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Interactions between nutrition and exercise: diet, supplementation and sports performance

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Supervisor

Prof. Marco Bruno Luigi Rocchi

Co-Advisor

Prof. Francesco Lucertini

Ph.D. Candidate

Dott. Stefano Amatori

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Sam: This is it.

Frodo: This is what?

Sam: If I take one more step, it'll be the farthest away from home I've ever been.

Frodo: Come on, Sam. Remember what Bilbo used to say:

"It's a dangerous business, Frodo, going out your door. You step onto the road, and if you don't keep your feet, there's no knowing where you might be swept off to."

(J.R.R. Tolkien, The Lord of the Rings – Part 1: The Fellowship of the Ring)

Abstract

This Doctor of Philosophy (Ph.D.) thesis presents five papers I have worked on in these three years, related to the topic "nutrition, supplementation and sports performance", and to the interactions between these. The thesis could be ideally divided in three main topics:

1. The effects of physical exercise on dietary behaviours
2. The contribution of the sports supplements for the sports performance
3. The prevalence of weight-control practices for the performance optimisation.

Specifically, in the first two chapters of the thesis, an experimental, and a longitudinal observational study are presented, which both of them aimed to investigate the influence of physical exercise on dietary behaviours. In the first one, a sample of college students was involved in a specific training period lasted for nine weeks, during which their diet was daily monitored. In the second one, the effect of self-administered exercise on food choices has been explored during the Covid-19 lockdown.

On chapters three and four, the focus moved to the topic of 'sports supplements'. A cross-sectional study is presented which explored the supplements consumption in professional and recreational beach volley athletes, taking part in a national championship. Then, in a randomized-controlled trial, we aimed to investigate the performance-enhancing effect of a commercially available supplement, mainly containing carbohydrates and branched-chain amino acids.

Lastly, in chapter five is presented an observational study which explored the rapid weight-loss strategies applied by a sample of professional and amateurs boxers, in order to lose weight before the competition.

Some future research questions have risen, that will likely bring toward a better understanding of these complex relationships, with a common goal: improve health and optimise sports performance.

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- Donati Zeppa, S.; Sisti, D.; **Amatori, S.**; Gervasi, M.; Agostini, D.; Piccoli, G.; Bertuccioli, A.; Rocchi, M.B.L.; Stocchi, V.; Sestili, P. High-intensity Interval Training Promotes the Shift to a Health-Supporting Dietary Pattern in Young Adults. *Nutrients* 2020; 12, 843. doi: 10.3390/nu12030843 | Impact Factor: 4.546; Category: Nutrition & Dietetics; Quartile: 1st; Rank: 17/89.
- **Amatori, S.**; Donati Zeppa, S.; Preti, A.; Gervasi, M.; Gobbi, E.; Ferrini, F.; Rocchi, M.B.L.; Baldari, C.; Perroni, F.; Piccoli, G.; Stocchi, V.; Sestili, P.; Sisti, D. Dietary Habits and Psychological States during COVID-19 Home Isolation in Italian College Students: The Role of Physical Exercise. *Nutrients* 2020; 12, 3660. doi: 10.3390/nu12123660. | Impact Factor: 4.546; Category: Nutrition & Dietetics; Quartile: 1st; Rank: 17/89.
- **Amatori, S**.; Sisti, D.; Perroni, F.; Impey, S.; Lantignotti, M.; Gervasi, M.; Donati Zeppa, S.; Rocchi, M.B.L. Which are the Nutritional Supplements Used by Beach-Volleyball Athletes? A Cross-Sectional Study at the Italian National Championship. *Sports* 2020; 8(3):31. doi: 10.3390/sports8030031 | Impact Factor: N/A; Category: N/A; Quartile: N/A; Rank: N/A.
- Gervasi, M.; Sisti, D.; **Amatori, S**.; Donati Zeppa, S.; Annibalini, G.; Piccoli, G.; Vallorani, L.; Benelli, P.; Rocchi, M.B.L.; Barbieri, E.; Calavalle, A.R.; Agostini, D.; Fimognari, C.; Stocchi, V.; Sestili, P. Effects of a commercially available branched-chain amino acid- alanine- carbohydrate-based sports supplement on perceived exertion and performance in high intensity endurance cycling tests. *Journal of the International Society of Sports Nutrition* 2020; 17(6). doi: 10.1186/s12970-020-0337-0 | Impact Factor: 5.068; Category: Sport Sciences; Quartile: 1st; Rank: 6/85.
- **Amatori, S.**; Barley, O.R.; Gobbi, E.; Vergoni, D.; Carraro, A.; Baldari, C.; Guidetti, L.; Rocchi, M.B.L.; Perroni, F.; Sisti, D. Factors influencing weight loss practices in Italian boxers: A cluster analysis. *International Journal of Environmental Research and Public Health* 2020; 17, 8727. doi:10.3390/ijerph17238727. | Impact Factor: 2.468; Category: Public, Environmental & Occupational Health; Quartile: 2nd; Rank: 58/193.

Not included in the thesis

- Gervasi, M.; Gobbi, E.; Natalucci, V.; **Amatori, S.;** Perroni, F. Descriptive Kinematic Analysis of the Potentially Tragic Accident at the 2020 Austrian MotoGP Grand Prix Using Low- Cost Instruments: A Brief Report. *International Journal of Environmental Research and Public Health*, 2020; 17(21), 7989. doi: 10.3390/ijerph17217989 | Impact Factor: 2.468; Category: Public, Environmental & Occupational Health; Quartile: 2nd; Rank: 58/193.
- De Barbieri, I.; Sisti, D.; Di Falco, A.; Galeazzo, M.; **Amatori, S**.; Rocchi, M.B.L.; Perilongo, G. Relationship-based care model in pediatrics: a randomized controlled trial to implement the parents' perception of the quality of nursing care. *Journal of Advanced Nursing* 2020; 00, 1-12. doi: 10.1111/JAN.14585 | Impact Factor: 2.561; Category: Nursing; Quartile: 1st; Rank: 6/123.
- Perroni, F.; Migliaccio, S.; Borrione, P.; Vetrano, M.; **Amatori, S**.; Sisti, D.; Rocchi, M.B.L.; Salerno, G.; Del Vescovo, R.; Cavarretta, E.; Guidetti, L.; Baldari, C.; Visco, V. Can haematological and hormonal biomarkers predict fitness parameters in youth soccer players? A pilot study. *International Journal of Environmental Research and Public Health* 2020; 17, 6294. doi: 10.3390/ijerph17176294 | Impact Factor: 2.468; Category: Public, Environmental & Occupational Health; Quartile: 2nd; Rank: 58/193.

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- Bertuccioli, A.; Rocchi, M.B.L.; Morganti, I.; Vici, G.; Gervasi, M.; **Amatori, S**.; Sisti, D. Streptococcus salivarius K12 in pharyngotonsillitis and acute otitis media – A meta-analysis. *Nutrafoods* 2019; 2, 80-88. doi: 10.17470/NF-019-0011 | Impact Factor: N/A; Category: N/A; Quartile: N/A; Rank: N/A.
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- Gervasi, M.; Calavalle, A.R.; **Amatori, S**.; Grassi, E.; Benelli, P.; Sestili, P.; Sisti, D. Post-activation potentiation increases recruitment of fast twitch fibers: a potential practical application in runners. *Journal of Human Kinetics* 2018; 65: 69-78. doi: 10.2478/hukin-2018-0021 | Impact Factor: 1.664; Category: Sport Sciences; Quartile: 3rd; Rank: 61/83.
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- Gervasi, M.; Sisti, D.; **Amatori, S**.; Andreazza, M.; Benelli, P.; Sestili, P.; Rocchi, M.B.L.; Calavalle, A.R. Muscular viscoelastic characteristics of athletes participating in the European Master Indoor Athletics Championship. *European Journal of Applied Physiology*, 2017; 117 (8): 1739–1746. doi: 10.1007/s00421-017-3668-z | Impact Factor: 2.580; Category: Sport Sciences; Quartile: 2nd; Rank: 28/81.

List of Conference Presentations

Oral Presentations

- **Amatori, S.**; Sisti, D.; Serra, T.; Donati Zeppa, S.; Calavalle, A.R.; Sestili, P.; Rocchi, M.B.L.; Gervasi M. A multidimensional approach to monitor fatigue in indoor cycling. XI SISMES National Congress – Bologna, 27-29 September 2019
- Sisti, D.; **Amatori, S.**; Serra, T.; Gervasi, M.; Rocchi, M.B.L. A new non-linear approach in the determination of VT_2. X SISMEC National Congress – Roma, 18-21 September 2019
- Sisti, D.; **Amatori, S.**; Gervasi, M.; Sestili, P.; Calavalle, A.R.; Rocchi, M.B.L. Postural sway alterations linked to a high intensity interval training session. SISMEC & Ass. A. Liberati Congress – Roma, 7-8 November 2018
- Gervasi, M.; Sisti, D.; **Amatori, S.**; Andreazza, M.; Benelli, P.; Sestili, P.; Rocchi, M.B.L.; Calavalle, A.R. Muscle tone and viscoelastic characteristics of athletes participating at European Master Athletics Indoor Championship 2016. VIII SISMES National Congress – Brescia, 29 September – 1 October 2017

Posters

- **Amatori, S.;** Donati Zeppa, S.; Preti, A.; Gervasi, M.; Gobbi, E.; Rocchi, M.B.L.; Baldari, C.; Perroni, F.; Stocchi, V.; Sestili, P.; Sisti, D. Dietary habits and psychological states during COVID-19 home isolation: the mediation effect of physical exercise in Italian college students – SISMEC Intermediate Congress – Online only, 12-13 November 2020
- **Amatori, S.**; Sisti, D.; Lantignotti, M.; Gervasi, M.; Bertuccioli, A.; Donati Zeppa, S.; Calavalle, A.R.; Rocchi, M.B.L. Nutritional supplement practices of beach volleyball players of the Italian National Championship: a cross-sectional study. European Sport Nutrition Society Congress – Madrid, 15-16 November 2019
- Donati Zeppa, S.; Gervasi, M.; Agostini, D.; Sisti, D.; **Amatori, S.**; Piccoli, G.; Sestili, P.; Guescini, M.; Stocchi, V. Effect of endurance training protocol and Friliver® Performance supplementation on gut microbiota phyla. European Sport Nutrition Society Congress – Madrid, 15-16 November 2019
- **Amatori, S.**; Sisti, D.; Lantignotti, M.; Gervasi, M.; Bertuccioli, A.; Donati Zeppa, S.; Calavalle, A.R.; Rocchi, M.B.L. Nutritional supplement practices of beach volleyball players of the Italian National Championship: a cross-sectional study. XI SISMES National Congress – Bologna, 27-29 September 2019
- Gervasi, M.; Sisti, D.; **Amatori, S.**; Donati Zeppa, S.; Annibalini, G.; Piccoli, G.; Vallorani, L.; Rocchi, M.B.L.; Barbieri, E.; Calavalle, A.R.; Agostini, D.; Stocchi, V.; Sestili, P. Effects of a branched-chain amino acids-alanine- supplementation intake in high intensity endurance cycling tests. European College of Sport Science Congress – Prague, 3-6 July 2019
- Sisti, D.; **Amatori, S.**; Bensi, R.; Calavalle, A.R.; Gervasi, M.; Lauciello, R.; Rocchi, M.B.L. Baskin – A basketball-based sport for disabled athletes: a role weight's analysis. X SISMES National Congress – Messina, 5-7 October 2018

Definition of Abbreviations

AIC: Akaike information criterion
BCAA: Branched-Chain Amino Acids
BIC: Bayesian information criterion
BMI: Body Mass Index
CHO: Carbohydrate
CK: Creatine Kinase
CNS: Central Nervous System
COVID-19: Coronavirus Disease 19
ES: Effect Size
FFM: Free-Fat Mass
HIEC: High Intensity Endurance Cycling
HIIT: High Intensity Interval Training
HR: Heart Rate
HR-QoL: Health-related Quality of Life
ID: Identity Code
IV: Independent Variable
KMO: Kaiser-Meyer Olkin
LCA: Latent Class Analysis
LTs: Lactate Thresholds
MICT: Moderate-intensity continuous training
PANAS: Positive and Negative Affect Scale
PHQ-9: Patient Health Questionnaire 9
PL: Placebo
RDA: Recommended Dietary Allowance
REC: Recovery
RPE: Rate of Perceived Exertion
RWLQ: Rapid Weight Loss Questionnaire
RWLS: Rapid Weight Loss Score
SF-12: 12-item Short Form Health Survey
SPR: Sprint
SSABIC: Sample-size adjusted BIC
SU: Supplemented
TRIMP: Training Impulse
Trp: Tryptophan
TTE: Time To Exhaustion
VO_2max: Maximal Oxygen Consumption
W_{LT}: Power at lactate threshold
Wpeak: Peak power

Introduction

Nutrition and physical exercise are two pillars of wellness on which a correct lifestyle is based [1]. These two elements are in a relationship of mutual influence: indeed, if it is known that diet can have a significant impact on sports performance, less is known about the influence of exercise on dietary behaviours. The latter has been proposed as a gateway towards healthier food choices [2]. However, the physiological and psychological mechanisms that underlie this phenomenon are not yet fully understood, as neither are the effects of different types of training. In this context, for the study presented in the first chapter of this thesis we sought to determine the effects of nine weeks of high-intensity interval training on dietary patterns, in a sample of previously untrained college students. Out experimental hypothesis – based on the previous literature – was that exercise might lead to healthier food selection. This study got published on *Nutrients* journal, with the title: *High-intensity Interval Training Promotes the Shift to a Health-Supporting Dietary Pattern in Young Adults* [1].

Given the expertise made with, and the results of, the above-reported study, we aimed to investigate the interactions between self-administered exercise and dietary behaviours during the forced home-isolation period due to the COVID-19 pandemic. The lockdown (intended as home-confinement) has been reported to induce severe mood disturbances, such as depression and anxiety [3]. Mood disturbances may negatively impact both nutrition and physical exercise [4]. On a unique condition as the one the world lived in the first months of 2020, we hypothesised that people who voluntarily self-performed physical exercise at home, could have followed a healthier diet, and that exercise may have played a protective role against the adverse effects of the mood disturbances. This study, entitled "*Dietary Habits and Psychological States during COVID-19 Home Isolation in Italian College Students: The Role of Physical Exercise*" is presented in the second chapter of this thesis, and it was published in November 2020 on *Nutrients* journal [5].

In the world of competitive sports, in conjunction with training and nutrition, it is widely accepted that the ingestion of some nutritional supplements could enhance sports performance [6]. Supplements may help, support and improve performance through several mechanisms. However, given the high variability of performance models among different sports, supplements may not work in the same way for all the disciplines. Explore and describe the practices adopted by the athletes is the first step, in order to examine and possibly improve the consumption of such products in specific sports or situation. In order to pursue this goal, in chapter three is presented a cross-sectional observational study we conducted, investigating the supplements consumption habits of a sample of professional and recreational beach volley athletes. This study was published on *Sports* journal, entitled "*Which are the Nutritional Supplements Used by Beach-Volleyball Athletes? A Cross-Sectional Study at the Italian National Championship*" [6].

Besides exploring the habits of the athletes regarding supplementation, it is fundamental to determine the efficacy of these products on improving performance, through rigorous randomised, placebo-controlled, double-blind trials. In chapter four, is presented an experimental study we conducted, aiming to investigate the performance-enhancing effects of a commercially available sports supplement, containing carbohydrates, branched-chain amino acids, and l-alanine. In this study, which got published on the *Journal of the International Society of Sports Nutrition* with the title "*Effects of a commercially available branched-chain amino acid-alanine-carbohydrate-based sports supplement on perceived exertion and performance in high intensity endurance cycling tests*" [7], the effects after both an acute and a chronic supplementation have been investigated.

Alongside with nutrition and supplementation, specific strategies are usually applied in some sport disciplines in order to improve performance. For example, it is common practice for combat sports' athletes to lose significant body weight in a short amount of time leading up to a competition, to gain an advantage by being paired with a smaller opponent, a practice colloquially named as 'weight-cutting' [8]. These rapid weight-loss strategies may represent a health-risk factor for the athletes. In the observational study presented in chapter 5, entitled "*Factors Influencing Weight Loss Practices in Italian Boxers: A Cluster Analysis*", we explored which are the factors which might influence the use of the weight-cutting practices by a sample of professional and amateurs Italian boxers. This paper was published in the *International Journal of Environmental Research and Public Health* [9].

Chapter 1

High-intensity interval training promotes the shift to a health-supporting dietary pattern in young adults [1]

Abstract: A healthy lifestyle is based on a correct diet and regular exercise. Little is known about the effect of different types of exercise on dietary preferences. To address the question of whether high-intensity interval training (HIIT) could modulate the spontaneous food choices, an experimental study was carried out on thirty-two young, healthy normal-weight subjects. The spontaneous diet of each subject has been monitored over nine weeks of indoor-cycling training, divided into three mesocycles with an incremental pattern: total energy intake, macronutrients and micronutrients have been analysed. A two-way mixed model has been used to assess differences in dietary variables; a principal factor analysis has been performed to identify sample subgroups. An increased energy intake (+17.8% at T3; $p<0.01$) has been observed, although macronutrients' proportions did not vary over time, without differences between sexes. An increase of free fat mass was found in the last mesocycle (+3.8%), without an augment of body weight, when, despite the increased training load, a stabilization of energy intake occurred. Three different subgroups characterized by different dietary modifications could be identified among participants that showed a common trend to a healthier diet. Nine weeks of HIIT promoted a spontaneous modulation of food choices and regulation of dietary intake in young normal-weight subjects aged 21-24. Importantly, this life-period is critical to lay the foundation of correct lifestyles to prevent metabolic diseases and secure a healthy future with advancing age.

1.1. Introduction

Physical exercise and nutrition (diet) are the two pillars of wellness on which the correct lifestyle guidelines are based, although it is undeniable that only a small proportion of the population in industrialized countries follows an active and correct lifestyle. Physical inactivity and an incorrect diet are involved in the aetiology of obesity. As hypothesized and subsequently verified at a preliminary level in the mouse model by Koch et al. [10] the change in energy transfer capacity that occurs through exercise capacity can be considered the central mechanistic determinant in the division between disease and health, where the continuous cellular and molecular reorganization characteristic of living organisms constitutes a dynamic evolutionary strategy providing a selective advantage to subjects capable of developing and maintaining a better health condition.

Regular exercise is currently adopted along with diet and eating habits modification to counteract obesity, even if further studies are needed to clarify if they work synergically but remain separate strategies, or if they track together and share the same neurobiological substrates and pathways. The wellness effects of physical exercise are already well known, improving the control of appetite-regulating hunger-satiety and psychological mechanisms. The appetite and hunger are suppressed following exercise, in particular in the immediate post-exercise state [11]; this effect, known as "exercise-induced anorexia", is linked to the suppression of orexigenic hormones, such as ghrelin, and the increase of satiety hormones, such as peptide YY [12]. It is known that regular exercise positively influences mood and self-control in several areas and effectively counteracts binge

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[1] Published: Donati Zeppa, S.; Sisti, D.; Amatori, S.; Gervasi, M.; Agostini, D.; Piccoli, G.; Bertuccioli, A.; Rocchi, M.B.L.; Stocchi, V.; Sestili, P. High-intensity interval training promotes the shift to a health-supporting dietary pattern in young adults. *Nutrients* 2020; 12, 843. doi:10.3390/nu12030843.

eating also in response to negative emotions [13,14]. Mechanisms involved in hunger–satiety are better regulated in active people in which eating in response to negative emotions is less frequent. Furthermore, even if energy request during physical exercise can lead some subjects to augment caloric intake, an implementation in exercise causes a negative energy balance, also due to improved homeostatic control of appetite [15]. Several studies suggest that regular physical activity can positively affect eating behaviour and food choices in a healthy direction, helping in correcting inappropriate long-term eating habits [16].

This relationship between physical exercise and diet opens the possibility to consider exercise as a gateway behaviour that could lead to dietary behaviour changes [2]. To authors' knowledge, the available studies investigating the relationship between exercise and dietary behaviours were characterized by short duration protocols (days or weeks), and studies of longer duration (several months) are lacking [17]. Furthermore, the influence of exercise on dietary preferences is dependent on the type, method, frequency, duration and intensity of exercise performed; the sample sizes and characteristics may also affect the results [17]. Recently, Joo et al. [18] reported that aerobic exercise of longer duration or higher intensity led to different dietary patterns, even though the trend was toward healthier diets in any case.

Traditionally, physical activity to improve health in the non-athletes population have been characterized by constant low- to moderate-intensity exercise. However, in the last decade, growing evidence proposed that vigorous exercise may produce more benefits than moderate-intensity exercise alone; in this context, high-intensity interval training (HIIT) is considered one of the most effective activity to improve cardiorespiratory and metabolic function and maximize health outcomes [19]. Buchheit and Laursen [20] defined HIIT as "repeated short-to-long bouts of a rather high-intensity exercise interspersed with recovery periods". The exercise intensity during a HIIT session can be individualized, such that two subjects may train at different speeds or power (external load), but with the same relative intensity, as %VO_2peak or %HRmax (internal load). This implies that HIIT is an exercise mode that could be applied and finely tuned to different types of populations [19]. HIIT can be manipulated by several different parameters, such as duration and intensity of work phases, number of series and repetitions, work to rest ratios, etc.; indeed, distinct protocols can differently affect the adaptive responses to the exercise [21].

In this study, we investigated whether a HIIT program could play a role in modulating spontaneous food choices and how such food choices were more or less healthy. The aim was to evaluate if a specific incremental indoor cycling HIIT program was able, in itself, to modify eating habits without a dietary counselling. This might pave the way to the development and implantation of effective intervention programs to maintain lifelong health and prevent metabolic pathologies. The experimental study was carried out on young subjects in health and normal weight.

1.2. Materials and Methods

1.2.1 Study Design

This was a within-subject training study. Dietary habits of a sample of young, healthy subjects were evaluated before, during and after a period of high-intensity interval training.

1.2.2 Participants

Thirty-two healthy collegiate (20 males: age 22.6 ± 1.7 years, height 175.5 ± 6.5 cm, weight 67.9 ± 9.9 kg, BMI 22.0 ± 2.7 kg/m^2; 12 females: age 21.5 ± 0.8 years, height 159.5 ± 4.7 cm, weight 52.8 ± 5.2 kg, BMI 20.6 ± 1.2 kg/m^2) were recruited. The exclusion criteria were major cardiovascular disease risks, musculoskeletal injuries, upper respiratory infections, smoking and consumption of any medicine in the past three months. With respect to nutritional habits, exclusion criteria were lactose intolerance, celiac disease, food allergies and particular dietary regimens (vegetarian, vegan, ketogenic, palaeolithic, intermittent fasting or less common diets).

The subjects were asked to answer a set of questions, taken from Malek et al. [22], regarding the mode, frequency, duration, length of time performing habitual physical activity, and intensity of the exercise performed. All participants performed not more than one 60 min leisure walking or jogging session per week, in the three months before the start of the study. The maximal oxygen consumption (VO_2max) of the participants was directly measured with an incremental ramp test (as described below); VO_2max values were 42.0 ± 5.9 ml/kg/min for men and 32.2 ± 6.5 ml/kg/min for women, in line with normative values of non-active young people already reported in the literature [23,24].

The participants were advised to maintain their dietary routine and to abstain from using dietary supplements, nutraceuticals or drugs, over the study period. The participants were also instructed to refrain from all training activities except the sessions included in the experimental design. Following a medical health-screening, all participants provided written informed consent to participate in the study, which was approved by the Ethical Committee of the University of Urbino Carlo Bo, Italy (02/2017, date of approval July 10, 2017) and was conducted in agreement with the Declaration of Helsinki for research with human volunteers (1975).

1.2.3 Training Protocol

Thirty-six indoor cycling training sessions over nine weeks were performed (see Figure 1.1). The training program was structured in three mesocycles, with an incremental pattern: both frequency and duration of the sessions were increased. Before the beginning of the training period, an incremental ramp test was performed by every subject, in order to set the individual training zones, based on the heart rate (HR) recorded at the first and second lactate thresholds (LTs); maximal oxygen consumption (VO_2max) was also assessed. For further information about training zones definition, see Gervasi et al. [7]. Each session was designed following the same training intensity distribution, based on a polarized model, with around 70% of session time spent in zone 1, 10% spent in zone 2 and 20% spent in zone 3, according to Seiler and Kjerland [25]. Each session was composed of a warm-up, a high-intensity interval exercise, and a cool down, as widely employed in the indoor cycling community. Intervals were performed at different intensities and durations, in order to match the polarized training intensity distribution (an example of a workout is presented in Figure 1.2). As a general guideline, intervals longer than 4 min were performed in zone 2, while intervals between 2 and 4 min were performed in zone 3. Recovery periods between intervals were performed in zone 1. During the training sessions, HR of each subject was monitored, and the values were projected onto the wall, as a percentage of maximal HR; the subjects were asked to maintain the same intensity zone of the instructor, based on their HR zones. All participants completed the whole training program; three subjects had to quit a training session before the end due to personal issues, but this was not considered a problem for the statistical analyses.

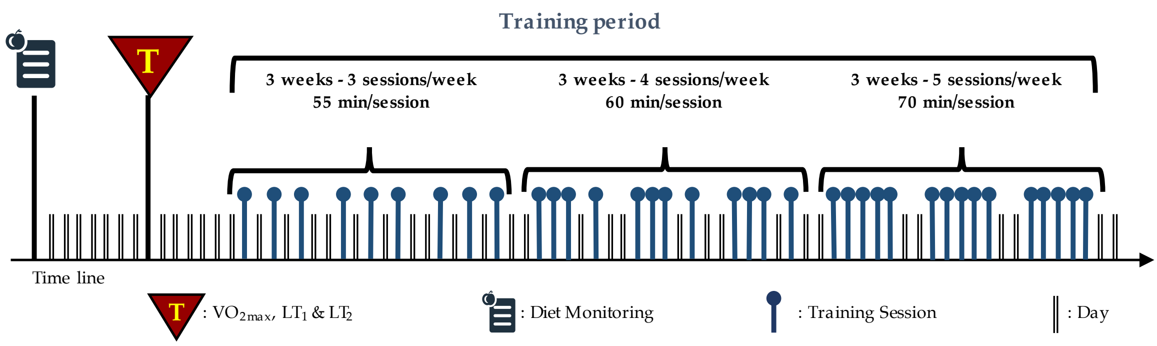

Figure 1.1. Structure of training period: nine weeks divided into three mesocycles. The frequency and duration of the sessions are also indicated. Modified with permission from Gervasi et al. [7].

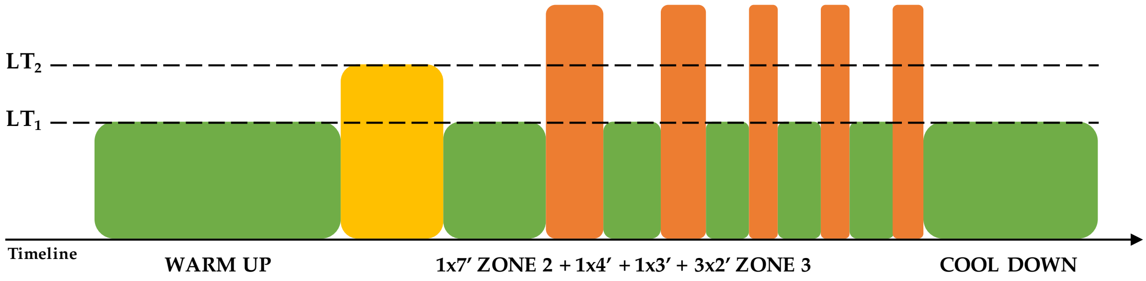

Figure 1.2. Example of a 70 min training session. LT: lactate threshold.

1.2.4 Body composition assessment

Weight and BMI (Body Mass Index) were measured for each participant. Body composition was assessed through electrical bioimpedance (Arkray BIA101 Sport Edition), at T0 before the beginning of the training, and subsequently after each mesocycle of training (T1, T2, T3). The measurements were taken in the morning next to the last session, after six or more hours of fasting. Percentage of Free-Fat Mass (FFM) was reported for each mesocycle.

1.2.5 Diet monitoring

Diet monitoring of subjects started two weeks before the start of training (T0) until the end of the training protocol (T3) and was performed by daily call interviews after dinner. In order to choose the most representative portions according to the indications of the subjects, a "PHOTOdietometer" was provided to each of them. The PHOTOdietometer is a visual scale which, by observing the different images, helps subjects to identify and objectively refer the portion of food taken according to volume, weight in grams and/or carbohydrate content. Information about mealtime and its characteristics in terms of cooking, seasonings and quantities were also provided. Data were collected and processed using MètaDieta software (METEDA Srl, San Benedetto del Tronto, Italy), and the following variables were considered for elaborations:

- total energy intake and macronutrient quantity;
- starch, soluble and insoluble fibre, glycaemic index and glycaemic load;
- saturated, monounsaturated, polyunsaturated fat, omega-3, Omega-6, cholesterol;
- iron, vitamin C, A, E.

1.2.6 Statistical Analysis

Descriptive statistics were performed using mean and standard deviation, where appropriate. Percentage variations were also reported in diet habits for each macro or micronutrient considered. In order to verify the weight, free-fat mass, macro- and micronutrient mesocycle-dependent changes, two ways mixed design (MANOVA for repeated measures) has been performed. Time was within-subjects 4 levels factor (T0, T1, T2, T3). T0 mean values were revealed during 2 weeks before starting training, T1 relative to the first mesocycle, T2 to the second mesocycle and finally T3 to the third one. Sex was two levels between groups factor. Contrasts are used to test for differences among the levels of a between-subjects factor; simple contrast compares the mean of each level (T1, T2, T3) to the mean of T0 level. Overall and partial Eta squared were used as effect size estimation. Mauchly's Test of Sphericity was performed; when epsilon was > 0.75, the Huynh-Feldt correction was applied, and when epsilon was < 0.75, the Greenhouse-Geisser correction was applied [26]. Wilks' lambda was considered as an appropriate multivariate test statistic. A Principal Axis Factor with a Varimax (orthogonal) rotation of delta (T3-T0) of 16 nutritional variables above considered was conducted on data gathered from participants. Kaiser-Meyer Olkin (KMO) measure of sampling adequacy was also performed; the minimum acceptable value for KMO is 0.6, although the ideal is over 0.70. Only factors with an eigenvalue of ≥1 were considered. The variance percentage accounted for by each component to the total variance was also reported. All elaborations were conducted with α=0.05. Elaborations and graphics were obtained using Prism 6 (GraphPad, La Jolla, California) and SPSS version 20.0 (SPSS Inc., Chicago, IL).

1.3. Results

1.3.1 Weight, Body Composition, Macro- and Micronutrients

Mauchly's Test of Sphericity indicated that the assumption of sphericity had been violated for all dependent variables, and therefore a Greenhouse-Geisser correction was used. The results showed no gender difference (time x sex interaction, Wilks' lambda=0.63; $F(57,215) = 0.634$, $p = 0.98$); this means that the effect of physical exercise over the three mesocycles is not different between sexes. A significant effect of time on all dependent variables (Wilks' lambda=0.153; $F_{(57,215)} = 3.314$, $p < 0.001$) was observed; this finding suggests that physical exercise led subjects to modify their diet. However, this modification had no effect on body weight, that did not change over time, either in males or females but, interestingly, the percentage of free-fat mass significantly increased at T3 (+3.8%, p<0.001), without differences between sexes (p=0.097) (Figure 1.3 and Table 1.1).

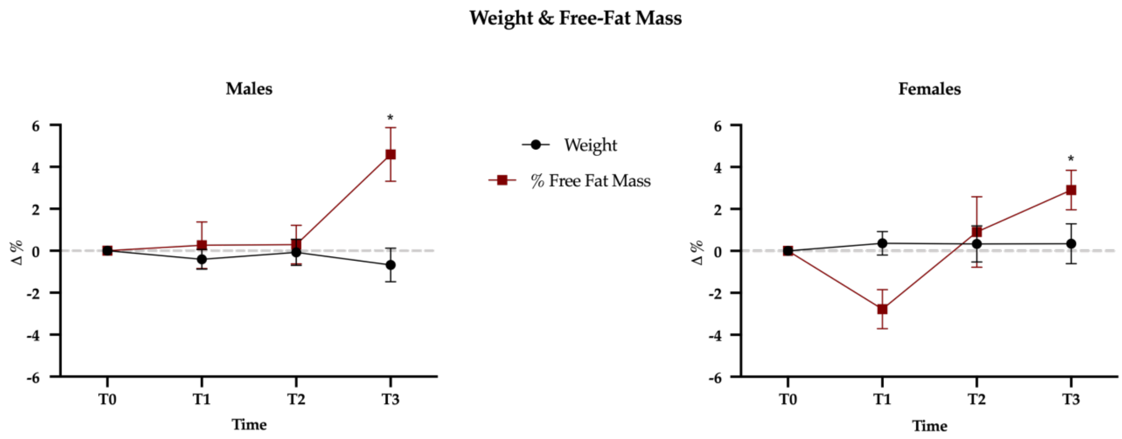

Figure 1.3. Weight and FFM variations between baseline (T0) and the three mesocycles (T1, T2, T3). Data are presented as percentage variations with standard errors.

Table 1.1. Variations between baseline (T0) and end of each mesocycle (T1, T2, T3) of body mass and FFM, macro- and micronutrients; results are split between males and females and presented as mean ± standard deviations. Data highlighted in bold are statistically significant (simple contrast analysis).

	T0		**T1**		**T2**		**T3**	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
Weight (kg)	68.2 ± 10.0	52.9 ± 5.4	67.8 ± 9.3	53.0 ± 5.3	67.9 ± 8.6	53.0 ± 5.6	67.4 ± 7.9	53.0 ± 5.5
Free-Fat Mass (%)	83.7 ± 5.9	75.0 ± 2.9	83.7 ± 5.9	72.9 ± 3.4	83.3 ± 4.6	75.7 ± 5.0	**87.3 ± 4.3**	**77.2 ± 3.8**
Energy (kcal)	2122 ± 663	1121 ± 189	**2266 ± 646**	**1317 ± 238**	**2432 ± 745**	**1541 ± 412**	**2375 ± 777**	**1531 ± 460**
Carbohydrate (gr)	270.2 ± 58.3	168.3 ± 33.6	284.2 ± 75.4	167.3 ± 23.9	**318.0 ± 106.1**	**222.0 ± 97.5**	**313.6 ± 99.8**	**216.2 ± 85.7**
Protein (gr)	83.4 ± 16.8	49.2 ± 6.3	**94.2 ± 26.6**	**55.5 ± 10.7**	**98.3 ± 29.5**	**62.3 ± 21.7**	**96.3 ± 29.5**	**62.5 ± 21.7**
Fat (gr)	77.9 ± 23.8	44.5 ± 14.9	86.0 ± 30.6	49.7 ± 14.3	**88.7 ± 31.5**	**59.8 ± 21.6**	**84.4 ± 35.1**	**63.4 ± 31.4**
Starch (gr)	129.9 ± 44.2	48.4 ± 15.5	132.8 ± 49.0	57.3 ± 17.1	**148.0 ± 58.1**	**77.6 ± 27.6**	**158.0 ± 61.1**	**81.7 ± 26.2**
Soluble Fibre (gr)	2.6 ± 1.2	1.0 ± 0.4	2.6 ± 1.2	1.1 ± 0.4	2.8 ± 1.4	1.1 ± 0.4	3.0 ± 1.6	1.5 ± 0.6
Insoluble Fibre (gr)	7.5 ± 3.4	3.2 ± 1.4	6.8 ± 3.3	3.5 ± 1.3	7.6 ± 4.3	3.6 ± 1.5	8.5 ± 6.6	4.8 ± 2.9
Glycaemic Index	55.3 ± 5.1	58.9 ± 7.3	54.5 ± 3.8	55.5 ± 4.3	55.9 ± 3.7	56.7 ± 5.7	**54.4 ± 3.4**	**56.5 ± 2.6**
Glycaemic Load	89.9 ± 27.4	47.7 ± 24.8	94.1 ± 24.1	48.8 ± 12.5	**110.1 ± 33.0**	**54.2 ± 14.8**	**113.1 ± 34.8**	**59.9 ± 16.7**
Saturated Fats (gr)	23.3 ± 10.9	11.7 ± 4.1	**25.1 ± 9.6**	**14.9 ± 4.9**	**25.9 ± 9.4**	**18.4 ± 7.2**	**24.3 ± 9.7**	**20.8 ± 13.4**
Monounsaturated Fats (gr)	27.5 ± 12.1	10.6 ± 6.4	**28.5 ± 12.3**	**14.1 ± 7.4**	**29.3 ± 11.2**	**16.5 ± 8.7**	**28.8 ± 13.3**	**16.7 ± 11.9**
Polyunsaturated Fats (gr)	8.4 ± 3.4	3.8 ± 1.1	9.0 ± 3.1	4.6 ± 1.6	**9.6 ± 2.8**	**5.8 ± 2.8**	**10.0 ± 4.3**	**6.2 ± 3.2**
Omega-3 (% kcal/kcal tot)	0.4 ± 0.2	0.5 ± 0.2	0.4 ± 0.1	0.5 ± 0.2	0.4 ± 0.1	0.5 ± 0.2	0.4 ± 0.1	0.5 ± 0.4
Omega-6 (% kcal/kcal tot)	3.0 ± 1.1	2.5 ± 1.0	3.0 ± 0.6	2.7 ± 0.8	3.1 ± 0.4	2.7 ± 1.2	3.1 ± 0.6	2.8 ± 1.0
Cholesterol (mg)	238.9 ± 73.8	116.4 ± 58.5	**217.1 ± 73.5**	**95.0 ± 22.9**	265.1 ± 65.1	88.6 ± 60.3	236.5 ± 66.9	86.6 ± 60.5
Iron (mg)	9.0 ± 2.9	4.2 ± 1.0	8.8 ± 2.6	4.9 ± 1.7	9.4 ± 2.9	5.8 ± 2.8	**9.8 ± 3.9**	**6.3 ± 3.8**
Vitamin C (mg)	68.1 ± 30.4	39.0 ± 21.8	60.3 ± 22.1	37.5 ± 18.4	66.2 ± 26.6	45.5 ± 22.0	80.7 ± 49.8	59.3 ± 35.4
Vitamin A (mcg)	1081.0 ± 537.9	512.7 ± 604.1	982.2 ± 433.4	590.7 ± 469.6	980.4 ± 458.1	666.5 ± 623.9	910.3 ± 507.6	540.6 ± 328.9
Vitamin E (mg)	8.4 ± 3.8	3.6 ± 2.5	8.3 ± 3.8	4.5 ± 2.7	8.8 ± 4.4	5.2 ± 2.8	8.7 ± 4.7	5.2 ± 3.9

Univariate tests showed significant association with time for the following nutrients: total energy intake (p<0.001), protein (p=0.001), carbohydrate (p=0.03), starch (p<0.001), glycemic index (p=0.02), glycemic load (p<0.001), total fat (p=0.02), saturated (p=0.009), monounsaturated (p=0.017), polyunsaturated (p=0.009) fat, cholesterol (p=0.004), iron (p=0.028), and vitamin C (p=0.032) (Figures 1.4 and 1.5).

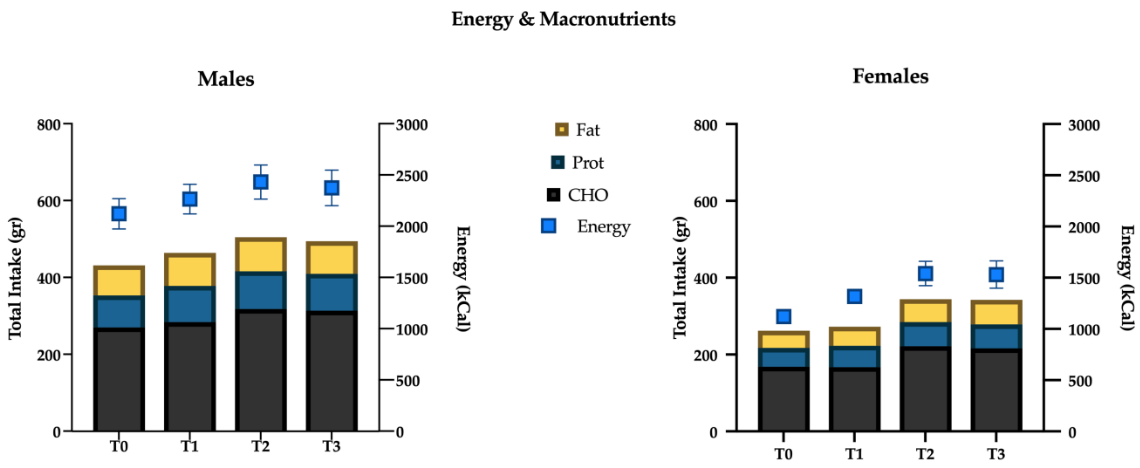

Figure 1.4. Energy intake (kcal) and macronutrients (gr) variations over time.

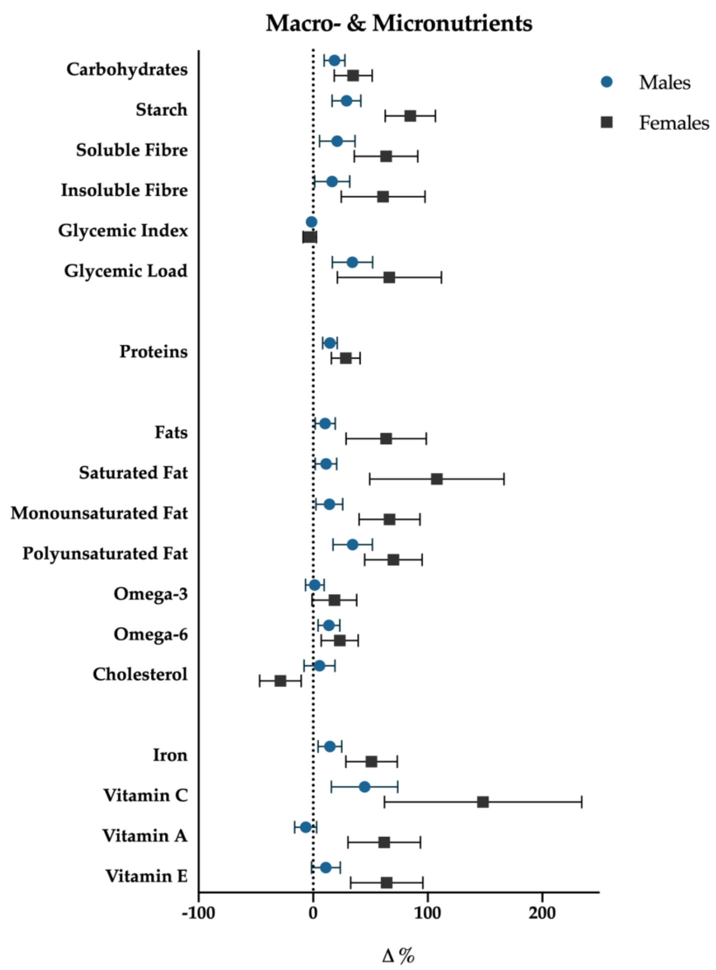

Figure 1.5. Macro- and micronutrients percentage variations (T3-T0); data are presented with standard error bars. Males are reported with blue dots, females with black squares.

For each of the above significant variables, considering simple contrast analysis among T0 values and subsequent mesocycles (T1, T2 and T3), it was possible to highlight the following results, which are depicted in Figure 1.5:

I) total energy intake increased significantly at the T1 mesocycle (+9.4%, p=0.001), and raised a +17.8% value at T3 (p<0.01); ii) negligible differences were found in the mean proportions of energy deriving from macronutrients intake in the different mesocycles: carbohydrate, fat and protein accounted for 49.6%, 33.1% and 17.6% over the entire experimental protocol, respectively;

II) the net intake of the macronutrients showed an overall increase in the course of the mesocycles. In particular protein intake increased at T1 (+12.9%, p=0.003) and reached a +18.5% value at T3 (p<0.01); fats and carbohydrates showed a similar trend with no significant increase at T1, +21.5% (p<0.01) and +19.2% (p<0.01) at T2, +19.4% (p=0.015) and +17.1% (p=0.036) at T3, respectively;

III) a more detailed analysis of lipid intake composition showed that saturated (T1: +12.4%, p<0.01; T2: +22.0%, p<0.01; T3: +21.4%, p=0.012) and monounsaturated fats (T1: +9.1%, p<0.01; T2: +15.8%, p=0.012; T3: +14.7%, p=0.023) increased in all the mesocycles, whilst polyunsaturated ones showed a significant increase only at T2 (+21.9%; p=0.016) and T3 (+27.4%, p=0.014). Although non-significant gender differences have been found with regard to total fat intake (p=0.25), females showed a slight positive trend in saturated fat consumption (p=0.07). Cholesterol showed a decrease at T1 (-11.1%, p<0.001), starch and glycemic load increased at T2 (+22.4%, p<0.01; +20.5%, p<0.001), and at T3 (+30.3%, p=0.001; +25.8%, p<0.001), respectively. Glycemic index decreased significantly in T3 (-2.6%, p=0.028). Finally iron (+17.7%, p=0.037) increased at T3.

1.3.2 Factor Analysis

The results of an orthogonal rotation (Varimax rotation) of the solution are shown in Table 1.2. The analysis yielded a three-factor solution; the Kaiser-Meyer Olkin measure of sampling adequacy was 0.785, above the minimum ideal value. Seven items loaded in Factor 1: these are all related to an increase in energy intake, due to augmented consumption of macronutrients (carbohydrates, proteins and fats), and Vitamin E. Three items loaded in Factor 2: increased intake of Omega-3, Omega-6 and Polyunsaturated fats. Finally, six items loaded in Factor 3, showing an increased intake of fibres, Vitamin A and C, starch and iron.

Table 1.2. Factor Analysis results.

Component	Factor 1	Factor 2	Factor 3
Δ Fat	**0.865**	0.278	0.043
Δ Protein	**0.821**	0.088	0.395
Δ Carbohydrate	**0.731**	-0.030	0.466
Δ Energy	**0.713**	0.037	0.517
Δ Monounsaturated fat	**0.694**	0.326	0.206
Δ Saturated fat	**0.647**	0.003	0.209
Δ Vitamin E	**0.629**	0.439	0.392
Δ Omega 6	0.140	**0.848**	0.206
Δ Omega 3	0.016	**0.714**	0.061
Δ Polyunsaturated fat	0.472	**0.697**	0.444
Δ Insoluble fibre	0.282	0.281	**0.857**
Δ Soluble fibre	0.135	0.286	**0.836**
Δ Vitamin C	0.275	0.043	**0.783**
Δ Vitamin A	0.268	0.161	**0.644**
Δ Starch	0.551	0.118	**0.583**
Δ Iron	0.515	0.168	**0.573**
Eigenvalues	**8.62**	**1.55**	**1.29**
% of total variance	**30.10**	**44.68**	**71.61**

1.4. Discussion

In this study, the influence of exercise training on habitual dietary patterns of young sedentary adults has been evaluated. Despite increased awareness, public health campaigns, and novel therapies, worldwide obesity in adults has greatly increased in the last ~40 years [27].

Lifestyle-based interventions focusing on diet and physical activity can produce a 7–10% drop in initial body weight and represent the cornerstone of weight-loss approaches [28]; however this condition is not maintained over time, with frequent weight gain relapses known as "yo-yo effect" [29]. A better understanding of the connection between the two integrated approaches, physical activity and eating behaviours, is necessary to prevent and limit the failure of long term- interventions. Notably, to authors' knowledge, little is known about how changes in one of these behaviours can support the long-term maintenance of the change in the other.

Physical exercise contributes to a negative energy balance by increasing energy expenditure, but the role of exercise on food intake is not yet fully understood. Changes in exercise behaviour can also influence nutrition habits by application of self-regulatory psychological resources across behaviours; in particular exercise can facilitate improved fruit/vegetable consumption in young adulthood, a life-period critical to lay the foundation, based on the adoption of a health behaviour, for the prevention of metabolic diseases and for securing a healthy future with advancing age [30]. Joo et al. [18] suggested that food choices differ depending on the type (intensity and duration) of the exercise performed. Despite this, to the authors' knowledge, scientific literature regarding the effects of different exercise modalities on dietary habits is still scarce.

HIIT has been proposed as an effective training modality to promote fat mass reduction while preserving free-fat mass, by increasing energy expenditure and improving appetite control

[31,32]. This potential anorexigenic effect does not seem to occur with moderate-intensity continuous training (MICT) [32]. HIIT has been reported to improve endurance performance to a greater extent than MICT, both in sedentary and recreationally active individuals. This may be due to an up-regulated contribution of both aerobic and anaerobic metabolism to the energy demand, a consequently enhanced availability of ATP and improved energy status of working muscles [33]. Moreover, interval exercise has been shown to produce a higher EPOC (excess post-exercise oxygen consumption) respect to moderate-intensity exercise; this is proposed to be one of the factors that induce higher weight-loss after interval exercise [34]. Importantly, different HIIT protocols may produce different adaptive responses to the exercise: training parameters such as duration and intensity of work and recovery phases can be manipulated to conveniently and precisely adapt HIIT to different sub-populations varying in age, gender, BMI and healthiness features [19].

1.4.1. Body composition

No changes in weight have been observed throughout the study; on the contrary, the free-fat mass showed an increase in the last mesocycle. The first six weeks of exercise, indeed, did not produce any change in body composition, but in the last three weeks – i.e. when the frequency of training augmented up to five sessions per week – an increase in muscle mass has been found. The increase in training load in the third mesocycle is not accompanied by a higher energy intake in comparison to the previous weeks, as happened comparing the first mesocycle with baseline, and the second mesocycle with the first. As previously reported by Scheurink et al. [35], the exercise-induced increased energy expenditure is followed by a compensatory increase over the long term; despite this, the not-augmented energy intake in the third mesocycle reported herein supports the hypothesis that a metabolic adaptation may occur. Furthermore, it is also possible that the influence of these factors may differ between sedentary subjects (which were enrolled in our study) and athletes by virtue of their particular physiological and psychological adaptations that include an improved ability to regulate energy balance and/or increased sensitivity to satiety signals [36].

The reduction of fat mass shown after the third mesocycle is in accordance with previous studies [37,38] that found a decrease in body fat mass only with higher training frequency (3 times/week vs. 1 or 2 times/week). It was concluded that the training frequency is associated with fat mass loss. Accordingly, an increase in free-fat mass after a HIIT period was reported by other Authors [21,39], even though a limitation of these two studies was that they focused on overweight and obese women. Finally and along the same line, HIIT has been demonstrated to be efficient in improving body composition in normal-weight subjects [40,41].

1.4.2 Energy and macronutrients intake

As previously discussed, an increase in the energy intake has been reported in the first two mesocycles of training respect to baseline control, but no further increase has been shown in mesocycle three; macronutrients proportions remained relatively stable over time. These results are consistent with previous data by Miguet et al. [32], who described an augmented energy intake after 16 weeks of training, in obese adolescents; moreover, these authors reported that - although the overall intake (grams) of macronutrients increased following the exercise intervention - the proportions remained approximately equal to pre-training. A slight increase in fat consumption was found in females [32]: notably we found a similar trend in women. High-intensity exercise has been shown to reduce the spontaneous energy intake during lunch and dinner following the exercise bout in obese adolescents, with a positive effect on the 24-hours energy balance [42]. This anorexigenic effect in obese adolescents has been confirmed by other authors [32,42] and this adaptation was due to peripheral (mainly gastro-peptides) and neurocognitive (neural responses to food cues) pathways [43]. Also lactate has been found to be a key suppressor of energy intake, maybe due to the interaction of this molecule with glucose sensors in the central nervous system, suggesting that high-intensity exercises may have greater anorexigenic effect as compared to low-intensity ones [44].

However, for the sake of completeness, Hazell et al. [39] did not find any change in overall energy nor macronutrients intake, after six weeks of sprint interval training respect to pre-training. Furthermore, the studies [45] focusing on the effect of physical exercise on appetite did not reach a definitive consensus: indeed, inconsistent results have been obtained regarding the effect of physical exercise to alter macronutrients and micronutrients intake. To this regard, it could be speculated that a major limitation in these studies likely contributing to the inconsistency of the results may reside in self-reported food diaries and questionnaires on food frequencies, together with short time data collection. Indeed, self-reported food diaries are susceptible to underreporting from participants, while food frequency questionnaires present a lower underreporting risk; moreover both these approaches are aimed to evaluate dietary intake of nutrients and foods over a given period, but are not reliable for the daily quantification of energy intake [46]. The 24-hour recall shows underreporting levels of about 11% [47], but using the same method for multiple days has been shown to reduce underreporting to 4%, after 3 days [48]. Furthermore, by comparison of total energy expenditure by doubly labelled water, 24-hour recalls result less underreporting than food frequency questionnaires [49]. In conclusion, the multiple-pass 24-hour recall method we used in a period longer than 3 months is likely to be the best strategy. Finally, it should be considered that any strategy used to record food intake may lead participants to modify their eating habits [46].

1.4.3 Factor Analysis

An augmented intake of all macronutrients characterised Factor 1: this led to an absolute increase in energy intake. So, this factor could be summarised as just "eat more". Factor 2 was defined by increased consumption of polyunsaturated fatty acids, especially omega-3 and -6: a greater intake of these nutrients could be explained by more fish, vegetable oils, nuts and seeds into the diet. Partially overlapping results were found in 2013 by Hiza et al. [50] who, analysing a one-day diet of a sample of 8272 subjects, reported greater consumption of oils of vegetable origin together with lower consumption of saturated fatty acids in females, compared to male subjects. Finally, Factor 3 was marked by higher consumption of fibres, vitamin C and A, starch and iron: these changes could be traduced to an augmented consumption of fresh fruit and vegetables and cereals. Johnson et al. [51] reported a common pathway between physical activity, fruit and vegetable consumption and mental well-being. Five or more servings of fruit and vegetables may be sufficient to reduce oxidative stress and provide other health benefits, without impairing training adaptations [52]. Bellisle [11] highlighted that active persons eat more and ingest more fruits and vegetables than less active ones, even though it is not known whether these food choices are driven by biological needs (e.g. replacement of glycogen) or elicited by social and psychological factors. Furthermore, maintaining a positive iron balance is essential to avoid the effects of iron deficiency and anaemia, and to support performance in athletes [53].

In order to determine and prescribe the best protocols to reach a healthy weight, better knowledge of the complex interaction between physical activity and eating behaviours is needed [54]. Behavioural changes occur when an external imposition, aimed at achieving a goal, is internalized by the subject. These changes are due to brain plastic processes and modification in neurobiological substrates, that provoke a neurocognitive remodelling. Physical activity and healthy choices can likely share the same pathways involving "top-down" inhibitory control, contributing to neurocognitive processes needed for effective behaviour changes [17]. Andrade et al. [15] demonstrated that women who received 12-months of behavioural intervention, including moderate and vigorous exercise had reduced the emotional overeating through eating self-regulation, suggesting that an active lifestyle can contribute to long-term weight management. Physical exercise can alleviate stress-induced unhealthy food choices [55]. Physical activity and dietary habits are likely interconnected and together could represent good strategies for sustainable weight management.

This study was subjected to some limitations. The sample size was small; however, to the authors' knowledge, this is the only study that daily monitored participants' diets during a structured training period. Another limitation of the present study might be that we did not specifically consider and monitor – as possible factors indicative of a "healthier dietary habit" – the presence of nutraceuticals in the diet. Indeed, although lacking an internationally accepted definition and official classification, nutraceuticals (i.e. a group of products that are more than food but less than pharmaceuticals) are increasingly considered as important constituents of a healthy diet [56]. The amount of nutraceuticals in the diet is mostly raised through the consumption of enriched foods or dietary supplements: here we asked our participants to abstain from taking dietary supplements and/or fortified foods to avoid a factor capable of influencing their instinctive diet preferences and complicating the analysis of food constituents. Hence, we deliberately excluded today's most common sources of nutraceuticals from our investigation. However, factor analysis of our data showed an increased consumption of omega-3 and -6 fatty acids, fibres, which are often referred to as "nutraceuticals'' [57]: in this light we can infer that their consumption tends to increase as an implicit consequence of a healthier food choice. Along this line, it would be interesting to see whether, in the absence of the specific prescription adopted herein, HIIT leads participants to increase dietary supplements/fortified foods consumption.

1.5. Conclusions

Physical activity and eating patterns are not independent and are closely related. Our results demonstrated that a nine weeks HIIT, progressively incremented in frequency and duration of the sessions, is capable of promoting a spontaneous and unaware modulation of food choices in young subjects, leading to a healthier diet. The factor analysis of macro and micronutrients evidenced three subgroups with different diet habit patterns: one that just "eats more", a second one that eats more fish, vegetable oils, nuts and seeds and a third one that eats more fresh fruit and vegetables and cereals. Although different subgroups have been identified, the overall trend goes towards a preference for healthier food choices. Behavioural changes involving physical activity and more healthy food choices are conditions sine qua non to treat and counteract obesity and related diseases.

Chapter 2

Dietary Habits and Psychological States during COVID-19 Home Isolation in Italian College Students: The Role of Physical Exercise [2]

Abstract: Social isolation has adverse effects on mental health, physical exercise, and dietary habits. This longitudinal observational study aimed to investigate the effects of mood states and exercise on nutritional choices, on 176 college students (92 males, 84 females; 23 ± 4 years old) during the COVID-19 lockdown. During 21 days, nutrition and exercise were daily monitored, and the mood states assessed. A factor analysis was used to reduce the number of nutritional variables collected. The relationships between exercise, mood and nutrition were investigated using a multivariate general linear model and a mediation model. Seven factors were found, reflecting different nutritional choices. Exercise was positively associated with fruit, vegetables and fish consumption ($p = 0.004$). Depression and quality of life were, directly and inversely, associated with cereals, legumes ($p = 0.005$; $p = 0.004$) and low-fat meat intake ($p = 0.040$; $p = 0.004$). Exercise mediated the effect of mood states on fruit, vegetables and fish consumption, respectively, accounting for 4.2% and 1.8% of the total variance. Poorer mood states possibly led to unhealthy dietary habits, which can themselves be linked to negative mood levels. Exercise led to healthier nutritional choices, and mediating the effects of mood states, it might represent a key measure in uncommon situations, such as home-confinement.

2.1. Introduction

The novel coronavirus SARS-CoV-2, responsible for the COVID-19 epidemic, was first identified in Wuhan (China) in December 2019. In Italy, the first official cases appeared in late February, and on 9 March, the Italian government placed about 60 million people in a de facto quarantine mode, being asked to live in home-confinement for several weeks, until 4 May.

In the last months, several articles have been published regarding the effects of the home confinement and social-isolation period on different domains: psychological states [58,59], physical activity [60], nutrition [61,62] or the integration of these [3,63-65]. Mood disturbances, such as anxiety, depression, anger and irritability, have been previously reported in several studies during quarantine periods, as reviewed by Brooks et al. [66]. Ammar et al. [58] conducted an online survey during the COVID-19 outbreak on over a thousand people in home confinement, reporting reduced mental well-being and satisfaction and increased depression and need for psychological support, compared to the pre-epidemic period. The increased adverse psychological effects were associated with a reduction in physical activity and an increase in unhealthy diet behaviours [3].

2.1.1. Mood and Nutrition

The association between diet and mood has been recently systematically reviewed by Arab et al. [67]. The food intake can be involved in regulating mood and emotions, and this can affect

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[2] Published: Amatori, S.; Donati Zeppa, S.; Preti, A.; Gervasi, M.; Gobbi, E.; Ferrini, F.; Rocchi, M.B.L.; Baldari, C.; Perroni, F.; Piccoli, G.; Stocchi, V.; Sestili, P.; Sisti, D. Dietary Habits and Psychological States during COVID-19 Home Isolation in Italian College Students: The Role of Physical Exercise. *Nutrients* 2020; 12, 3660. doi: 10.3390/nu12123660.

the food choices suggesting a bidirectional influence [68]. Indeed, low mood and poor nutrition are mutually connected, one influencing the other and vice versa [69]. For example, it has been reported an alteration in food choices, not always healthy, in response to different psychological states or stress conditions. For example, foods rich in fat and carbohydrate are commonly preferred to be consumed by depressed subjects [70]. On the flip side of the coin, healthy dietary patterns (e.g., Mediterranean diet) are associated with a reduced risk of depression and better mental health [71]. For instance, an inverse association between vegetables and fruits consumption with depression mood has been previously found [72]. Furthermore, the intake of nuts containing unsaturated fatty acids, polyphenols and vitamins may have protective effects against mood and cognitive disorders [73,74]. Lassale et al. [71] have been able to synthesise the link between diet quality, measured using a range of predefined indices, and depressive outcomes, concluding that adherence to a healthy diet, in particular a traditional Mediterranean diet, or avoiding a pro-inflammatory diet, appears to confer some protection against depression in observational studies. This provides a reasonable evidence base to assess the role of dietary interventions to prevent depression [71].

2.1.2. Exercise and Nutrition

The phenomenon known as "multiple health behaviour change" defines the interrelation between health-related behaviours. This concept can be summarised as: the higher the probability of one health-related behaviour, the higher the probability for the other [75]. Indeed, physical exercise and diet were reported to be strictly interconnected [76]. Joo et al. [18] reported a modification in the dietary patterns of young adults after 15 weeks of training. They highlighted that, even if different effects were produced depending on the duration and intensity of the exercise, the overall trend was towards healthier food selection. Donati Zeppa et al. [1] reported that high-intensity exercise promoted a spontaneous and unaware modulation of food choices, leading to a healthier diet in young adults. Several mechanisms have been proposed to explain this phenomenon, as improved appetite control and a reduction in emotional overeating [15]; healthy nutritional choices and physical exercise seem to share the same pathways which lead to effective behavioural changes towards a healthier lifestyle [17].

2.1.3. Mood, Exercise and Nutrition

The interaction between exercise, improved psychological states and healthier eating behaviours was previously proposed by Annesi et al. [77], who reported a reduction in negative moods, an increase in self-regulate eating ability, and a clinically relevant reduction in weight, after six months of moderate-intensity exercise in obese women. Regular exercise practice was shown to positively influence mood, self-efficacy and use of self-regulatory skills, key predictors of controlled eating [78]. However, the direction of relationships between psychological and behavioural variables is difficult to assess. Annesi and Mareno [77,79] used a mediation model to study these interactions, suggesting a psychological pathway between increased exercise and decreased emotional eating, so highlighting the role of exercise in facilitating nutrition changes.

2.1.4. The COVID-19 Context

Given the above, the effects of unique conditions—as it has been the COVID-19 outbreak—on psychological states, dietary behaviours and physical activity have been a topic of great interest. Antunes et al. [63] conducted a survey on a sample of Portuguese adults during the epidemic, reporting altered eating habits, with increased quantity and frequency of meals; contrasting results are presented regarding the attention to the food selection. Di Renzo et al. [80] investigated the impact of the COVID-19 pandemic on eating habits and lifestyle changes among the Italian population aged ≥12 years, reporting a higher adherence to the Mediterranean diet in the group aged 18–30 years, compared to the younger and the elderly population. Gallo et al. [64]

investigated the impact of isolation measures on energy intake and physical activity levels in Australian university students, reporting a reduction in physical exercise levels, and an increase in energy intake and snacking frequency. Interestingly, Romero-Blanco et al. [81] reported that—during home-confinement in Spain—university students that adopted a Mediterranean diet, also spent more time doing physical exercise. Ingram et al. [4] investigated the changes in health behaviours related to negative mood states in a sample of about four hundred adults during the COVID-19 quarantine in the UK, reporting changes in diet, sleep quality, physical activity levels and poorer mental health. Most of the studies above reported had cross-sectional designs, performed through questionnaires or online surveys. It was previously identified that surveys and self-reported food diaries with a short time of data collection are likely to underreporting from participants; however, the 24 h recall for multiple days significantly reduces this effect [1].

The present longitudinal observational study aimed to investigate the effects of mood states and exercise on nutritional choices of a sample of college students, during the period of forced home-isolation due to COVID-19 outbreak. A daily follow-up for three weeks has been done, recording both dietary habits and physical exercise of the participants.

2.2. Materials and Methods

2.2.1. Participants

A convenience sampling was used in this study. Participants were college students, pertaining to three different study courses (pharmacy, biology and sports sciences) at the Urbino University, following online classes during the data collection period. During the lockdown period, people were allowed to leave home only for health issues, to go to work or to buy food and other essential products (only one family member at the time): therefore, people's movements were limited to the minimum necessary. Bars and restaurants were closed, so people were forced to eat at home. They were invited to take part in the study through verbal explanation of the project during an online class, and later, an email was sent to each of them. All participants read and signed a written informed consent to take part in the study, which was conducted in accordance with the Helsinki Declaration. They were free to leave the study at any time, without giving any further justification. Once data was collected by the researcher, names were removed and an univocal identity code assigned to every participant, to secure anonymity. Ethics committee approval has been received by the Human Research Ethical Committee of the Urbino University (No. 31_2020).

2.2.2. Data Collection

Data collection was conducted during three weeks of home-isolation due to the COVID-19 pandemic in Italy, between 6–26 April 2020 (Figure 2.1). Before starting to record the diet, participants were asked to add in a pre-structured form their height and weight, if they were spending the isolation period at home with their families, with friends or alone, and if they were used to regularly practicing sports activity before the COVID-19 epidemics. Exclusion criteria were being on a particular dietary regimen (e.g., prescribed by a nutritionist), suffering from chronic diseases, being afflicted by COVID-19 before or during the period, and having a flu during the data collection period.

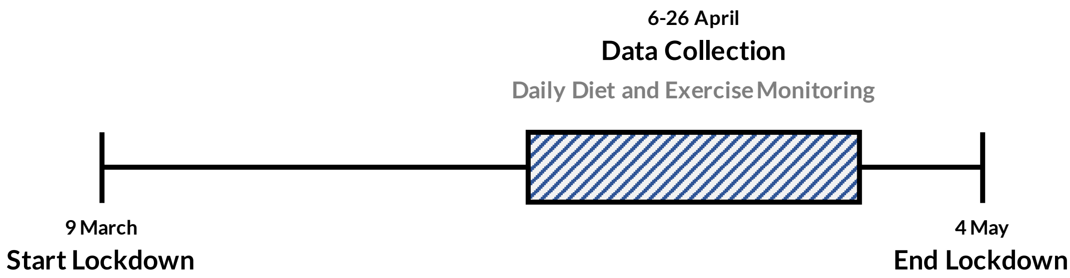

Figure 2.1. Study timeframe.

2.2.3. Dietary Habits

Participants were asked to daily fill a pre-structured food diary, in which they inserted each food or drink consumed during the whole 24 h, with the quantity (expressed in pieces, e.g., one banana, or grams, e.g., 100 g of pasta). A daily reminder to fill the food diary was sent to every participant during the study period. Diet monitoring has gone on for 21 days (three weeks). After the completion of the data collection, data were manually screened and uploaded on the MètaDieta software (METEDA Srl, San Benedetto del Tronto, Italy), used for the data processing. This software was previously used for monitoring the diet in university students [1]. It automatically calculates the total energy intake and the nutritional values, with macro- and micronutrients quantities, for each food or drink consumed during the day. The following variables were selected for the analyses: carbohydrates (glucides, starch, glycaemic index, glycaemic load), protein (animal-based protein, plant-based protein, protein value), lipids (animal lipids, plant lipids, mono-, polyunsaturated and saturated fats, Omega 3 and 6), fibre (soluble and insoluble), amino acids (tryptophan, phenylalanine, tyrosine) and vitamins (A, B6, B12, C, D, E, and folic acid). Furthermore, the distribution of energy intake among the different meals throughout the day has been evaluated.

2.2.4. Physical Exercise

On a separate sheet of the food diary, participants were instructed to add if they trained, with the duration in minutes and the perceived effort (RPE) using the modified CR-10 scale by Foster et al. [82], for each day. The CR-10 scale asked participants to rate their effort answering the simple question "How was your workout?" on a scale from 0 "rest" to 10 "maximal". The participants were already familiarised with the use of this scale; furthermore, verbal and written instructions on the use of the CR-10 scale and the diary have been provided to all the participants before the beginning of the study. Duration and intensity values were used to calculate the training load, as SessionRPE (duration × intensity) [83]. This method was reported to be superior to monitor training loads of fitness workout sessions compared to other heart-rate based methods [84]. The training load has been used as the measure of exercise performed for the statistical analyses. Due to the home isolation restrictions, people were not allowed to go out to do physical exercise; assuming that other forms of physical activities were minimal in this situation, only structured exercise sessions (e.g., runs or rides on treadmill or bike trainers, free-weight exercises) were taken into account.

2.2.5. Psychological Scales

2.2.5.1. Positive and Negative Affect Schedule (PANAS)

The PANAS is a tool that assigns scores based on feeling or emotion-related adjectives [85]. Wordings are rated as positive when it refers to positive emotions and positive interactions with others (e.g., interested, strong, proud). Wordings are rated negative when they involve negative experiences with the world and others (e.g., hostile, nervous, afraid). The brief 20-item scale was used in this study. On each adjective, respondents assign a score on a Likert-type scale ranging from 1 (very slightly) to 5 (extremely). This tool was previously used with a university student population [86]. The Italian validated version of the PANAS was used in this study [87].

2.2.5.2. Patient Health Questionnaire 9 (PHQ-9)

The PHQ-9 is a self-administered questionnaire aimed at surveying the nine main criteria for major depression [88,89]. Participants have to rate, on a 4-point scale (from 0 "not at all" to 3 "nearly every day"), how often they have been bothered by the nine criteria over the last two weeks (e.g., "Little interest or pleasure in doing things", "Feeling down, depressed, or hopeless", "Feeling tired or having little energy"). A global score was calculated as a sum of the scores on each item. This questionnaire was validated for depression screening in university students [90]. For this study, we used the validated Italian version of the scale [91]. An oft-used scoring threshold for the presence of depression is: 0–4 = none, 5–9 = mild, 10–14 = moderate, 15–19 = moderately severe, ≥20 = severe.

2.2.5.3. 12-Item Short Form Health Survey (SF-12)

The SF-12 is a self-report measure of the impact of health conditions on a person's everyday life [92]. It is used as a measure of health-related quality of life (HR-QoL), previously used with university students [93]. The tool covers eight main domains, and these domains can be grouped into two main areas: mental-depending HR-QoL (e.g., "How much of the time during the past 4 weeks have you felt calm and peaceful?") and physical-depending HR-QoL ("During the past 4 weeks have you accomplished less than you would like with your work or other regular activities as a result of your physical health?"). A global score of HR-QoL can also be calculated by summing the scores on each item. Higher scores on the SF-12 correspond to higher HR-QoL. In this study, we used the validated Italian version of the SF-12 [94].

2.2.6. Statistical Analyses

Descriptive statistics were reported for macronutrients and demographics characteristics. Mean (standard deviation) was used for demographic variables; median (first (Q1) and third (Q3) quartiles) for training frequencies (sessions/week), psychometric scales' scores and percentage of energy intake distributed in the different meals throughout the day (breakfast, morning snack, lunch, afternoon snack and dinner). From the list of macro- and micronutrients obtained from the Metadieta software, given the high number of variables collected, it was appropriate to reduce their number, minimising the loss of information. So, a Principal Axis Factor with a Varimax (orthogonal) rotation of 27 nutritional variables above considered was conducted on data gathered from participants. Kaiser–Meyer Olkin (KMO) measure of sampling adequacy was also performed; the minimum acceptable value for KMO is 0.6, although the ideal is over 0.70. Only factors with an eigenvalue of ≥1 were considered. The variance percentage accounted for by each component to the total variance was also reported. The factor score coefficient matrix was also computed. The resulting component score variables are representative of—and can be used in place of—original variables with an acceptable loss of information (1-total cumulative variance).

The internal consistency reliability index for the mood states questionnaire was quantified using Cronbach's alpha. Cronbach's alpha is a measure of internal consistency, that is how closely related a set of items are as a group. It is considered 'moderate' when it is >0.6 and 'good' when it is >0.7 [95].

In order to investigate whether psychological states and exercise could be associated with the nutritional choices, a multivariate generalised linear model was performed. The dependent variables were the factors obtained from factor analysis; participant's repeated measures (ID) was a random factor; the fixed factors was sex; finally, training load, mood scales (PANAS, PHQ-9, SF-12) and BMI were covariates. Pillai's trace statistic was used; Pillai's trace is more robust to departures from assumptions than Wilk's lambda, Hotelling's trace, and Roy's largest root. Subsequent univariate ANOVAs were also performed for each dependent variable (factors) considered.

Finally, in order to assess if exercise could mediate the effect of psychological states on nutrition, a mediation effect has been calculated. A mediated effect is also called 'indirect effect', and it occurs when the effect of the independent variables (psychometric scales) on the dependent variable (factors' scores obtained by factor analysis) is—as the name says—mediated by another variable: a mediator (training load). Mandatory condition to establish any mediation effect is that the independent variable (IV) must have a significant effect on the mediator. In mediation analysis, we considered as independent variables the same variables reported in GLM analysis. In mediation analysis, the following estimations are reported:

- Average causal mediation effect: it is the indirect effect of the IV (psychological states) on the DV (factors scores) that goes through the mediator (training load).
- Average direct effect: it describes the direct effect of the IV on the DV, without mediator effect.
- Total effect: it stands for the total effect (direct + indirect) of the IV on the DV.
- Proportion Mediated: it describes the proportion of the effect of the IV on the DV that goes through the mediator. It is calculated by dividing the mediation effect through the total effect.

All elaborations were conducted with $\alpha = 0.05$. Statistical analyses were performed using SPSS 22.0 (IBM, Armonk, NY, USA), except from the mediation effect that has been calculated with the *mediation* v.4.5.0 package [96] using R Studio v. 1.2.5033 software. GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA) has been used to build the figures.

2.3. Results

2.3.1. Sample Characteristics

A total of 250 students were invited to participate in the research. Of these, 176 (response rate: 70.4%) participants (23 ± 4 years old) voluntarily took part in this observational study; all the participants correctly fulfilled the three week daily food diary. Of these 92 were males (178.5 ± 6.3 cm, 73.1 ± 9.8 kg, 22.9 ± 2.6 kg/m^2) and 84 were females (164.6 ± 6.6 cm, 58.2 ± 7.5 kg, 21.4 ± 2.5 kg/m^2). 141 of them reported practising sports activity, at different levels (from beginner to professional), with a median of four training sessions/week (Q1: 3, Q3: 5). Almost all of them (except seven people) reported having spent the whole lockdown period in their respective residences, with their families.

2.3.2. Dietary Habits

Energy intake and macro- and micronutrients were analysed daily. Average energy intake of 1742 (±688) kcal was reported in males, and 1237 (±486) kcal in females. Macronutrients were distributed in the diet as 48% carbohydrates, 30% lipids and 22% proteins. Energy intake was spread out during the day as so: (median, Q1–Q3): breakfast 14.0% (5.8–22.0%), morning snack 0.0% (0.0–2.0%), lunch 38.0% (29.0–50.0%), afternoon snack 6.0% (0.0–13.0%) and dinner 33.0% (22.0–44.0%). In Figure 2.2 are reported the average macronutrients (in absolute value, gr) and energy intake (kcal), split for gender.

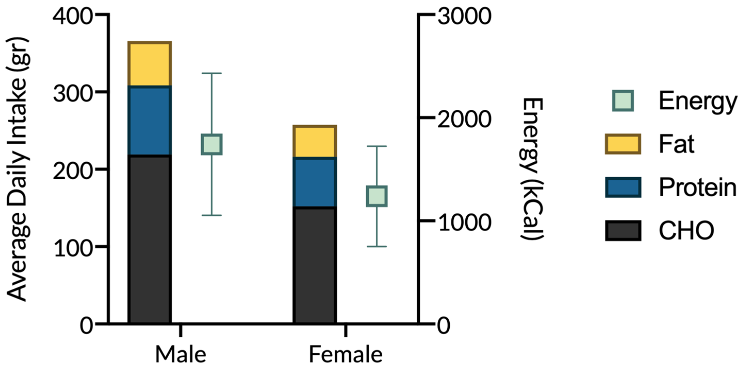

Figure 2.2. Daily macronutrients (gr) and energy (kcal) intake, for male and female.

2.3.3. Factor Analysis

A Principal axis factor analysis with varimax rotation has been conducted to assess the underlying structure in micro and macronutrients. The analysis yielded a seven-factor solution; the Kaiser–Meyer Olkin measure of sampling adequacy was 0.783, above the minimum ideal value. Table 2.1 presents the rotated component matrix, eigenvalues, and the percentage of variance explained; note that the seven components explain 77.1% of the variance.

Table 2.1. Factor analysis results. Only the components' values significantly related to each factor are reported.

	Factors						
Component	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Starch	0.883						
Carbohydrates	0.832						
Glycaemic load	0.821						−0.361
Plant-based protein	0.751			0.373			
Glucides	0.656		−0.450				
Vegetable fats		0.826					
Omega-6		0.747	0.362				
Monounsaturated fats		0.738	0.526				
Polyunsaturated fats		0.714				0.378	
Vitamin E		0.620		0.597			
Animal lipids			0.842				
Saturated fats		0.366	0.795				
Animal-based protein			0.659		0.483	0.378	
Protein value			0.559	0.377			
Vitamin C				0.767			
Folic acid				0.730			
Vitamin A				0.568			
Vitamin B6			0.357	0.555			
Insoluble fibre	0.368			0.549			0.520
Tryptophan					0.913		
Phenylalanine					0.903		
Tyrosine			0.395		0.830		
Vitamin D						0.864	
Omega-3						0.798	
Vitamin B12						0.537	
Glycaemic Index							−0.791
Soluble fibre	0.435			0.518			0.541
Eigenvalues	**8.64**	**3.94**	**2.52**	**1.97**	**1.36**	**1.23**	**1.13**
% of cumulative variance	**32.0**	**46.6**	**55.9**	**63.3**	**68.3**	**72.9**	**77.1**

Seven variables loaded in Factor 1, accounting for 32.0% of the variance; a high intake of carbohydrates characterised it with elevated glycaemic load (including both glucides and starch), plant-based protein and fibre. This factor may be mainly due to cereals, and secondly to legumes consumption. Six variables loaded in Factor 2, accounting for 14.6% of the variance; these were related to vegetable-fats consumption (monounsaturated, polyunsaturated, and saturated fats), vitamin E and Omega-6. This factor might be interpreted as dried fruits and vegetable oils intake, as olive oil. Nine variables loaded in Factor 3, accounting for 9.3% of the variance; it was characterised by a high intake of animal-based protein and lipids, with high protein value, saturated and monounsaturated fats, Omega-6, vitamin B6 and Tyrosine, but showed a negative load for glucides consumption. This Factor could be summarised by milk, its derivatives and high-fat meats. Nine variables loaded in Factor 4, accounting for 7.4% of the variance; it was characterised by a prevalence of hydrosoluble (B6, C and folic acid) and liposoluble vitamins (A, E), fibres and plant-based protein with a high protein value. These items lead to fresh vegetables and fruit consumption (in particular wheat germs). Four variables loaded in Factor 5, accounting for 5.0% of the variance; it was characterised by a significant amino acid intake (tryptophan, phenylalanine, tyrosine) and animal-based proteins. Given that this factor is not affected by fats, it could be hypothesised that lean (low-fat) meats represent it. Five variables loaded in Factor 6, accounting for 4.6% of the variance; with a high intake of Omega-3, vitamin B12, vitamin D, animal-based proteins, and polyunsaturated fats, this factor could be easily interpreted as fish consumption. Finally, four variables loaded in Factor 7, accounting for 4.2% of the variance; it was characterised by soluble and insoluble fibres, with negative weights for glycaemic index and load. This Factor could be explained with whole-grain cereals consumption and other vegetables rich in fibres. The above-described factors are graphically summarised in Figure 2.3.

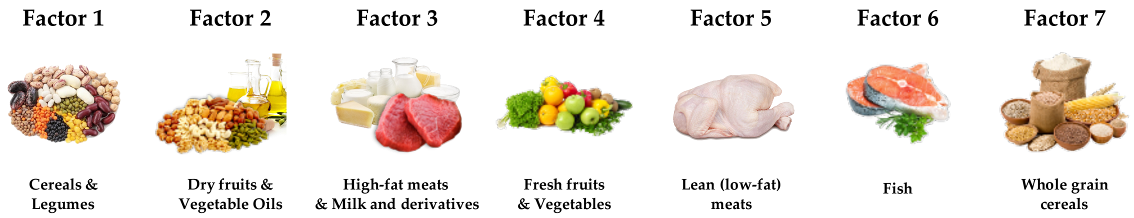

Figure 2.3. Factors possible explanations.

2.3.4. Physical Exercise

The exercise was daily monitored by calculating the training load as SessionRPE [83]. On average, participants trained 4.6 ± 3.3 times/week; the mean duration was 54 ± 41 min, with an RPE of 6.6 ± 1.8. As people were not allowed to go outside for training, exercise sessions mainly included indoor walks, runs or rides (on treadmill or bike trainers), rope jumping and free-weight exercises.

2.3.5. Psychological States

A total of 81 (88%) males and 78 (92.9%) females fully responded to the questionnaires.

The PHQ-9 scores had a median of 6 (Q1: 3, Q3: 8) for males and 7 (Q1: 6, Q3: 10) for females. A total of 28 males (30.4%) and 9 females (10.7%) were classified as 'none symptoms'. A total of 41 (44.6%) and 49 (58.3%) participants had mild symptoms. A total of 11 (11.9%) and 16 (16.1%) reported moderate symptoms. A total of 1 (1.1%) male and 3 (3.6%) females reported moderately severe symptoms, and only 1 female (1.2%) had severe symptoms of depression. Median for the PANAS positive affect score was 31 (Q1: 26, Q3: 37) for males and 27 (Q1: 23.75, Q3: 34) for females, while for the PANAS negative affect score median was 18 (Q1: 15, Q3: 23) for males and 23 (Q1: 18.75, Q3: 29) for females. The SF-12 score was calculated as the total score. The median for the SF-12 score was 39 (Q1: 35, Q3: 41) for males and 37 (Q1: 34, Q3: 40) for females.

The alpha for PHQ-9 was 0.69 (reasonable internal consistency reliability), whilst alpha for PANAS was 0.63 (minimally adequate internal consistency reliability); finally alpha was 0.78 (good internal consistency reliability) for SF-12.

2.3.6. Effects of Mood and Exercise on Nutrition

A multivariate analysis of variance was conducted to assess if gender, BMI, training load and mood states were significantly associated with the linear combination of seven latent factors deriving from factor analysis. No different effects of training volume (duration) or intensity (perceived exertion) have been found, so training load was considered as the only measure of exercise performed. Box's Test of Equality of Covariance Matrices showed a $p < 0.001$, so Pillai's trace statistic was used. A significant difference was found for all predictor variables: gender, with Pillai trace = 0.019, $F_{(2593,7)} = 7.02$, $p < 0.001$; ID, with Pillai trace = 1.474, $F_{(18193,861)} = 5.63$, $p < 0.001$; PANAS positive score, with Pillai trace = 0.008, $F_{(2593,7)} = 2.69$, $p = 0.009$; PANAS negative score, with Pillai trace = 0.006, $F_{(2593,7)} = 2.14$, $p = 0.037$; SF12, with Pillai trace = 0.007, $F_{(2593,7)} = 2.69$, $p = 0.009$; PHQ-9 total score, with Pillai trace = 0.009, $F_{(2593,7)} = 3.26$, $p = 0.002$; BMI, with Pillai trace = 0.008, $F_{(2593,7)} = 3.05$, $p = 0.003$ and finally training load, with Pillai trace = 0.008, $F_{(2593,7)} = 2.94$, $p = 0.004$. In subsequent ANOVA analysis, ID was always significant for all dependent variables; this implies that the nutrition pattern is a personal characteristic with a large effect size (eta squared = 0.211).

Follow up univariate ANOVAs indicated that BMI (t = 1.97; $p = 0.049$), PHQ-9 (t = 2.83; $p = 0.005$) and PANAS positive (t = 3.01; $p = 0.003$) were positively correlated to Factor 1, whilst SF-12 (t = −2.88; $p = 0.004$) and gender (male = 0, female = 1; t = −4.96; $p < 0.001$) was negatively correlated. Factor 2 was associated only with ID, as above reported. Considering Factor 3, gender (t = −2.61; $p = 0.009$), PANAS negative (t = −1.99; $p = 0.046$) and training load (t = −2.24; $p = 0.025$) showed negative correlations whilst BMI (t = 2.18; $p = 0.030$) showed positive one. Factor 4 was positively correlated only to training load (t = 2.88; $p = 0.004$). BMI (t = 2.48; $p = 0.013$), PHQ-9 (t = 2.06; $p = 0.040$) and PANAS positive (t = 2.11; $p = 0.035$) were positively correlated to Factor 5, whilst SF-12 (t = −1.98; $p = 0.048$) and gender (t = −2.11; $p = 0.035$) were negatively correlated. Factor 6 was positively correlated to PANAS negative (t = 2.57; $p = 0.010$) and training load (t = 2.13; $p = 0.033$) and negatively correlated to PHQ-9 (t = −2.31; $p = 0.021$). Finally, as Factor 2, Factor 7 was associated only with ID. These results are graphically presented in Figure 2.4.

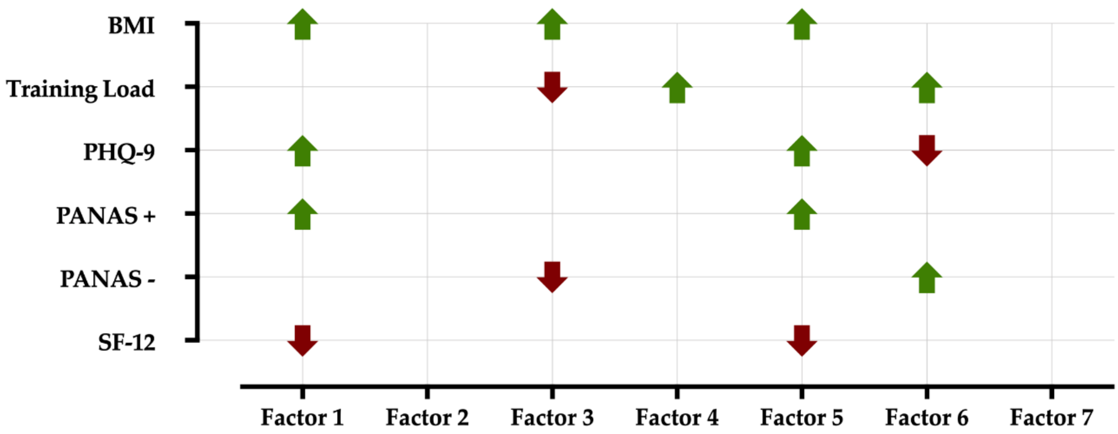

Figure 2.4. Interactions between Factors (dependent variables) and Body Mass Index (BMI), training load, and psychometric scales (PHQ-9, PANAS +, PANAS −, and SF-12). Green arrows show positive correlations, while red arrows negative correlations. Only significant interactions have been reported ($p < 0.05$).

2.3.7. Mediation Effect

The mediation effect of exercise on psychometric scales has been investigated. As presented in the hypothetical model in Figure 2.5, we assumed that the influence of mood (psychometric scales) on the nutritional choices (factors above presented), might be mediated by the training load.

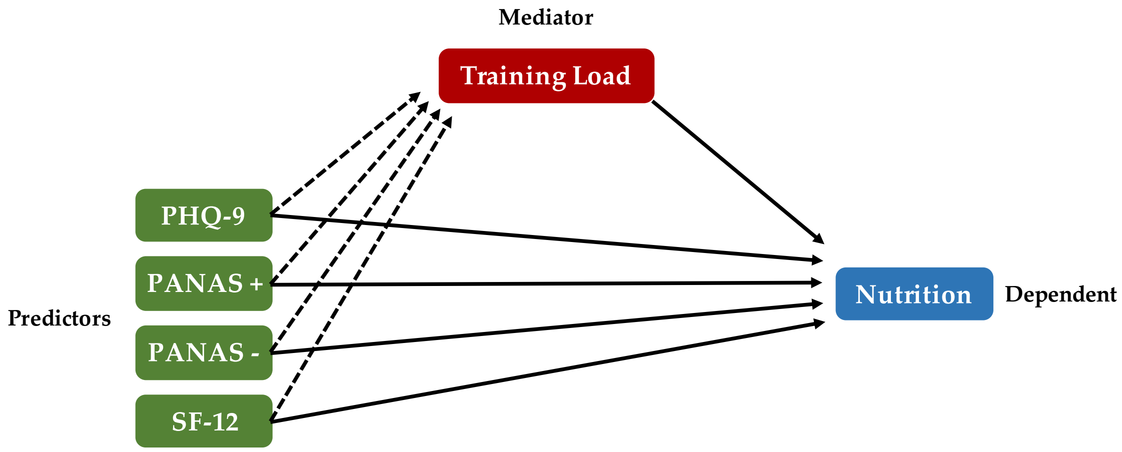

Figure 2.5. The theoretical model of the mediating effect of training load on the relationship between psychological states and nutritional choices. Solid lines represent a direct effect (predictors on dependent, mediator on dependent), while dashed lines represent the mediation effect.

This analysis has been carried out with the *mediation* R package, with training load as mediating factor, psychometric scales (PHQ-9, PANAS +, PANAS − and SF-12 scores) as independent variables, and the seven factors as dependent variables. Firstly, the mediator has been regressed onto the independent variables; the training load was significantly correlated to each psychological scale ($p < 0.05$). The effect of mood (measured through the psychometric scales) on nutritional choices (factors' scores) was mediated via the exercise performed, only for Factors 4 (fresh fruit and vegetable) and 6 (fish), as illustrated in Figure 2.6. For Factor 4, the mediation effect was −0.0014 (95% CI: −0.0029–0.00; $p = 0.024$) for PHQ-9 score, 0.0012 (95% CI: 0.0004–0.00; $p < 0.001$) for PANAS positive score, 0.0008 (95% CI: 0.0002–0.00; $p = 0.004$) for PANAS negative score, and −0.0017 (95% CI: −0.0031–0.00; $p < 0.001$) for SF-12 score. The proportion of the effect of the mood on Factor 4 that goes through the exercise was 2.7% for PHQ-9 ($p = 0.024$), 9.0% for PANAS positive ($p < 0.001$), and 3.8% for PANAS negative ($p = 0.004$) scores respectively. The model accounted for 4.2% of the variance in Factor 4 (R^2: 0.0417, $p < 0.001$). For Factor 6, the mediation effect was −0.0018 (95% CI: −0.0039–0.00; $p = 0.03$) for PHQ-9 score, 0.0016 (95% CI: 0.0008–0.00; $p < 0.001$) for PANAS positive score, 0.0011 (95% CI: 0.0002 –0.00; $p = 0.008$) for PANAS negative score, and −0.0022 (95% CI: −0.0039–0.00; $p < 0.001$) for SF-12 score. This means that the proportion of the effect of the PANAS positive scores on Factor 6 that goes through the exercise was 22.2% ($p = 0.044$). The model accounted for 1.8% of the variance in Factor 2.6 (R^2: 0.0184, $p < 0.001$).

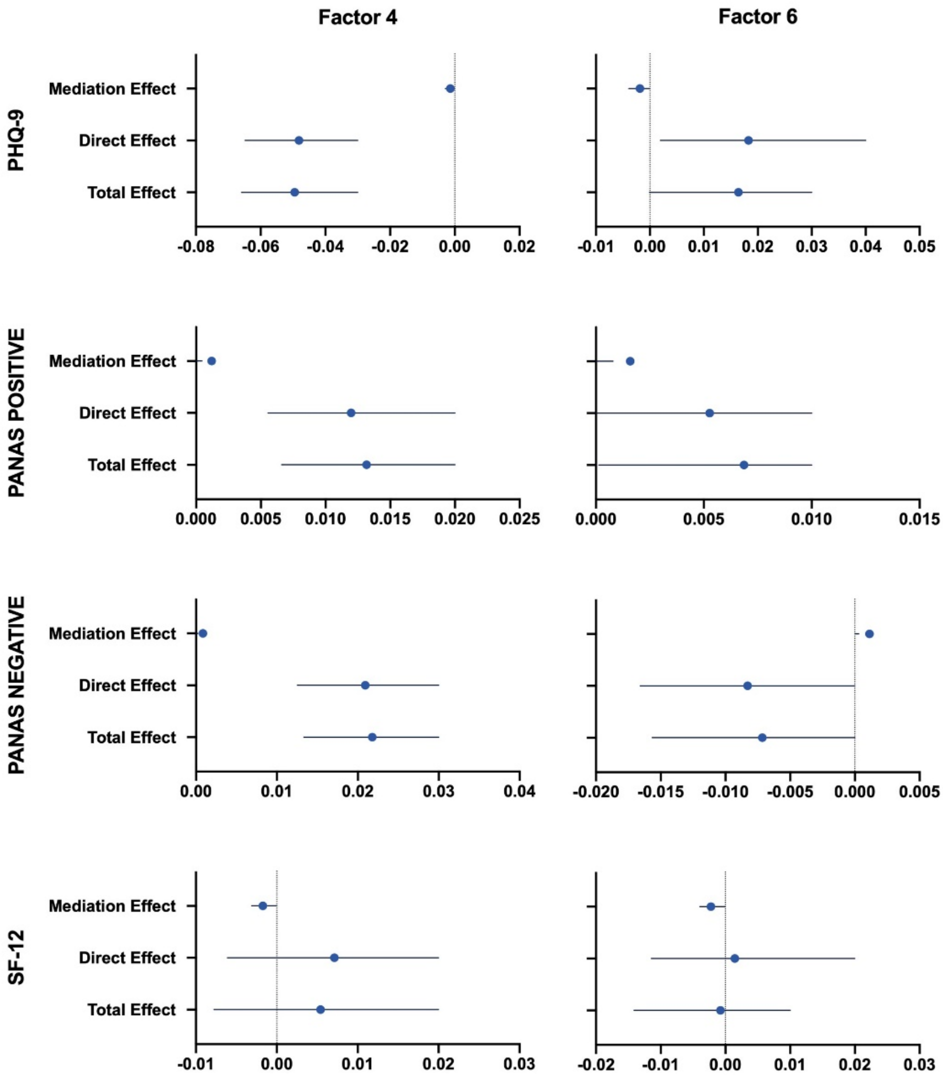

Figure 2.6. Plots of mediation, direct and total effects of training load on psychometric scales (PHQ-9, PANAS +, PANAS −, SF-12) for Factors 4 (fruit and fresh vegetables) and 6 (fish).

2.4. Discussion

This longitudinal observational study aimed to investigate the effects of mood states and exercise on nutritional choices during the COVID-19 home-confinement period. Based on the previous literature, it is known that both mood and exercise might affect nutritional choices [1,69]. We assumed that these effects are not independent, but exercise might mediate the mood-food relationship, so determining changes in nutritional choices [79].

2.4.1. Effect of Exercise on Nutrition

The combination of a healthy diet and physical exercise is the foundation of a healthy lifestyle. Exercise contributes to a negative energy balance by increasing energy expenditure, but the mechanisms which induce modifications in the dietary habits have not yet been completely elucidated. Indeed, research suggests that the role of physical exercise in stimulating psychosocial changes, which leads to better eating, might be more important than the effect of increased energy expenditure [79]. It has been proposed that exercise could influence nutrition habits by application of self-regulatory conducts [15], improved mood states and increased self-efficacy

[77], being suggested as a gateway behaviour for healthful eating [2]. In our study, physical exercise has been shown to negatively correlate with Factor 3 and positively correlates to Factor 4 and 6. This means that in people who exercise more, have a higher consumption of fresh fruit, vegetable and fish, and a lower intake of fat-rich meats and milk-derivatives, the results suggest that physical exercise appears to mediate healthier dietary habits. This is in accordance with the results obtained by Donati Zeppa et al. [1], which showed that nine weeks of high-intensity interval training promoted a spontaneous modulation of healthy food choices and regulation of dietary intake in young normal-weight college students, aged 21–24. Jayawardene et al. [30] also highlighted that exercise could favour fruit and vegetable consumption in young adulthood. Romero-Blanco et al. [81] conducted a study among university students during home-confinement due to COVID-19 in Spain, and evidenced a positive correlation between a Mediterranean diet (known to be rich in fruit and vegetables) and physical exercise. Physical exercise and healthy food choices seem to facilitate each other, via improved self-regulatory strategies and intentions (e.g., planning to perform physical exercise), indicating potential transfer effects between these behaviours [30,75,97]. Although exercise has been observed to mainly influence fruit and vegetable consumption [79], it could be argued that the above-mentioned transfer effects may also influence other nutritional choices, such as increasing fish intake or limiting meats or milk-derivatives, known to be rich in saturated fats.

2.4.2. *Effect of Mood on Nutrition*

In our sample, 13% of males and 19% of females reported moderate to severe symptoms of depression (PHQ-9 $\geq$ 10), a result that is in accordance with the known higher prevalence of depression in women than in men [98,99]. Quarantine situations have been evidenced to negatively impact on mental health, in particular by promoting insomnia, depression, anxiety, irritability, stress-related disorders and anger [100,101]. Poorer mental health, a partial consequence of COVID-19 lockdown, possibly led to less healthy diets, which can themselves be linked to negative mood levels, such as anxiety and depression [102], in a vicious circle [4]. Indeed, associations between diet and psychological disorders—such as depression and anxiety—have been previously reported [103].

Higher scores on PHQ-9 (i.e., higher depression scores) are linked to a high intake of Factor 1 (mainly characterised by cereals and legumes) and Factor 5 (e.g., low-fat meats), and low consumption of Factor 6 (e.g., fish) consumption. The results of PHQ-9 scores are coherent with those reported for SF-12 (i.e., better quality of life), where higher scores were associated with lower consumption of Factor 1 and 5. There is a known link between mood and carbohydrates, with many people eating palatable high carbohydrate/high-fat foods when in a poor mood [104]. Indeed, carbohydrate-rich foods could be a way of self-medicating depression [105], and the effect of carbohydrate cravings may be even stronger when people eat food with a high glycaemic index. A carbohydrate-rich meal tends to raise serotonin levels, which imbalance contributes to the development of depressive symptoms [106]. Therefore, it would make sense that people with more severe depressive symptoms (likely associated with a lower perceived life quality) tend to have a diet rich in carbohydrates. Conversely, there is some, although controversial, evidence that eating fish might reduce the risk of depression [107,108]. In general, the more enriched in fruits and vegetables and poorer in fat and in red meat a diet is, the lower the chance of showing symptoms of depression [107]. However, people perceiving their quality of life as already good were less likely to consume foods popularly considered "healthy" since, in all likelihood, they did not feel the need of correcting with the diet their health or psychological status.

Furthermore, higher scores in the positive affect scale (PANAS positive) were associated with a higher intake of Factor 1 and 5. This could be explained by the model proposed by Christensen L. [109]: it has been proposed that there is a reciprocal interactive relationship between carbohydrate cravings and mood. Emotional distress causes a preference and craving for carbohydrate-rich foods, and these may have a positive reinforcing effect on mood, explaining the higher PANAS positive score. However, legumes consumption has been previously related to lower stress levels and better positive affects [110]. Finally, although negative affect (PANAS negative scores) have been associated with a reduction in Factor 3 (i.e., high-fat meat and milk-derivatives) and an increase in Factor 6 (i.e., fish), research relating meat consumption with mood and life quality is inconclusive, with contrasting results; so, more research is needed to investigate these relationships [110]. However, it might be suggested that this effect depends on a subgroup of people with modest depression preferring a diet with fish and less high-fat meat and milk-derivatives as a strategy to deal with their symptoms.

Diet and mood can mutually influence each other through the gut–brain axis, in which the gut microbiome plays an important role in maintaining mental health [111]. Brain areas and neurotransmitters and neuropeptides that are involved in both mood and appetite likely play a role in mediating this relationship [112].

2.4.3. Exercise as a Mediator between Mood and Nutrition

The indirect effects of exercise on mood scales (PHQ-9, PANAS positive and negative, SF-12) and nutritional choices (factors) were studied using a mediation model. Although some other studies reported that changes in diet and physical exercise had a clear link to negative mood states [4], to the authors' knowledge only a few studies used a mediation approach to investigate this relationship [77,79]. Results supported that exercise partially mediated the relationship between the four psychological domains (PHQ-9, PANAS positive and negative, SF-12) and the Factor 4 (fruit and vegetable consumption) and 6 (fish consumption). This is in accordance with the study published by Annesi et al. [79], who claimed that changes in physical exercise mediated the relationships between mood and increased fruit and vegetable consumption. Mediation may still exist in the absence of a significant association between dependent and independent variables, as reported by MacKinnon et al. [113]. Indeed, in our case, none of the psychological scales was associated with Factor 4, and only PHQ-9 and PANAS negative were associated with Factor 6, respectively, negatively and positively (Figure 2.4). MacKinnon et al. [113] and Kenny [114] described inconsistent mediation when the mediator acts as suppressor variable, in one of the following cases: indirect effect and total effect are opposite in sign, the estimate of the total effect is closer to zero than the direct effect, or the direct effect is larger than the total effect [115]. Given the above, exercise could be a suppressor variable that buffers the interaction between SF-12 and Factor 4 and 6, PHQ-9 and PANAS negative and Factor 6. The mediation effects of exercise in the links of PHQ-9 and SF-12 on nutritional Factors might suggest that some participants actively changed their lifestyle to deal with the health and psychological anticipated impact of the lockdown and tried to prevent it by increasing their physical exercise and modifying their diets.

This work has some strengths and limitations. The main strength point is that diet monitoring was conducted for three-weeks using a 24 h recall method, instead of using simple surveys. Moreover, to our best knowledge, this is one of the first attempts to investigate the complicated relationship between dietary habits, psychological states and physical exercise using a mediation model. A limitation of the paper is that psychometric scales were collected only once during the monitoring period. Furthermore, we do not have data regarding the dietary habits pre-quarantine, which could have been useful in order to assess changes in nutrition and lifestyle behaviours consequent to the home-isolation. However, some effects of the Italian lockdown might reveal later than the short duration of this trial was able to observe. Another limitation is represented by the convenience sampling method, used for the recruitment of the participants. Future studies could focus on the psychological and physiological mechanisms underlying the changes in dietary habits following different training modalities.

2.5. Conclusions

In this paper, the analysis of nutritional choices, physical exercise and mood in college students during COVID-19 outbreak highlighted the importance of physical exercise on dietary habits. Indeed, exercise showed to have both a direct effect, influencing fresh fruit and vegetables and fish consumption, and an indirect effect on nutritional choices, counterbalancing the impact of negative psychological states on the dietary habits. Poorer mental health possibly led to unhealthy diets, which can themselves be linked to negative mood levels, in a vicious circle. Overall, exercise led to healthier nutritional choices and, mediating the effects of mood states; it might represent a key measure in particular situations, such as a home-confinement due to a pandemic.

Chapter 3

Which are the nutritional supplements used by beach-volleyball athletes? A cross-sectional study at the Italian National Championship [3]

Abstract: Beach volleyball is an intermittent team sport played under high temperature and humidity. Given that some nutritional supplements can enhance sports performance, this study aimed to evaluate the quantity and the heterogeneity of the nutritional supplementation practices of amateur (n=69) and professional (n=19) beach volley athletes competing in the Italian National Championship; an online form was used to collect data about the supplementation habits. The latent class analysis was used to find sub-groups characterised by different habits regarding supplements consumption. The most frequently used supplements (more than once a week) are vitamins B and C (39.2% of athletes), protein (46.8%), and caffeine (36.9%). The latent class analysis revealed three different sub-groups of athletes: the first class (56.7%) included athletes who were used to take very few supplements, the second class (17.0%) was characterised by higher consumption of supplements and the third class (26.2%) was in the middle between the others two. Groups were characterised not only by the quantity but also by the category of supplements used. Our results highlighted a high heterogeneity in supplementation habits. A pragmatic approach to supplements and sports foods is needed in the face of the evidence that some products can usefully contribute to enhance performance.

3.1. Introduction

Athletes have to train as hard as possible with optimal adaptation and recovery, to remain healthy and injury-free, to achieve a physique that is suited to their event, and to perform at their best on the day(s) of peak competitions [116]. Beach volleyball is an intermittent team sport played by two teams of two players on a sand court divided by a net [117]. It is characterised by frequent high-intensity efforts interposed by short recovery phases [118]. The performance involves jumps (e.g. attacking, serving, blocking), short sprints, changes of direction and diving digs [119]. During a single set, Palao et al. [118] observed that defenders and blockers performed an average of 27 and 31 jumps, respectively. In addition, moving on sand increases energy cost compared to moving on the solid ground [120]. Beach volleyball is played under demanding environmental conditions: Zetou et al. [121] reported that during over 50 matches analysed in an official tournament, the mean air temperature was 33.6 °C (max 38 °C) and mean humidity was 56% (max 75%). Bahr & Reeser [122] reported that in the 19% of the World Tour tournaments between 2009 and 2011, wet-bulb globe temperature (that combine temperature, humidity, wind speed and solar radiation) exceeded 32°, that is considered the upper limit for physical activity in hot and humid conditions (US Navy 'black flag' condition). Players have to play up to three matches (42.2 ± 9.8 min per match; ranging from 30 to 64 min) per day with small intervals between games and for three days in a row [123]. The extended duration of exposure of the

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players to the sun and to high temperatures are important factors that increase the risks of dehydration and thermal stress.

In conjunction with training and nutrition, numerous evidence demonstrate that the appropriate ingestion of some nutritional supplements can enhance sports performance [124]. A number of excellent reviews have evaluated the performance-enhancing effects of most supplements in endurance sports [124-126]. On the contrary, less attention has been paid to the performance-enhancing claims of supplements in the context of team-sport performance; only a few papers in the literature summarised the effects of sports supplements in this field [127,128]. Even if some generic evidence is present for carbohydrate [129] and protein [130] supplementation in team sports, supplements that enhance performance in some disciplines may not work in the same way in others (and vice versa). For example, nutritional supplements that have been demonstrated to improve continuous exercise performance may not improve intermittent exercise performance. According to Maughan et al. [124] nutritional supplements can be classified as dietary supplements, sport nutrition products and ergogenic supplements. The category of dietary supplements mainly comprises micronutrient supplements such as vitamins and minerals, but also essential fatty acids. Such dietary supplements may promote the athletes' general health through the prevention and treatment of nutrient deficiencies [126]. Sport nutrition products mainly contain macronutrients, such as carbohydrates, proteins and fat, and include sports drinks, recovery drinks, energy bars, etc. The category of ergogenic supplements mainly comprises products with performance-enhancing claims, such as caffeine and creatine [131]. Furthermore, some supplements may be used for multiple functions. For example, carbohydrate supplements are used to enhance performance in many events via the provision of fuel substrate [132] or to support the immune system [133].

Proposed factors responsible for performance decrements during high-intensity periods of play during team-sports include, among others, limitations to energy supply and metabolite accumulation, that limit performance by causing fatigue, loss of concentration and consequent reduction of skills over the course of the event [125,134]. Supplements that offset the influence of these limiting factors are able to improve the recovery of both sprint and jump performance and to speed the recovery of athletes following matches or training, subsequently improving team-sport performance [127]. In fact, performance enhancement, prevention of nutritional deficiencies, better body composition, immune system enhancement, and recovery from training and injury are some of the known reasons why athletes use supplements [124].

To our knowledge, no studies have been conducted investigating the use of nutritional supplements by beach volley athletes. Thus, the purpose of this study was to evaluate the quantity and the heterogeneity of the nutritional supplementation practices of amateur and high-performance beach volley athletes participating in the Italian National Championship. Aspects of dietary supplementation that were evaluated included supplement use, reasons for supplementation, and sources of supplementation information.

3.2. Materials and Methods

This was an observational cross-sectional study involving amateur and professional beach volley athletes competing in the Italian National Championship in the 2018 season. After a verbal and written explanation of the experimental design of the study, approved by the local Institutional Review Board, written informed consent was obtained from the athletes, and a trained research assistant sent an online form (Google Form) by email to every athlete. Participants responses were anonymous, as no identifiable details were collected online. In addition, all players were fully accustomed to the procedures used and were informed that they could withdraw from the study at any time.

3.2.1. Participants

At the final of the Italian National Championship (Catania, 2018), all the 134 athletes participating at the competition were recruited for participation in the study. The response rate was 86.6%, as 116 athletes that were approached took part in the study. Of these, 28 athletes (20.9%) were excluded because they did not answer correctly to the questions. 88 athletes (65.7%) were then included in the final analyses. Of the 88 athletes, 40 (45.5%) were males, and 48 (54.5%) were females. Height was 190.4 ± 6.5 cm for males, 174.3 ± 5.4 cm for females; weight was 84.8 ± 7.3 kg for males, 63.0 ± 5.4 kg for females. The mean age of the athletes was 28.1 ± 6.0 (min 18; max 47) years. Only 3 subjects (age = 41; 46; 47 years) were over the age of 40. Additional demographic characteristics of the subjects were collected, education level, occupation, years of sports practice, level of competition and sponsorship by a sports supplements producer company. Participants characteristics are shown in Table 3.1.

Table 3.1. Socio-demographic characteristics of respondents (n=88).

Variable	Frequency (n)	Percentage (%)
Sex		
Males	40	45.5%
Females	48	54.5%
Age		
18-20 years	7	8.0%
21-25 years	26	29.5%
26-30 years	28	31.8%
31-35 years	17	19.3%
> 35 years	9	10.2%
Education Level		
High School	24	27.3%
Bachelor Degree	34	38.6%
Master Degree	30	34.1%
Occupation		
Athlete	19	21.6%
Independent Worker	23	26.1%
Dependent Worker	28	31.9%
Student	18	20.4%
Duration playing beach volley		
< 5 years	29	33.0%
5-10 years	45	51.1%
> 10 years	14	15.9%
Level of competition		
National	80	90.9%
International	8	9.1%
Sponsored		
Yes	8	9.1%
No	80	90.9%

3.2.2. Procedures and instruments

The procedure for data collection that was used in the current study has been used before [135]. The online form was divided into two main sections. The first section captured information on participants' socio-demographic and sports history-related characteristics. The second section consisted of questions about athlete nutritional supplement use, including the type of supplements used, frequency of consumption, personal motivations for use, and the sources of sports nutrition information. Subjects were asked to indicate the frequency of use on a 3-points

Likert Scale (0 = <1 time/week, 1 = 1 to 2 times/week; 2 = >2 times/week), of each of the following supplement: carbohydrate, protein, branched-chain amino acids (BCAA), caffeine, glutamine, creatine, carnitine, sodium bicarbonate, beta-alanine, probiotics, phosphates, vitamins (B, C, D, E), calcium, iron, omega-3, omega-6 and herbals. At the end of the list, the variable "Other" was present to give the possibility to the subject to add any other supplement. Moreover, subjects were asked to answer "yes" or "no" if they were used to use each of these products during the match: sports drinks, electrolytes, energy drinks, sports bar and sports gel. Finally, subjects were asked to select one (or more) option from a list, about where they search/take information about the nutritional supplements and their personal motivation to their use.

3.2.3. Statistical analysis

SPSS Statistics 22.0 was used for analysis, and a $p<0.05$ was considered statistically significant. Continuous variables, such as age and years of sports practice, were categorised in classes and the data are presented as absolute frequencies and percentages. Latent class analysis (LCA) was performed with R 3.5.3 and poLCA package version 1.4.1 [136]. It has been used to derive a profile of beach volleyball athlete from the supplement items above reported. LCA identifies (latent) subgroups of individuals (i.e. classes) who share common features, whereas factor analysis identifies unobserved common dimensions accounting for the correlations among observed variables. LCA posits that a heterogeneous group can be reduced to several homogeneous subgroups by evaluating and then minimising the associations among responses across multiple variables, and tests for the existence of discrete groups with a similar symptom or item endorsement profile [137]. LCA estimates two parameters: 1) the likelihood of endorsement of a given item for individuals in a particular class; and, 2) the class membership probabilities. Since in LCA no a priori assumptions are made concerning the number of latent classes, LCA model selection was conducted according to fitting indices such as G2 likelihood ratio ($-2*\ln(L)$), the Akaike information criterion (AIC), the Bayesian information criterion (BIC), and the sample-size adjusted BIC (SSABIC) [138]. For each of these indexes, lower values indicate better fit. Chi-square test was used to assess associations among supplements used and socio-demographic characteristics (gender, age, level of education, occupation, years of beach volley practice, level of competition and sponsorship); Cramer's V was calculated as a measure of association.

3.3. Results

Complete results of the Dietary Supplement, Sports Nutrition Products and Ergogenic Aids categories are reported in Table 3.2. None of the athletes reported using any supplements other than those already listed a priori in the survey.

Table 3.2. Type and frequency of use of nutritional supplements.

	< 1 time/week	1-2 times/week	≥ 3 times/week
Dietary Supplements			
Vitamin C	60.8	16.5	22.8
Vitamin B	60.8	20.3	19.0
Vitamin D	67.9	16.7	15.4
Vitamin E	67.5	15.0	17.5
Calcium	70.1	14.3	15.6
Iron	72.7	14.3	13.0
Omega-3 Fatty Acids	74.0	15.6	10.4
Omega-6 Fatty Acids	87.5	9.7	2.8
Herbals	95.9	2.7	1.4
Sports Nutrition Products			
Protein	53.2	23.4	23.4
BCAA	62.5	18.8	18.8
Carbohydrate	66.2	18.2	15.6
Ergogenic Aids			
Caffeine	63.2	22.4	14.5
Glutamine	82.9	11.8	5.3
Creatine	81.6	9.2	9.2
Carnitine	91.9	5.4	2.7
Sodium Bicarbonate	94.4	4.2	1.4
Beta-Alanine	89.0	6.8	4.1
Probiotics	91.9	5.4	2.7
Phosphates	93.2	5.5	1.4

BCAA = Branched Chain Amino Acids.

3.3.1. Latent Class Analysis

The 3-classes solution has been chosen on the basis of fitting indexes; AIC, SSABIC and G2 likelihood-ratio were lower in the 3-classes solution than in the 2-classes one, whilst BIC was higher in the 4-classes solution respect to the 3-classes one (Figure 3.1).

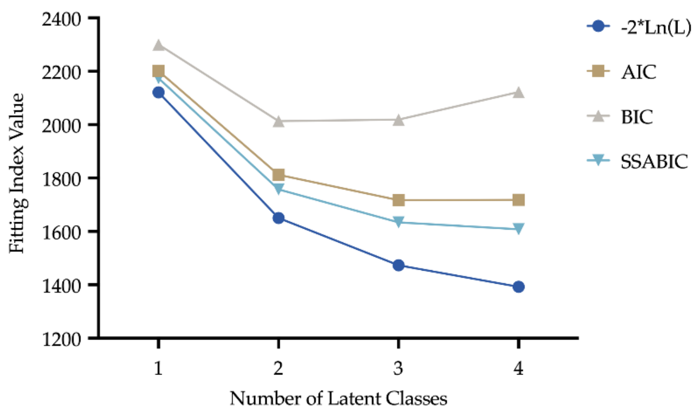

Figure 3.1. Latent Class Analysis scores (lower values indicate a better fitting). -2*ln(L): G2 Likelihood ratio; AIC: Akaike information criterion; BIC: Bayesian information criterion; SSABIC: Sample-size adjusted BIC.

In this solution, a first class (LC_1) has been found, which included 50 athletes (56.7%) characterised by taking very few supplements; the participants included in LC_1 had a 96.6% probability of taking supplements less than one time per week. The second class (LC_2, including 15 athletes, 17.0%) were characterised by higher consumption of supplements; the probability of the participants taking supplements 1 or 2 times per week was 15.0% and 41.3% more than 2 times per week. The third class (LC_3, including 23 athletes, 26.2%) was characterised by a higher probability of use supplements with respect to LC_1, but lesser than LC_2. In fact, it can be highlighted that the differences in supplements intake habits between group 2 and 3 were found in particular regarding to use of dietary supplements (all vitamins, calcium, iron and herbals) and of some ergogenic aids (caffeine, carnitine, phosphates and sodium bicarbonate), with LC_2 subjects were more likely to use these supplements frequently. Although the difference was not significant between LC_2 and LC_3, a trend can be noticed in higher use of carbohydrate, protein, BCAA and Omega-6 by LC_2 group. Complete results are reported in Table 3.3.

Table 3.3. Latent Class Analysis results: values represent the probability of response for members for each class.

Class Sizes:		56.7%	17.0%	26.2%					
		LC_1	**LC_2**	**LC_3**			**LC_1**	**LC_2**	**LC_3**
Dietary Supplements									
Vitamin B	1	**96.0%**	*0.0%*	*39.2%*	Protein	1	**84.1%**	*13.3%*	*34.7%*
	2	*2.0%*	20.0%	**52.1%**		2	*9.9%*	**33.3%**	**34.9%**
	3	*2.0%*	**80.0%**	*8.7%*		3	*6.0%*	**53.3%**	**30.4%**
Vitamin C	1	**96.0%**	*0.0%*	*39.4%*	BCAA	1	**90.0%**	*40.0%*	*30.6%*
	2	*2.0%*	*0.0%*	**52.0%**		2	*6.0%*	*13.3%*	**43.3%**
	3	*2.0%*	**100.0%**	*8.7%*		3	*4.0%*	**46.7%**	**26.1%**
Vitamin D	1	**100.0%**	*6.7%*	*52.3%*					
	2	*0.0%*	13.3%	**47.7%**	**Ergogenic Aids**				
	3	*0.0%*	**80.0%**	*0.0%*	Beta-Alanine	1	98.0%	73.3%	87.0%
Vitamin E	1	**100.0%**	*6.7%*	*48.0%*		2	*2.0%*	**13.3%**	**8.7%**
	2	*0.0%*	*0.0%*	**52.0%**		3	*0.0%*	**13.3%**	**4.3%**
	3	*0.0%*	**93.3%**	*0.0%*	Glutamine	1	94.0%	*66.7%*	78.3%
Calcium	1	**100.0%**	*20.0%*	*52.3%*		2	*6.0%*	**20.0%**	**13.0%**
	2	*0.0%*	*6.7%*	**43.3%**		3	*0.0%*	**13.3%**	**8.7%**
	3	*0.0%*	**73.3%**	*4.3%*	Creatine	1	100.0%	*46.7%*	74.0%
Iron	1	**100.0%**	*20.0%*	61.0%		2	*0.0%*	**26.7%**	**13.0%**
	2	*0.0%*	13.3%	**39.0%**		3	*0.0%*	**26.7%**	**13.0%**
	3	*0.0%*	**66.7%**	*0.0%*	Caffeine	1	**84.0%**	*33.3%*	56.6%
Omega-3	1	**98.0%**	*40.0%*	*56.7%*		2	*8.0%*	20.0%	**43.4%**
	2	*2.0%*	**26.7%**	**30.3%**		3	*8.0%*	**46.7%**	*0.0%*
	3	*0.0%*	**33.3%**	**13.0%**	Carnitine	1	100.0%	*73.3%*	91.3%
Omega-6	1	100.0%	*60.0%*	87.0%		2	*0.0%*	**13.3%**	**8.7%**
	2	*0.0%*	**26.7%**	**13.0%**		3	*0.0%*	**13.3%**	*0.0%*
	3	*0.0%*	**13.3%**	*0.0%*	Phosphates	1	100.0%	86.7%	87.0%
Herbals	1	100.0%	86.7%	95.7%		2	*0.0%*	**6.7%**	**13.0%**
	2	*0.0%*	**6.7%**	**4.3%**		3	*0.0%*	**6.7%**	*0.0%*
	3	*0.0%*	**6.7%**	*0.0%*	Sodium	1	100.0%	80.0%	95.7%
					Bicarbonate	2	*0.0%*	**13.3%**	**4.3%**
Sports Nutrition Products						3	*0.0%*	**6.7%**	*0.0%*
	1	**98.0%**	*26.7%*	*39.3%*	Probiotics	1	94.0%	93.3%	91.3%
Carbohydrate	2	*2.0%*	**20.0%**	**43.4%**		2	*2.0%*	**6.7%**	**8.7%**
	3	*0.0%*	**53.3%**	**17.3%**		3	**4.0%**	*0.0%*	*0.0%*

1 = <1 time/week; 2 = 1-2 times/week; 3 = >2 times/week; LC = Latent Class; BCAA = Branched-Chain Amino Acids. Values that are substantially higher for the segment than for the total sample are highlighted in bold. Values that are substantially lower for the segment than for the total sample are highlighted in italics.

3.3.2. Athletes characteristics and nutritional supplement use

Use of protein (χ^2 = 10.282, p = 0.036, V = 0.365) and BCAA (χ^2 = 15.527, p = 0.004, V = 0.441) supplements was significantly greater in males than females. In fact, more than 35% of women say they do not use BCAA, and 25% do not even take proteins. For all the other predictive variables used (age, level of education, occupation, years of beach volley practice, level of competition and sponsorship) no significance was found for any of the supplements used ($p>0.05$).

3.3.3. Supplement intake during a competitive beach volleyball match

The results of the answer to the question "Do you normally use these supplements during the game?" showed that almost half of athletes (45.5%) use sports drinks (in the form of powder or ready to drink liquid), electrolytes (as powder sachets or tablets) (37.2%), and energy drinks (ready-to-drink liquid or concentrated shot) (36.7%) during the match. Athletes reported using carbohydrates in the form of bars and gels, 22.4% and 20.3% respectively.

3.3.4. Sources of information and athletes' motivation for supplement use

Nutritionists/dietitians were the main sources of information for the use of supplements (31.6%), followed by internet and teammates (15.8% for both), physicians (12.3%), pharmacists (12.3%), coaches (7.0%) and only a few athletes claimed to have taken information from specialist magazines (5.3%). Most athletes use supplements with the aim of improving performance (32.5%), preventing nutritional deficiencies (25.0%) and improving recovery (20.0%). As a minor percentage, they are used as a supplement to the diet (7.5%), to improve health, reduce stress, improve the immune system, improve self-perception and increase muscle mass (2.5% for all the items). Relative frequencies (%) of these items are graphically reported in Figure 3.2 and 3.3.

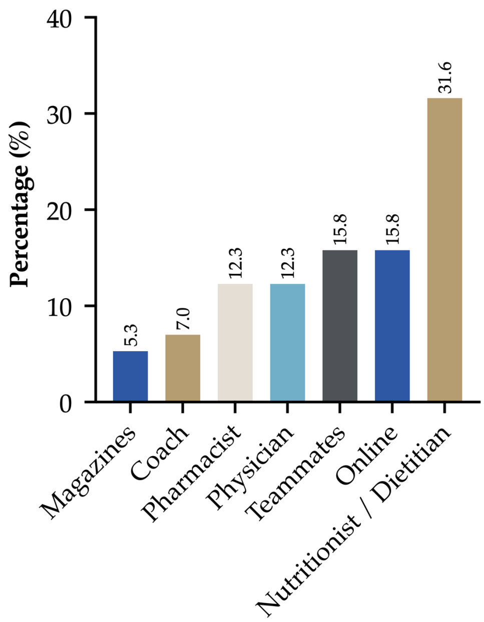

Figure 3.2. Athletes' sources of information regarding supplements.

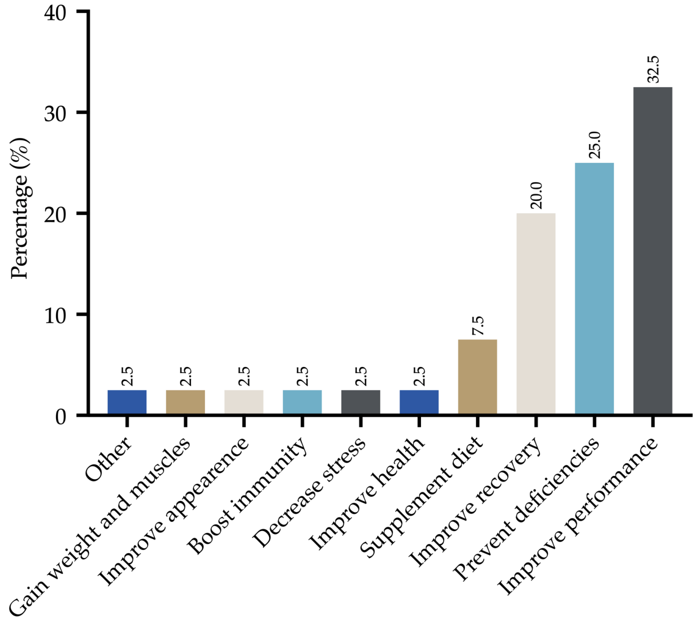

Figure 3.3. Athletes' motivations to use supplements.

3.4. Discussion

To our knowledge, this is the first study that aimed to analyse the quantity and the heterogeneity of the nutritional supplementation practices of amateur and high-performance beach volley athletes. The main result of our study was that three main sub-groups were found using an LCA, each group defined by a different behaviour regarding supplements consumption (both type and frequency of assumption); these results highlight a high heterogeneity in the consumption of the supplements among beach-volley athletes.

Training, recovery and nutrition are the essential components of the sport performance. Once these basic factors are accounted for, the use of some evidence-based nutritional supplements may help athletes to improve performance [139]. Many athletes have a "win at all costs" mentality and will choose to use a supplement regardless of the possible side effects. Furthermore, many of the effects reported for some supplements are anecdotal and have not been supported by scientific evidence (e.g. carnitine or glutamine) [126,140]. The "more is better" philosophy when applied to supplements consumption, may lead to adverse effects, often due to inappropriate patterns of use by athletes (i.e. mixing and matching different products without regard to total doses nor possible negative interactions between ingredients) or by the lack of safety of the products. In addition, common supplements have been found to contain undeclared prohibited substances, that may cause a positive doping outcome [124].

Only a few studies investigated the supplements consumption habits of volleyball/beach volleyball athletes. Zapolska and colleagues [141] reported the nutritional supplements consumption habits of a sample of 17 professional volleyball athletes. The use of sports supplements was declared by 89% of the respondents. The most popular supplements were: protein (71%), carbohydrate (24%), vitamins and minerals (82%), amino acids (76%) and stimulants (47%), including coffee (65%) and caffeine tablets (41%); also BCAAs, creatine and glutamine were consumed. Zetou et al. [142] explored the common practices of 47 beach volleyball players (both elite and non-elite) regarding fluid, supplements and nutrition intake during a tournament. Interestingly, they reported that only very few players (about 25% of their participants) took supplements or other aids, most of them did not follow a systematic diet before their games and, most surprisingly, they were not aware of the association between dehydration and performance (and they did not have any plan concerning the intake of fluids during their games). The findings described from Zetou and colleagues are consistent with our data as 45.5% of subjects normally use sports drinks, 37.2% take electrolytes, and 36.7% of energy drinks during their matches. Our results showed that a large part of the interviewed athletes (56.7%) do not take any type of supplement, as reported from the LCA. However, LCA also showed a group of athletes (LC_2; 17.0%) that reported taking – more or less frequently – almost every supplement included in the study, despite few of the supplements demonstrating an evidence-based efficacy in improving sports performance (e.g., sports drinks, carbohydrate, creatine, caffeine, β-alanine, etc.) [126]. We did not report any difference related to the athlete's status (professional vs. recreational); however, this result is may not be reliable, due to the very low number of elite athletes included in our sample. Our results are in accordance with those reported by Zetou et al. [142], who did not find any difference in nutrition practices or fluid intake between elite and non-elite athletes, but in contrast with Wardenaar et al. [143], who found some differences in supplements use between elite and non-elite athletes of several disciplines.

Even when there is a robust literature on sports supplementation, it may not cover all applications that are specific to an event, environment, or individual athlete [125]. According to the ISSN Exercise & Sports Nutrition Review Update [126], there is a category of supplement with strong evidence to support efficacy, mainly composed by sports food (sports drink, bars and gels, electrolytes, …), and performance-enhancing supplements (caffeine, beta-alanine, creatine, sodium bicarbonate and phosphate). Despite generic evidence supporting their use, it is suggested that these supplements would be best used with an individualised and event-specific protocol. If limiting to the world of team sports, such as beach volleyball, the list of supplements that have a scientifically proven effect on athletes' performance narrows further. After a literature search on the principal databases (PubMed and Sport Discus), no studies have been found that investigated the efficacy of specific supplements in beach volley. Lamontagne-Lacasse et al. [144] studied the effect of creatine supplementation on jumping performance in elite volleyball players, concluding that creatine could increase the height in repeated jumps, attenuating the magnitude of muscular fatigue and offering a potential advantage during a match. Mielgo-Ayuso et al. [145] reported that oral iron supplementation prevented iron loss and enhanced strength in female volleyball players during the competitive season. Pfeifer et al. [146] did not find any difference in performance after a low-dose caffeine and carbohydrate supplement intake during a volleyball competition, even if, as stated by the authors, the dose of supplement (54g carbohydrate plus 100mg caffeine, divided into two separate doses) that was used may not have been sufficient to produce a positive effect.

In regard to Dietary Supplements consumption (mainly vitamins and minerals), research demonstrated that if an athlete is deficient, exercise capacity may be reduced; despite this, performance improvements due to vitamins/minerals supplementation when athlete's status is adequate have not been reported [126]. Although a direct link between vitamins/minerals supplementation and performance is lacking, several nutrients (such as vitamin C and D) have been suggested to help athletes stay healthy, in particular during intense training periods. The athletes involved in heavy training period may have higher Recommended Dietary Allowance (RDA) of some vitamins; obtaining adequate vitamins through use of supplement appears to be a prudent behaviour for some athletes [147].

3.4.1. Source of information and motivation to use

Athletes' supplementation practices are often guided by family, friends, teammates, coaches, the Internet, and retailers, rather than sports dietitians and other sport science professionals [125]. A good percentage of the athletes reported to take information from a professional figure (nutritionist or physician), but the remaining 56.1% are not. Specifically, our results showed that 31.6% of the interviewed athletes reported having received a consult by a nutritionist/dietitian and the 12.3% by a physician; the 15.8% reported asking information about supplements from internet and teammates, and 7.0% from the coach. Following advice from coaches, teammates, internet or non-scientific magazines may lead to bad supplement choices [148] and could present significant potential risks for the athlete health and performance. Our results are in accordance with Braun et al. [149] which showed that the motivations for supplement use include enhancement of performance or recovery, improvement or maintenance of health, an increase in energy, compensation for poor nutrition, immune support, and manipulation of body composition. In our study, 32.5% reported using nutritional supplements to improve performance, to prevent deficiencies (25.0%) and to enhance recovery (20.0%), as reflected by the most frequently used supplements (vitamins, proteins, BCAAs, carbohydrates, caffeine, glutamine and creatine). Nevertheless, Petróczi et al. [150] reported that, despite the frequent use of dietary supplements, athletes have misconceptions about their effectiveness; indeed, the authors did not observe agreement between athletes' rationale and behaviour in relation to their nutritional supplement use. According to some other studies, there is a large population of athletes who report incorrect information about the supplements they use [151]. It is important that coaches, nutritionist, and athletes above all, make reference to the more recent sports nutrition and supplementation guidelines, published by the American College of Sports Medicine [125], the International Society of Sports Nutrition [126], and the International Olympic Committee [124].

The conditions in which beach volley matches are played make it a unique discipline that needs to be further studied. In particular, the environmental conditions (very hot and high humidity) highlight the need for proper integration and maintenance of fluid replacement to compete at best. The development of hyperthermia during exercise in hot ambient conditions is associated with a rise in sweat rate, which can lead to progressive dehydration if fluid losses are not minimised by increasing fluid consumption. Dehydration of even 2% body mass might impair sports performance [152,153]. Fluid replacement recommendations should be adapted at the particular environmental situation (temperature and humidity) of each match-day. Understandably, this study was subject to some limitations. The sample size was quite low and very variable results were likely, with consequences difficult to make a reliable synthesis. Most athletes use supplements because they believe their diet is not satisfactory; in this study, we did not study the content of athletes' diets and for this reason, we were not able to make observations on the relationship between the use of supplements and the adequacy of the nutrients of the diet of each individual. For these reasons, further large-scale studies are recommended to ascertain the quality and the heterogeneity of the nutritional supplementation practices of beach volley athletes.

3.5. Conclusion

Team sports share the common feature of intermittent, high-intensity efforts, but experience marked variability of game characteristics between sports, and positions/playing styles within the same sport. This creates a diversity of physiological challenges and nutritional needs for team sport athletes [154]. According to the aim of this study, as to explore the common supplementation practices of beach volley athletes, our results highlighted a high heterogeneity in the supplementation habits: most of them did not use supplements in a structured way, using them occasionally and often without a scientific rationale behind. It is appreciable that most athletes rely on professional figures (nutritionist and/or physicians) for their nutrition and supplementations protocols; an even greater reliance on these professional figures could increase the intake of some evidence-based supplements and limit their use of other (without proven scientific efficacy), thus improving the effects on their performance and health status. A pragmatic approach to supplements and sports foods is needed in the face of the evidence that some products can usefully contribute to a sports nutrition plan and/or directly enhance performance.

Chapter 4

Effects of a commercially available branched-chain amino acid-alanine-carbohydrate-based sports supplement on perceived exertion and performance in high intensity endurance cycling tests [4]

Abstract: Sports nutritional supplements containing branched-chain amino acids (BCAA) have been widely reported to improve psychological and biological aspects connected to central fatigue and performance in endurance exercise, although the topic is still open to debate. The aim of the present study was to determine whether the intake of a commercially available BCAA-based supplement, taken according to the manufacturer's recommendations, could affect the rating of perceived exertion (RPE) and performance indexes at the beginning (1d) and end of a 9-week (9w) scheduled high intensity interval training program, with an experimental approach integrating the determination of psychometric, performance, metabolic and blood biochemical parameters. This was a randomized double-blind placebo-controlled study. Thirty-two untrained, healthy young adults (20 males and 12 female) were enrolled. A high-intensity endurance cycling (HIEC) test was used to induce fatigue in the participants: HIEC consisted in ten 90 s sprints interspersed by ten 3 min recovery phases and followed by a final step time to exhaustion was used. In parallel with RPE, haematological values (creatine kinase, alanine, BCAA, tryptophan, ammonia and glucose levels), and performance indexes (maximal oxygen consumption - VO_2max, power associated with lactate thresholds - W_{LT1}, W_{LT2} and time to exhaustion - TTE) were assessed. All subject took the supplement (13.2 g of carbohydrates; 3.2 g of BCAA and 1.6 g of L-alanine per dose) or placebo before each test and training session. Dietary habits and training load were monitored during the entire training period. The administration of the supplement (SU) at 1d reduced RPE by 9% during the recovery phase, as compared to the placebo (PL); at 9w the RPE scores were reduced by 13% and 21% during the sprint and recovery phase, respectively; at 9w, prolonged supplement intake also improved TTE and TRIMP. SU intake invariably promoted a rapid increase (within 1 h) of BCAA serum blood levels and prevented the post-HIEC tryptophan:BCAA ratio increase found in the PL group, at both 1d and 9w. There was no difference in dietary habits between groups and those habits did not change over time; no difference in glycemia was found between SU and PL. VO_2max, W_{LT1} and W_{LT2} values improved over time, but were unaffected by supplement intake. On the whole, these results suggest that i) the intake of the BCAA-based commercially available supplement used in this study reduces RPE as a likely consequence of an improvement in the serum tryptophan:BCAA ratio; ii) over time, reduced RPE allows subjects to sustain higher workloads, leading to increased TRIMP and TTE.

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4.1. Introduction

Amino acids are thought to enhance athletic performance in several ways, for example modifying fuel utilization during exercise and preventing mental fatigue and overtraining [155]. A recent (2017) position stand of the International Society of Sports Nutrition [156] states that the three branched-chain amino acids (BCAA), leucine, isoleucine, and valine are unique among the essential amino acids for their roles in protein metabolism, neural function, blood glucose and insulin regulation. It has been suggested that the Recommended Dietary Allowance (RDA) for sedentary individuals (considering that BCAAs occur in nature in a 2:1:1 ratio, leucine: isoleucine: valine) should be 45 mg/kg/day for leucine and 22.5 mg/kg/day for both isoleucine and valine; this RDA is even higher for active individuals [157]. Moreover the European Food Safety Authority indicated an amount recommendation between 3g and 12g per day (higher dose may lead to ammonia build-up) [158]. Supplementation with BCAA has been proposed as a possible strategy to limit the development of central fatigue [159], in particular, in endurance events [155]. Central fatigue, which pertains to the central nervous system (CNS), is a complex phenomenon arising under conditions of low energy availability [160,161], ammonia accumulation in blood and tissues [162], and changes in neurotransmitter synthesis - in particular, an increase in serotonin and a decrease in dopamine - which causes a state of increasing tiredness during exhaustive exercise [163]. The presence of elevated cerebral serotonin levels observed in rats under fatigue [164], is the basis of a well-accepted theory to account for the onset/increase of central fatigue in humans as well. Indeed, during prolonged sustained exercise, an increased brain uptake of the serotonin precursor Tryptophan (Trp) has been observed in humans [165,166]. This theory has recently been bolstered by Kavanagh et al. [167], whose study based on paroxetine administration in humans demonstrated the influence of serotonin availability in increasing central fatigue under prolonged maximal contractions. The ability of BCAA to compete with Trp in crossing the blood brain barrier led us to hypothesize that BCAA supplementation could reduce cerebral serotonin synthesis, thus preventing/delaying the onset of central fatigue during prolonged exercise [168,169].

In addition to BCAA, other amino acids reputed to play a role in maintaining performance during endurance exercise are often included in sports supplements. Among these, L-alanine (Ala, another component of the product tested in the present study) is thought to support performance through several mechanisms [170], including the prevention of an exercise-induced decrease in many gluconeogenic amino acids and hence a metabolic profile that enhances performance [171]. Ala is consumed in quantities of 3 to 4 g/day on average in a typical diet; however, no studies have assessed the long-term effects of its supplementation in humans alone or combined with BCAA [172].

Carbohydrates (CHO) also play an important role in supplementation in the course of endurance events, increasing and/or maintaining energetic substrate availability [173], preventing and/or delaying hypoglycaemia and its deleterious effects on brain functions and cognitive performance, and promoting direct anti-fatigue brain responses through the activation of sweet taste oral receptors [159].

In light of these findings, researchers have turned their attention to the study and development of supplements containing BCAA alone or combined with specific substances (such as CHO), assessing the efficacy of their association [169,174]. Several recent investigations have shown BCAA supplementation to positively affect prolonged exercise under specific conditions. In particular, BCAA were shown to positively impact the rating of perceived exertion (RPE) [168] and performance [175,176]. However, due to the great heterogeneity of the experimental protocols and formulations used, the results of these studies are not always unequivocal; hence, the actual efficacy of BCAA – used alone or combined with other components - remains a much debated issue [169,177,178].

This uncertainty may generate confusion and/or false expectations regarding the efficacy of these sport supplements. To shed light on this issue, it is important to perform highly controlled and randomized studies as well as to develop and validate specific and reliable test procedures capable of determining the actual efficacy of supplements intended for use in sports after both short and long term intakes [124]. To this end, a recent study [179] validated a variable high intensity protocol followed by a time to exhaustion (TTE) endurance capacity test (namely high intensity endurance cycling test, HIEC) as a reliable and sensitive method to assess both performance and fatigue, providing a stable platform for the comparative analysis of the effects of different nutritional interventions. HIEC can be performed either at the beginning or at the end of training periods and protocols. In the present study, we applied HIEC to a 9-week program based on High Intensity Interval Training (HIIT), a widely used protocol to improve specific variables of endurance performance [180,181]. It is worth noting that, to date, to the best of our knowledge, no study has tested the effects of the consumption of a commercially available and established BCAA-alanine-CHO based supplement on HIEC over a medium-long endurance training period.

The first aim of this randomized double-blind placebo-controlled study was to determine whether, the single or prolonged intake of a commercial BCAA, Ala and CHO formula (Friliver® Performance, FP, Dompè Farmaceutici Spa), taken according to the manufacturer's recommendations, affects RPE [182], performance indexes (maximal oxygen consumption, VO_2max; peak power, Wpeak; power at lactate thresholds, W_{LT1} and W_{LT2}; and TTE) and relevant serum blood markers (creatine kinase - CK, Ala, BCAA, Trp:BCAA ratio and glycemia) in young adults, at the beginning (1d) and at the end (9w) of a 9-week indoor cycling HIIT [179]. The second aim was to verify whether a prolonged supplementation may help participants to comply with the required training load during a 9w HIIT program with progressively increasing volume.

4.2. Materials and Methods

4.2.1. Participants

Thirty-two healthy university students (20 males: age 22 ± 1.7 years, height 175.5 ± 6.5 cm, weight 68.2 ± 10.9 kg, BMI 22 ± 2.7 kg/m^2; 12 females: age 21 ± 0.9 years, height 159.5 ± 4.8 cm, weight 52.5 ± 5.3 kg, BMI 21 ± 1.2 kg/m^2) were recruited. The exclusion criteria were: major cardiovascular disease risks, musculoskeletal injuries, upper respiratory infection, smoking and consumption of any medicine or protein/amino acid supplement in the past three months. All participants, assessed with a specific questionnaire, performed no more than one 60 min leisure walking or jogging session per week in the three months preceding the start of the study; their VO_2max values at baseline were in line with- and thus confirmed - their low level of training (see Table 4.2). The participants were advised to maintain their dietary routine, and to abstain from using additional dietary supplements during the study period. They were also instructed to refrain from all training activities except for the sessions included in the experimental design. Subjects were asked to refrain from the consumption of alcohol, hypnosedative drugs and beverages containing caffeine on the two days prior to the trial. Following a medical health-screening, all participants provided written informed consent to participate in the study, which was approved by the Ethics Committee of the University of Urbino Carlo Bo, Italy (02/2017, date of approval July 10, 2017) and was conducted in accordance with the Declaration of Helsinki for research with human volunteers (1975).

4.2.2. Study Design

This was a randomized double-blind placebo-controlled trial (2/2017, conducted according to Good Clinical Practice). In order to ensure balance, randomization for permuted blocks (n = 4) was used. Stratification was used to ensure equal allocation by gender to each experimental condition. Study design was structured as follows: metabolic/performance (VO_2max, Wpeak, W_{LT1}, W_{LT2} and TTE), biochemical (BCAA, Ala, Trp, CK serum and glucose blood levels) and RPE data were acquired before (1d) and after (9w) the incremental training period.

4.2.2.1. Supplement and Supplementation Regimen

FP (Dompè Farmaceutici Spa, Milan, Italy, see Table 4.1 for the formulation) was taken 1h before HIEC and each training session according to the manufacturer's recommendations. BCAA and Ala content per single dose is within the range recommended by European Food Safety Authority and comparable to the dosage used in other studies [158,172,183]. The PL group ingested a non-caloric placebo that was identical in packaging, appearance and taste to the actual supplement. FP and PL were dissolved in 500 ml of still water and ingested before each training session; neither FP nor PL was taken on rest days. Over the entire study period, the SU group received an average daily dose (total amount of each amino acid in FP/duration in days of the study) of 0.91 g leucine, 0.46 g valine, 0.46 g isoleucine and 0.91 g alanine. Importantly, as verified by the qualified medical specialist (P.B.), none of the participants experienced any side effects or adverse events as a result of the FP or placebo ingestion.

Table 4.1. Composition of Friliver Performance®

	Per dose	Per 100 g
Energy	71 kcal (304 kJ)	355 kcal (1520 kJ)
Total Carbohydrate	13.2 g	66 g
Sucrose	11.6 g	58 g
Polyalcohol	1.6 g	8 g
L-Leucine	1.6 g	8 g
L-Alanine	1.6 g	8 g
L-Valine	0.8 g	4 g
L-Isoleucine	0.8 g	4 g
Citric acid	1.06 g	5 g
Orange flavour	0.8 g	4 g

4.2.2.2. Incremental Test

Prior (3 days before) to the pre- and post- training experimental sessions, each subject performed an incremental test to assess individual VO_2max, Wpeak, W_{LT1} and W_{LT2}. Male subjects started cycling on an electronically-braked ergometer (SRM Italia, Lucca, Italy) at 75 W, and power output was increased by 25 W every 3 min, whereas female subjects started at 50 W, and power output was increased by 20 W every 3 min. All subjects continued increasing power output until volitional exhaustion or cadence dropped below 60 rpm [184,185]. In the absence of specific literature, intervals were set at 3 min, which represents an appropriate compromise with previous data on incremental exercise test design [186,187]. Oxygen consumption was monitored breath-by-breath using a Cosmed K4b2 metabolimeter, (COSMED, Rome, Italy) and values of heart rate (HR) (assessed with a Polar RS-800 HR monitor, POLAR, Kempele, Finland) were recorded continuously; VO_2max was calculated according to Robergs et al. [188]; blood lactate was measured before starting the test and in the 15 s before the end of each stage using a Lactate-Pro (portable blood lactate meter, Arkray, Kyoto, Japan) on micro blood samples drawn from the tip of the index finger. As already experimented in a previous study [189], and according to Seiler et al [190] lactate blood levels ([La]) were used to calculate the power at lactate thresholds of [La] 2.0 mmol /L (W_{LT1}) and [La] 4.0 mmol/L (W_{LT2}) and then identify the three HR training intensity zones. The scheme was: zone 1: [La]<2.0 mmol/L; zone 2: 2.0<[La]<4.0 mmol/L; zone 3: [La]>4.0 mmol/L [191,192]. Wpeak was calculated as follows: Wpeak = Wf + [(t/D x P)], where Wf is the power output during the last completed stage, t is the duration of the last uncompleted stage, D is the duration of each stage in seconds (=180 s) and P is the incremental increase in power output with every stage [193].

4.2.2.3. Rating of Perceived Exertion

RPE was determined with the 0-10 OMNI-cycle scale, which combines mode-specific pictorial illustrations with a numerical rating format, using a procedure described in the literature [194,195]. A standard definition of perceived exertion ("the subjective intensity of effort, strain, discomfort, and fatigue that was felt during exercise") and instructional sets for the OMNI scale were read to the subjects immediately before the exercise test [195]. The initial exercise anchoring procedure was illustrated and performed during the incremental test (see "Incremental Test" section). Participants were asked to point to their RPE on the OMNI-cycle scale, which was in full view at all times during testing.

4.2.2.4. HIEC Test

The HIEC test was performed on a power meter-provided bike "Technogym Group Cycle™ Connect" (Technogym S.p.A., Cesena, Italy). To preliminarily calculate the individual workload, a modified O'Hara protocol [196] based on Wpeak was adopted. Following a warm up stage (four 5 min continuous progressive increments at a workload corresponding to 50%, 60%, 65% and 70% Wpeak), participants performed ten 90 s sprints (SPR) at 90% Wpeak, separated by 180 s recovery (REC) at 55% Wpeak. The subjects capable of completing all the 10 SPR recovered for an additional 3 min at 55% Wpeak, and then performed a final TTE step at 90% Wpeak. Exhaustion was defined as the inability to maintain power output within 5 W of the target output for 15 s despite verbal encouragement; no feedback on elapsed time was provided. TTE was taken as a performance marker. Subjects were asked to maintain the same predefined cadence throughout the HIEC regardless of the power output variations (from 90 to 55% Wpeak) introduced by the operator at each REC/SPR change [179]. Subjects were asked to provide their RPE 10 s before the end of each of the warm up, SPR and REC steps [182]. Immediately after the incremental test, three days before the experimental session, the subjects performed a shortened version of the HIEC test so that they would be familiar with the test [179].

4.2.3. Design of the 1d and 9w Experimental Training Sessions

The 32 subjects were divided in 4 groups of 8, and they performed the HIEC test on two consecutive days (2 groups per day). On the experimental day, subjects in the first group arrived at the laboratory at 06.00 AM, two hours before the test, in a fasted state. The second group of the day arrived two hours later in a fasted state. All subjects had a standardized breakfast consisting of 400 ml of fruit juice and servings of jam tart adjusted according to gender caloric needs (90g for females and 135g for males; total breakfast calories: 612-794 kcal, 119.6-150.6 g CHO, 6-8.4 g Protein, 11.4-16.9 g Fat). Breakfast total calories represented about 30% of the Total Energy Intake, calculated using the FAO equation, with a coefficient of 1.55 (male) and 1.56 (female) to take into account the Physical Activity Level (light activity) [197]. The design of the experimental session is shown in Figure 4.1.

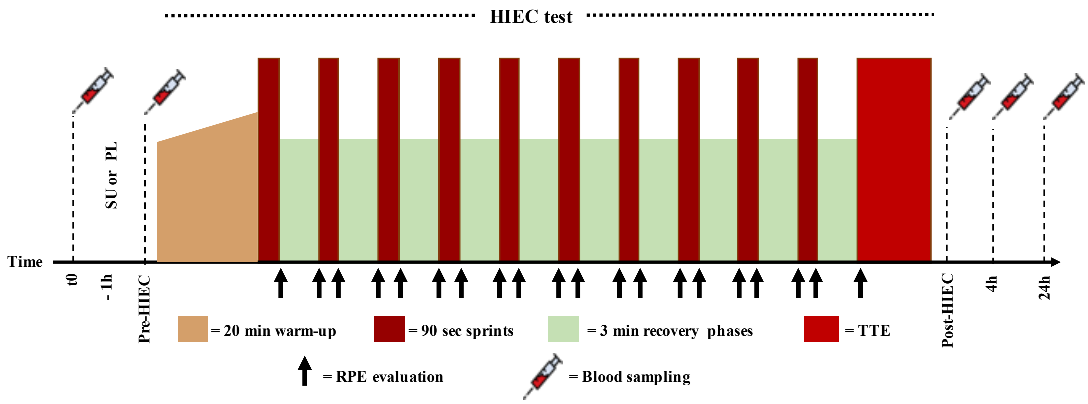

Figure 4.1. Design of the experimental sessions at 1d and 9w. The experimental sessions were performed in the morning. 1 h after breakfast, participants had their first blood draw immediately before the consumption of SU or PL; after another 1-h interval, a second blood sampling was performed immediately before the beginning of the HIEC (Pre-HIEC). In the course of the HIEC, RPE was repeatedly evaluated as indicated by the arrows. Further blood samples were collected immediately, at 4 and 24h after the completion of the HIEC.

4.2.3.1. Blood Sampling and Analysis

Venous blood samples (5 ml) were obtained from the antecubital vein and collected in BD Vacutainer® SST™ blood collection tubes (BD diagnostic preanalytical systems, Milan, Italy) 1h after breakfast (immediately before FP or PL ingestion) (T0), 1h after ingestion (immediately before exercise) (pre-HIEC), immediately post exercise (post-HIEC), after 4h and 24h. Serum was obtained from clotted blood by centrifugation at 1000g at 4°C for 15 min and stored at -80°C for later analyses. Serum CK activity was measured at pre-HIEC, post-HIEC, 4h and 24h by a standardized commercially available colorimetric enzymatic assay (BioVision, Vinci-Biochem, Italy). Ammonia levels at T0, pre and post-HIEC were measured using a commercially available assay (Sigma Ammonia Assay Kit, Sigma-Aldrich, USA). The serum blood levels of BCAA, Ala, total and free Trp were determined at T0, pre and post-HIEC, by HPLC according to Stocchi et al. [198]. The intra and inter-assay confidence interval for CK kit is ≤10.0% for both values; for ammonia determination assay kit 4-7 and 5-8% values, respectively.

4.2.3.2. Glycemia Assessment

Blood glucose was measured by a portable glucometer (MyStar Extra, Sanofi) [199] at the following times: T0 in the fasted state; immediately and 30 min after breakfast; before the intake of FP or PL (1h after the standardized breakfast); 30 min after intake of SU or PL; and immediately before and after the HIEC test.

4.2.4. Training Protocol

Thirty-six indoor cycling training sessions were performed over a 9w period (see Figure 4.2). The training sessions were divided into three mesocycles, as follows:

- First: three 53.1 ± 1.3 -min sessions per week over a 3-week period;
- Second: four 59.1 ± 1.2 -min sessions per week over a 3-week period;
- Third: five 68.2 ± 1.4 -min sessions per week over a 3-week period.

The 32 subjects were divided in two groups of 16 and trained by two expert instructors with the aim of following the same training program. Each session was choreographed based on conventional principles (. warm-up, systematic high intensity interval exercise, and cool-down) widely used in the indoor cycling community [200]. The training program of each session was designed following the same intensity distribution, based on a polarized model, with around 70% of the session time spent in zone 1, 10% spent in zone 2 and 20% spent in zone 3 (see "*Incremental Test*" section for zone determination), according to Seiler and Kjerland [191]. During the training sessions, the HR of each subject (instructor included) was monitored and recorded using a Polar Team Pack 2 (POLAR, Kempele, Finland). HR values were projected onto the wall, as percentage of maximal HR (% HR_{max}), and the subjects were asked to maintain the same intensity as the instructor.

One hour before each training session, the subjects of the SU group ingested a single dose of FP, while the subjects of the PL group ingested the placebo.

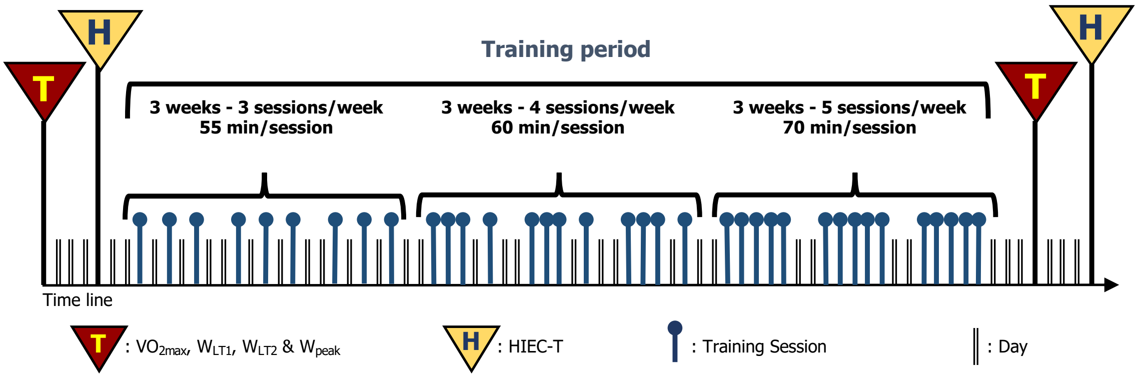

Figure 4.2. Structure of training period: nine weeks divided into three mesocycles (three weeks each). The frequency and the duration of the sessions are also indicated. Key: VO_2max, maximal oxygen consumption; W_{LT1} and W_{LT2}, power at lactate thresholds; Wpeak, peak power; HIEC-T, high intensity endurance cycling test.

4.2.5. Training Load Analysis

Lucia's TRIMP [201] was used to calculate the Training Load for each session. The concept of Lucia's TRIMP integrates total volume, on the one hand, and total intensity relative to the intensity zones, on the other. Briefly, the score for each zone is calculated by multiplying the accumulated duration in the zone by a multiplier for that particular zone (e.g. 1 min in zone 1 is given a score of 1 TRIMP (1 × 1), 1 min in Zone 2 is given a score of 2 TRIMP (1 × 2), and 1 min in Zone 3 is given a score of 3 TRIMP (1 × 3); the total TRIMP score is then obtained by summing the results of the three zones [201]. Finally, the mean TRIMP scores of each mesocycle performed by the SU and PL groups were compared.

4.2.6. Diet & Diet Tracking

During the entire training period, subjects' nutrition was monitored daily (by call interviews, always carried out after dinner) and data were collected and processed using MètaDieta software (METEDA S.r.l., San Benedetto del Tronto, Italy); macronutrients and total energy intake for experimental and control groups were finally compared in order to exclude differences in nutritional habits.

4.2.7. Statistical Analysis

Descriptive statistics were performed using means and standard deviations. Homogeneity between groups was tested using the unpaired t- test. Daily protein, fat, carbohydrate and total caloric intake were compared between groups; the t-test and Cohen's effect size (ES) [202] were used to quantify differences. For Cohen's d, an ES of 0.2–0.3 was considered a "small" effect, around 0.5, a "medium" effect, and 0.8 to infinity, a "large" effect [202]. The time series of the RPE analysis were performed using the HIEC test values for each of the four conditions (1d SPR, 1d REC, 9w SPR, 9w REC) comparing the PL and SU groups. For each of the four conditions, differences between slopes and intercept (SU vs PL) were tested using the statistical approach according to Dupont and Plummer [203]. Furthermore, in all experimental conditions, SPR RPE values were plotted against delta RPE (SPR - REC) in order to verify the degree of recovery in REC steps. Two-way ANOVA with interaction was used in 1d and 9w conditions to test the association among group partnership (SU vs PL) and gender, used as predictive binary factors, and TTE, used as a dependent variable. Partial eta-squared (η^2) values were used as ES. VO_2max, Wpeak, W_{LT1} and W_{LT2} were compared between 1d and 9w training using ANOVA for repeated measures, using groups as a between categorical predictive factor. According to Cohen (1988) [202], an η^2 ranging from 0.02 to 0.13 was considered a "small" effect, from 0.13 to 0.26 a "medium" effect, and higher than 0.26 a "large" effect . TRIMPs were compared between groups as the mean of sessions of each mesocycle. TRIMP comparison was performed using a two-way ANOVA with interaction, followed by the LSD post-hoc test. Similarly, glucose levels were compared at different measurement times. Finally, CK, total BCAA, alanine, total Trp, free Trp, Trp:BCAA and ammonia levels were compared using a two-way ANOVA for repeated measures. For CK levels, time (pre-HIEC, post-HIEC, 4h and 24h CK levels) was within factor, and group membership (SU vs PL) was between factor. Contrast analysis for differences between two consecutive measures (post-HIEC vs pre-HIEC; 4h vs post-HIEC; 24h vs 4h) versus the group was performed. CK levels were also plotted versus TRIMP values during HIEC, and correlation analysis was performed. For total BCAA, alanine, total Trp, free Trp and Trp:BCAA, time was within factor (t0, pre-HIEC and post-HIEC) and group membership was between factor. All statistical analyses were performed using Excel or SPSS 20.0; the significance threshold was fixed at 0.05.

4.3. Results

4.3.1. Baseline Anthropometric, Metabolic and Biomechanical Variables

Anthropometric, metabolic and biomechanical variables of participants were assessed before the beginning of the experimental session as reported in Table 4.2. No differences were found between the two groups in the tested parameters.

Table 4.2. Anthropometric, metabolic and biomechanical variables of the participants at baseline; Mean, standard deviations and p values for group are reported.

	Supplemented Group (n=16)		Placebo Group (n=16)		Group (*p*)
Participants	Males=10	Females=6	Males=10	Females=6	
Age (yr)	22.1 ± 2.2	20.6 ± 1.0	21.0 ± 1.0	20.5 ± 0.7	0.322
Height (cm)	173.9 ± 6.0	157.2 ± 4.4	177.1 ± 6.8	161.8 ± 4.3	0.072
Weight (kg)	69.2 ± 12.7	50.3 ± 4.8	67.2 ± 6.9	54.6 ± 5.2	0.726
BMI (kg/m^2)	22.8 ± 3.4	20.3 ± 1.2	21.4 ± 1.6	20.8 ± 1.4	0.584
HR_{max} (bpm)	197.3 ± 8.2	197.5 ± 5.0	199.0 ± 7.8	199.7 ± 4.7	0.458
VO_2max (ml/kg/min)	42.6 ± 10.4	35.0 ± 8.5	43.9 ± 4.5	28.1 ± 3.1	0.315
W_{peak} (watt)	212.5 ± 33.9	146.7 ± 23.4	230.0 ± 28.4	133.3 ± 23.4	0.844
W_{LT1} (watt)	76.1 ± 11.3	57.7 ± 23.8	73.9 ± 22.3	40.8 ± 14.0	0.312
W_{LT2} (watt)	127.1 ± 23.4	88.2 ± 23.5	138.8 ± 27.6	74.9 ± 16.6	0.928

4.3.2. Diet Monitoring

Daily caloric intake over the study period was virtually identical for both groups: 1944 ± 876 kcal in the SU group vs 2043 ± 947 in the PL group, with no significant difference (t test; $p>0.05$); ES showed a negligible effect (ES=0.07).

Daily CHO, fat and protein intakes, supplemented vs placebo group were 49.1% vs 51.1%; 33.4% vs 32.4%; 17.4% vs 16.9%, respectively. No differences in specific macronutrient intake were found between groups (t test; $p>0.05$); a very small, negligible effect size was observed for carbohydrates, fats and proteins: 0.12, 0.07 and 0.06, respectively.

4.3.3. VO_2max, Wpeak and Power at Lactate Thresholds at 1d and 9w

All these variables, namely VO_2max, Wpeak, W_{LT1} and W_{LT2}, were significantly different in pre vs post 9w training as shown in Table 4.3. For all variables, p values were < 0.001. Results indicate that all post training values were significantly greater than pre-training ones, with partial $\eta^2 > 0.484$ (large effect). The effect of SU intake was not significant ($p>0.05$) for all dependent variables.

Table 4.3. VO_2max, Wpeak, W_{LT1} and W_{LT2}*, in SU and PL groups at 1d and 9w.

	Supplemented Group			**Placebo Group**		
	1d	9w	Δ%	1d	9w	Δ%
VO_2max	39.73 ± 10.18	44.58 ± 6.67	+12%	37.95 ± 8.82	42.93 ± 5.54	+13%
Wpeak	187.81 ± 44.20	231.56 ± 48.91	+23%	193.75 ± 54.79	239.06 ± 56.01	+23%
W_{LT1}	69.21 ± 18.69	103.64 ± 32.95	+50%	64.83 ± 27.12	91.03 ± 26.56	+40%
W_{LT2}	112.50 ± 29.85	155.56 ± 35.34	+38%	114.83 ± 39.59	156.51 ± 40.66	+36%

* VO_2max: maximal oxygen consumption ($ml*kg^{-1}*min^{-1}$); W_{peak}: power peak (watt); W_{LT}: power at Lactate Threshold (watt); Δ%: percentage difference in average values.

4.3.4. Perceived Exertion During HIEC Test

RPE values, measured during the 20 min warm up of the HIEC tests increased progressively, showing a very similar trend in the PL and SU groups in both 1d and 9w periods (Figure 4.3, respectively). During the 10 SPR, each of them followed by a REC step, RPE showed an upward trend characterized by a sawtooth pattern in all the conditions tested. As expected, the RPE values reached the maximum at the end of the TTE step (11 points on OMNI cycle scale). Hence, only RPE values starting from 20 min (the end of warm up) to 65 min (prior to TTE phase) were considered for further analyses (data highlighted in grey box).

4.3.4.1. Perceived Exertion at 1d (Pre training HIEC Test)

The linear regression equation of the curve built on SPR steps' data in PL group was $RPE_{HIEC}=0.508$ time+3.937 ($r^2=0.98$) vs $RPE_{HIEC}=0.398$ time+4.501 ($r^2=0.99$) in the SU group. Intercepts ($p=0.163$) and slopes ($p=0.086$) were not significantly different. The linear regression equation of REC steps' data in the PL group was $RPE_{HIEC}=0.463$ time+4.033 ($r^2=0.97$) vs $RPE_{HIEC}=0.344$ time+4.013 ($r^2=0.99$) in the SU group. Intercepts were not significantly different ($p=0.742$), whereas, interestingly, slopes were ($p=0.001$). This would imply that in REC steps, the SU group showed a lower RPE (Figure 4.3).

4.3.4.2. Perceived Exertion at 9w (Post training HIEC Test)

The linear regression equation of SPR steps' data in the PL group was: $RPE_{HIEC}=0.338$ time+5.657 ($r^2=0.93$) vs $RPE_{HIEC}=0.247$ time+5.354 ($r^2=0.98$) in the SU group. Slopes, unlike intercepts ($p=0.079$), were significantly different ($p=0.017$), suggesting that in the SPR phase, the SU group showed a lower RPE. The linear regression equation of REC steps' data in the PL group was: RPE_{HIEC}=0.246 time+5.513 (r^2=0.92) vs RPE_{HIEC}=0.221 time+4.452 (r^2=0.97) in the SU group. Slopes were not significantly different (p=0.371), while an extremely significant difference was found between intercepts (p<0.001). This implies that in the REC steps, the SU group showed a systematically lower RPE (Figure 4.3).

On the whole, RPE values increased linearly over the execution time of HIEC in both the SU and PL groups (Figure 4.3). Notably, the extent of the increment was significantly lower in the SU group than it was in the PL group in all the conditions tested (Figure 4.3), with the only exception of the 1d pre-training SPR phase (Figure 4.3); the lowest increment was observed in the 9w post-training REC phase.

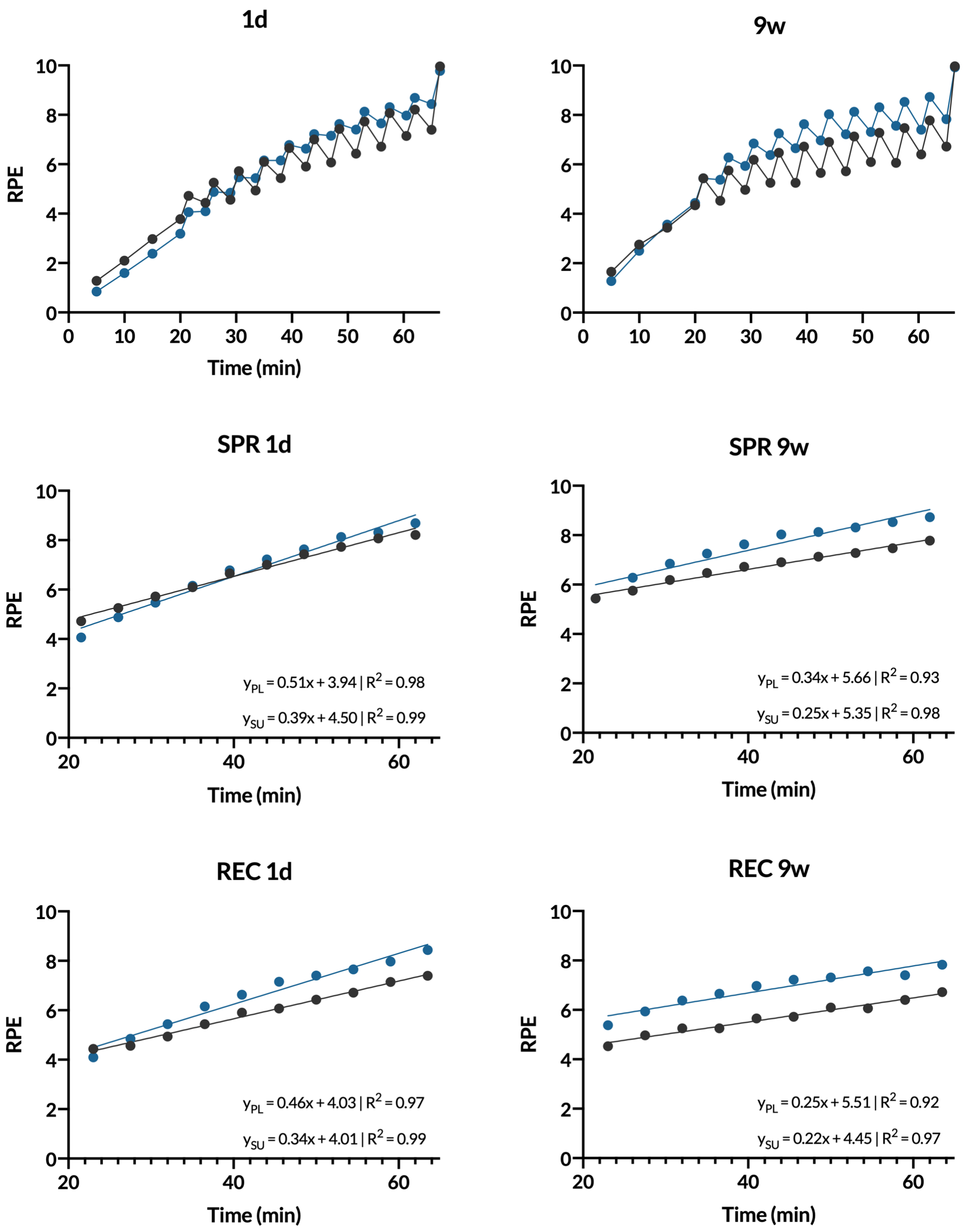

Figure 4.3. Perceived exertion rate (RPE) values versus session time; on the top whole RPE time series; in the middle RPE values in sprint (SPR) steps at pre-training (1d) and post training (9w) stages, respectively; on the bottom RPE values in REC steps at 1d and 9w, respectively. Black circles refer to SU and blue circles to the PL group. Modified with permission from Gervasi et al. [7].

That SU group experienced a more efficient recovery than the PL group,. a lower REC-associated RPE, which can be better appreciated in the scatter plots of Figure 4.4 showing the differences (Delta) between the SPR- and REC- RPE values as a function of the RPE recorded at the end of each of the SPR steps.

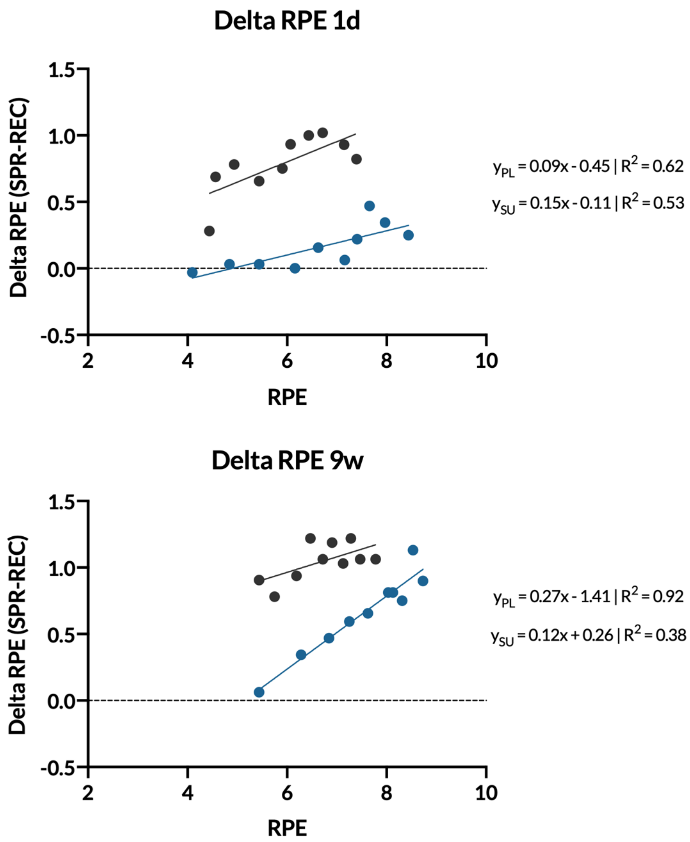

Figure 4.4. RPE reduction after the completion of each REC step in the SU and PL groups. The RPE differences are expressed as Delta RPE, which represent the difference between the RPE measured at the end of each SPR and at the end of its subsequent REC step. Delta RPE are plotted against the absolute RPE (on the x-axis) measured at the end of each corresponding SPR step. Upper and lower panels show 1d and 9 w, respectively. Black circles refer to SU and blue circles to the PL group. Modified with permission from Gervasi et al. [7].

Furthermore, after 9w, the means of the RPE scores in the SU group were reduced compared to the PL group by 13% in the SPR and by 21% in the REC phases; notably, even after the first administration of FP at 1d, RPE during the REC phase decreased by 9% compared to the PL group (Figure 4.5).

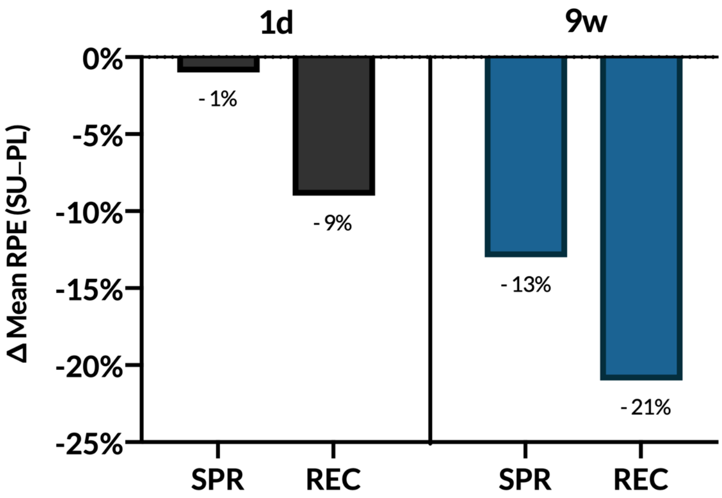

Figure 4.5. Difference between the mean RPE scores of SU vs PL groups. RPE were measured during the 1d (left, in black) and 9w (right, in blue) HIEC sessions. The 1d SPR, 9w SPR, 1d REC and 9w REC columns were calculated from the data points in Figure 4.3. Modified with permission from Gervasi et al. [7].

4.3.5. Performance During HIEC Test: Time To Exhaustion

TTE values were determined and taken as reliable performance parameters [204,205]. Analysis of the 1d data failed to reveal significant differences between groups (371±147 s for SU; 359 ± 177 s for PL; $p>0.05$). On the contrary, with regard to 9w, data showed that the mean TTE was significantly longer for the SU group (517±210 s) than for the PL group (321±214 s) ($p=0.025$), with partial $\eta^2=0.201$ (medium effect); the interaction effect was also significant ($p<0.05$).

4.3.6. Training Load Analysis

TRIMP represents a recognized parameter to express the extent of training load [206]. TRIMP values were compared between groups in the course of the training period, which was divided into three different three-week mesocycles (first mesocycle: 1-3 weeks; second: 4-6 weeks; third: 7-9 weeks) characterized by progressively increasing training loads (both in terms of the frequency and duration of the sessions). During the first mesocycle (3 sessions/week of 53.1 ± 1.3 min) subjects averaged 98.4 ± 4.9 TRIMP (SU) and 97.9 ± 4.1 (PL) per session (total TRIMP per mesocycle: 886 in SU, 881 in PL); during the second mesocycle (4 sessions/week, of 59.1 ± 1.2 min), subjects averaged 97.9 ± 5.4 TRIMP (SU) and 96.5 ± 7.1 (PL) per session (total TRIMP per mesocycle: 1175 in SU, 1158 in PL); no differences in these mesocycles were found between groups (post-hoc LSD test; $p>0.05$). Notably, during the last mesocycle (5 sessions/week of 68.2 ± 1.4 min) TRIMP values were significantly higher (post-hoc LSD test; $p=0.014$; ES=0.6, large effect) in the SU group than they were in the PL group, with averages of 109.4 ± 5.7 vs 104.1 ± 6.4 per session, respectively (total TRIMP per mesocycle: 1641 in SU, 1561 in PL). Data are shown in Figure 4.6.

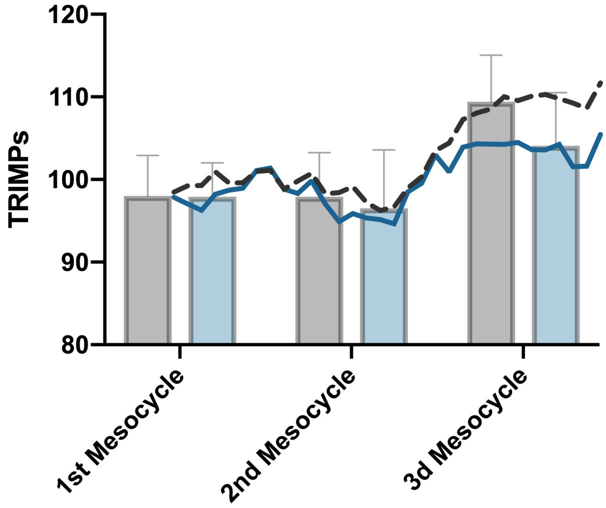

Figure 4.6. Training loads in the PL and SU groups as a function of mesocycles and training progression. Bars represent the mean training impulse (TRIMP) associated with the corresponding mesocycle in the PL (blue columns) and SU (black columns) groups (standard deviations are reported). Mesocycles and weeks are reported on the x axis. Dashed black line (SU) and solid blue line (PL) were obtained using a 5-day moving average. Modified with permission from Gervasi et al. [7].

4.3.7. Serum Creatine Kinase (CK)

Serum blood CK levels changed over time in the SU and PL groups at both 1d and 9w measurements ($p<0.001$). At 1d, CK levels showed an increase in the post-HIEC, followed by a progressive decrease before returning to basal values after 24h. At 1d, group partnership (SU or PL) did not show a different trend of CK concentration (time x group interaction; $p=0.568$). On the contrary, at 9w SU vs PL group showed a different trend of CK concentration (time x group interaction; $p=0.017$). A contrast analysis for determining differences between two consecutive measures showed that the SU group was different from the PL group in “post-HIEC vs pre-HIEC ($p=0.048$)” and “4h vs post-HIEC ($p<0.047$)”. In other words, CK levels were significantly higher only in the SU group in the post-HIEC at 9w, while in all the other conditions, no significant differences could be identified. However, after 4h, the SU group [CK] was no longer significantly different ($p>0.05$) from the PL group. Data are shown in Figure 4.7.

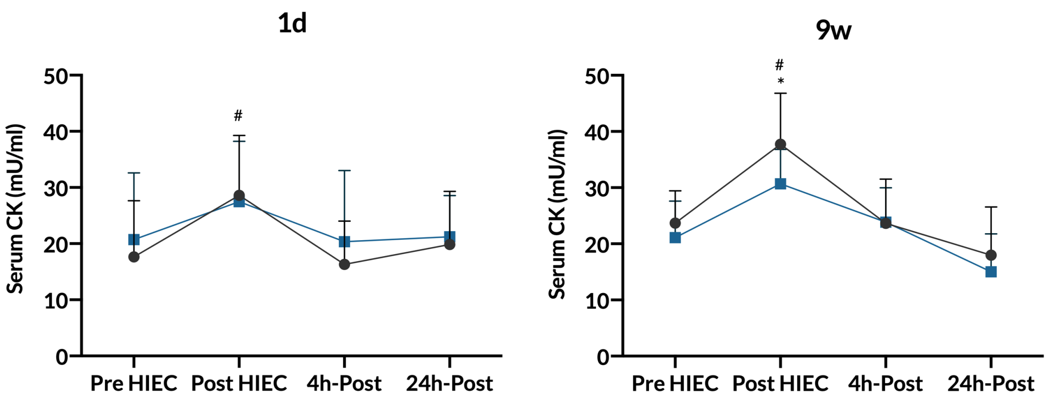

Figure 4.7. Creatine kinase (CK) serum blood levels. CK was determined at the indicated time points at 1d (left) and 9w (right) in the SU (black dots) and PL groups (blue dots); * p<0.05 as compared to PL; # p<0.05 as compared to an earlier time point. Modified with permission from Gervasi et al. [7].

4.3.8. Serum Blood Levels of BCAA, Ala, Trp, Ammonia and Ratios of Free Trp:BCAA

Blood samples were collected immediately before (T0), 1 h after the ingestion (pre-HIEC) of FP or PL, and at the end of the HIEC test (post-HIEC). HPLC analysis of serum blood samples (Figure 4.8) showed that total BCAA concentrations ([BCAA]) before the ingestion of FP or PL powder at both 1d and 9w were similar, and that at pre-HIEC they increased significantly only in the SU group (p<0.05). [BCAA] measured at post-HIEC decreased significantly in the SU group at 1d and 9w, though to a lesser extent in the latter case.

Pre- and post-HIEC plasma levels of total Trp and free Trp were also determined and are shown in Figure 4.9: no significant difference (p>0.05) was found in the total Trp values both as a time- or group- function; free Trp levels increased significantly in post-HIEC compared to pre-HIEC, both at 1d (p=0.001) and at 9w (p=0.003), while no significant change was detected between groups (p>0.05).

Regarding Trp:BCAA ratios, at pre-HIEC they were consistently higher in the PL group than they were in the SU group. At 1d, notwithstanding the time-related increase in both groups (pre- *vs* post-HIEC), the PL group was characterized by a higher ratio than the SU group; interestingly, at 9w a statistically significant increase could be found only in the PL group.

Ala serum blood levels ([Ala]) reached slightly higher levels only in the SU group at 1d and 9w pre-HIEC phase (p=0.06; Figure 4.11), while in post-HIEC at both time points [Ala] significantly increased in the PL as well as in the SU group (p<0.05), with the latter characterized by a slightly higher increment at 9w *vs* PL.

Finally, serum ammonia levels at 1d were 40.4±18.0 μM SU *vs* 43.6±23.2 μM PL at T0; 49.1±22.1 SU *vs* 42.4±20.3 PL at pre-HIEC; 121.0±78.6 SU *vs* 111.3±61.2 PL at post-HIEC. At 9w similar values were observed with T0 levels of 43.6±21.5 μM SU and 43.3±24.5 μM PL; 49.3±20.6 SU *vs* 42.0±20.4 PL at pre-HIEC; 121.1±67.5 SU *vs* 108.7±51.6 PL at post-HIEC. Statistically significant differences were found only in pre-HIEC *vs* post-HIEC (p<0.05).

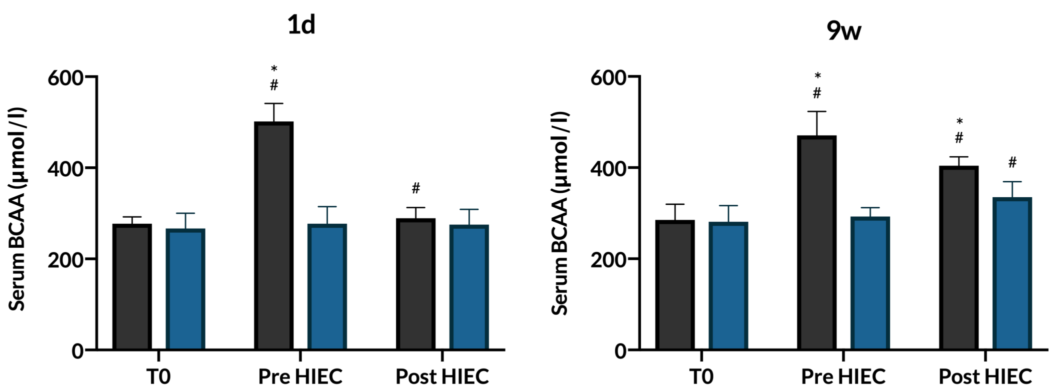

Figure 4.8. Branched chain amino acids [BCAA] serum blood levels. [BCAA] (total amount of Leu, Isoleu and Val concentrations) were determined prior to (T0) FP or PL powder ingestion, 1 h after (pre-HIEC) and at the end of the HIEC test (post-HIEC). Left and right panels show analyses performed at 1d and 9w respectively. Values for the SU (black bars) and PL (blue bars) groups are reported, with mean and standard deviations. * p<0.05 per group; # p<0.05 per time. Modified with permission from Gervasi et al. [7].

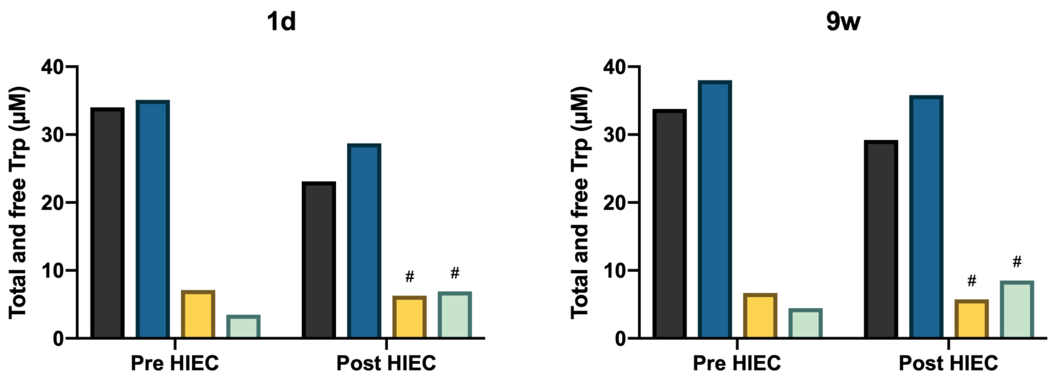

Figure 4.9. Total and free Trp plasma concentrations. Trp levels were determined at pre-HIEC and at post-HIEC. Left and right panels show analyses performed at 1d and 9w, respectively. Key: blue bars show total Trp in the PL group; black bars, the total Trp in the SU group; light green bars, the free Trp in the PL group; yellow bars, the free Trp in the SU group. Data are reported as means ± standard deviation. # $p<0.05$ per time. Modified with permission from Gervasi et al. [7].

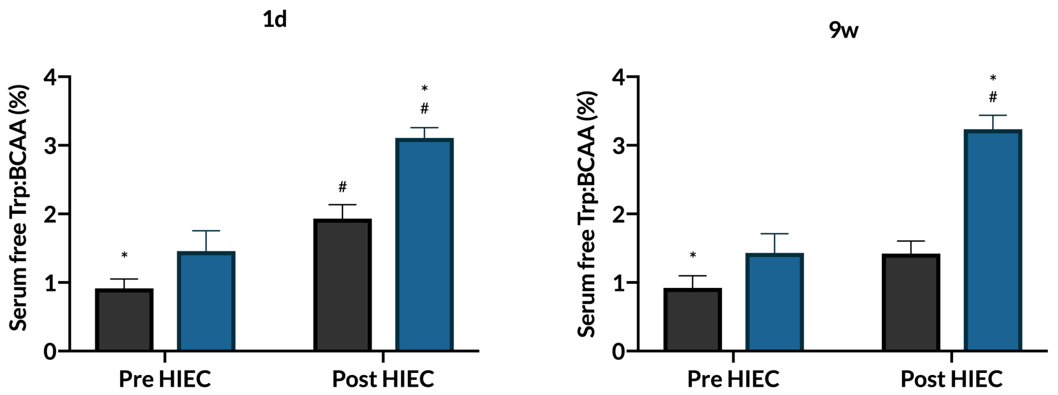

Figure 4.10. Free Trp to BCAA ratios. Free Trp and BCAA levels were determined and their ratios were then calculated in both the FP and PL groups. Trp:BCAA ratios before (pre-HIEC) and after HIEC test (post-HIEC) are shown. Left and right panels show analyses performed at 1d and 9w, respectively. Values for the SU (black bars) and PL (blue bars) groups are reported as means with standard deviations. * $p<0.05$ between groups; # $p<0.05$ between time points. Modified with permission from Gervasi et al. [7].

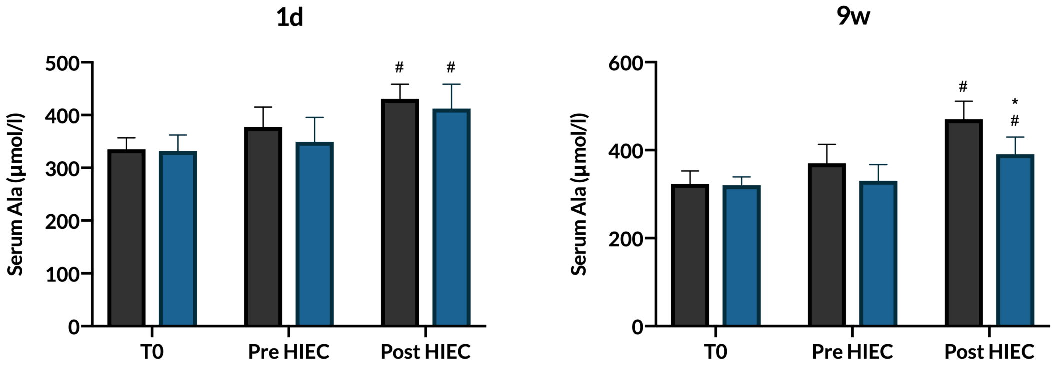

Figure 4.11. Ala serum blood levels after SU or PL ingestion and post-HIEC. Left and right panels show analyses performed at 1d and 9w, respectively. Values for the SU (black bars) and PL (blue bars) groups are reported as means with standard deviations. * $p<0.05$ between groups; # $p<0.05$ compared to an earlier time point. Modified with permission from Gervasi et al. [7].

4.3.9. Glycemia

Glycemia was determined prior to breakfast (4.8±0.1 and 5.3±0.2 mM in SU vs PL respectively, p>0.05) and at different time points up to the end of HIEC test. As expected, 30 minutes after breakfast, glucose levels increased (9.4±1.5 and 8.5±1.8 mM in the SU and PL groups respectively) and decreased thereafter, approaching basal levels (5.7±0.5 in SU vs 5.6±0.6 mM in PL; p>0.05). No further significant difference between groups was observed post-HIEC (6.1±0.2 vs 5.8±0.6 mM in SU and PL respectively; p>0.05).

4.4. Discussion

The effects of FP – an established, commercially available sports nutritional supplement containing BCAA, Ala and CHO – on RPE, performance and the capacity to sustain physical training were investigated in a group of 32 healthy young subjects enrolled in a randomized double-blind placebo-controlled trial. Along with RPE and performance values, a number of relevant nutritional and biological parameters were also determined. Notably, to the best of our knowledge, this is the first study adopting a validated and reliable HIEC protocol [207] for these purposes. Indeed, other protocols have been used to determine similar end-points in the past [208,209], but it is worth noting that they had not been previously and specifically validated.

The major finding of this study is that a single intake of FP is capable of attenuating RPE, and that its prolonged 9w consumption according to manufacturer's recommendations not only augments RPE-attenuating capacity, but also improves TTE and TRIMP, which both reflect the capacity to sustain training loads. HPLC analysis of blood sampled 1 h after FP ingestion, unlike the sample taken 1 h after PL administration, showed a significant increase in BCAA levels. This finding indicates that BCAA are rapidly absorbed after oral ingestion of FP, and that their increased serum blood concentration is likely related to the above-mentioned effects on RPE, TTE and TRIMP.

Following the first intake, the SU group showed lower RPE values only in the HIEC REC phases, while a significant RPE reduction was found following a chronic (9w) intake also in the high intensity SPR phases. Furthermore, both acute and chronic intake caused a significantly more rapid decrease in RPE observed between the SPR and corresponding REC phases compared to PL. It is worth noting that, unlike previous studies on BCAA and RPE [168,210], by virtue of the particular design of the HIEC test, this is the first investigation in which RPE associated with

SPR or with REC phases was separately quantitated. This allowed us to determine that FP significantly accelerated the reduction of RPE during the recovery phases compared to PL.

As regards Trp levels, we only found a slight although significant exercise-dependent variation in free-Trp between pre- and post-HIEC, an effect in line with the data reported and discussed by other Authors [211,212].

Our results indicate that serum blood circulating Trp:BCAA ratios increase after HIEC in PL, and that FP consumption invariably prevented this effect. Similar qualitative and quantitative results have been observed in previous studies [168,183] on BCAA supplementation and RPE in exercising young adults. Under the conditions we observed in the PL group, namely an increased Trp:BCAA ratio, Trp is supposed to be more available for brain uptake, thus promoting an augmented synthesis of serotonin [177]; on the contrary, a significantly lower Trp:BCAA ratio, which we did observe in the SU group, is thought to antagonize brain Trp uptake, thus limiting serotonin synthesis and availability [213]. According to the widely held belief linking brain serotonin increases with central fatigue development [161,168], this sequence of events might have contributed to the lower RPE values we observed upon acute and/or prolonged FP supplementation. Since in our conditions Trp blood levels increase, some concern might be raised with regard to its conversion, through the kynurenine pathway, into correspondingly higher levels of the excitotoxic quinolinic acid and kynurenine [214]. However, as discussed by Fernestrom et al. [215], even under conditions of supplementation with extra-Trp, no effect attributable to quinolinic acid toxicity have never been observed in humans. In addition, physical exercise has been shown to prevent per se the eventual brain entry of Trp-derived kynurenine [216] as well as to attenuate the activity of the kynurenine pathway [217,218].

Ammonia cerebral uptake and concentration are known to increase in humans during prolonged exercise [166], thus augmenting central fatigue by altering cerebral energy metabolism and neurotransmission [162]. However, although HIEC promotes an increase in serum ammonia levels, we did not find differences between the SU and PL groups at any of the considered time points (T0, pre-HIEC and post-HIEC). This finding, in keeping with data from the literature [169], might depend on the relatively low dose of supplemented BCAA.

With regard to the higher [Ala] upon FP ingestion, we can only speculate on its relevance based on the literature. Supplemental Ala has been shown to exert a positive influence on the anaplerosis of the tricarboxylic acid cycle, on muscle glycogen storage, energy synthesis and on the regulation of ammonia metabolism, transport and excretion [219,220]. Along these same lines, although we have no direct evidence, higher [Ala] could exert a converging role in support of the effects on RPE observed herein.

Regarding glycemia, we did not find any variation between the two groups in the glycemic values of pre- and post-HIEC tests, suggesting that the extra CHO of FP do not significantly modify blood glucose prior to or after testing compared to PL. In this regard, it should also be considered that in our setting both groups had ingested a breakfast containing 120-150 g of CHO 1 h before HIEC, that is approximately tenfold the amount of CHO contained in FP. In light of these considerations, the CHO contribution to the functional and metabolic outcomes described thus far is probably limited. Indeed, a recent study by O'Hara et al. [221], using the same experimental setting we adopted in the present investigation, showed that the intake of 40 g of CHO (galactose or glucose) in one litre of water, taken 30 minutes before HIEC, did not modify the RPE or the TTE compared to the placebo.

Finally, with respect to the possible direct effects of CHO on RPE, only in studies in which CHO were given during – and not prior to (as in our case) – endurance exercise have such effects been observed [222]. On the whole, it can be inferred that in our conditions CHO hardly affect RPE through direct central interactions.

With regard to performance, most of the studies on BCAA-containing supplements have failed to find any significant improvements [210,223] nor did we find any differences in terms of relevant metabolic parameters (VO_2max and Power at Lactate Thresholds) between SU and PL, either upon single (1d) or prolonged (9w) supplementation. However, even though TTE did not improve after the first, acute intake of FP, it did increase significantly following the 9w supplementation. This observation is in line with those of Kephart et al. [176], showing that, although in a different experimental settings, 10-week BCAA supplementation results in increased peak/mean power in well-trained cyclists. Interestingly, the same study also reported a significant increase in serum blood [BCAA] and a consequent improvement in the circulating Trp:BCAA ratio, hence suggesting that performance enhancement could be related to a central fatigue-mediated mechanism [176]. Considering that our SU group did not show any improvement in metabolic parameters or free-fat mass (not shown), we also suggest that the TTE increase might be related to the stable attenuation of RPE rather than to ergogenic or anabolic effects.

With regard to the ability to sustain training loads, our results showed that TRIMP were the same in both groups with work volumes per week < 240 min. Interestingly, at higher work volumes (ca. 350 min in the third mesocycle) TRIMP values were significantly higher in the SU than in the PL group. In this regard, it is worth considering that higher TRIMP expresses an increased ability to sustain exercise at high HR values, while lower TRIMP reflects the relative inability to exercise under the same conditions.

Several studies report that the inability of athletes to increase their HR for a given load is indicative of an overreaching state [224,225]. Again, in accordance with the serotonin theory of central fatigue, chronic elevation in brain serotonin levels has been causally associated with the development of an overtraining state and related symptoms, culminating in decreased performance [226]. Although it is mere speculation, the improved Trp:BCAA ratios afforded by FP supplementation could also explain the enhanced capacity to sustain higher training loads in SU athletes.

Elevation of serum blood CK within 24/72h post-exercise is recognized as a marker of muscle damage caused by intense eccentric and resistance training [227,228], and its severity also depends on exercise intensity [229]. BCAA supplementation, under specific circumstances (high dosage,. 12-20 g/day for at least 10 days starting one week before challenging exercise) has been shown to prevent the elevation of serum CK levels following a continuous, submaximal exercise test, thus suggesting that it may attenuate muscle damage [175,230]. Our testing conditions also involved 10 sprints and a TTE phase performed at 90% of Wpeak, and could reasonably result in some muscle damage. However, despite the exhaustive protocol adopted, we did not find serum CK variations ascribable to muscle damage. Indeed, CK level increases were transient and returned rapidly (4h) to baseline values, showing no variations thereafter (24h) in in either the SU or PL group. On the other hand, we found that after 9w of supplementation, the transient post-HIEC increase in CK was significantly higher in the SU group than it was in the PL group, an effect that could be accounted for by the higher training load of the SU group. In spite of this more consistent serum CK increase, 4h after completion of HIEC, the SU group recovered to the same baseline values as the PL group. These results suggest that the transient CK increase in our conditions is not indicative of muscle damage, but is rather an expression of the higher training load [231].

On the whole, our data suggest that the higher TRIMP values found in SU subjects at 9w reflect their enhanced capacity to sustain training, whose volume may consequently increase over time leading to better performance than that achieved by PL subjects. Reduction in RPE, which was observed from the very beginning of the test period, is likely to play a pivotal role in the progressively enhanced capacity to sustain higher training volumes. The main limitation of the present study, as well as of similar ones, lies in the use of a multi-ingredient supplement, which makes it difficult to determine the relative impact of each component on the tested markers: as a consequence, ascertaining which of the ingredients had what effect or if there was a synergistic interaction among the ingredients remains an open question. On the other hand, the strength of this study resides in the fact that it details a multi-technique experimental approach that could be applied, in the future, to directly compare the efficacy of formulations containing different constituents (such as caffeine, electrolytes, β-alanine etc.) in attenuating RPE. This would be important because, at present, it is very hard to compare the effects of different sport supplements with different formulations on RPE because they have been studied using non-homogeneous experimental designs and approaches [232].

4.5. Conclusions

The main findings of this study are that the consumption of FP (a commercially available nutritional supplement containing BCAA, Ala and CHO) according to the producer's suggestions reduces RPE at all the time points tested and that, over a 9w-intake, also improves TTE and TRIMP. Although it was not possible to specifically address mechanistic issues, the effects we observed are in keeping with the theory of RPE sensitivity to serum blood Trp:BCAA ratio, while the contribution of metabolic effects seems negligible. The prolonged intake of FP, which promotes a reduction in RPE and recovery times, can enhance the capacity to sustain higher training loads and ultimately improve endurance performance. Importantly, these effects occur without affecting dietary habits and caloric intake.

Chapter 5

Factors Influencing Weight Loss Practices in Italian Boxers: A Cluster Analysis [5]

Abstract: It is common practice in combat sports that athletes rapidly lose body weight before a match, by applying different practices—some safer and others possibly dangerous. The factors behind the choice of practices utilised have not been fully studied. This study aimed to investigate the weight loss strategies used by Italian boxers and to look at the difference between higher and lower risk practice adaptors. A modified version of a validated questionnaire has been sent to 164 amateur (88%) and professional (12%) boxers by email. A heatmap with hierarchical clustering was used to explore the presence of subgroups. Weight loss strategies were used by 88% of the athletes. Two clusters were found, defined by the severity of weight loss behaviours. Professional fighters, high-level athletes and females were more represented in Cluster 2, the one with more severe weight-loss practices. These athletes were characterised by a higher weight loss magnitude and frequency throughout the season and reported being more influenced by physicians and nutritionists, compared with the boxers in Cluster 1. Not all the weight loss practices are used with the same frequency by all boxers. The level of the athlete and the boxing style have an influence on the weight-cutting practices.

5.1. Introduction

Combat sports have been rapidly growing in popularity over the past two decades, with ~25% of Olympic gold medals being in some form of combat sport, as well as professional sports (e.g., boxing, mixed martial arts and kickboxing) drawing millions of spectators [233]. In the 2021 Olympic Games, there will be six combat sports, with the new entry of karate. In combat sports, athlete body mass is verified at an official 'weigh-in', and they are then divided into categories by their body weight, in order to ensure competitors of similar size [8].

It is common practice for athletes to lose significant body weight in the days/weeks leading up to the competition to gain an advantage by being paired with a smaller opponent: this practice is colloquially referred to as 'weight-cutting'. This weight-loss can be achieved by several different strategies, including energy intake restriction (gradual dieting and fasting), total body fluid reduction (restricting fluid intake, increasing sweat response (heated wrestling, plastic suits, saunas and spitting) and pseudo extreme/abusive medical practice (laxatives, diet pills, diuretics, enemas, sporting bulimia (vomiting)) [234,235]. Some of these could be dangerous for the athletes' health: for example, moderate and severe dehydration increases the risk of acute cardiovascular problems, such as heart stroke and ischaemic heart disease. Furthermore, a severe dehydration could potentially increase the risk of brain injury due from head trauma induced by the strikes. Other potential risks related to weight-cutting practices include suppressed immune function, changes to insulin sensitivity and hormonal imbalances [8].

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The use of weight-cutting has been observed to be highly prevalent (60–80%) across a wide range of combat sports including Brazilian jiu-jitsu, boxing, judo, mixed martial arts (MMA), muay-thai, kickboxing, taekwondo, wrestling and karate [234-236]. While the majority of athletes utilise gradual dieting and increased energy expenditure through exercise, there is a significant portion of athletes that report using more potentially dangerous methods (such as severe heat exposure), though the precise magnitude is unclear [234-237]. Furthermore, while previous research has reported more broad data on weight-cutting in combat sports, there is a paucity of data examining the potential differences in factors between athletes that choose to use more potentially dangerous methods of weight loss than those who focus on the less extreme methods. Factors such as competitive level, weight class and who influences their decision making may explain such choices. Developing a deeper understanding of weight-cutting in combat sports is essential, as the practice has been associated with negative health outcomes (including several deaths worldwide) [8,238] as well as impaired performance [234,239-241], although this is a topic of debate [242,243].

Boxing is one of the most popular combat sports worldwide. Statistics showed that, in 2017, in the United States the number of participants in boxing (of all ages and categories) exceeded six million people; in the UK in 2018 around eight hundred thousand were reported to be participating in boxing [244]. In boxing, research has failed to observe any positive or negative relationship between weight-cutting and competitive performance [243,245]. Despite no clear evidence that weight-cutting improves performance, the practice has been found to be highly prevalent, with research reporting >92% of all boxers to engage in some form of weight loss for competition [234,235]. However, the research has not conducted an in-depth exploration of the potential factors influencing athletes who chose to utilise higher-risk methods. Such an exploration is important, as it will allow organisers and regulatory bodies to better tailor communication to athletes and coaching staff when trying to manage extreme weight loss practices.

The primary aim of this cross-sectional study was to investigate the common weight-cutting strategies used by a sample of professional and amateur boxers competing in Italy. A secondary aim was to investigate if clusters existed regarding the strategies and the frequency of the rapid weight loss behaviours.

5.2. Materials and Methods

5.2.1. Participants and Study Design

A total of 164 subjects (144 males and 20 females) were recruited for this cross-sectional study. All participants were active boxers, both amateur (Olympic-style boxing) and professionals. They were recruited from several clubs in the period between July and November 2019; the Italian Boxing Federation, through the regional managers and coaches of the National team, helped to reach a bigger number of athletes. Participation was voluntary, and the data collected were completely anonymous; after a verbal explanation of the study, participants signed informed consent. For the forty-eight under 18 participants, consent to participate was signed by their legal guardians. The study was conducted in accordance with the Helsinki Declaration, and the approval to conduct the survey was received by the Ethics Committee of Urbino University (no approval number has been provided since it was not mandatory, given that no sensitive data were collected in the study).

5.2.2. Survey

A snowball sampling technique, often used in populations which are difficult for researchers to access, has been used in order to reach a larger number of athletes. Then, an online survey (Google Forms) has been sent via email to every participant who decided to take part in the study. The link to the survey has been sent to each participant by only one researcher, and the mailing list was protected by a password in order to not be accessible from others. The survey was based on the 'Rapid Weight Loss' questionnaire (RWLQ) of Artioli et al. [246], that has been already used in other similar studies [234,247,248]. It was composed of two sections: in the first one, personal data were collected, like age, gender, sports experience (level, category, number and level of matches) and weight-loss history (e.g., how many kilos were lost before a match, how many times weight was cut in one season). On the second section, subjects were asked to indicate—using a four-level Likert scale (0 = never done; 1 = almost never; 2 = sometimes; 3 = always)—if they applied each of the following 14 weight-cutting strategies (gradual weight-loss, increased training volume, water restriction, wear a plastic suit during the day/night, wear it during training, skip meals or fasting, to train in a heated environment, sauna, diet pills, diuretics, laxatives, to vomit, to spit saliva). The RWLQ provides a validated RWL score (RWLS) that allows a quantitative measure of the aggressiveness of the weight-cutting behaviours. On a latter section of the survey, not included in the original version of Artioli et al. [246], subjects were asked to indicate, on a three-level Likert scale (1 = no influence; 2 = moderate influence; 3 = big influence), in which extent the following figures may influence their behaviours regarding weight-cutting strategies: physician/nutritionist, master, physical coach, family, other boxers/teammates.

5.2.3. Data Analysis

The *pheatmap* R package (Manufacturer, City, Country) [249] was used to create a heatmap of the 14 weight-cutting strategies, with the *euclidean* distance and *complete* method. Hierarchical clustering analysis was performed on the subjects, with the relative dendrogram. Elbow and silhouette methods were used for determining the optimal number of clusters, using the *fviz_nbclust* function of the *factoextra* R package (Manufacturer, City, Country) [250]. A Permanova has been conducted using the function *adonis2* of the *vegan* R package [251] to check the statistical difference between the two clusters. A chi-square test has been used to test differences in weight-cutting strategies used and in participants characteristics between the two clusters, applying the false discovery rate correction (Benjamini–Hochberg) (*FSA* R package (Manufacturer, City, Country) [252]). Results are presented with $p(\chi^2)$ and Cramer's V. V-values should be interpreted as >.5 = high association, 0.3 to 0.5 = moderate association, 0.1 to 0.3 = low association, 0 to 0.1 = little or no association. A Mann–Whitney test has been used to test the difference in weight-cutting history and RWLS between the two clusters. All the analyses have been performed using SPSS 26.0 (IBM, Armonk, NY, US) or R Studio 3.6.2 (RStudio PBC, Boston, MA); GraphPad Prism 8 (GraphPad Software, San Diego, CA, US) has been used to build figures.

5.3. Results

5.3.1. Participants Characteristics

A sample of 164 athletes of various levels took part in this survey. Of them, 144 (88%) were males and 20 (12%) were females, 48 (29%) participants were under 18, 145 (88%) were amateur athletes (it means that they compete in Olympic-style boxing) and 19 (12%) were professional boxers; 29 athletes (18%) included in this sample have won a medal in an international competition (tournaments, Worlds or Olympic Games). Athletes who had won or had medalled at an international competition were deemed "highest calibre," those who won or had medalled at a national competition were deemed "moderate calibre," and all others were classified "lesser calibre" for the analyses, as previously done by Reale et al. [247]. Of the 164 athletes, only 20

(12%) reported to have not ever applied acute weight loss strategies before a match. Detailed characteristics of the sample are reported in Table 5.1.

Table 5.1. Participants characteristics. Data are reported as means ± standard deviations. For asymmetric values, median (min-max) have been used. Where applicable, absolute frequencies (percentage) are reported.

	Males (n=144)	**Females (n=20)**	**Total (n=164)**
Height (cm)	175.9 ± 7.1	164.2 ± 7.4	174.5 ± 8.1
Weight (kg)	70.7 ± 11.8	56.1 ± 6.1	68.9 ± 12.2
Age (years old)	23.4 ± 6.4	19.2 ± 5.5	22.9 ± 6.4
Education Level			
Primary School	24 (16.7%)	5 (25.0%)	29 (17.7%)
High School	96 (66.7%)	9 (45.0%)	105 (64.0%)
Bachelor Degree or >	24 (16.7%)	6 (30.0%)	30 (18.3%)
Age started boxing	16.4 ± 4.9	14.4 ± 4.4	16.2 ± 4.9
Age started competing	18.4 ± 4.9	16.8 ± 4.4	18.2 ± 4.9
Training sessions (n/week)	5.9 ± 2.5	6.2 ± 2.3	6.0 ± 2.5
Training volume (h/week)	11.2 ± 7.3	10.5 ± 3.2	11.2 ± 6.9
Boxing Category			
Amateur (Olympic-style boxer)	126 (87.5%)	19 (95.0%)	145 (88.4%)
Professional	18 (12.5%)	1 (5.0%)	19 (11.6%)
Boxer Level			
Highest calibre	22 (15.3%)	7 (35.0%)	29 (17.7%)
Moderate calibre	27 (18.8%)	9 (45.0%)	36 (22.0%)
Lesser calibre	95 (66.0%)	4 (20.0%)	99 (60.4%)

5.3.2. Clustering & Heatmap

A heatmap (Figure 5.1) was built with the reported frequencies of the 14 different weight cutting strategies, by each of the 164 subjects. A two clusters solution has been found to be the best, using both Elbow and Silhouette methods; it can be graphically noted by the dendrogram. Cluster 1 comprises 133 subjects (81%), while the other 31 subjects (19%) belong to Cluster 2. The two clusters significantly differ one from the other (Permanova test; $F_{(1,162)}$= 32.57, p<.0001).

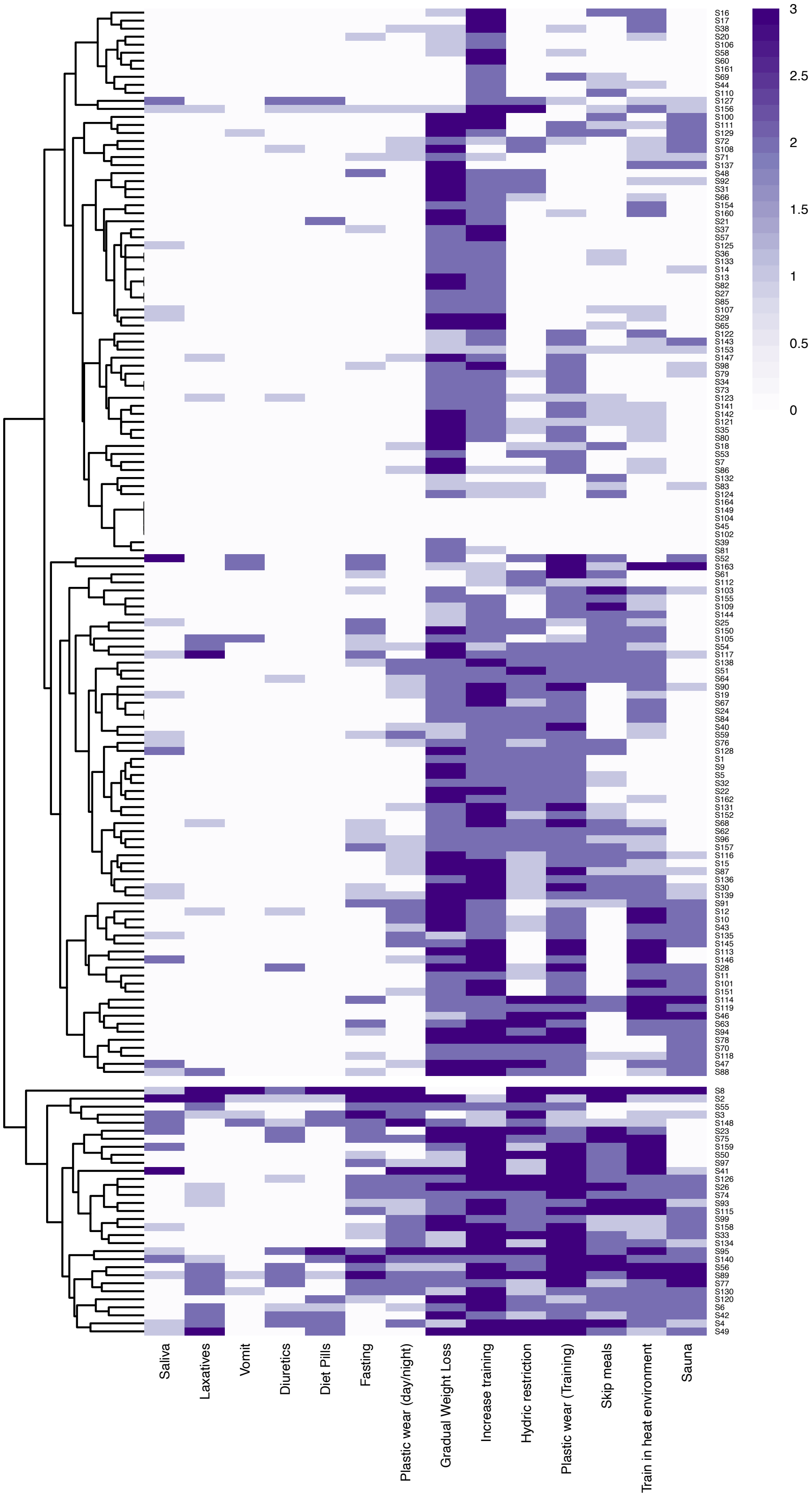

Figure 5.1. Heatmap with weight-cutting strategies (in columns) and subjects (in rows). An empty line separates the two clusters (Cluster 1 at the top, Cluster 2 at the bottom). White colour is associated with the 'never used' answer, while purple represents the 'always' answer.

In Table 5.2, the subjects' responses to the specific questions on weight-cutting habits are reported, divided for the two clusters. 20 athletes (12.2%) reported no lifetime experience of weight loss before competition. As it can be noted, the two clusters differ for the maximal number of kilos loss before a single match in the entire career (p = 0.003), how many times a subject cut their weight in the previous season (p = 0.016), the average weight loss before a competition (p = 0.012), and the number of kilos usually regained in the week after the competition ($p < 0.001$); so, the severity of rapid weight loss behaviours is higher in the subjects pertaining to the Cluster 2.

Table 5.2. Subjects responses to weight loss specific questions in the two clusters. Data are reported as mean (min–max).

Weight-Cutting History	**C1 (*n* = 133)**	**C2 (*n* = 31)**
Most weight lost for a competition (kg)	4.5 (0–17)	6.4 (2–20) *
Number of times of weight cutting in the previous season (times)	1.6 (0–11)	2.5 (0–9) *
Weight usually lost for a competition (kg)	1.5 (0–8)	2.6 (0–10) *
Number of days over which weight is usually lost (days)	12.1 (0–40)	17.2 (2–60)
Age at which began to cut weight for competitions (years)	18.5 (11–34)	17.8 (11–28)
Weight typically regained in the week after the competition (kg)	2.0 (0–10)	4.2 (2–15) *

* $p < 0.05$ (Mann–Whitney with false discovery rate correction).

In Figure 5.2, the frequency of use of the different strategies in the two clusters is reported. The two clusters significantly differ on all the strategies analysed (χ^2 test; $p < 0.05$), except for the 'Gradual Weight Loss' ($p = 0.529$). It is interesting to note that, with the practices becoming more dangerous to health, the difference between the two clusters becomes more visible.

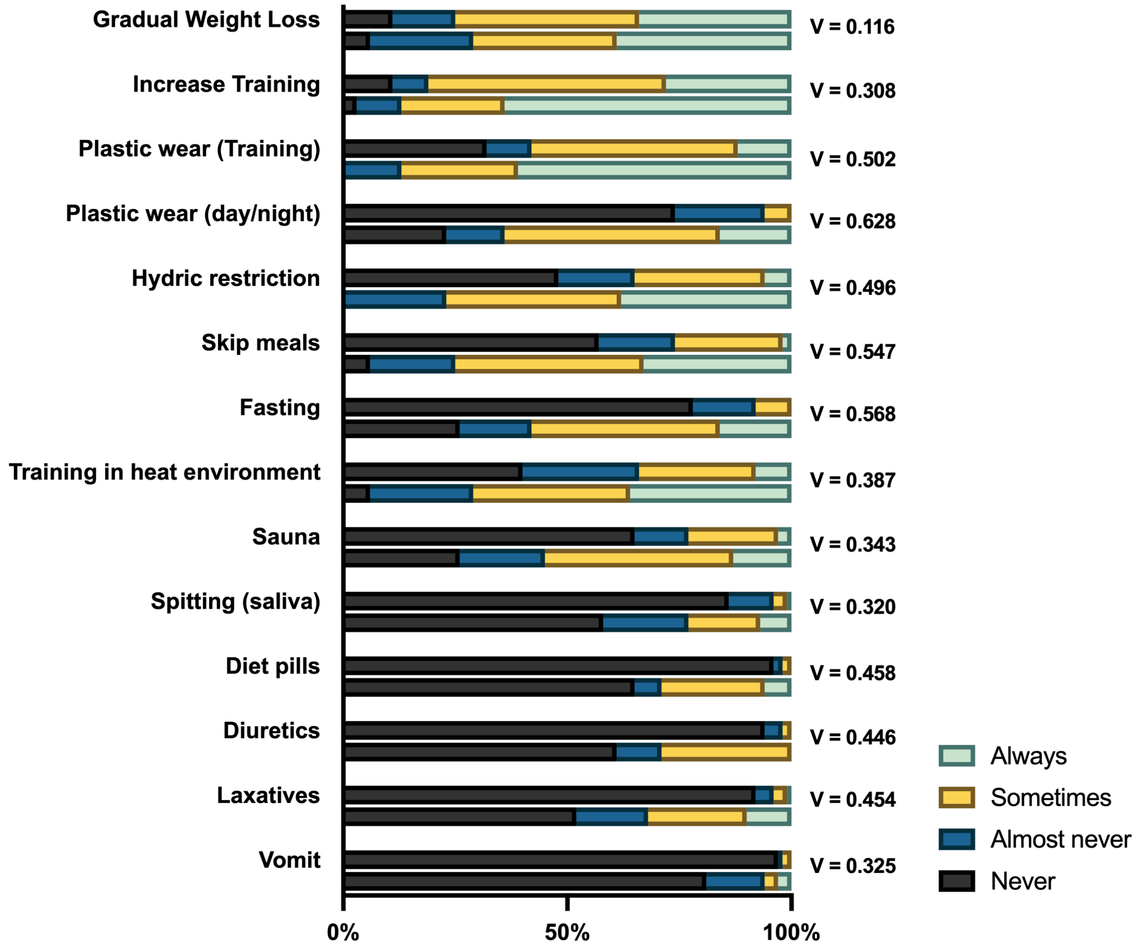

Figure 5.2. Frequency of use of the different weight-cutting strategies in Cluster 1 (upper bars) and Cluster 2 (lower bars), for each weight-cutting method. Association strength is reported as Cramer's V.

5.3.3. Rapid Weight Loss Score

The above-reported results (Table 5.2 and Figure 5.2) are well summarised by the 'Rapid Weight Loss Score' (RWLS), that was significantly higher in the Cluster 2, respect to the Cluster 1 (Mann–Whitney U = 302, $p < 0.0001$), as reported in Figure 5.3. The RWLS provides a measure of the severity of the weight-cutting behaviours. RWLS had a mean of 18.4 (±7.9) in the Cluster 1, and of 35.3 (±10.2) in the Cluster 2.

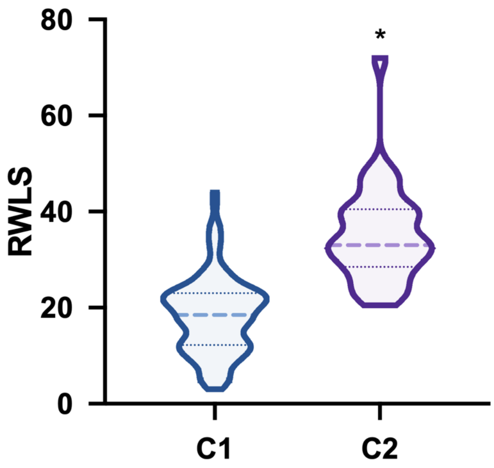

Figure 5.3. RWLS in the two clusters (C1 and C2). The thick line represents the median, while the thin ones represent first and third quartiles. Star indicates statistically significant difference.

5.3.4. Socio-Demographic Differences Between the Two Clusters

In Table 3, the socio-demographic differences between the two clusters are reported. This analysis has been selected in order to explore associations between some dependent variables, such as age, gender, education level, boxing category and level, and people of influence, and the two clusters as predictive factors. The variables which are dependent on the clusters are: gender, with a higher percentage of females pertaining to the Cluster 2; the category, with a higher prevalence of professional athletes in Cluster 2, in respect to the amateur ones; the level of the boxers, with higher calibre athletes (subjects who achieved a medal in an international competition) more present in Cluster 2. Regarding the people who might have an influence on choosing the weight-cutting strategies, only physicians/nutritionists seem to have an impact on these patterns, with the athletes in Cluster 1 reporting a lesser influence on their choices by these figures. It is worth noting that there is no difference in the age classes between the two groups.

Table 5.3. Socio-demographic differences between the two clusters. Data are reported as n (% within-cluster).

	Cluster 1 (n=133)	Cluster 2 (n=31)	p(χ^2); Cramer's V
Age			
<18 years old	37 (27.8%)	11 (35.5%)	p=.398; V=.066
>18 years old	96 (72.2%)	20 (64.5%)	
Gender			
Males	120 (90.2%)	24 (77.4%) *	p=.05; V=.153
Females	13 (9.8%)	7 (22.6%) *	
Education Level			
Primary School	23 (17.3%)	6 (19.4%)	
High School	85 (63.9%)	20 (64.5%)	p=.923; V=.31
Bachelor Degree or >	25 (18.8%)	5 (16.1%)	
Category			
Amateur (Olympic-style)	122 (91.7%)	23 (74.2%) *	p=.006; V=.215
Professional	11 (8.3%)	8 (25.8%) *	
Boxer Level			
Highest calibre	19 (14.3%)	10 (32.3%) *	
Moderate calibre	29 (21.8%)	7 (22.6%)	p=.048; V=.192
Lesser calibre	85 (63.9%)	14 (45.2%)	
People who may have influence			
Physician / Nutritionist			
No / Small Influence	69 (51.9%)	8 (25.8%) *	p=.007; V=.247
Moderate Influence	12 (9.0%)	8 (25.8%) *	
Big Influence	52 (39.1%)	15 (48.4%)	
Coach			
No / Small Influence	33 (24.8%)	8 (25.8%)	p=.609; V=.078
Moderate Influence	17 (12.8%)	2 (6.5%)	
Big Influence	83 (62.4%)	21 (67.7%)	
Physical Trainer			
No / Small Influence	60 (45.1%)	14 (45.2%)	p=.171; V=.147
Moderate Influence	13 (9.8%)	0 (0.0%)	
Big Influence	60 (45.1%)	17 (54.8%)	
Family			
No / Small Influence	86 (64.7%)	19 (61.3%)	p=.845; V=.045
Moderate Influence	9 (6.8%)	3 (9.7%)	
Big Influence	38 (28.6%)	9 (29.0%)	
Other boxers			
No / Small Influence	89 (66.9%)	21 (67.7%)	p=.689; V=.067
Moderate Influence	15 (11.3%)	2 (6.5%)	
Big Influence	29 (21.8%)	8 (25.8%)	

* stars indicate rows whose column percentages are significantly different from each other.

5.4. Discussion

In this cross-sectional study, the common rapid weight loss strategies used by a sample of In this cross-sectional study, the common rapid weight loss strategies used by a sample of professional and amateur boxers were investigated and the presence of clusters have been explored relating to the severity (type and frequency) of each practice used. In this study, 88% of the participants reported having used rapid weight loss strategies during their careers. The strategies which can be applied to achieve a rapid weight loss are various, from the safer (e.g., gradual weight loss, increased training volume) to the most severe and potentially dangerous ones, including body water manipulation (fluid restriction, glycogen depletion, induced dehydration), sweating methods (both passive sweating, e.g., sauna and heated rooms, and exercise-induced sweating) and gut content manipulation (food restriction, use of laxatives, diuretics, vomiting) [8,235]. All these practices are not used with the same frequency by all boxers. Indeed, the main result of the study is the presence of two clusters of boxers in our sample, characterised by the severity of the weight-cutting practices they are used to apply. The cluster with more severe weight loss practices has a higher proportion of professional fighters, high-level athletes and females, and is also marked by a higher number of kilos usually lost before a match and regained in the week after that, and a higher number of times the subject cut the weight in the previous season.

Regarding the weight loss strategies, Reale and colleagues [247] reported that boxers favour the more gradual approaches to weight loss with respect to other combat sports. Indeed, the most used practices in our sample were the gradual weight-loss, increased training volume, and other practices linked to augmented sweat rate. These practices are the same reported to be most prevalent by Barley et al. [234]. Although infrequently reported, harmful practices such as vomiting and use of banned diuretics or laxatives remain concerning. The weight loss history and the magnitude of weight loss in our boxers are comparable with those reported in the study conducted by Barley et al. [234]. Indeed, the average weight loss before a competition and the weight regained in the week after the match are similar in the two studies, although slightly lower in the present sample; the boxers in our study were used to lose weight in almost half of the days before competition than those reported in the study by Barley et al. [234]. The effect of the duration of weight loss practices, its relationship with the severity of the practices used, and its consequences to subsequent weight regain, need to be further explored.

The heatmap (Figure 5.1) shows that the usage frequency of different weight-cutting strategies is not the same in all the subjects. Indeed, two clusters of subjects have been found in our sample. Cluster 1 is characterised by the use of gradual weight loss and increased training as the most prevalent strategies, while Cluster 2 had a greater presence of those strategies which can be referred to as gut content manipulation (use of laxatives, diuretics, vomiting and spitting saliva), that would provide a greater risk to athlete physical health [238]. The athletes in Cluster 2 had a greater magnitude of total weight loss compared with those from Cluster 1 (2.8 ± 2.2 kg and 1.7 ± 1.2 kg, respectively) and a higher frequency of weight cutting throughout a competitive season (Table 2). A greater amount of weight loss would plausibly result in an increased risk of impaired physical performance [234,240,253]. However, it is unclear if such reductions in physical performance are outweighed by the benefit of competing against smaller opponents; such complications may contribute to the lack of clear benefit or detriment on performance as a result of weight cutting [233]. These results confirm that there is a significant population of athletes within boxers that disproportionately use higher-risk strategies when trying to lose weight for competitions.

A higher proportion of females is present in Cluster 2, with respect to Cluster 1. To the authors' knowledge, no studies have deeply investigated the differences in weight-cutting strategies between male and female fighters, and this should be an important topic for future research. It should be noted the absence of significant differences in the presence of under18 athletes in the two clusters. This means that a good proportion of the youth athletes (about 22%) pertaining to Cluster 2 apply the same dangerous weight-loss practices as adults. The authors believe that this point deserves attention, as practices like manipulation of the gut content (use of diuretics, laxatives, or vomiting) represent a risk for the fighters wellbeing [8], in particular for youth. Future research should look to better understand the motivations of such athletes and their prevalence in other combat sports to help regulators and organisers when trying to design mitigation strategies for weight-cutting practices.

A higher proportion of top-level boxers (athletes who at least won or acquired a medal at an international competition) was present in the Cluster 2. A higher prevalence of extreme weight loss practices in higher calibre athletes has been previously reported by Reale et al. [247]. Higher calibre athletes apply more severe weight-cutting strategies, but it could be argued that they also have access to more support staff and thus would feel more comfortable to attempt difficult weight cuts. This is potentially confirmed by the greater reliance of Cluster 2 subjects on physicians and nutritionists (Table 3). Overall, the coach seems to have the biggest influence on the boxers' choices in both clusters; this result is in accordance with those previously reported by Reale et al. [247], whilst Barley et al. [235] reported a high influence also of other boxers (partners or opponents). Another important difference between the two clusters is represented by the boxing category, as amateur/Olympic-style vs. professional boxing, with pro boxers that are more represented in Cluster 2. These two boxing styles follow different rules as per match duration, protective gear and scoring. The amateur boxing tournaments feature several matches over several days, unlike professional boxing where fighters have several weeks (and sometimes months) between bouts. Furthermore, professional boxing is also known as "prizefighting", because matches are fought for a purse that is divided between the boxers as determined by contract, whilst amateur boxers cannot fight for money. Competing for a low number of times per year, and fighting-for-pay, professional boxers could be inclined to use more risky strategies to cut the weight before the matches. Furthermore, the time between weigh-in and the competition differs between amateur/Olympic-style and professional boxing. As for the Italian regulations, in amateurs, the weigh-in is the same day as the competition, while in professionals the day before [254]. A longer time between the weigh-in and the match could allow the athletes to apply more severe weight-loss strategies in order to lose more weight, having more time to recover and refuel before the match. It is worth noting that research has focused more on Olympic-style boxing, while professional boxing has been less investigated.

This study provides an overview of the weight-cutting strategies used both in amateur and professional boxing. These results could be useful for teams and Federations, in order to develop educational programs which could improve the knowledge and awareness of coaches and athletes regarding the risks involved with rapid weight loss and the recommended procedures to do that safely and adequately. The inclusion of physicians and nutritionists in the decision process regarding the weight loss strategies used could improve the safety of these procedures, reducing the health risks for the athletes. These findings also highlight the lack of information about weight-cutting practices in female and youth boxers, populations that surely need further consideration. However, the study presents some limitations: first of all, the use of an online survey to collect the data. Although this relies on self-reported data, a sufficiently large total athlete sample was recruited, and a highly validated combat sports survey was used in an attempt to mitigate this confounder. Additionally, the results are relative to the Italian context, and so the results generalisability cannot be taken for granted. Lastly, we did not collected data regarding the time between the weigh-in and the match, information that could have been useful for a better understanding of the phenomenon.

5.5. Conclusions

The cluster analysis put a focus on the characteristics of fighters who apply more severe practices (i.e., losing greater amounts of body weight as well as using methods such as fluid restriction, heat exposure and gut content manipulation), showing that professionals and higher-level fighters are more prevalent in Cluster 2. Fighters pertaining to this cluster also cut their weight more frequently during a competitive season, accompanied by a higher number of kilograms lost each time, and then regained to a greater extent after the match. Coaches are the most influential figures for the athletes, even if those in Cluster 2 are also conditioned by physicians and nutritionists. Possible future research could monitor a group of high-level athletes for the weeks before and after their matches, in order to have some reliable data on real behaviours and to avoid the use of potentially less reliable (self-reported) survey data.

General Discussion and Conclusions

In the present thesis, some of the complex relations between exercise, nutrition and sports performance have been investigated, from different points of view. It has been shown how exercise and nutrition are deeply interconnected, with one inducing changes in the other, and vice versa.

From a health perspective, we showed in Chapter 1 and 2 how exercise plays a role as a gateway behaviour, leading to the adoption of healthier food choices. However, these studies open several future research questions: the effect of different training modalities may induce nutritional changes in different directions, increasing or reducing the consumption of different food categories. Furthermore, the psycho-biological mechanisms underlying these changes are not yet completely understood, although some explaining models have been proposed in the literature (e.g. shared pathways and neurocognitive processes leading to behavioural changes). To explain and understand these mechanisms, might be extremely helpful in order to optimise the combined prescription of exercise and diet, for different populations (for example, in athletes, to facilitate weight control during the off-season, or in clinical populations, such as obese people in order to improve weight loss).

From a performance perspective, it is widely known that nutrition has an essential role in exercise performance. In this relationship between nutrition and exercise, sports supplements have a substantial role, and their consumption is increasing year by year. In Chapter 3, the use of sports supplements in the context of a national beach-volley competition has been explored. To know the habits of the athletes of determined sports is the first step to eventually make corrections, or give them suggestions in order to optimise their practices. Indeed, has been often reported in the literature a lack of knowledge and awareness of athletes regarding their supplement use. It is also important to explore their motivation for consuming determinate supplements in order to develop products based on the needs of the athletes and the single disciplines. In addition, it must be kept in mind that some supplement categories are sport-dependent, meaning that they may not work in the same way on different disciplines (e.g. endurance vs team sports); said so, it is essential to test their efficacy in improving performance in specific settings. In Chapter 4, we tested the performance-enhancing effect of a commercially available product in the context of high-intensity indoor cycling. It must be also noted that most of the commercial sports supplements are composed of multiple ingredients, whose interactions are often unknown or unexplored. Thus, it is important, apart from studying the single molecules, also conducting studies in the real setting.

Research in this field would benefit from considering the different populations involved in sports practice: males vs females, youth vs adults, professional vs recreational, etc.. When conducting studies over a single population, the results could not be necessarily extendable to others. In Chapter 5, for example, when exploring the rapid weight loss practices of a sample of boxers, we highlighted the absence of information about categories such as youth, or female athletes. This, of course, opens the doors to future studies.

"The characteristic of scientific progress is our knowing that we did not know."

Gaston Bachelard (1884-1962)

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