, 48 h after exercise β-hydroxybutyrate was also lower than immediately post-exercise levels. After exercise, fatty acids are also needed for the replenishment of muscle phospholipid and triglycerides stores, and regeneration of damaged muscle fibers (Nikolaidis et al., 2008). Moreover, and despite the marked inconsistency observed in blood lipids responsiveness to exercise, a reduction of total cholesterol and LDL is a common observed change (Leon & Sanchez, 2001). Indeed, another study (Nikolaidis et al., 2008) that used eccentric knee flexion exercise to induce muscle damage, also found a decrease in total cholesterol and LDL on the second day after de EIMD protocol. In addition, cholesterol is an essential component of biological membranes; along with phospholipids, it is the most abundant lipid on the membranes (Lars Bastiaanse et al., 1997). Therefore, the decrease in the
cholesterol fractions from the plasma might be a consequence of the cholesterol incorporation in the new/regenerated cell membranes.

The increase in total proteins after exercise has also been reported elsewhere (Gravina et al., 2011; Kratz et al., 2002). One possible explanation for this phenomenon is related to the dehydration caused by exercise (Kratz et al., 2002), leading to a decrease in plasma volume and, consequently, to an increase in protein concentration. However, in our study, no changes were detected for plasma volume after exercise, suggesting that other mechanisms might have caused this increment in serum proteins. Indeed, an elevated lymph flow, from contracting muscle into the vascular compartment, with high protein content, can contribute to the increased protein values observed after exercise, and also to the maintenance of plasma volume during long-term exercise (Convertino, 1987; Kratz et al., 2002).

The kidney, heart, and liver seemed to preserve their normal function in response to the applied protocol. Serum creatinine is the most widely accepted measure of renal function (Banfi et al., 2012). The common reference range for the general population and for athletes is <1.5 mg·dL\(^{-1}\) (Kratz et al., 2004). In our study, creatinine concentration did not differ over time, and all participants showed serum levels within the recommended range. Regarding urea, a time main-effect was observed, but no pairwise comparisons were detected, making unclear the changes over time. A beverage-main effect was also detected, but after correction for changes from baseline the results suggest for non-significant intra-individual differences in pre-exercise values rather than differences in beverages recovery response. Taken together, these results suggest maintenance of renal clearance throughout the protocol. The maintenance of low cTnI values over time, without differences between beverages, excludes the occurrence of myocardium injury in these athletes. All the measurements remained <0.02 ng·mL\(^{-1}\), well below the upper limit of the normal range (0.4 ng·mL\(^{-1}\)) (Kratz et al., 2004). Our results support the findings of other studies (Siegel et al., 2001; Smith et al., 2004), in which cTnI did not raise with
strenuous exercise. Hepatic injury could also be excluded since alkaline phosphatase values were maintained over time, similar to what was seen elsewhere (Gravina et al., 2011). Alike to that verified for cTnI, none of the participants at any time point presented alkaline phosphatase levels above the upper reference value of 120 U·L\(^{-1}\) (Kratz et al., 2004). Alanine aminotransferase (ALT), also considered a marker of liver damage, was also assessed during our study (data not shown). But, in the context of EIMD, the interpretation of ALT serum concentration should regard its release from muscle (Nathwani et al., 2005). Nevertheless, all measurements were under the upper recommended reference of 35 U·L\(^{-1}\) (Kratz et al., 2004), supporting the non-occurrence of hepatic damage indicated by alkaline phosphatase levels. Therefore, based on these data, we can suggest that the applied exercise protocol did not injure any of these three organs.

To our knowledge, this was the first study to compare the metabolic recovery from EIMD between beverages with different natures. Taking into account that no kidney, heart or liver injury was detected during the course of this study, changes related to altered functioning of these organs can be ruled out. Therefore, the detected variations in the evaluated metabolic markers might be attributed to the applied protocol. Considering this, we can conclude that both beverages, independently of their commercial or homemade nature, led to similar recovery patterns from EIMD regarding the studied metabolic biomarkers. This represents important and useful information for athletes. They may be allowed to select the nature of the recovery beverage based on price (food is usually cheaper), convenience (food usually takes longer to prepare), and contamination risk with prohibited substances in sport (Sousa et al., 2014), rather than on differences in the metabolic response.
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REFERENCES


Carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures. *American Journal of Clinical Nutrition, 72*(1), 106-111.


CHAPTER III

4. DISCUSSION

5. CONCLUSIONS
4. Discussion

The main findings of our studies were:

1. The majority of athletes reported to have used one or more NS over the previous year. Multivitamins/minerals were the most frequently consumed supplement, the main cited reason to use NS was to accelerate recovery, and physicians were the preferable advisors regarding NS usage. Type of NS consumed was found to be different between gender and age groups, and weekly training hours. Age and gender also seem to influence reasons to use NS and sources of information. Non-scientifically supported choices revealed a lack of reliable information and appropriate nutritional strategies (e.g. use of multivitamins/minerals and glutamine);

2. After adjustment, NS users showed a higher intake from food, for at least one gender, for energy and for 7 of the 17 studied nutrients. The highest PMI were seen for vitamins D and E, calcium, folate, and magnesium. After adjustment, NS users, irrespective of gender, reported lower PMI for calcium, and female users for magnesium;

3. Athletes using NS were different from non-users on a variety of sociodemographic and sporting characteristics, namely gender, age, BMI, parental education, type of sport, and training load (weekly hours of training and gym). After adjustment, supplement usage was associated with being ≥18 years old, performing individual sports, and >2 h gym/week. Independently of confounders, NS users reported a higher intake of meat, eggs, and yogurt, and a lower intake of processed meat, vegetable oils, margarine, chips, and fast food;
4. The ingestion of a commercial or a homemade recovery beverage, with similar nutritional content, led to similar recovery patterns from EIMD regarding:
   a. Muscle damage (CK, myoglobin, lactate dehydrogenase, aldolase, aspartate aminotransferase, and alanine aminotransferase);
   b. Functional recovery (eccentric peak torque of the quadriceps, and countermovement jump height);
   c. Soreness markers (general muscle soreness, and soreness from palpation);
   d. Inflammation (white blood cells, neutrophils, monocytes, basophils, eosinophils, lymphocytes, interleukin-6, tumour necrosis factor-α, and C-reactive protein);
   e. Oxidative stress (total antioxidant status, protein carbonyls, uric acid, and glutathione reductase);
   f. Metabolic parameters (glucose, total proteins, non-esterified fatty acids, β-hydroxybutyrate, triglycerides, total cholesterol, low-density lipoprotein, high-density lipoprotein, creatinine, urea, alkaline phosphatase, and cardiac-specific troponin I).

4.1 Nutritional supplements usage

To our knowledge these were the first studies to assess NS usage among high-level Portuguese athletes, and to compare nutritional information from food between athletes using and not using NS (studies I to III). The current data demonstrated that supplement usage was a common practice among athletes, similarly to what occurs in other countries (Dascombe et al., 2010; De Silva et al., 2010; Diehl et al., 2012; Lun et al., 2012). Additionally, the supplement most frequently taken was multivitamins/minerals which is also consistent with the choices of athletes from other regions (Braun et al., 2009; Dascombe et al., 2010; Lun et al., 2012). Main reasons to resort to supplements included performance- and health-related motivations, similar to what is
currently reported by athletes as the most commonly cited motives (Maughan et al., 2011). In Portugal, supplementation might be perceived by athletes as a medical intervention, since physicians were reported as the main source of information for NS usage.

Interestingly, we denoted that associations between types of consumed NS and underlying reasons to use them were not always in agreement with scientific information on the effects of certain substances. Examples of this were the associations found between multivitamins/minerals and ‘have more energy/reduce fatigue’, and glutamine and ‘stay healthy’. This behaviour was firstly reported by Petróczi and Naughton (2007). The incongruence observed in both studies is preoccupant since denotes poor understand regarding NS proven effects. Moreover, we found that athletes using NS reported a higher nutritional intake from food and a lower PMI for some micronutrients. Taking into account that ‘stay healthy’ was one of the most cited reasons to take NS, and multivitamins/minerals were the most used NS, these finding re-enforce the lack of congruence between rationale and practice. Athletes who were using NS seemed to be the ones who were already more concerned about nutrition-related aspects and with least need to supplement their diet with macro- and/or micronutrients. Therefore, professionals working with athletes, and athletes themselves, should review and question NS usage. Give our results, it is urgent to provide athletes with accurate information on scientifically proven effects of NS, in order to make informed and conscious choices about the usage of supplements. Additionally, our results showed that athletes might also benefit from a quantitative and qualitative nutritional approach focusing on nutritionally-dense foods, since the amount of ingested proteins seemed higher than recommended, that of CHO was near the lower borderline, and a high PMI for several micronutrients was observed.

Moreover, we found that athletes using NS had a propensity for healthier food choices. They reported higher intake of foods perceived as healthier, such as nuts, pulses, fish, skimmed-milk, yogurts, and tea, and lower intake of fat-
rich foods such as olives, vegetable oils, and margarine, processed meat, chips, and fast food. Even with confounders’ adjustment, NS users were more likely to ingest yogurt and less likely to consume processed meat, vegetable oils, margarine, chips, and fast food. These results are congruent with the idea that athletes taking supplements are more self-concerned. Indeed, these results are aligned with those from general population (Bailey et al., 2011; Beitz et al., 2004; McNaughton et al., 2005; Reinert et al., 2007), in which NS users have presumably healthier diet characteristics. Importantly, and to our knowledge, this relationship in sports context had never been made. We also found that athletes using NS also reported higher consume of foods with sports relevance, such as good food sources of protein (fish, meat, eggs, skimmed milk, and yogurt, with meat, eggs, and yogurt ingestion being associated with NS usage even after adjustment for confounders) and CHO (pasta and marmalade/jam), and also coffee, which is a food source of the ergogenic aid caffeine (Burke, 2008).

We also concluded that being male, ≥18 years old, having a BMI ≤22 kg·m⁻², being descendent of parents with ≤9 years of education, performing individual sports, and >2 h gym/week was associated with NS usage. Even after adjustment, supplement usage was associated with being older, performing individual sports, and >2 h gym/week. Therefore, we concluded, as others (Dietz et al., 2014; Erdman et al., 2007; Giannopoulou et al., 2013), that some sociodemographic and sporting characteristics are important determinates of NS usage among athletes. Knowing the characteristics associated with NS usage may help sports and health professionals to identify an alleged or future NS user. Professionals working with athletes may opt to query them regarding these parameters, enabling the development (or not) of a self-directed supplement scheme based on scientific evidence.
4.1.1 Methodological considerations: strengths and limitations

Athletes were also asked about the brand of the supplements, the intake dosage, and the frequency of usage. Unfortunately, this information was not possible to be analysed accurately, mainly due to the time extent of data gathering. This particular study characteristic also did not permit to collect information regarding energy expenditure and biochemical data. Moreover, athletes were asked about what type of supplements they consumed and general reasons to use supplements, as an all. Consequently, we could only statistically associate the underlying reasons for usage with NS type. Additionally, in our studies both punctual and habitual users were considered as NS users, which may constitute a limitation. Other possible limitation is the different number of athletes per sport and age group which might have biased the results in favour of the most represented groups. Nevertheless, important information may also come from smaller groups.

Additionally, it is known that athletes may have difficulty to quantify the portion size in the FFQs (Magkos & Yannakoulia, 2003). Still, the FFQ method for nutrient intake assessment has been considered as remarkably robust (Kushi, 1994), and it can be satisfactorily used for the dietary assessment of athletes (Magkos & Yannakoulia, 2003). Indeed, this type of questionnaire has been widely administered in studies with the similar aim of evaluate dietary intake of athletes (Di Cagno et al., 2012; Nikic et al., 2014; Soric et al., 2008). Moreover, FFQ are one of the most common tools used to examine dietary intake in long-term studies. Importantly, they can cover seasonal variations, and might be superior to other methods, namely multiple 24-hour dietary recalls and food records, to monitor rarely consumed foods (Illner et al., 2010). Nevertheless, results from FFQs should be cautiously interpreted since multiple food records are a preferred method for more precise dietary estimates (Shim et al., 2014). The questionnaire utilized in our study has an acceptable validity (Lopes, 2000; Moreira et al., 2003; Pinto et al., 2010) and has been already
used in Portuguese adults (Oliveira et al., 2009; Pinto et al., 2009), and adolescents (Leite et al., 2007; Moreira et al., 2014).

It is true that the evaluated time-length may have brought some issues but it also was a great opportunity to collect information about the long-term NS usage and the athletes' dietary intake during the same period of time. The number of studies doing this simultaneous approach is scarce, and the information that arose from the combination of the 2 questionnaires is of great and emerging interest. Moreover, this study was performed with a considerable high number of high-level athletes from several different sports, which gave the opportunity to add some novel information about this particular and privilege group of athletes.

4.1.2 Future directions

Since our studies permitted a 12-months approach, future studies may opt to assess shorter periods of time. This will allow a better understanding on what type of supplements athletes choose to use at a given period of the season, the specific reasons to use each one, frequency of consumption, and ingested amount. Among other information, this approach will simultaneously permit to study the reasons to consume each NS and the athlete's knowledge regarding NS. Furthermore, if shorter periods of time are assessed, food and physical activity records might be applied, and biochemical analysis could be performed.

Additionally, it would be useful to have more knowledge regarding the consumption of NS within each speciality of certain sports, namely athletics (long- and middle-distance running, sprints, hurdles, jumps, throws, combined events, and race-walking), swimming (competitive swimming, synchronized swimming and open-water swimming) and cycling (road cycling, off-road
cycling, and BMX riding), and also within weigh-classes in the case of sports with weight categories, such as boxing.

4.2 Recovery beverages for exercise-induced muscle damage

In the past, the main goal of sports nutrition was to support the increment of training load by preventing illness, injury, and chronic fatigue, in order to permit greater performance improvements (Maughan & Burke, 2011). Nowadays, nutrition strategies can allow athletes to train smarter, i.e. maximize training adaptation, allowing a similar level of fitness with less training (Maughan & Burke, 2011). One of these strategies is the post-exercise recovery meal, which is determinant to the replenishment of endogenous substrate stores, repair and reconditioning of skeletal muscle cells (Beelen et al., 2010). Indeed, dietary strategies that enhance the recovery from the stressful impact of exercise, can help maintaining performance for subsequent exercise sessions (Beelen et al., 2010). In our study (I), the Portuguese athletes reported ‘accelerate recovery’ as the first reason to use NS, denoting the importance of recovery strategies for these athletes. Moreover, this specific reason was shown to be associated with the consumption of protein supplements, sport drinks, and vitamin C.

The rationale to include protein and CHO in the recovery meal after EIMD has been extensively described previously, particularly in study IV. Briefly, muscle protein accretion is believed to be required to optimize repair and reconditioning after EIMD (Beelen et al., 2010). Proteins/ amino acids are fundamental to stimulate muscle protein synthesis (MPS), synergistically with exercise, especially resistance type, and are essential for muscle protein accretion (Phillips, 2014). Additionally, the hyperaminoacidemia will promote a rise in insulin which suppresses muscle protein breakdown (MPB), leading to a more positive muscle protein balance (MPS >> MPB) (Phillips, 2014). Regarding CHO, even though they may have little (by assisting the insulin rise
and attenuating muscle protein breakdown) or no impact on protein turnover when ample doses of protein are provided (Phillips & van Loon, 2011), CHO can promote muscle glycogen re-synthesis (Jentjens & Jeukendrup, 2003). Beyond the advantages of initiating a subsequent bout of exercise with higher CHO stores (Jentjens & Jeukendrup, 2003), low muscle glycogen levels may have a negative impact on muscle protein synthesis (Creer et al., 2005), and promote muscle protein breakdown (Lemon & Mullin, 1980). Moreover, even high intensity short-duration exercise relies heavily on CHO (MacDougall et al., 1999). Therefore, the ingestion of CHO, along with proteins, after demanding exercise has been recommended to optimize performance (Beelen et al., 2010; Phillips, 2012). According to the state of art (Phillips, 2011), our approach (studies V to VII) was based on a protein dose of 20–25 g to maximize muscle MPS. More recently (Moore et al., 2014), a dose of 0.24 ± 0.06 g·kg⁻¹ was suggested as the relative to body weight protein amount that better stimulated MPS in young adults. In our studies (studies V to VII), we used 0.26 g protein·kg⁻¹·h⁻¹, which is closely akin to these latest recommendations.

It is largely accepted that acute high-intensity resistance exercise can induce oxidative stress due to ROS production, resulting in oxidation of multiple compounds (Finaud et al., 2006; Hudson et al., 2008). Besides ROS negative consequences, they have a central role regarding training-induced adaptations (S. K. Powers et al., 2011). Whether or not athletes benefit from antioxidant supplementation remains controversial, since arguments in favour and against its use still exist (S. Powers et al., 2011). These arguments were described and discussed in study IV. By now, there is limited evidence to support the recommendation of antioxidant supplementation; in fact, the avoidance of an excessive antioxidant supplementation have been recommended (S. Powers et al., 2011). Rather than rely on supplementation, athletes should focus their attention in consuming a well-balanced and energetically adequate diet, which can provide antioxidant-rich foods. In our studies (V to VII), we used a vitamin C dose of 60.8 ± 1.0 mg·h⁻¹ which was the calculated amount contained in the 100 g of strawberries included in the MS.
From the available literature (study IV), it seemed that the provenance of nutrients – commercial or natural – might not largely interfere with the recovery process. Our paper went in line with Luc van Loon’s opinion (van Loon & Gibala, 2012) that after exercise the focus should be placed on getting an ample amount of protein, rather than on the type and nature of protein. In a real-life situation, when it comes to the recovery meal, athletes need to choose conscientiously between a supplement and food (not between a supplement/food and placebo). Although it was pertinent and its importance previously denoted (Cockburn et al., 2008), to date, the compared recovery effect of food with an equivalent nutritional supplement had never been done. Therefore, our main aim with studies V to VII was to compare 2 recovery beverages of different natures, and obtain information that could be transposed to practice. Today, with our studies, we have scientifically proven evidence that an NS and food, with similar nutritional content, led to similar recovery process in an ample range of parameters. Now, athletes and athlete support personnel can make informed and scientifically driven choices regarding the nature of the recovery meal. Given our results, the choice between NS and food for the post-exercise meal might be based on other characteristics than on the recovery outcome. Obviously, other studies should be done to confirm our results. Nevertheless, the hypothesis that a supplement and a homemade meal, with similar nutritional content, would lead to similar recovery outcomes from EIMD is now an open road, where the first step has already been given.

4.2.1 Methodological considerations: strengths and limitations

Since we wanted to approach a real-life issue, i.e. the athlete’s choice regarding the nature of the recovery meal (supplement or food), we recruited athletes, and not just active individuals, to participate in our study. Since participants were accustomed to regular exercise and were unlikely to exhibit the level of muscle damage following an acute bout of exercise of untrained
As already mentioned, our main aim was the comparison between the two beverages. Therefore, we only performed 2 trials. Although the absent of placebo group might be considered a limitation, several others studies (Breen et al., 2010; Cermak et al., 2009; Luden et al., 2007; Romano-Ely et al., 2006; Wojcik et al., 2001) used the same approach when the purpose was to compare beverages. Moreover, adding a control group would imply an increased washout period for, at least, 8 weeks (Nosaka et al., 2005), due to repeated bout effect, if a crossover design was maintained. The repeated bout effect phenomenon refers to the protective adaptation for a subsequent bout of exercise, where a repeated bout of a similar eccentrically-based exercise results in markedly reduced symptoms of damage compared to the initial bout (McHugh, 2003). Our trials were performed at the beginning of the summer season, in order to be feasible for athletes to respect the period of no strenuous exercise – 2 days prior and during the 3 days of each trial – and to not interfere with their training programme. If the washout period had been prolonged for 2 months (twice, to account for the 3 groups), we would not have been capable to respect athletes’ training programme. This would tremendously impair the recruitment process and would probably increase the dropout. Other option would have been to recruit more athletes, in order to create 3 different groups: one for each beverage, and a placebo one. However, this strategy would involve the (almost impossible) recruitment of, at least, 30 athletes, with similar training level and analogous sport specificity. Given the mentioned alternatives, we opted to compare solely the 2 recovery beverages, which was the main aim of the studies. Additionally, other study (Cockburn et al., 2008) has already compared the effect of CHO–P beverages with a control group, using a similar exercise protocol.
4.2.2 Future directions

Future studies may test other potential options to be included in the recovery meal, namely foods rich in protein, carbohydrates, antioxidants and/or anti-inflammatory nutrients. Examples include meat, fish, eggs, soy, bread, pasta, rice, potatoes, beans, fruit, tea, and nuts. Moreover, it would be interesting to investigate the intake of food after other types of physical exertion beyond strength exercise, namely endurance and intermittent high-intensity workouts.

Additionally, given that the used single-leg exercise protocol did not seemed sufficient to induce an inflammatory systemic response, future works may opt to develop other protocols that require a bigger muscle mass involvement. Other possible option is to measure markers of local muscle inflammation. Moreover, although the applied exercise protocol was suitable to induce muscle damage, it does not represent movements that would be typically performed by exercising individuals. Therefore, future research could focus on physical exertions that can be more directly translated to the real-world.

There is a vast potential for other parameters to be analysed regarding the impact of food strategies on exercise recovery. Possible options are the measurement of other cytokines, F2-isoprostanes, superoxide dismutase, glutathione peroxidase, insulin, and cortisol. In addition, the investigation of potential mechanism may be other line for future research.

Lastly, the participants included in our study were young adult male athletes. Future studies should investigate the impact of these strategies in other age groups and also in females.
5. Conclusions

Our results added new information to the sports nutrition field. The findings of our studies allow us to conclude that:

1. NS consumption among Portuguese athletes was widespread;

2. There is an urgent need to provide athletes with education and access to scientific and unbiased information, so they can make assertive and conscientiously choices regarding the utilization of NS;

3. Athletes consuming supplements were those with a better nutritional intake;

4. Athletes taking NS were probably the ones who would least benefit from it;

5. Supplement users were different from non-users on sociodemographic and sporting characteristics;

6. Food choices of NS users differed from those of non-users and seemed to be both health- and sport-driven;

7. Beverages different in nature (commercial or homemade), but similar in nutritional content, led to similar recovery patterns from EIMD.
References


