

UNIVERSITY OF NICOSIA

Integral physiological responses to nutritional periodization: from  
sleep to the gut microbiome, physical fitness and exercise  
performance

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PhD (Doctor of Philosophy) in Nutrition and Dietetics

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School of Life and Health Sciences

June / 2023



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A thesis submitted to the University of Nicosia  
in accordance with the requirements of the degree of  
PhD (Doctor of Philosophy) in Nutrition and Dietetics  
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## Abstract

Over the years there is a rapid increase in the prevalence of inadequate sleep and its detrimental consequences. However, the effect of a long-term nutrition intervention on sleep optimization has not been studied yet. We first conducted a systematic review, meta-analysis and meta-regression regarding the impact of carbohydrates on sleep. It was found that acute interventions with decreased intake of carbohydrate (CHO) quantity favored N3 stage sleep proportion and duration, whilst increased dietary CHO intake prolonged REM stage sleep. The acute manipulation of the CHO quality did not show any significant effect on sleep stages, however affected measures of sleep continuity. The intervention part of the present PhD project examined whether a long-term carbohydrate periodization intervention could optimize sleep, and affect body composition, athletic performance and gut microbiome. Forty-two healthy, physically active male volunteers were recruited and participated in this one-month randomized-controlled nutrition and exercise intervention. The intervention lasted four weeks and consisted of three groups: i) Sleep Low- No Carbohydrates (SL-NCHO): participants consumed all their carbohydrate intake at regular intervals prior to the evening exercise training session, ii) Sleep High- Low Glycemic Index (SH-LGI) and iii) Sleep High- High Glycemic Index (SH-HGI): Carbohydrate intake was spread evenly throughout the day both prior (60% of total CHO intake) and after (40% of total CHO intake). The SH-LGI and SH-HGI groups differentiated in the evening carbohydrate quality, consuming either LGI or HGI foods, respectively. Alongside, participants performed a supervised standardized exercise program combining resistance exercise and HIIT sessions. Participants' nutritional status, sleep-related measures, body composition, athletic performance, as well as stool and blood samples were collected at baseline and at the end of the intervention to assess the intervention effect on sleep, body composition, exercise performance and gut microbiome, collectively. The results revealed that sleep initiation, continuity and duration were improved in all trials ( $p < 0.005$ ). However, sleep duration variability reduced significantly only in SL-NCHO and SH-LGI trials ( $p < 0.005$ ), but not in the SH-HGI trial ( $p > 0.05$ ). Deep sleep was increased more in individuals with inadequate sleep duration and low sleep efficiency in the SL-NCHO trial, compared to good sleepers ( $p < 0.05$ ). Accordingly, sleep duration increased more in poor sleepers in the SH-LGI trial, than in good sleepers ( $p < 0.05$ ). Body fat mass, fat-free mass, as well as athletic performance, were significantly improved in all trials ( $p < 0.05$ ). In contrast, visual reaction time performance improved more in individuals with low sleep efficiency and low self-reported sleep quality in the SH-HGI trial, compared to good sleepers ( $p < 0.05$ ). Overall, complex relationships appeared between exercise nutrition and sleep, while gut microbiome regulation mediated these correlations ( $p < 0.05$ ). Considering those findings, it was shown for the first time that a one-month nutrition intervention in combination with exercise training largely affected

sleep, body composition and athletic performance in trained individuals. It is essential that lifestyle medicine and relevant interventions will be under further investigation, in terms of sleep and overall health improvement.

**Keywords:** Carbohydrate Periodization, Glycemic Index, Glycemic Load, Polysomnography, Actigraphy, Body Composition, Athletic Performance, Cardiorespiratory Fitness, Visual Reaction, Counter Movement Jump, Gut microbiota



## **Dedication**

The present thesis is dedicated to my wonderful family for their unconditional support in every way to achieve my dreams. To my father, for always being by my side, supporting me and giving me the greatest example of what a good-hearted man is. To my mother, for taking care of me beyond my dreams and always being there when I was scared. To my brothers, for always protecting me and being my secret power. To my nephew, who teaches me everyday how beautiful and meaningful is life.

And to life. The beautiful life that brought me hope.

P.S. Mom, dad, we made it!



## **Acknowledgements**

The present thesis is the result of the great support of many people to whom I owe great gratitude. In 2017 I joined the University of Nicosia family, studying on the MSc in Sports Nutrition/Dietetics and Nutrition Intervention. Since then, I received great support from the University and I will always be grateful for making me Dream Big and think UNIC.

First and foremost, I would like to thank my Doctoral Advisory Committee. I would like to express my utmost gratitude to my supervisor, Prof. Christoforos Giannaki for always being there, no matter the day, no matter the time. The present thesis wouldn't be possible without him. Thank you for giving me self-confidence when I needed it. My deepest gratitude extends to Prof. Eleni Andreou. I consider myself very lucky for your support and guidance. Of course, I could not miss out Prof. George Aphas, for his support, laboratory leadership and encouragement throughout the completion of this thesis. Thank you all for your priceless assistance and mentoring over these years.

I would like to especially thank Prof. George Sakkas. Dear professor, I deeply thank you for guiding me with your precious experience, extensive knowledge and ethics. Thanks must also go to Prof. Panayiotis Bariotas, for tutoring me with invaluable information with regards to sleep recording, monitoring and scoring. I would also thank Prof. Kyriacos Felekis, Prof. Myrtani Pieri and their team for helping me with blood and stool sample analysis. Moreover, my great appreciation extends to Ms Savoula Ghobrial, Dr. Monica Nikitara and Mr Evangelos Latzourakis for their help on blood draws. Of course, I could never forget Ms Natalia Papageorgiou and Ms Athena Aristotelides, students of Nutrition and Dietetics program, for their priceless help with nutrition and exercise data collection.

In my very humble opinion, the best thing that happened to me all these years is the new people I had the chance to meet. I realized once again that people are priceless and love is the driving force for everything. Unfortunately, this section is very little to recognize all my friends, colleagues and all the new people that contributed to this thesis. Among them, all the volunteers who participated in the experimental studies. Dear volunteers and friends, none of this research would have been possible without you. I can never thank you enough.

The acknowledgment section would never be complete without expressing my deep appreciation to Mr Aris P. Hadjipanayi, UFIT Fitness Centre Manager and friend. Thank you for believing in me and for helping me countless times, and for everything you provided me with, all these years. I hope this thesis will make you proud of me.

Dear Professors, Colleagues and Friends, thank you all for everything, I consider myself blessed for meeting you. You made this journey UNIC.

## **Declaration**

This dissertation/ thesis is submitted in partial fulfilment of the requirements for the University of Nicosia University Degree of PhD of Nutrition and Dietetics with title: “Integral physiological responses to nutritional periodization: from sleep to the gut microbiome, physical fitness and exercise performance”. The regulations for the degree are set out in the University of Nicosia Calendar and are elaborated in a practice manual known as House Rules for the Study of PhD Degrees at University of Nicosia.

### **Supervisor’s Declaration**

I confirm that, to the best of my knowledge:

- √ the research was carried out and the thesis was prepared under my direct supervision.
- √ except where otherwise approved by the Academic Administration Committee of University of Nicosia, the research was conducted in accordance with the degree regulations and house rules;
- √ the thesis represents the original research work of the candidate.
- √ the contribution made to the research by me, by other members of the supervisory team, by other members of staff of the University and by others was consistent with normal supervisory practice.
- √ external contributions to the research are acknowledged.

Supervisors: Dr Christoforos Giannaki, Dr Eleni Andreou, Dr George Aphamis

Date: 8/6/2023

### **Candidate’s Declaration**

I confirm that:

- √ this thesis represents my own work;
- √ the contribution of the supervisor and others to the research and to the thesis was consistent with normal supervisory practice.
- √ external contributions to the research are acknowledged.

Candidate: Angelos Vlahogiannis

Date: 8/6/2023

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## Abbreviation Index

AASM	American Academy of Sleep Medicine	mg/kg	Milligrams per kilogram of bodyweight
ANOVA	Analysis of Variance	miRNA	Micro RNA
ANS	Autonomic Nervous System	NREM	Non Rapid Eye Movement
BIA	Bioelectrical Impedance Analysis	OSA	Obstructive Sleep Apnea
BMI	Body Mass Index	PAL	Physical Activity Level
BPM	Beats Per Minute	PSG	Polysomnography
CHO	Carbohydrates	PSQI	Pittsburgh Sleep Quality Index
CMJ	Countermovement Jump	REE	Resting Energy Expenditure
DCI	Daily Caloric Intake	REM	Rapid Eye Movement
DRI	Daily Recommended Intake	RER	Respiratory Exchange Ratio
ECG	Electrocardiogram	RM	Repetition Maximum
ECW	Extra Cellular Water	ROL	REM Onset Latency
EEG	Electroencephalography	RPM	Revolutions per Minute
EMG	Electromyogram	SCFAs	Short Chain Fatty Acids
EOG	Electrooculogram	SE	Sleep Efficiency
ESS	Epworth Sleepiness Scale	SFI	Sleep Fragmentation Index
FAO	Food and Agriculture Organization	SH-HGI	Sleep High - High Glycemic Index
FFM	Fat- Free Mass	SH-LGI	Sleep High - Low Glycemic Index
FM	Fat Mass	SL-NCHO	Sleep Low - No Carbohydrates
FSS	Fatigue Severity Scale	SOL	Sleep Onset Latency
g/kg	Grams per kilogram of bodyweight	SWS	Slow Wave Sleep
GH	Growth Hormone	TBW	Total Body Water
GI	Glycemic Index	TDEE	Total Daily Energy Expenditure
GL	Glycemic Load	TIB	Time In Bed
HGI	High Glycemic Index	Trp	Tryptophan
HIIT	High- Intensity Interval Training	TSH	Thyroid-Stimulating Hormone
HR <sub>max</sub>	Maximum Heart Rate	TST	Total Sleep Time
ICW	Intra Cellular Water	VO <sub>2max</sub>	Maximum Oxygen Uptake
IIV	Intra-Individual Variability	VRT	Visual Reaction Test
LGI	Low Glycemic Index	WASO	Wake After Sleep Onset
LNAAs	Large Neutral Amino Acids	W <sub>max</sub>	Maximum Watt Output

## List of Publications

- **Vlahoyiannis, A.**, Giannaki, C. D., Sakkas, G. K., Aphas, G., & Andreou, E. (2021). A Systematic Review, Meta-Analysis and Meta-Regression on the Effects of Carbohydrates on Sleep. *Nutrients*, 13(4), 1283.
- **Vlahoyiannis, A.**, Sakkas, G. K., Manconi, M., Aphas, G., & Giannaki, C. D. (2020). Athletes' sleep assessment: from lifestyle to pharmacological interventions and vice versa. *Sleep Medicine*.
- **Vlahoyiannis, A.**, Sakkas, G. K., Manconi, M., Aphas, G., & Giannaki, C. D. (2020). A critical review on sleep assessment methodologies in athletic populations: factors to be considered. *Sleep Medicine*.
- **Vlahoyiannis, A.**, Aphas, G., Bogdanis, G. C., Sakkas, G. K., Andreou, E., & Giannaki, C. D. (2020). Deconstructing athletes' sleep: A systematic review of the influence of age, sex, athletic expertise, sport type, and season on sleep characteristics. *Journal of Sport and Health Science*.

## **CHAPTER 1: INTRODUCTION**





## 1.1 Rationale of Research

The rapid evolution in the sports nutrition field drastically increased the available knowledge and opened new horizons for both the improvement of athletes' health and performance, while elucidating potential physiological patterns behind these improvements [1]. Towards this direction, a carbohydrate periodization protocol was risen, named “training high – sleep low”, and has been tested as a tool to augment exercise adaptations in multi-physiological systems [2].

Beyond several established physiological adaptations [3], the results of the effects of the “train high -sleep low” strategy on athletic performance enhancement are inconclusive [2]. Moreover, the effect of this carbohydrate periodization model has been understudied with regards to sleep or gut microbiome -both key regulators of health and performance in athletes- [4,5]. Since carbohydrate manipulation is one of the key sports nutrition strategies to improve athletic performance, the study of relevant interventions bears of high significance for several reasons. As was shown previously, the majority of athletes experience several sleep issues, falling below the age-specific sleep recommendations [6]. However, it has been demonstrated that exercise does not impair sleep by itself [7] and acute post-exercise nutrition including acute carbohydrate manipulation, could elevate the exercise-induced sleep-optimizing effect, with further benefits on the following morning's performance [8].

To our knowledge, no-long term nutrition intervention for sleep optimization has been studied yet. Therefore, it is still relatively unknown whether a long-term carbohydrate periodization protocol could optimize sleep and alter gut function in a way that athletic performance and body composition will also be enhanced. This would allow to elucidate further potential interrelations and biological pathways underlying these adaptations.

## 1.2 Purpose of the Research

The purpose of this study is to examine the effect of long-term carbohydrate periodization protocols in combination with regular exercise training on sleep initiation, maintenance and architecture, physical performance, body composition, gut microbiome and miRNA in healthy trained individuals.

*Primary Objective:* What would be the effect of a long-term carbohydrate-periodization protocol on sleep-related parameters?

*Secondary Objectives:*

- i) What would be the effect of a long-term carbohydrate-periodization protocol on exercise performance?
- iii) What would be the effect of a long-term carbohydrate-periodization protocol on body composition?

- ii) What would be the effect of a long-term carbohydrate-periodization protocol on gut microbiome?
- ii) What would be the effect of a long-term carbohydrate-periodization protocol on circulating miRNAs?
- iv) Would gut microbiome synthesis differentiate with regard to potential alterations in sleep-related parameters?
- iv) Would circulating miRNAs differentiate with regards to potential alterations on sleep-related parameters?
- v) Would potential long-term alterations in sleep impact athletic performance and body composition?
- vi) Does the effect of the examined intervention varies between poor and good sleepers?

### **1.3 Significance of Thesis**

Sleep is a lifelong element for health and wellbeing. Nevertheless, alongside the dramatically increased prevalence of inadequate sleep patterns, no data exist about the long-term physiological effect of non-pharmacological and easily applicable lifestyle interventions. In this sense, the study of a long-term carbohydrate manipulation intervention to investigate potential effects on sleep parameters, including sleep initiation, maintenance and architecture, would not only be novel, but of utmost importance. The current PhD project moves one step further, aiming to investigate both gut microbiota and circulating miRNAs, a relatively new research field that has not been extensively investigated yet. Therefore, this project seeks to further study potential physiological mechanisms that may explain the possible effects of nutrition and exercise on sleep and exercise performance in healthy individuals.

To the best of our knowledge, limited data examine integrative physiological adaptations that may promote sleep. Assessing all these physiological systems after the proposed nutrition intervention will provide valuable data to understand how the human body functions. Taking into account that one-third of the human population is not able to meet their sleep needs, future sleep-optimizing interventions may be designed for high-risk populations for sleep disorders, such as adolescents, the elderly and athletes and thus prevent impairments of health including diabetes.

## **CHAPTER 2: LITERATURE REVIEW**



## **2.1 Sleep Physiology**

### **2.1.1 Definition of Sleep**

Sleep is an actively modulated and metabolically discrete state, the cornerstone of health, recovery, and regeneration. Notably, humans spend about one-third of their lifespans sleeping or trying to fall asleep. For many years, sleep was under extended investigation regarding its effects on brain function [9] and its consequences on cardiovascular health (especially in the case of sleep disorders). In the last decades, research related to exercise and nutrition sciences revealed evidence that sleep has a direct impact on recovery [10,11] and can also affect a wide range of physiological parameters that could affect recovery indirectly, such as hormone secretion, immune system, and exercise performance.

It is difficult to identify sleep as the absence of wakefulness and consciousness or the suspension of sensorial processes, as these could also occur during pathological states. Instead, sleep is more accurately described as the combination of the activation of specific neurons in selective brain areas and the passive withdrawal of afferent stimuli to the brain. Hence, in contrast to several pathological states characterized by decreased consciousness, sleep is a constantly, reversible and actively regulated state that serves to enforce rest and fasting, supporting the optimization of metabolic processes at the appropriate phase of the 24-h cycle [12].

### **2.1.2 Sleep Initiation, Maintenance and Architecture**

Sleep initiation and maintenance are often portrayed as a “two-process” model, that includes the i) homeostatic and ii) circadian processes. This is because sleep maintenance and sleep initiation are controlled by numerous homeostatic influences that are inversely related to both time spent awake and the circadian effects entrained in the 24h cycle. More explicitly, the homeostatic process comprises mechanisms that are accumulated during wakefulness and dispersed during sleep. Whilst the second process in the model of sleep, the circadian process, is largely defined as a 24-hour physiological response to both environmental factors and neural oscillators. Altogether, this two-process model significantly stimulates sleep initiation and maintenance in diverse experimental protocols.

To initiate and maintain sleep, the ascending reticular activating system, a critical subcortical neural network, must be suppressed. Thus, transitions between sleep and wake are regulated by a distinct subcortical network. It has not been fully determined which molecular triggers the onset of sleep, but extracellular adenosine may play a key role. This is because adenosine levels collectively increase during wakefulness and are reduced as sleep progresses [13]. In this sense, this could be justified by the alarming effects of coffee and theophylline that are attributed to

their adenosine antagonist properties. Nonetheless, the possibility that other molecules play substantial signaling roles in sleep initiation and maintenance cannot be eliminated yet.

In addition to sleep onset, sleep maintenance is facilitated by several sleep phases, meaning that sleep architecture is comprised of a series of sleep stages. Hence, sleep on its own is not a homogenous construct. Humans switch between four stages of sleep during sleep maintenance, and the following stages that usually occur are the N1 and N2 sleep stages (commonly called “light sleep”), the N3 sleep stage (commonly called “deep sleep”), and the REM sleep phase (from the words: rapid eye movement) [14]. The rhythmic alternation between non-REM (NREM) and REM stages are distinctive characteristics of sleep architecture. These alterations can also be directed by the switching between activity and inhibition of specific neurons within the brainstem [15,16]. These three different states of brain activity during sleep, that is vigilance or wakefulness, NREM, and REM sleep, guided researchers to study and quantify these brain variations via electroencephalography (EEG), which measures the activity of neurons in the cortex [17].

From a historical perspective, Loomis and colleagues (1937) were able to distinguish these recurrent rotations of the stages during sleep on an EEG device [18]. The REM sleep phenomenon was explained fifteen years later by Aserinsky and Kleitman [19]. Within the next eight years, Berger discovered REM atonia, a condition in which muscle tone is impaired during REM sleep [20].

Such thought-provoking findings pointed in the right direction since REM sleep is differentiated from wakefulness mainly due to the slight or absent movement during sleep. This is because the EEG characteristics of REM sleep are comparable to those of the vigilance state. On the other hand, the vigilance state is characterized by low-amplitude activity and increased frequency [21,22] as opposed to NREM EEG which demonstrates a generally higher amplitude and lower occurrence. Specifically, four different types of brain EEG characteristics occur during sleep: delta waves (5-4Hz), theta waves (4-8 Hz), alpha waves (8-12 Hz) and beta waves (13- 20 Hz) [14]. Considering these four types of sleep waves, sleep is primarily categorized as REM sleep and NREM sleep [14].

The American Academy of Sleep Medicine (AASM) Scoring Manual has identified that 75-80% of sleep time among adults is NREM [14]. The Rechtschaffen and Kales sleep manual, which was published in 1968, is regarded as a fundamental aspect of modern sleep medicine [23]. It is grounded on EEG and it divides NREM sleep into four stages: N1, N2, N3, and N4 [23]. Yet, the revision of the classification by the AASM in 2007 and its later amendments, unified the N3 and N4 sleep stages, resulting in today's subdivision of sleep in N1, N2 and N3 NREM stages [14]. It is to note that considering the relative research that was conducted before the revision of this thesis, the results of the studies reporting the N4 sleep stage, will be referred

as is. In keeping with sleep architecture, the N1 and N2 sleep stages are typically referred to as “light sleep” while N3 is “deep sleep” or slow wave sleep (SWS). Interestingly, approximately 48-63% of a person's time is spent in light sleep (N1: 3-8%, N2: 45-55%), while 15-23% is spent in deep sleep. REM sleep, on the other hand, consists of relatively 20-25% of total sleep time (Table 2.1.2.1).

Moreover, different changes occur from one stage to another. For example, advancement from the N1 stage to the N2 stage typically takes 10-12 minutes whilst transition from N2 to N3 can take 30-60 minutes. The transition from light to deep sleep is accompanied by a decrease in physical and eye movements and NREM and REM sleep stage interchange cyclically from four to six times during one regular sleep. More specifically, a cycle can persist from 90 to 100 minutes and SWS accounts for most of the first two sleep cycles. But, as SWS declines or is nonexistent, and REM sleep rises, this pattern varies.

**Table 2.1.2.1.** Summary of sleep architecture.

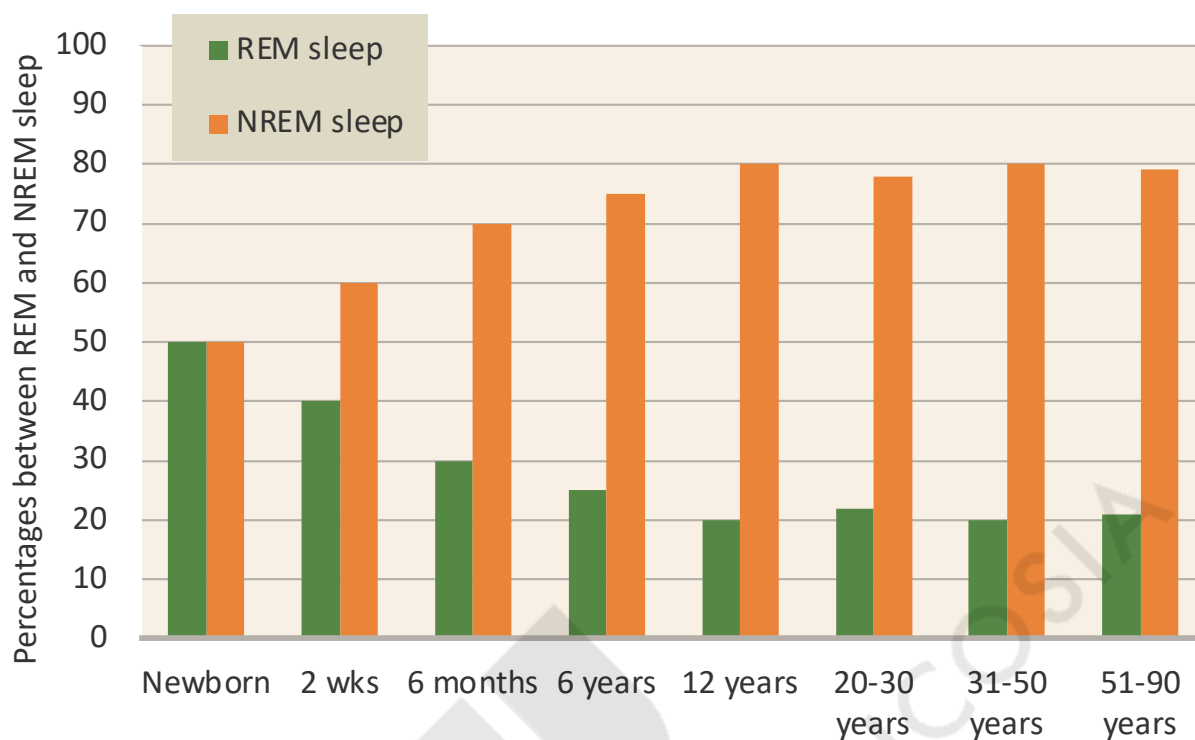
Sleep state	% Total Sleep Time
NREM sleep	75-80
N1 stage	3-8
N2 stage	45-55
N3 stage	15-23
REM sleep stage	20-25

REM: Rapid Eye Movement; NREM: Non-REM

### 2.2.3 Sleep across the Human Lifespan

Sleep quantity develops and changes throughout the lifecycle of a human being. Newborns can spend up to 16 hours per day sleeping in short, scattered naps [24]. Afterward, sleep duration decreases to 10 hours a day during childhood, when most children stop napping [25]. In adolescence, sleep declines further and ranges from 7.3-8.5 hours a day [26]. From adolescence onwards, a pattern of shortened sleep duration is repeated [27], which only stops once ageing is reached. However, for the elderly, the proportion of total sleep time to total time in bed -also known as sleep efficiency- is negatively impacted. This is because sleep arousal within the elderly population is extremely common during nocturnal sleep. To counterpoise this lack of sleep, the elderly instinctively nap during the day, therefore maintaining their total sleep time. In the same pattern, sleep architecture also evolves across the lifespan. A baby often enters REM sleep right after going to bed. Nevertheless, despite this, by three months, the NREM & REM sleep patterns are achieved and closely resemble the ones in adults. In addition, deep sleep percentage appears to significantly decrease with ageing, while on the other hand REM sleep

percentage does not seem to change [28]. Figure 2.1.3.1 depicts REM and NREM sleep patterns during the lifecycle.



**Figure 2.2.3.1.** Percentage of REM and NREM sleep during lifespan. Adopted and modified from Roffwarg HP, Muzio JN, Dement WC, Ontogenic development of the human sleep-dream cycle, Science 1996, 152(3722); 604-619

### 2.2.4 Sleep in Athletic Populations

Despite regular exercise being proposed as an impactful non-pharmacological approach to sleep disorders due to its proven imperative for good sleep [29,30], athletes frequently experience poor sleep and sleep disturbances [31]. Remarkably, growing evidence indicates that athletes experience inadequate levels of both sleep quantity and quality [32]. This is of utmost importance since inadequate sleep can interfere with athletes' health, performance and ability to recover [31].

It has been reported that athletes' sleep duration plateaus at roughly 7.2 hours of sleep per night as opposed to the recommended 8 hours of sleep per night. Moreover, the inadequate sleep pattern in athletes is not limited to one age group. In fact, adolescent athletes have reported an average of 6.3 hours of sleep per night. This metric falls significantly below the 8 hours of sleep proposed by the AASM guidelines. Even though adolescent athlete sleep increases from 6.3 hours to 7.1 hours per night in young adulthood it barely reaches the minimum value of the AASM guidelines. Although these guidelines are advised to the general public, athletes' sleep

demands may be underestimated because regeneration demand is greater in athletes and sleep extension has been proven that could accelerate recovery and performance [33]. Indeed, there are reports that athletes report feeling fully recovered when their sleep duration exceeds 8 hours of sleep per night [34].

A momentous indicator of athletes' sleep quality is their sleep efficiency which is calculated as a percentage of their actual sleep time to their total sleep period [31]. Remarkably, both sleep duration and sleep efficiency are decreased in athletes, independently of athletic expertise or age group. Yet, a major concern is that decreased sleep duration is not found to be attributed to difficulties initiating sleep -that is increased sleep onset latency- but rather to difficulties maintaining sleep since it is revealed that athletes experience continuous wake bouts -that is increased wake after sleep onset- throughout nocturnal sleep [31]. To conclude, sleep duration and quality are both important variables to consider when studying interventions that aim to optimize athletes' health and performance, as they can significantly affect muscle growth and recovery.

With regards to sleep architecture, even if exercise can induce significant sleep-stage alterations [35], athletes' sleep on normal occasions seems not to differ dramatically from the general population [31]. Like in the general population, NREM sleep stages consist of almost 80% of an athlete's sleep, mirroring the typical sleep of an average adult. More distinctively, an athlete's sleep on average has been shown to consist of 23.1% REM sleep, 9.7% N1, 48.7% N2 and 18.5% N3. Nonetheless, sleep architecture may alter significantly under certain circumstances, such as after ultra-endurance competitions or when sufficient recovery is needed, by increasing the N3 sleep stage. [36].

## **2.2 Nutrition and Sleep**

Nutrition can facilitate recovery both directly and indirectly through tissue restoration and sleep optimization because nutrient distribution and periodization are related to hormones associated with sleep [37]. Sleep-related hormones and peptides can optimize recovery by modulating the body's circadian rhythm and impacting sleep initiation and maintenance [38].

### **2.2.1 Macronutrients Modulation and Sleep**

Since all nutrients may exhibit synergistic effects, it is hard to investigate the effect of a single macronutrient on sleep. Nevertheless, the acute effect (four days) of a high-protein diet showed decreased wake episodes during night sleep [39]. It was further found that an intervention of 1.5g/kg of protein improved sleep when compared to a diet consisting of 0.8g/kg protein intake [39]. These supportive results were duplicated to a degree in obese sedentary individuals



experiencing a caloric deficit [40]. Specifically, in that study, a diet with a higher extent of protein (20% vs 10%) displayed a lower score on the global sleep score of the Pittsburg Sleep Quality Index (PSQI) showcasing the potential that higher protein diets can have on sleep quality improvement [40].

With regard to amino acids, as the building blocks of protein, the majority of research is limited to tryptophan, with minor research conducted for glycine as well. Tryptophan (Trp) has been lengthily studied for its influence on sleep in healthy and clinical populations [41]. There is an emerging body of research showing that besides seriously insomniac patients Trp encourages sleep in infants, healthy adults, and mild insomnia patients [41]. High doses of Trp have shown significant results, yet even doses of up to 1g have resulted in valuable sleep trends [41,42]. Measures of subjective sleep, as well as sleep latency, are affected, and doses above 1g potentially increase REM sleep but have no effects on NREM sleep [42]. On the other hand, glycine is another popular amino acid tested for its impact on subjective sleep quality [43]. In fact, 3g of glycine prior to sleep suggested an enhancement in subjective sleep quality and decreased deep sleep latency, however, more studies are needed.

Carbohydrates have received substantial attention from researchers due to their relationship with brain function [44] and their consequential interrelationship between sleep and glucose homeostasis [45,46]. Early studies on pre-bed carbohydrate quantity consumption have shown significant effects on sleep architecture [47]. Specifically, in 1981, Porter and Horne investigated the effect of three pre-bed meals with different compositions on sleep architecture: a) Glucose and Fried potatoes (a high carbohydrate diet HCHO), b) Crispbread salad and butter (a low carbohydrate diet LCHO), c) three tablets of methylcellulose (a zero-carbohydrate diet ZCHO) [47]. The study reported that consuming an HCHO pre-bed meal decreased the N4 sleep stage and REM sleep slightly increased when compared to the LCHO group. Shifting to a high-carbohydrate dietary plan for four consecutive days as an acute nutritional manipulation intervention, showed significant results on sleep onset latency reduction [39]. Yet, later studies showed that the consumption of high peri-workout carbohydrates did not affect sleep time in overreached athletes, nor did it alleviate the decrease in aerobic performance caused by intensified training [48].

As carbohydrates and sleep have been proven to be closely related, not only quantity, but the quality of carbohydrate intake has been under extended investigation as well. The early study of Porter and Horne was of great importance not only because they illustrated the significant effect of carbohydrates on sleep architecture [47], but because it was the first study that, even unwittingly, modified the glycemic index/load of the tested pre-bed meals [47]. Setting the limitations of a day's food consumption in the study aside, this study pioneered in indicating the impact of carbohydrate quality on sleep architecture. Thirty years later Afaghi and

colleagues extended Porter and Horne's results by investigating the impact of the glycemic index and the timing of pre-bed meals and their influence on sleep patterns [49]. Specifically, Afaghi and colleagues discovered that compared to a meal with a low glycemic index (LGI), a high glycemic index meal (HGI) resulted in reductions in SOL, and this result was more noticeable four hours before bedtime rather than one hour before bedtime. Comparable results were found when similar meals were given as evening post-workout meals, with promising effects on both sleep and the next day's performance in recreational athletes [8]. Additionally, Jalilolghadr and colleagues showed that a pre-bed HGI or LGI drink can influence sleep patterns in children [50]. Specifically, the HGI drink consumption 1 hour pre-bed was found to facilitate higher NREM and total arousal indices.

On the other hand, constraining carbohydrates and replacing them with dietary fat has been shown to significantly impact sleep-related parameters as well. Epidemiological evidence in females enrolled in a cross-sectional study, suggests that dietary fat ingestion was negatively correlated to nighttime sleep [37]. The same study also reported that napping is related to fat intake. According to other research, adolescent girls' total fat intake is inversely related to REM sleep, in contrast to boys [51]. Despite this, these results are questionable since the correlation coefficients of significant correlations were very low. In clinical trials, an isoenergetic, low-carbohydrate diet increased REM latency without affecting sleep time or architecture [52]. Also, healthy, good sleepers whose carbohydrate intake was further reduced to 1% of their total daily caloric intake exhibited a surge in SWS sleep percentage and a decrease in REM sleep percentage [53]. Overall these results suggest a potential role of high-fat low carbohydrate lies in shifting from REM sleep stage, in favor of deep sleep.

This is very interesting, since an increase in dietary fat and protein intake, for example, can invigorate the release of cholecystokinin [54,55], which, in turn, can increase subjective sleepiness [55] and N3 sleep stages [56,57]. Such suggestions are thought-provoking, as roughly 70% of growth hormone (GH) pulses—a peptide hormone that stimulates anabolism—concur with N3 sleep and GH secretion is proportionally correlated to N3 sleep stage duration [58]. Hence, relevant interventions are needed since there is growing evidence emphasizing on how body restoration and sleep optimization can be impacted by nutrition.

Collectively, the majority of these interactions between nutrients and sleep are mediated by a variety of molecules that are directly modulated by both dietary macronutrient intake, as well as nutrition as a whole (Table 2.2.1.1.) [59]. For instance, l-tryptophan availability within the brain (an antecedent of 5-hydroxytryptamine [45]), can be altered by a series of physiological responses that are triggered when dietary carbohydrates and proteins are manipulated. This is important because 5-hydroxytryptamine can be converted to melatonin which is an essential hormone for sleep initiation and maintenance [60]. Not only that, but a rise in l-tryptophan

availability is associated with an increase in REM sleep stage [61] which reinforces brain repair and reorganization processes [62]. Hence, both sleep initiation and sleep architecture could theoretically be altered by macronutrient availability and distribution, however, no long-term nutritional interventions are available to date.

**Table 2.2.1.1** Dietary Impact on sleep-related measures.

Dietary Factor	Effect on Sleep Quantity & Continuity				Effect on Sleep Stages	
	TST	% SE	SOL	WASO	N3	REM
HGI pre-bed meal	↑	↑	↓			
HCHO Diet						↑
LCHO Diet					↑	
Low Sodium Diet	↓			↑		
Tart Montmorency	↑	↑	↓	↓		
Cherry Juice						
Caffeine	↓	↓	↑			↓
GABA			↓			
Alcohol	↓	↓	↓	↑	↑	↓
Hydration	↓					

TST: Total Sleep Time; SE: Sleep Efficiency; SOL: Sleep Onset Latency; WASO: Wake After Sleep Onset; N3: Sleep Stage; REM: Rapid Eye Movement Sleep Stage; HGI: High Glycemic Index; HCHO: High Carbohydrates; LCHO: Low Carbohydrates; GABA: Gamma-aminobutyric acid

### 2.2.2 Micronutrients Modulation and Sleep

Beyond macronutrients, vitamins and minerals have been shown to have a significant impact on sleep-related parameters. Firstly, it has been shown that sleep efficiency is remarkably lower in individuals with vitamin D deficiency [63]. In the same line, lack of Vitamin D has also been linked to sleep disorders like OSA [64]. Still, the relationship between BMI and vitamin D should not be overlooked, since it may be a significant confounding factor mediating the relationship between vitamin levels and sleep efficiency. Secondly, retinol, a fat-soluble vitamin has demonstrated the ability to influence the molecular pathway of sleep regulation [65]. Thirdly, it has been suggested that vitamin E may benefit sleep quality, potentially due to its antioxidant activity that can counteract the oxidative stress related to sleep disorders [63].

Overall, it is interesting that insufficient vitamin consumption was found to coincide with short sleep duration in children and adolescent athletes [66].

With regards to minerals, magnesium, even though in cross-sectional studies there is a relationship between sleep-related parameters and magnesium status, the results of clinical trials are contradicting, highlighting the need for further research [67]. In the same line, even though zinc is proposed as a sleep regulator, the evidence is still limited [68]. Interestingly, early studies have shown that sodium intake potentially impacts sleep patterns [69]. Notably, a low sodium diet (a total of 500mg sodium) for three days, increased nighttime plasma norepinephrine levels and impaired sleep quality. Hence, participants following a low-sodium diet had an increase in awakenings during nocturnal sleep, a decrease in REM sleep and less overall sleep time.

### **2.2.3 Dietary Supplements and Sleep**

While some supplements have anecdotally been described as sleep aids or sleep disturbers, there is still no conclusive scientific evidence to support these claims. For example, valerian and glycine can improve subjective sleep quality but have no significant impact on quantitative sleep-related measures [70,71]. Contrarily, supplementation of 100 mg of melatonin 30 minutes before bedtime for four weeks enhanced sleep quality and efficiency in resistance-trained athletes [72]. In this sense, tart cherry juice, which is rich in melatonin, is another promising food supplement that is constantly under investigation. According to the results of a two-week study on elderly people, brand-named tart cherry juice supplementation stimulated improvement in insomnia by decreasing wake after sleep onset [73]. Nevertheless, total sleep time, sleep efficiency and sleep onset latency were found not to be significant. Controversially, a randomized, double-blind, placebo-controlled study found that tart cherry juice was effective in improving sleep efficiency, time spent in bed and total sleep time as well [74].

On the other hand, there are dietary supplements that are widely known to interfere with sleep but at the same time are extensively studied as ergogenic supplements, commonly used by athletes, such as caffeine [75,76]. Overall, caffeine is shown to impair both sleep duration and efficiency [76]. Nevertheless, there are data that caffeine may disrupt sleep architecture as well, reducing deep sleep [76]. According to Miller et al. , caffeine consumption approximately 6.5 hours before bedtime disrupted a plethora of sleep indices like sleep onset latency, sleep efficiency, REM, and total sleep time, even if it benefited afternoon performance in male cyclists and triathletes [77]. Hence, recent data recommend that a regular caffeinated beverage should be consumed at least 8.8h hours prior to the usual bedtime [76].

On the opposite though, caffeine is shown to ameliorate the decrease in skill tests detected in elite rugby players after partial sleep deprivation [78]. Nevertheless, there were no significant

differences in ergogenic effects when caffeine was administered in low doses (91mg/kg) or high doses (5mg/kg). Ali et al reported analogous results in females taking a caffeine supplement [79]. Likewise, there was no difference between high and low-dose acute creatine supplementation in offsetting sleep-deprivation-related decreases in the skill set of elite rugby players [78]. Yet, due to the already stated caffeine's impact on sleep, its use in the evening should be cautious [77].

## **2.3 Exercise and Sleep**

Exercise and sleep share a reciprocal relationship [80]. It is well known that sleep quality is typically linked to regular exercise and that sleep can reduce the gravity of symptoms in patients suffering from sleep disorders [81]. In general, regular exercise beneficially affects sleep parameters in non-athletic populations (healthy and diseased populations). On the other hand, it seems that athletes, is a group of the population that experiencing sleep problems and inadequate sleep. Different exercise training types, training intensities and volumes, frequency and timing of exercise have been reported to have an impact in athletes' sleep and recovery. Paradoxically, the phenomenon of insufficient sleep is common among athletes, and it could cause inadequate restoration of the body. As such, there is a growing amount of research investigating the sleep habits of athletes and the impact of exercise on sleep, but only a few nonpharmacological approaches to optimize athletes' sleep.

### **2.3.1 Exercise Intensity, Frequency and Sleep Physiology**

As the dependent variable of intensity, exercise type is reviewed in this section, mainly by studying the mechanisms by which ATP production is sustained (e.g., oxidative pathways or glycolysis accompanied by lactic acid production).

In contrast to former sleep hygiene recommendations that discouraged exercise in specific time intervals prior to usual bedtime, recent data show that aerobic exercise does not have a negative effect on sleep duration in either short [7] and long-term [82] interventions. In this sense, a strong line of research states that sleep-related parameters are not impacted by aerobic exercise intensity of up to 75% [83,84]. The duration and continuity of sleep are also not affected by medium-intensity continuous training in controlled experiments [7]. Notably, these results were consistent post a 4-week intervention [82]. In contrast to aerobic exercise, there are less data regarding resistance training, even though it appears to positively impact anxiety and depressive symptoms, as well as sleep quality [85]. In addition, when strength training is done twice daily, sleep does not seem to be affected [86].

Nonetheless, research indicates an enhancement in sleep quality and reductions in fatigue when exercise intensity is increased up to a certain point [82]. One possible cause for such results is

the heat produced by the body throughout the exercise. This is because the human blood vessels dilate when body temperature rises to upsurge the supply of blood to the peripheral muscle tissues. During sleep, the body initiates the same process to decrease body temperature and maintain homeostasis [87]. Another possible cause for the effect of exercise on sleep-related parameters is that sleep initiation and maintenance are highly correlated with fatigue during the daytime following exercise, underlying exercise's effects on body recovery [88].

On the other hand, increasing enormously either exercise intensity or frequency, may actively disturb sleep patterns. There is consistent evidence that sleep continuity is disturbed, and sleep architecture changes as the duration of aerobic exercise increases, such as during an extreme-duration event. Specifically, sleep architecture appears to favor the N3 sleep stage as opposed to REM sleep [35,36], demonstrating that tissue restoration after extreme endurance activities is linked to N3 sleep. On the opposite, athlete's rest days are associated with a decrease in the N3 sleep stage and an increase in REM sleep compared to normal training days that include high-intensity exercise [89]. Overall, data for training and N3 show a mutual relationship [90], and as such reduced recovery needs could result in this reduction in N3 sleep [89,91].

Not only exercise intensity, but the frequency of exercise sessions is associated with sleep-related alterations. More specifically, a constant inverse relationship exists between sleep efficiency and exercise frequency/intensified training. When training frequency in the microcycle is extended to six days a week there is an inclination for sleep efficiency to decline [86]. Such results were replicated in a study including cyclists whose increased training frequency [48]. In a real-world example, sleep efficiency and duration decreased gradually because of prolonged intensified exercises and overreaching [48,92]. Overreached athletes have a hard time recovering due to the sympathetic tone that is increased during deep sleep [93]. Therefore, overreaching and overtrained athletes are more likely to experience poor self-perceptions of sleep quality and impaired athletic performance [94]. Considering the above, athletes who undergo periods of intense training are likely to experience sleep disturbances as well as decreased exercise performance [95].

### **2.3.2 Exercise Timing and Sleep Physiology**

The timing of exercise could also significantly affect sleep modulation under certain circumstances. Even though the general representation is that exercise aids sleep optimization, research indicates that morning exercise is not directly related to sleep initiation and duration compared with evening or late-night exercise. Overall, training sessions in the morning have not shown a significant impact on sleep quality or architecture [90,96]. However, when morning exercise was tested on a sample with sleep disorders for two weeks sleep duration was

significantly improved, highlighting exercise as a non-pharmacological aid for sleep improvement [97].

Shifting exercise to the early evening seems not to significantly impact nocturnal sleep duration [84,98]. As soon as, the exercise is transferred to the evening until 3 hours before the usual bedtime, the results show that exercise could also improve sleep efficiency [7]. Towards this direction, there is data suggesting that exercise in the evening enhances deep sleep in the first sleep cycle and reduces sleepiness during the day [96]. The rise in N3 sleep post-late-night exercise was found to be stable in both females and males without any variation in other sleep factors [99]. Alternatively, sleep architecture could be affected by shifts in exercise time, as REM sleep would decrease [90].

Overall, the investigation of the effect of exercise on sleep should be cautious, since several factors can affect these relationships, such as the individual's chronotype, that may alter the effect of exercise timing on sleep. More specifically, individuals with morning or evening chronotypes have indicated diverse time-dependent responses to exercise [100]. Nevertheless, again when biological chronotype is controlled, exercise seems to promote better sleep efficiency [7].

### **2.3.3 The effect of Sleep on Exercise Performance**

Recently, the scientific community has concentrated on many methods to enhance athletes' sleep quality because it is crucial for both performance and health [101,102]. Overall, in actual competition scenarios, competitors who slept inadequately the evening before an international sporting event had lower chances of victory [103]. It was discovered that throughout the competition, the netball teams that placed in the top two places slept for an hour longer on average, and reported better subjective sleep, but did not differ from other teams in terms of sleep efficiency [104]. Towards this direction, the sharp growth in publications on PubMed over the past ten years that contain the keywords “athlete AND sleep AND recover”, demonstrates the increased interest in this specific field. Notably, only 60 publications were found for the years 2002-2012, but more than 600 publications were retrieved for the years 2013-2023.

With regards to aerobic performance, according to epidemiological research healthy adults who self-reported better sleep quality on the PSQI, had higher  $VO_{2max}$  and  $W_{max}$  values on a stationary bicycle than the rest of the population [105]. However, similar outcomes are not replicated in athletes [106,107]. Self-reported sleepiness was not the primary factor in determining how tired junior athletes felt throughout typical soccer practice [106]. Adult athletes' pre-competition sleep is frequently disturbed, according to research [107-109]. However, a cross-sectional study of marathon runners found no evidence of a connection between performance and self-reported sleep habits the night before a competition [107].

The restriction of sleep duration has been shown to greatly impact aerobic performance. Both partial or total sleep restriction has been adversely associated with exercise, which requires sustained and stable performance, plus cardiorespiratory effort [110-114]. Specifically, aerobic performance significantly decreased after 30 hours of no sleep while effort perception increased [115]. In this study, participants underwent a treadmill performance test that included a 30-minute pre-load at 60% of  $\text{VO}_2$  max and a 30-minute self-paced distance test. Individuals who had been sleeping deprived covered 187 meters less on average versus people in a controlled environment.

Increased physiological demand and lactate accumulation and a reduced time to exhaustion [112] have proved to negatively impact athletic performance because of partial sleep deprivation. Such a decline in performance could be endorsed by two changes. Neuromuscular fatigue is translated as the perception of longer effort [116] or the alternating of the aerobic pathways [111]. Due to the disruption of muscle glycogen regenerating following sleep restriction, energy substrate use may be one of the reasons for reduced athletic performance [117]. The depletion of glycogen impacts both muscle foundation and stamina since glucose uptake is crucial for athletic performance [118].

On the other hand, sleep extension in the form of a single 60-minute nap did not have any apparent effects on  $\text{VO}_2$ max or running economy during a gradual treadmill test to voluntary exhaustion [119]. Pre-bedtime red light exposure was a different strategy for enhancing athletes' aerobic performance via sleep optimization [120]. In a study of 20 female professional basketball players, participants were exposed to red light for 30 minutes each night for 14 days in a study from China. Researchers discovered that morning plasma melatonin levels increased, and that self-reported sleep quality had improved. At the same time, endurance performance—as determined by a self-paced 12-minute distance test—improved.

With regards to the research on anaerobic performance, the most common research setup setting is the investigation of varying lengths of sleep deprivation to the anaerobic performance of healthy participants. The strength of compound exercises like the bench press, leg press, and deadlift reduced when just three consecutive nights of three hours of sleep were obtained, while isolation movements like the biceps curl did not change [121]. Additionally, following sleep loss, the feeling of exertion increased substantially during a set of 20 repeats of each of these activities. In the same line, after inducing five hours of advanced simulation of traveling to disturb sleep, it was shown that none of the sleep-related interruptions such as decreased sleep time and sleep efficiency had an impact on Wingate's test performance [122]. These findings confirm earlier research by Mougin and colleagues, who found that athletes who were slightly sleep deprived during the Wingate test did not differ in terms of peak power, mean power output, or peak velocity [123]. Similar findings are shown following 24 hours of total sleep



deprivation [124-126]. In the same line, weightlifters' volume load or training intensity during a normal weightlifting program involving snatches, clean and jerks, and front squats were unaffected by lack of sleep [125]. Total sleep deprivation for one night does not affect hand-grip strength [124].

Interestingly, this relationship between sleep and anaerobic performance has been shown to be regulated by the timing of exercise testing. For example, early morning anaerobic performance in the Wingate test after complete sleep loss was identical to regular sleep [126]. However, test results in the afternoon demonstrated that all anaerobic performance indicators, including mean and peak power, declined [126]. Thus, there seems that partial or total sleep deprivation may affect afternoon—but not morning anaerobic performance [126,127].

With regards to cognitive performance of athletes, it has been shown that the brain adjusts to mild or moderate sleep deprivation and modulates cognitive function to maintain performance even at low levels [128]. However, this adaptation in brain activity is insufficient to maintain performance when sleep is severely limited (just under three hours a day) [128]. Adolescents' self-reported sleep problems were linked to a higher felt effort level when engaging in cognitive tasks, according to a cross-sectional study [129]. Also, it has been demonstrated that sleep affects motor skills including tandem gait [130].

Furthermore, sleep restriction has a detrimental effect on other athletic performances, such as accuracy [131]. Sleep deprivation seems to have an impact on sports-specific skills, such as servicing tennis accuracy [131], and even caffeine administration is not able to alleviate the unfavourable impact of sleep deprivation on serving accuracy [131]. Long-term sleep deprivation tends to affect the nervous system's activity by increasing sympathetic activity, decreasing parasympathetic activity, and altering the sensitivity of the spontaneous baroreflex, which is essential for maintaining homeostasis in the cardiovascular system [132]. However, it is fascinating to highlight that overtraining produces significant changes in the parasympathetic and sympathetic nervous systems [133]. As a result, this is another way through which poor performance may be related to insufficient sleep [134].

Different exercise methodologies may be too responsible for some of the studies' contradictory results, which do not conclusively demonstrate a major impact of sleep deprivation on athletic performance [4]. Altogether, though, there might be a few speculative biochemical mechanisms that are not properly understood, but there appears to be a significant amount of evidence that links inadequate sleep to poor exercise performance.

#### **2.3.4 The effect of Sleep on Post-Exercise Recovery**

Through its impact on several molecules, including hormones and cytokines, sleep is essential for the preservation and regeneration of skeletal muscle function. Sleep may hasten tissue

repair following exercise-induced muscle soreness, both exercise-induced and injury [135]. On the other hand, athletes' ability to recover from a single bout of exercise proved to be decreased by one night of partial sleep deprivation [11].

It has been repeatedly demonstrated that poor sleep following a stimulation that damages skeletal muscle can impair recovery and adaptation to exercise [135]. This might be explained by the fact that sleep deficit interferes with muscle recovery by increasing catabolic hormone secretion and decreasing anabolic hormone secretion [135,136]. For instance, testosterone is well documented for its effects on muscular hypertrophy [136] and muscle regeneration after exercise [137,138]. Sleep deprivation can lower testosterone by up to 15%; to comprehend how much testosterone is reduced, consider that normal ageing can cause a reduction of up to 1% every year [139,140].

Even though acute increases in anabolic hormones might not have an impact on muscle protein synthesis [141], chronically low testosterone levels and rising blood cortisol levels can have a significant impact on the amount of muscle degradation and restoration [46,142,143]. It is important to note that this series of physiological adjustments also happens in older people and particular disease states and circumstances, including cachexia [135]. As a result, as exercise duration and intensity rise, insufficient muscle restoration—even following an acute sleep deficit—could be harmful [10,11]. Overall, however, there is a link between inadequate sleep and exercise-induced damages [144], which includes those that depend on the immune system or other biological functions such as muscle plasticity.

Both sleep and circadian rhythm have an impact on inflammatory activity during nocturnal sleep [145], although it might be difficult to interpret the pertinent data. Neither complete nor partial sleep deprivation has persistently elevated circulating inflammatory marker levels [146]. Contrarily, numerous studies have shown that even a small amount of sleep deprivation can trigger the activation of inflammatory signaling pathways [145,147-149]. These findings indicate that even though these mechanisms are active, they may not translate immediately into an increase in inflammatory peptides.

When exercise-induced muscle soreness and insufficient sleep are combined, relative to sleep duration inflammatory responses, may be highlighted. For instance, it has been demonstrated that when paired with an exercise protocol that causes exercise-induced muscle injury, insufficient or no sleep increases blood IL-6 levels [150]. Furthermore, it has been demonstrated that greater levels of C-reactive protein (CRP) and IL-6 are linked to sleep problems and increased sleep length variability [151]. Particularly, it has been discovered that IL-6 is essential for hypertrophic signaling and muscle recovery [152]. However, since IL-6 causes weariness and sleeplessness, there is a theory that connects peripheral tissues with the central nervous system [153]. A potentially crucial option for the clinical population, such as in cachectic

patients, is targeted suppression of IL-6 signaling [154]. Long-term increased levels of IL-6 may also lead to skeletal muscle atrophy [154]. Generally, chronic inflammation is crucial because it is known to be associated with a number of serious disorders [155].

## **2.4 Gut Microbiome and Health**

### **2.4.1 Gut Microbiome Physiology**

The human body harbours approximately 100 trillion microbes, mostly a wide range of bacteria, as well as fungi, protists and viruses' ancients and protozoa, with bacteria being comparatively superior [156-158]. In fact, it is estimated that humans host as many microbial cells as human cells [159]. Weighting only about 0.3% of the human body, microbes interact with their micro-environment, having a great impact on human biological processes. Bacteria impact on human physiology is a result of their genes, and interestingly, more than 99% of the genes in the human body are microbial. However, less than 10% is similar between any two individuals [160]. This lays the foundation of two significant terms: alpha and beta diversity. Alpha diversity measures microbiome diversity in a single sample, and beta diversity represents the similarity or dissimilarity of two communities.

The exact amount and number of species that are present in the human body change dynamically across the lifespan [161]. Since the early stages of pregnancy, the fetus is exposed to numerous bacteria from the amniotic fluid that can be hosted in the fetus' gut. Nevertheless, bacteria colonization does not begin until birth. During birth, the bacteria that are present in the mother's uterus cover the baby and can potentially colonize the baby's gut. Hence, babies born through a c-section have been shown to have a different gut microbiota compared to their naturally born counterparts. Specifically, infants born with normal childbirth show a high concentration of lactobacilli in the first days. This particular element is related to the high degree of occurrence of lactobacilli in the vulva region [162]. In contrast, infants born by cesarean section are not colonized by microorganisms of the genus *Bacteroides*, but by optional anaerobic microorganisms, such as microorganisms of the genus *Clostridium* [163]. Literature shows that the microbial fecal analysis in embryos born with normal childbirth showed that their microbiome is 72% identical to the microbiome found after microbial stool analysis in the mother. In contrast, infants born by cesarean section showed a microbiome resemblance of only 41% to the mother's microbiome [164].

Nevertheless, not all bacteria are beneficial for the human body, and in some extreme cases, serious diseases like cholera can develop from bacteria. For this reason, the natural defense of a newborn is boosted via breastfeeding. Breast milk acts in several ways to protect infants'

health. Firstly, it contains many antibodies that aid in the baby's immune defense. Breastmilk also has human milk oligosaccharides, that promote the development of certain bacteria species, specifically the population of Bifidobacterium. Later, during growth, the introduction of new foods is accompanied by new microbia in the gut. Not only that but new foods will provide a substrate for the microorganisms to develop, to support growth and gut microbiota diversity. After approximately three years of age, the gut microbiota is similar to that of an adult [165-167]. During adulthood, relative stability is observed in the gut microbiome. However, several factors can alter the composition of the human microbiome. The comparison of the gut microbiome of young people, elderly people and with people reaching 100 years of life, showed that changes in microbial diversity and intestinal microflora do not follow a linear correlation with age but show great similarity between young people and people up to the age of 70, while it varies greatly in people approaching 100 years of life. It is observed, therefore, that the microbiome does not change sharply for more than four decades in the individual's life, unless strong factors influence it, but shows a marked change thereafter 36. Nevertheless, even if 99,9% of genes are similar for all human beings, only 10% of the microbiome is similar between two individuals [160].

Increased diversity in the composition of the microbiome is observed not only among individuals but also between different parts of the human body. Interestingly, bacteria colonize various parts of an organism beyond the GI tract, the extra-intestinal organs. These include the skin, oral and nasal cavities and vagina, however, their concentration in these areas is very small compared to the intestinal tract [168]. Specifically, for the intestinal tract, there is an increased microbiome variety, which is perfectly normal and is usually related to the chemical and physical composition of each part of the gastrointestinal tract. In more detail, it has been shown that the oral cavity contains a high number of bacteria ( $10^{12}$ ). The stomach carries about  $10^3$ – $10^4$  bacteria, the duodenum  $10^5$ – $10^6$  and the last part of the ileum  $10^8$ – $10^9$  bacteria (per gram of tissue or faeces). A greater number of bacterial cells has been found in the large intestine, with  $10^{12}$  bacteria per gram of intestinal tissue. Not only that but the variety of bacteria is greater in the large intestine than that in the small intestine.

Samples from the small intestine are composed of the species of Firmicutes and Actinobacteria, while Bacteroidetes and Lachnospiraceae of Firmicutes are present largely in samples from the colon. This is partly attributed to the fact that in the small intestine, there are more acidic conditions and a higher oxygen concentration than in the large intestine [169]. Therefore, the small intestine exhibits a microbial environment in which anaerobic bacteria could optionally prevail, being resistant to the combination of bile acids and antimicrobial agents, while they can take advantage of simple carbohydrates that are present in the small intestine. Bile acids are secreted from the bile duct in the duodenal region and strongly affect the types of bacteria

that colonize that part of the gastrointestinal tract [170]. In addition, the shorter time of passage of food in the small intestine compared to the colon affects the ability to attach to the tissues and even the mucosa of the small and large intestines.

In ileostomy samples, bacterial diversity was lower in the small intestine than in the colon, while many species of the genera Proteobacteria and Clostridium were identified. Analysis of gene expression showed that they participate in central metabolism and cellular carbohydrate entry pathways [171]. The largest and densest composition of microbes was observed in cultures from the colon and the cecum. These microbes are responsible for the catabolism of polysaccharides that have not broken down. The low concentration of antimicrobials and the lack of simple carbohydrates facilitates the growth of anaerobic bacteria involved in the degradation of polysaccharides, such as the families of Bacteroidaceae and Clostridiaceae [172]. Moreover, the colon's anatomy contributes to the presence of certain bacteria. Specifically, folding the walls of the colon creates folds in which specific bacteria grow [173]. Along the human small intestine and large intestine, there are specialized epithelial cells, called goblet cells, which secrete mucus, which can cover either partially or throughout the intestinal epithelium, creating a layer between the tissue and the intestinal lumen [174]. Therefore, the mucous layer creates an environment that protects against the growth of special microbes. However, some bacteria can penetrate inside the mucus and be in direct contact with the epithelium Field [175]. Hence, the microbiome shows marked changes along the gastrointestinal tract which are related to its site and individual structural features [176].

Overall, the dominant bacteria of the gut microbiome are in descending order Bacteroidetes, Firmicutes, Proteobacteria, Fusobacteria, Actinobacteria and Verrucomicrobia, with the top two making up over 90% of the intestinal microbiome. With regards to genera, the Bacteroidetes breed consists mainly of the genera Bacteroides and Prevotella, the Firmicutes breed of more than 200 genera, such as Clostridium (by 95%), Lactobacillus, Bacillus, Enterococcus and Ruminococcus, the Proteobacteria breed from the Escherichia, Shigella, Desulfovibrio, Bilophila and Helicobacter, the Fusobacteria breed from the genus Fusobacterium, the Actinobacteria breed mainly from Bifidobacterium and finally the Verrucomicrobia breed from Akkermansia [158,177]. However, the composition of the intestinal microbiome varies largely, and it is modified individually by several factors [158]. Interestingly, any change in a person's eating habits can modify the composition of the intestinal microflora within 24 hours, while returning back to existing eating habits will restore the intestinal microbiome to its original composition within 48 hours to retain the hosts' health [178,179].

A plethora of vital functions of the gut microbiome towards the host justifies its importance for the maintenance of physiological function, both of the intestine and the whole human body as well [158,180], and yet several potential mechanisms are not discovered. First, gut microbiota

plays a key role in digestion. Gut microbiota can break down complex carbohydrates since bacteria have certain types of enzymes that the human body does not. The intestinal microbiome contributes to the digestion and metabolism of nutrients that would otherwise not be usable by the body, such as specific types of carbohydrates (cellulose, hemicellulose, resistant starch, pectin, oligosaccharides, fibre, lignin). These carbohydrates are converted into absorbable metabolites, short-chain fatty acids (SCFAs). SCFAs, in turn, interact with the host by increasing the available energy and communicating with the brain and thus affecting host metabolism, appetite and food intake, factors related to the phenomenon of obesity [156,158,180,181]. Also, it is very important for vitamin syntheses, such as B vitamins, biotin, thiamine, cobalamin, riboflavin, pantothenic acids, vitamin K and folic acid [158,181]. These vitamins are released in the intestine, where they are absorbed and used in several biochemical pathways. Secondly, there is a two-way communication between the gut and central nervous system (CNS), also known as the gut-brain axis. On the one hand, the CNS can affect the gut microbiome through metabolic and endocrine pathways, as well as through the release of signalling molecules, such as cytokines and peptides. On the other hand, the gut microbiome can influence the CNS through products produced by the digestion of nutrients (SCFAs, [156,158]secondary bile acids (2BAs) and tryptophan metabolites -further discussed below-), which cross the gastro-intestinal barrier, enter the bloodstream and pass through the blood-brain barrier, affecting host behaviour [168,182]. For example, bacteria like *Corynebacterium* produce metabolites like serotonin, with profound effects on the brain's physiology and sleep. Not only that, but other metabolites as well, such as GABA, interacts in other ways with the nervous system. As for brain physiology, it is very interesting that certain gut bacteria are linked with sleep patterns, as will be discussed in the following chapter. Sleep-inducing compounds called muramyl peptides are produced by gut microbiota, and vice versa, poor sleepers are shown to have a certain gut microbiome profile. Moreover, gut microbiota acts protectively through several mechanisms that support the immune system. Bacteria occupy the intestinal surfaces and thus prevent the colonization of pathogenic microorganisms, participate in the elimination of toxic substances, and finally, by producing SCFAs, strengthen the intestinal mucosal barrier and seem to have anti-inflammatory and tumor suppressive properties. [156,158,180,181].

For bacteria to perform well and be beneficial for the host, it has been shown that a healthy lifestyle, including adherence to healthy nutritional patterns, exercise and sleep program can promote "symbiosis", a term often used to describe the increase of "beneficial" bacteria promoting health. Specifically, this mutually beneficial relationship between bacteria and the host, called symbiosis, has a ratio of bacteria to human cells of 1.3:1 [157,183]. However, on the other hand, disease, genetics, or lifestyle, in general, can impair human microbiota, promoting what is called "dysbiosis". Dysbiosis is a term to describe imbalances in the gut bacteria, that

could directly or indirectly impair health. Taking into account the number of beneficial functions of the intestinal microbiome for both the intestinal and whole human body, it is profound that any rapid change in the composition or function of the intestinal microbiome, called dysbiosis, could trigger alterations in neurotransmitter production, leading to overeating and weight gain [184], resulting in adverse effects on the health of the host [168].

There is growing evidence that a healthy lifestyle, including adequate and healthy sleep patterns, adherence to a healthy eating plan and regular physical activity impact on the gut microbiome in a certain way, favouring certain taxa. However, it is suggested that more clinical trials should be conducted to elucidate if lifestyle changes will promote specific gut microbiome changes and if these will be accompanied by health-related improvements in the specific physiological human systems.

#### **2.4.2 The effect of Nutrition on Gut Microbiome**

Diet is probably the most established modulator that shapes gut microbiome in both positive and negative ways [178], with significant alterations that can take place within 24h of dietary change [178]. There are two distinct routes by that nutrition affects microbiota: i) by increasing or decreasing the population of specific species of bacteria or ii) by regulating the metabolites produced in the intestine [158]. For example, oligosaccharides, as mentioned above, are metabolized in the large intestine, mainly by the genus *Bifidobacterium*, towards the formation of SCFAs. In breastfed infants, there is an increase in the Actinobacteria breed and inhibition in the development of Firmicutes and Proteobacteria, probably due to the positive correlation observed between the presence of oligosaccharides in infant milk and *Bifidobacterium* [158,168,182]. Hence, since the early stages of life, humans' eating habits play a key role in shaping the gut microbiome.

##### *Energy Intake*

Most of the macronutrients are absorbed before entering the large intestine, where the majority of microbiota is located [185]. However, it is estimated that 15% of carbohydrates and 5-35% of proteins enter the colon, representing between 10-30% of total ingested energy [186,187]. It is microbiota that accelerates energy turnover, by contributing to digestive efficiency and preventing all this energy from leaving the body through stools [185]. In this sense, it is very interesting that energy intake can modulate gut microbiota and vice versa. Comparing a lower with a higher caloric diet composed of a similar ratio of macronutrients it has been shown that the higher caloric load correlated with increases in Firmicutes spp and decreases in Bacteroidetes spp [188]. Notably, not only does energy intake alter the gut microbiome, but the gut microbiome altered energy harvesting by increasing energy absorption by approximately

150 calories. On the other hand, even though the data is limited, it has been shown that malnutrition is accompanied by impaired gut microflora development and especially a reduction in the relative abundance of Bifidobacterium and Lactobacillus spp [189]. Nevertheless, since gut microbes and energy intake can have an impact on each other, even if the evidence is yet growing, energy balance should not be overlooked when the gut microbiome is examined both in the general population and athletes.

### *Protein Intake*

Although the study of a specific macronutrient in isolation concerning its effect on the gut microbiome is difficult, there is evidence that dietary protein may induce both functional and compositional alterations to the gut microbiome [190]. With regards to the quality of protein intake, usually categorized as animal vs plant protein, early studies have shown that a high-meat diet may induce decreases in Bifidobacterium and an increase in Bacteroides and Clostridium compared to the group that consumed a vegetarian diet [191]. Later, the relationship between protein consumption and gut microbiome formation was further discriminated according to the source of proteins (animal protein, whey protein and pea protein). It was found that individuals who consumed animal protein prevailed an increase in Alistipes, Biophilina and Clostridium and a decrease in Roseburia and possibly Bifidobacterium [178,179,192]. In contrast, in individuals who consumed non-meat protein sources (milk, peas) there was an increase in Bifidobacterium and Lactobacillus and a decrease in Bacteroides and Clostridium [193-195]. Nevertheless, in an applied case scenario, a diet high in animal protein could be potentially also high in fat, with the latter also being a modifying factor of the gut microflora [179]. As a result of increased bile secretion after five days of a high protein/high-fat diet, Alistipes, Biophilina and Bacteroides are increased. Collectively, all increased protein intake may lead to increased sulfide production [196], a compound associated with ulcerative colitis [197]. In the same line, it has been suggested that protein intake is inversely related to alpha diversity in runners [198] but the data are conflicting [5,199]. As stands out from the relevant literature, protein may impair gut microbiota in athletes that do not meet the suggested macro and micronutrient intake but benefit those who do. Towards this direction, it is suggested that the effect of protein intake in gut microbiota is subject not only to protein intake but to the ratio of protein intake and protein-fermenting bacteria [200]. Notably, Bacteroides and Propionibacterium have a considerable proteolytic capacity [201], converting host enzymes, proteins and mucin into amino acids [202]. Although the data are limited, there is evidence that protein metabolism from bacteria into polypeptides may contribute to amino acid turnover in the mammalian gut [203]. However, as with other substances such as SCFAs discussed below, these procedures may be beneficial but both harmful for the host. For example, proteolytic fermentation may produce both beneficial



chemical compounds and toxic substances in the case of the putrefaction [196,204]. Hence, the evidence is not yet conclusive about the possible relations of protein to the gut microbiome and health. On the other hand, microbial-derived lysine contributes actively to the whole body protein pool [205], and this in athletes could be more than important since increasing essential amino-acid bioavailability can influence hematopoiesis, which in turn will increase oxygen-carrying capacity and cardiorespiratory fitness [206].

### *Fat Intake*

As with protein, dietary fats and especially specific fats have a remarkable impact on gut microbiota. Animal studies show that rodents that fed with lard showed metabolic dysfunction, accompanied by increases in Bacteroides. On the other hand, fish oil consumption acts as a safeguard against metabolic dysfunction [207]. In general, it has been shown that a diet high in saturated fatty acids leads to an increase in Bacteroides, Bilophila and Faecalibacterium prausnitzii, while a diet high in unsaturated fatty acids leads to an increase in Lactobacillus, Streptococcus, Bifidobacterium and Akkermansia muciniphila species [208-210].

According to dietary fat quantity, a diet rich in fat is shown to decrease Lactobacillus and Streptococcus, and increase Clostridiales and Bacteroides [192,208,211]. On the other hand, a low-fat diet could increase the abundance of stool Bifidobacterium [208]. Interestingly, a high-fat diet seems to impair intestinal permeability, since gut microflora contributes to the integrity of the intestinal mucosal barriers [212]. Hence, by reducing Bifidobacterium spp, and simultaneously over-activating the endocannabinoid system [213,214], a high-fat diet alters microbes in the gut in such a way that leads to the overgrowth of gram-negative pathogens, promoting LPS absorption across the intestinal barrier [215] and a cascade of physiological responses who induce a low-grade systemic inflammation [216] as related to obesity.

### *Carbohydrate Intake*

As it is clear, the effect of carbohydrates on the gut microbiome is profound since especially non-digested carbohydrates will ultimately modify bacteria in the gut since bacteria have more enzymes to break down carbohydrates than humans [217]. Beginning with the digested carbohydrates, including glucose, fructose, sucrose, and lactose, they have been shown to actively alter gut microbiota [179]. Increasing the dietary intake of digested carbohydrates (glucose, fructose and sucrose) by increasing dates consumption, resulted in an increase in Bifidobacterium and a decrease in Bacteroides, while when further combined with an increased lactose consumption there was a decrease in Clostridium and a decrease in Lactobacillus [218-220]. Diets high in complex carbohydrates as correlated with the abundances of Prevotella [221,222]. However, this specific specie is also increased in several health conditions such as

depression [223], hypertension [224] or insulin resistance [225]. This is usually attributed to the fact that the *Prevotella* genus also consists of several pathogenic strains, hence this bidirectional effect on the human health [226].

With regards to indigestible carbohydrates, so-called prebiotics, including fibre and resistant starch, modify the gut microbiome in a beneficial to the host way. Prebiotic sources, in addition to supplementation, are soybeans, inulin, raw wheat and barley, raw oats and non-digestible oligosaccharides, such as fructans, polydextrose, fructooligosaccharides (FOS), galactooligosaccharides (GOS), xylooligosaccharides (XOS) and arabinosaccharides (AOS). [168,179,227]. Studies have shown that reduced indigestible carbohydrate consumption decreases the abundance of microorganisms that colonize the intestine, while on the contrary, their increased consumption leads to an increase in their abundance and variety [179]. A high-fibre and high-prebiotics diet leads to an increase in *Lactobacillus* spp. and *Bifidobacterium* spp. and a decrease in *Clostridium*, while some specific prebiotics has an additional effect on the reduction in *Enterococcus* [228-237]. The positive correlation between fibre consumption and *Lactobacillus* spp. and *Bifidobacterium* spp has been shown also in a recent meta-analysis conducted in healthy individuals [238]. Furthermore, inulin administration decreases the Firmicutes / Bacteroidetes ratio (F / B) and increases the genus *Bifidobacterium*, in such a way that its action could potentially aid to fight obesity. Finally, resistant dextrin causes an increase in the genera *Akkermansia* and *Prevotella*, which contributes to fat and carbohydrate metabolism optimization through various mechanisms [239].

The effect of indigestible dietary fibres extends its effect not only in gut microbiome formation but underlying the metabolic role of microbiota to harvest energy from the dietary intake of the host [240], in a bidirectional way, which will ultimately lead to an increase in the abundance of certain species. Towards this direction, some microbes (especially Firmicutes) have enzymes to digest non-digestible dietary fibers into SCFAs, which play important role in energy regulation and homeostasis, having an indirect effect on obesity treatment and development [241,242]. There are several potential explanatory mechanisms behind this relationship, and at first glance, SCFAs provide individuals with an additional energy source, and as such should contribute to obesity development and not obesity treatment. Indeed, in literature, increased levels of SCFAs are reported in obese children versus nonobese counterparts, outlining a positive correlation between z-score and Firmicutes and a negative correlation between z-score and Bacteroidetes. Suggested explanatory mechanisms include SCFA's effect on adipose tissue via the action of GPRs [243,244], as well as a result of increased conversion of amino acids into SCFAs [245], indicating increased amino acid catabolism.

However, contrary to this concept of SCFA promoting obesity via increased energy turnover, SCFA participates in a cascade of physiological responses, such as interacting with the FFA2

and FFA3 receptors to secrete glucose-stimulated insulin from the b-cells of pancreases and release hormones that suppress appetite [246]. More specifically, butyrate and propionate are ligands for GPR41, an SCFA receptor who expressed in PYY and GLP-1 secretion, suggesting their direct effect on the energy homeostasis [247].

### *Other Nutrients*

In recent years, there has been a tendency for artificial sweeteners to replace sugar consumption. Artificial sweeteners include saccharin, sucralose, and aspartame. However, from a study done in mice, artificial sweeteners can potentially modify the composition of the intestinal microbiome in the opposite way to natural sweeteners, which can lead to adverse effects. More specifically, there may be a decrease in *Bifidobacterium*, *Clostridium* and *Lactobacillus* and an increase in *Bacteroides* [248]. Some animal studies show that artificial sweeteners may adversely affect gut microbiome composition, and further interfere with the microbiota's effect on energy homeostasis [249]. For example, Splenda (McNeil Nutritionals, LLC, Fort Washington, PA) administration to rats [250], sachharin [251] or acesulfame-potassium, disrupt gut microbiome and may activate energy harvesting pathways [252]. Nevertheless, the specific responses of the gut microbiome to artificial sweeteners are largely unknown and further studies are needed.

Regards to probiotics, which are usually contained in foods enriched with lactobacilli, bacteria that are naturally present in some foods such as milk and yogurt with beneficial effects on the gastrointestinal system [179]. Studies showed that there is an increase in the colonization of bacteria and more specifically in aerobic and anaerobic bacteria, in the genera *Bifidobacterium*, *Lactobacillus* and *Streptococcus*, while at the same time, there is a decrease in the number of coliforms, *Escherichia coli* and *Helicobacter pylori* [179,253-257].

Another nutrient that has been previously shown to affect the gut microbiome is polyphenols. Polyphenols include catechins, flavonoids, anthocyanins, and phenolic acids, while fruits, vegetables, seeds, tea, cocoa derivatives, and wine are their natural source [179]. Polyphenol consumption is related to an increase in *Bifidobacterium* and *Lactobacillus* and a decrease in *Bacteroides*, *Clostridium*, *Salmonella typhimurium* and *Staphylococcus aureus* [176,179,218,258-263].

### *Dietary Patterns*

Following the above, the investigation of the effect of every single macronutrient on the gut microbiome is hard and as such, a collective way to study gut microbiome alterations in humans is by assessing an individual's adherence to a certain dietary pattern, with its subsequent implications on the gut microbiota.

For example, a typical Western-style dietary plan consists of an increased intake of animal proteins, saturated fats and sugars and reduced fibre intake. As a consequence, this dietary pattern is associated with dysbiosis that stimulates inflammation, through the proliferation of pro-inflammatory substances [264]. The way that a Western diet affects the gut microbiome is through a reduction the bacterial abundance, in *Bifidobacterium*, *Lactobacillus*, *Eubacteria* and *Firmicutes* and a simultaneal increase in *Bacteroides*, *Enterobacteria*, *Bilophila* and *Alistipes* [158,178,179,222,265-267]. Notably, the western type diet can decrease specific bacteria with anti-inflammatory properties, such as *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, and *Roseburia spp.* [264]. Similarly, a gluten-free diet seems to affect the gut microbiome as a Western diet. More specifically, it decreases the bacterial colonization of *Bifidobacterium*, *Lactobacillus*, *Prevotella*, *Eubacteria* and *Roseburia*, while increasing *Enterobacteria* and *Escherichia coli* [179,268,269].

On the other hand, the vegetarian diet, which is typically described by the increased consumption of plant-based products, and as such increased dietary fibre intake, has been shown to increase bacterial colonization and reduce the concentration of *Bifidobacterium* and *Bacteroides* [158,179]. In the same line, the Mediterranean Diet, a diet rich in monounsaturated and polyunsaturated fatty acids, polyphenols, antioxidants and fibre, has been shown to increase the gut bacterial load of *Bifidobacterium*, *Lactobacillus*, *Prevotella*, *Eubacteria*, *Roseburia*, *Bacteroides* and the production of SCFAs, while a decrease in the species *Clostridium* [179,201,270,271]. These alterations in the gut microbiome are considered of utmost importance as they are directly related to the health of the host. On the contrary, reducing gut bacteria limit the benefits of the intestinal microbiome and consequently leads to dysbiosis [158,168,272,273]. In conclusion, the effect of diet composition on the gut microbiome can actively modify the gut microbiome in such a way that several physiological mechanisms can be indirectly affected. On the one hand, they may lead to a positive energy balance, inflammation and thus weight gain and obesity, while also contributing to the pathophysiology of various diseases, such as Type 2 Diabetes Mellitus and cardiovascular disease. On the other hand, can act protectively both against obesity and against the manifestation of other diseases. [179,264,274].

### **2.4.3 The effect of Exercise on Gut Microbiome**

Physical activity is another important modifying factor for the intestinal microbiome. More specifically, it affects bacterial diversity, both in terms of the number and the taxes that colonize the human gastrointestinal tract. [158,275]. Studies have been conducted to investigate the effect that exercise has on the microbiome, sampled athletes and non-athletes and could be categorized by both acute vs long-term, or by type and intensity.

Overall, there is a positive correlation between fitness levels with bacterial diversity and with levels of specific species, such as Firmicutes levels, while a negative correlation has been observed between fitness levels and Bacteroidetes species abundance. This relationship is stronger in athletes compared to non-athletes [158,275,276]. In addition to the beneficial role of exercise as a whole in the intestinal microbiome, its characteristics, such as type and intensity, are additional modifying factors in the composition of the intestinal microbiome.

Regarding the type of exercise acutely, a study conducted by Morita and colleagues in 2019, showed that after an exercise with aerobic type exercise, there is an increase in the genus *Bacteroides* and a decrease in the genus *Clostridium*, while after muscle strengthening type of exercise, the genus *Clostridium* is increased [277]. Regarding the intensity of exercise, a recent study conducted by Torquati and her colleagues in 2022, showed that different intensity exercise alters the intestinal microbiome differently. More specifically, in the first group that followed moderate intensity and longer exercise, elevated levels of *Bifidobacterium*, *Escherichia*, *Akkermansia muciniphila* and bacteria that produce butyric acid of the classes *Lachnospirales* and *Clostridium* Cluster IV were obtained. On the other hand, in the 2nd group of higher exercise intensity, there was also an increase in butyric acid-producing bacteria, this time from the *Erysipelothrichales* and *Oscillospirales* classes, as well as an increase in the species *Methanobrevibacter smithii* and *Negativibacilli* spp. [278]. In the same line, Zhao et al [279] showed that after a half-marathon, *Lentisphaerae* and *Acidobacteria* abundance increased -its function in the gut is yet unknown-, as well as *Coriobacteriaceae* and *Succinivibrionaceae* – involved in hormonal and bile salts metabolism. In the same line, both after Boston Marathon [280], as after the ultramarathon and rowing event [5], the relative abundance of the genus *Veillonella* increased. Very interesting is the fact that through the methylmalonyl-CoA pathway, *Veillonella* species can metabolize lactate into SCFA, acetate and propionate [280]. Hence it is suggested that an increased abundance of the *Veillonella* species promotes an alternative way to process lactate, by converting it into SCFAs, and re-entering circulation while simultaneously inhibiting the Cori cycle [5]. This effect lies upon strong evidence, since inoculation of these marathoners' stool samples that mentioned before in mice, showed significant improvement to the time of exhaustion of mice, reducing inflammatory cytokines [5]. Hence, even acutely, the gut microbiome is affected by bouts of physical exertion, and evidence is that this alteration is of benefit to the host, improving physical capacity.

Investigating the effect of chronic exercise intervention, it is showed not only that exercise modulates actively gut microbiome, but exercise-induced alterations are strongly related to alterations in body composition. Specifically, Allen and colleagues showed that exercise stimulates an increase in bacteria that produce SCFAs, more specifically *Clostridiales*, *Roseburia*, *Lachnospiraceae*, and *Erysipelotrichaceae*, which play a key role in the beneficial

effects caused by modification of the intestinal microbiome on host health [158,276]. These changes however were largely reversed after the wash-out period, underlying the importance of exercise on gut microbiota modulation. This study supports the concept that modulation of the gut microbiome may require a continual stimulus in order not to alter temporarily [281]. Chronic exercise interventions in the elderly show decreases in Clostridioides, while the opposite occurred for Oscillospira, which in turn were related to cardiometabolic risk factors [282]. In a long-term exercise intervention that lasted six months and consisted of both different exercise modalities and intensity, it was shown that beta diversity changed in all exercise groups compared to the control, with the vigorous exercise group experiencing increased alpha diversity at 3 months, compared to control [283]. More specifically, Bacteroidetes increased and the ratio of Firmicutes to Bacteroidetes decreased. This is very important since an increase in Bacteroidetes can potentially play a key role in converting complex sugars and protein degradation [284]. In this study, another finding was the decrease in Clostridium and Blautia genera, which in turn play a key role in whole body immune response, and specifically, Blautia can increase proinflammatory cytokines release [285]. Lastly, in a recent study, Liu et al. examined the effect of exercise intervention, combining both aerobic and resistance training in pre-diabetic overweight males showed that not all subjects responded well to the intervention [286]. Interestingly, 70% of individuals that did respond well to the treatment had distinctly different gut microbiome profiles than the 30% of individuals that did not respond to the intervention. Again, when transplanting the microbiome of both responders and not responders in mice, showed a causal relationship between gut microbiome in glycemic control and insulin sensitivity [286]. Taking all together both research in humans and mice points toward individualized exercise interventions through gut microbiome screening that will aim specifically into maximizing exercise effect on specific physiological parameters.

#### **2.4.4 Gut Microbiome and Sleep**

Over the last years, literature shows that gut microbiota extends beyond its interaction with diet to a series of physiological regulatory processes. As was already noted there is a complex communication network between the gut and brain: the Brain–Gut Microbiota axis, referred to as BGMA. The latter has three main pathways that allow for bidirectional communication of brain and gut microbiota: i) via the immune system, ii) the neuroendocrine system and iii) vagus nerve [287]. Both studies in animals and humans point toward the role of BGMA on sleep regulation, as several neurotransmitters and hormones distribute in the intestine have both effects on the brain and gut [288-290].

There are indirect implications that gut microbiota may be associated with sleep. Animal studies show that d-serine, a non-essential amino acid may regulate sleep [291]. In fruit flies, this amino

acid may be produced in the intestines or brain. However, when researchers edited the genes that controlled the production of D-serine in the brain, they found no changes in sleep habits. In contrast, when they repeated this procedure to inhibit D-serine production in the intestines, fruit flies slept less. Notably, the biochemical process of D-serine biosynthesis is regulated by enzymes that are produced by bacteria, such as *E.Coli*, that are found in the lower intestine [292]. Furthermore, D-serine may regulate platelet serotonin receptors, by increasing their sensitivity to the serotonin [293]. However, most of the research in humans according to D-serine is focused on the brain and it is unclear if a quantity of D-serine is produced in the gut by its microbiota and if this may affect serotonin, sleep or any other neurophysiological processes.

In humans, the available data that support the link between sleep and gut microbiome is limited to observational correlations between specific taxa and sleep-related measures. A study conducted by Smith and colleagues showed that bacterial diversity and the presence of the Bacteroidetes and *Firmicutes* species were positively correlated with sleep efficiency and total sleep time. Moreover, the *Bacteroidetes* species were negatively associated with sleep interruptions and the specie *Actinobacteria* was negatively associated with the number of awakenings, hence sleep fragmentation. At the taxa level, there seems to be a negative correlation between *Lachnospiraceae*, *Corynebacterium* and *Blautia* and impaired sleep but either no correlation or positive relationship between *Verrucomicrobia* and *Lentisphaera* with sleep-related measures and sleep quality [294,295]. Nevertheless, in the literature, there are several positive correlations between sleep efficiency and gut microbiome diversity [294,296]. More specifically, increased *Blautia* and *Ruminococcus* (*Firmicutes* gender), decreased *Prevotella* (*Bacteroidetes*) and higher alpha diversity is commonly present in good sleepers' gut microbiome [297], underlying the positive association between *firmicutes/bacteroidetes* ratio and sleep quality. In the same line, a positive association was found between PSQI scores and *Lentishpaerge* and *Verrucomicrobia* [295]. As for sleep quantity, when discriminating between bad (<6h per night) sleepers and normal sleepers (6-8h per night), it was shown that *Bacteroidetes*, *Firmicutes* and *Pseudomonadaceae* were increased and *Lachnospiraceae*, *Sutterellaceae* and *Rikenellaceae* were decreased in poor sleepers [298]. In a meta-analysis of more than 50.000 participants, it was concluded that dysbiosis may precede and play a key role as a stressor that induces systemic inflammation [299,300], which in turn was associated with self-reported sleep disturbance [146].

However, all these observational studies in humans have only illustrated a relationship between the gut microbiome and sleep, and up to date, there is no causal relationship between the impact of gut microbiota on sleep since there are no targeted clinical trials.

Despite the great scarcity of sleep optimization intervention studies and clinical trials that induce partial sleep deprivation show that impaired sleep could modify the intestinal microbiome, called dysbiosis, mainly affecting the gut-brain axis [288,301] and further disturbing sleep, causing a vice circle [302,303]. It has been shown that partial sleep deprivation may result in increased levels in the *Firmicutes/Bacteroidetes* ratio, higher abundance in *Coriobacteriaceae* and *Erysipelotrichaceae* and lower abundance in *Tenericutes* [304]. This decreased bacterial diversity after sleep deprivation, alongside decreases in the genera *Prevotella*, *Sutterella*, *Alloprevotella*, *Anaeroplasma* and *Elusimicrobium*, most of which are involved in the production of SCFAs, cause intestinal dysbiosis, and disrupts the intestinal barrier with various negative outcomes [305]. Overall, there are many findings in both animals and humans, that indicate a plethora of changes in gut microbiota, that in some cases are inconsistent [304,306,307]. Nevertheless, different sleep deprivation protocols, intervention methods and experimental settings may lead to these inconsistencies. However, sleep deprivation impairs the gut microbiome by reducing its diversity and by disrupting gut homeostasis.

Nevertheless, BGMA seems not only to affect sleep continuity but sleep architecture as well. In animal studies, the prescription of antibiotics to deplete the gut microbiome axis, showed altered sleep-wake architecture, with the light phase to be occupied by shortened NREMS duration and the dark phase to be occupied by prolonged NREMS duration – a phase of sleep in mice-, that is indicative of the reduced amplitude of behavioural circadian rhythmicity [308]. Interestingly, there were significant decreases in intestinal 5-HT levels, confirming the previous hypothesis that -at least in animal studies- 5-HT that is secreted primarily by intestinal cells is a key mediator of the relationship between gut microbiota and sleep.

## **2.5 The biology of miRNAs**

### **2.5.1 miRNAs' role in Health**

The human body consists of various cell types, such as muscle, bone, or skin cells. Even though each one of the cells has different structures and functions, they contain similar components, like the nucleus, that contain DNA. All the information that is arranged into segments of DNA -called genes- encodes instructions for cell development and function. Although every cell contains identical DNA copies, they are differentiating through gene silencing. Gene silencing contributes to each cell's "specialization", by reading only the necessary for each cell's characteristic structure and function. Ultimately, all this information is translated into proteins via messenger RNAs. In this pathway, microRNAs are used for gene silencing. Hence, miRNA length is approximately 20 nucleotides and consists of a class of short non-coding RNAs [309].



Overall, they participate actively in cells' regulation, development and function by hindering messenger RNA's action and contributing to that sense contributing to gene silencing [309,310]. Obviously, miRNA dysregulation can cause serious health implications for the human body such as cancer and heart disease [311].

As for proteins, microRNAs are coded through genes that are contained in DNA found in the nuclei. Each gene is transcribed by an RNA polymerase2, and in that way, a regulatory or a messenger RNA is produced. In the same way, it is produced a primary microRNA, with a typical hairpin loop structure, which will become a final microRNA after being processed in several steps. First DGCR8 protein recognizes the double-stranded stem, and the enzyme Drosha binds to DGCR8 to form a microprocessor complex that will cut the RNA into a smaller precursor microRNA. Then the precursor RNA is exported into cytoplasm where it will function by inactivating messenger RNAs of one or multiple genes through the Exportin5 transporter. In the next step, the precursor RNA will form a short double-stranded microRNA molecule, cleaved by an RNase protein called Dicer. After, Argonaute protein Ago2 will interact with dicer to bind microRNA and release the one stand. The other strand -called the guide strand- will interact with AGO2 and additional proteins, forming the RNA-induced silencing complex (RISC). This complex is guided through the target to silence one or multiple genes.

There are two ways of action of miRNAs for gene silencing. The miRNAs target a messenger RNA with a complementary sequence, enabling base pairing. Hence, binding to the 3'-untranslated region (3'UTR) of the target mRNAs, can regulate both mRNAs stability and protein synthesis [309,310]. After pairing RISC with messenger RNA, the one way of action is for the protein in the RISC to cut messenger RNA, which will be finally destroyed by the cell. On the other way, the translation is inhibited by preventing the ribosome's subunit to bind to the mRNA. In both ways, the messenger RNA is not translated into a protein and hence the gene is silenced.

The nomenclature of miRNA has been developed according to its structure and origin. A precursor microRNA (precursor miRNA or pre-miRNA) is denoted by the prefix "mir" followed by a hyphen and a number, with the latter indicating the chronological order of its name. For example, mir-123 is likely to have been discovered before mir-456. A mature miRNA is denoted by the prefix "miR". MiRNAs with almost identical sequences -other than one or two nucleotides- are denoted by an additional lowercase letter, e.g. miR-133a and miR-133b. Precursor miRNAs from which identical miRNAs are derived but located at different locations in the genome are denoted by a hyphen and a numerical patch in addition. For example, miRNAs, has-mir-133a-1 and hsa- mir-133a-2 both produce the has-miR-133a miRNA. The kind of origin of miRNA is denoted by a three-letter prefix. E.g., hsa-miR-133 a

is a human miRNA. Two miRNAs derived from opposite ends of the same pre-miRNA are additionally denoted by the patches 3p and 5p.

The miRNAs were not discovered before the 90s, and as such, there are major parts of miRNA's pathway that still need to be investigated [311]. However, there have been more than 2000 different human miRNA species (<http://www.mirbase.org/>), regulating more than 30% of genes [312]. Their crucial role is apparent in the cell, coordinating DNA repair, differentiation, metabolism, and apoptosis [309,310]. It goes without saying that dysregulation of certain miRNAs could be catastrophic for the health [313], underlining the great potential for medicine in both biomarkers as key treatments for various diseases. At the same time, miRNAs are constantly associated with pathophysiological conditions, such as muscle dysfunction, impaired immune response, disorders in the Central Nervous System, metabolic and cardiovascular diseases as well as cancer [314].

Interestingly, significant amounts of miRNA were also found extracellularly [315]. Specifically, miRNAs were apparent in several biological fluids such as urine, tears, breast milk, amniotic fluid, blood plasma, saliva, and semen [316-319]. In contrast to common RNA species (mRNA, rRNA and tRNA), these miRNAs were greatly stable under extreme conditions and variations in pH, temperature, and extended storage [320]. Early studies from Valadi and colleagues [321] that founded the ground for the next discoveries, reported that in vitro intracellular miRNAs are transported to the extracellular environment by exosomes, and nowadays it is suggested that may be a way of cell-cell communication [322]. This theory emerged after the detection of miRNAs in peripheral blood microvesicles [323], indicating that when miRNA is in extracellular space, it is protected by encapsulation into membrane-vesicles, underlying the existence of intercellular and interorgan communication system [320,321,324].

Following this line of thought, in recent years, there has been a growing investigation of miRNAs as biomarkers in various pathological conditions. The first time miRNAs were used as biomarkers were in 2008 by Lawrie et al. to examine diffuse large B cell lymphoma in patients' serum, and since then, their possible use as biomarkers has been reported in the literature for many diseases [325]. Hence, an area of great interest is growing in the patterns and alterations of extracellular miRNAs in health and diseases, including cancer and cardiovascular disease [324].

This relatively new class of biomolecules have several advantages suggesting that they could be biomarker candidates in a wide range of physiological conditions. As already stated, they are apparent in several body fluids, and they are very stable in unstable pH and temperature conditions. Hence, they could be easily accessible from the extraction of liquid biopsies of blood, urine, and body fluids by non-invasive methods. In addition, there is a high specificity, in terms of the type of origin of tissues or cells, as well as a high specificity, in terms of

differences in expression levels between physiological and pathological conditions. Not only that but technologies for detecting nucleic acids already exist, and the development of new techniques takes very little time and a fairly low cost, as they can be measured by quantitative polymerase chain reaction [326]. Yet, another advantage of miRNAs lies in the potential to be used as multi-point biomarkers for accurate diagnosis, guided treatment, and evaluation of response to lifestyle or drug treatments, even as novel therapeutic targets. Nevertheless, research into miRNAs as biomarkers is still in its early stages, so currently, due to differences in sample collection, storage, RNA isolation and several more, most of the available research is inconclusive and lacks repeatability.

### **2.5.2 miRNAs and Nutrition**

Recent findings have shown that there is a bidirectional regulation of miRNAs and nutrients. In addition to endogenous synthesis, mature miRNAs can be obtained from dietary sources and on the other hand, endogenous synthesis can be modified by bioactive compounds that are accumulated through the diet [327]. This is also referred to as nutrigenomics, the study of gene expression in response to epigenetic responses. As it is natural, these observations have far-reaching implications and are very promising for future investigations. Following this line of thinking, it is indicated that miRNAs are implicated in metabolism regulation, such as lipid metabolism and insulin sensitivity [328]. In addition, the role of miRNAs is extended as biomarkers of nutritional status. For example, there have been identified nine specific miRNAs in the circulation, were downregulated in response to the zinc depletion [329]. Notably, after zinc levels returned to normal, the miRNA signature was reversed. Moreover, it was very interesting the fact that the same miRNAs that responded to zinc depletion are also associated with inflammation [329]. Nevertheless, more miRNAs may suggest adequacy in other nutrients, such as vitamin D [330]. In this sense, the novel potential effect of nutrients on miRNA profile is further discussed below.

#### *Carbohydrates, dietary fibre and miRNA*

Carbohydrates are the major component of human dietary intake and even though there are no long-term clinical trials to investigate the effect of carbohydrate periodization on miRNAs regulation, there are several animal and observational studies that underline potential relationships between them. Carbohydrates contribute to a plethora of physiological procedures and their structure separates them into monosaccharides, oligosaccharides, and polysaccharides, with prominent evidence of their effect on miRNA expression.

The main monosaccharide in the human body is glucose, which consists of the building blocks for oligo- and polysaccharides. In animal studies, glucose supplementation has been shown to

increase miR-199 family expression in pancreatic beta cells [331]. In humans, their oral glucose intake is related to increased circulating levels of miR-375 [332]. Both verify that glucose intake is related to the expression of miRNAs in vitro and in vivo. Nevertheless, glucose is regulated by insulin and glucagon in an antagonistic way between these hormones. Towards this direction, when glucose homeostasis is impaired, miR-495 expression is shown to be reduced, potentially promoting cardiac fibrosis, by favouring inflammation, cell differentiation and collagen accumulation [333]. Vice versa, after high glucose treatment, miR-493-5p expression increased in stem cells [334].

Combining 2-20 monosaccharides results in the formation of oligosaccharides, which may be used for energy production after degradation, or act as dietary fibre, regulating gut microbiota as discussed earlier. Notably, miRNAs are also related not only to glucose homeostasis but to microbiome regulation [335], potentially contributing to reducing chronic diseases risk [336,337]. As for polysaccharides, the relative miRNA research is focused on indigestible forms that contain beta-glycosidic bonds. For example, green tea polysaccharides elevate miR-9 levels, and can potentially reduce lipopolysaccharide-induced inflammation [338]. In the same line, there is evidence that astragalus polysaccharide derived from the root of astragalus membranaceus can be effective by attenuation of several related to impaired glucose physiological responses in retinal pigment epithelium cells by suppressing miR-196 and miR-204 [339,340]. Moreover, astragalus polysaccharide has been shown to attenuate lipopolysaccharide-induced inflammation as well, by modulating the miRNA expression [341,342].

Towards this direction, more recent studies investigated the relationship between miRNA and dietary fibre. In vitro studies showed a decrease in miR-19b, miR-590-5p and miR-495 expression and an increase in miR-29a, miR-31 and miR-142-5p expression after administration of galacto-oligosaccharide (GOS), an oligosaccharide present in mother's milk [343]. Moreover, alginate oligosaccharide showed promising effects in downregulating miR-29b, prevailing antitumor effect and reducing aneurysm recurrence [344]. In this line, indigestible carbohydrates, and their metabolites have been reported to modulate miRNA expression in colon cancer cell proliferation as well. Specifically, it is indicated that butyrate - as an indigestible carbohydrate metabolite- changes miRNA levels and as such could be implicated in the regulation of colon cancer cell cycle, proliferation, and apoptosis [345].

### *Protein and miRNA*

Recent research has revealed that protein, as well as its building blocks, amino acids, are related to the miRNA regulation [346]. The relationship between protein intake has been shown to begin since the early stages of life [347]. There is evidence in animal studies that a low-protein

maternal diet affects negatively both the mother and offspring [348]. Specifically, a low-protein diet is correlated with the upregulation of miR-375 in the pancreas, combined with impairments in insulin secretion and glucose homeostasis in offspring [348]. These findings were duplicated when a maternal low-protein diet was prescribed even in the last week of pregnancy, via overexpression of miR-143 and miR-219 [349]. Overall, even if these results are derived from animal studies, it is suggested that a low-protein maternal diet could trigger pathological mechanisms that could result in impaired glucose homeostasis by regulating miRNA expression. On the other hand, it should be noted that even a high-protein maternal diet can negatively impact glucose metabolism [350]. Not only that, but a high-protein maternal diet could upregulate miR-24-1-5p, which in its turn, is related to osteoblast maturation and impairs offspring's bone health [351]. On the other hand, it is of utmost importance that protein intake can regulate miRNA profile in such a way that is related to the pathophysiological effects of muscle atrophy in the elderly as well [352]. It is known that muscle function in the elderly is improved when requirements for protein intake are met, while on the other hand, inadequate intake can result in muscle atrophy and oedema [353]. With regards to miRNAs, miR-1, miR-486, miR-23a, miR-23b, miR-26a, miR-148b, let-7b, and let-7g were downregulated after protein supplementation in adults with lower limb immobilization. Interestingly though, muscle atrophy might be attenuated by miR-26a expression and MuRF-1 downregulation [346]. Amino acids are crucial for skeletal muscle metabolism also [354]. Essential amino acid supplementation is showed upregulate miR-499, miR-208b, miR-23a and miR-1 while suppressing the expression of atrophy-related myostatin and myocyte enhancer factor 2C mRNA expression [355]. Interestingly, even if resistance exercise is known to attenuate muscle atrophy, supplementation of amino acids and carbohydrates leads to downregulation of miR-1-3p and miR-208a-5p that inhibit muscle protein synthesis, regardless of exercise condition [356], highlighting the effect of amino acids metabolism on regulating miRNAs expression related to muscle atrophy.

#### *Fat and miRNA*

Dietary fat consumption is necessary for the human body, involving to a wide range of vital procedures. With regards to miRNAs expression, in animal studies, it has been shown that a high-fat, high-cholesterol diet differential miRNAs expression, downregulating and upregulating several miRNAs [357]. Specifically, it has been shown that after a high-fat diet, mir-495 is upregulated, impairing glucose metabolism, and promoting inflammation [345]. Similarly, more than fifty miRNAs have been linked with prostate cancer progression in mice after a high-fat diet [358], and miR-29b is suppressed after a high-fat diet, increasing the risk for impaired cardiac recovery and hypertrophy after a cardiac event [359]. Additionally, ox-LDL

treatment downregulated the level of miR-217-5p and promoted human aortic endothelial cell apoptosis [360]. Moreover, high-fat diets trigger a cascade of reactions that ultimately underline a strong relationship with colorectal cancer [361], starting with the release of bile acids. Afterward, they are metabolized into deoxycholic acid by gut microflora, which in turn has been shown to decrease mir-199a-5p expression, promoting colorectal cancer [361], whilst on the other hand, upregulating miR-199a-5p expression can amend tumor cell growth. Even though there is scarcely available research in humans, a study of 11 healthy adult women showed that a high-fat breakfast was associated with the regulation of 33 circulating miRNAs, [362] underlying the relationship of miRNAs and dietary fat consumption, pointing towards the need for clinical trials in humans.

### *Micronutrients and miRNA*

Vitamins are essential micronutrients that take part in metabolism, without providing direct energy. Recent studies show various effects on altering the miRNAs expression [363].

With regards to fat-soluble vitamins, in a model of glucose-induced epithelial progenitor cells in in vitro study, vitamin D supplementation resulted in an alteration of 45miRNAs [364]. More specifically, in another study, the upregulation of miR-146b-5p and miR-140-5p and downregulation of miR-322-5p, miR-335-5p, miR-409-3p, and miR450b-5p has been observed after 1,25-(OH)<sub>2</sub>-D<sub>3</sub> administration [365]. In humans, vitamin D increased miR-126-3p regulation, reducing TNF- $\alpha$  secretion and thus inflammation in women with preeclampsia [366]. In the same line, 136 miRNAs have been differently regulated after vitamin D supplementation for 12 months, compared to baseline [330], an underlying promising potential field of research with regards to vitamin D and miRNA regulation of inflammation, bone metabolism and cancer progression. In addition, there is more evidence about fat-soluble vitamins and miRNAs, such as vitamin E. In animal studies, after 6 months of vitamin E depletion, miR-122a and miR-125b expression decreased, related to lipid metabolism and cancer/inflammation respectively [367]. Another animal study showed that vitamin E was related to miRNA expression while modifying lipid metabolism and fat tissue deposition [368]. Similarly, vitamins A and K may be related to miRNA alterations, with several potential health-related effects that need to be investigated in the clinical trial [369].

With regards to water-soluble vitamins, B12 is considered probably one of the most important B complex vitamins. It is shown that B12 supplementation is related to increased placental miR-16 and miR-21 [370]. These miRNAs are related to fetal development and inversely related to low offspring weight. Similarly, adipose tissue and blood samples' levels of B12 are related to 11 miRNAs regulation with a potential role in glucose homeostasis [371]. Moreover, miRNAs may play an important role in vitamin C antioxidant activity [372].

With regards to minerals, there are reports that miRNAs mediate several biochemical functions of both macro- and micro minerals Fields[363]. In animal studies, hypertensive rats fed following a high sodium diet showed a decrease in expression of miR-24 (a miRNA involved in moderating renal injury) [373], while another study showed increased miR-25 expression (linked with heart injury) [374].

Furthermore, there are primary indications for the relationship of miRNAs regulation in association with several other micronutrients such as selenium [375], iron [376] and iodine [377], alongside potassium, phosphorus, zinc, copper, silicon and cadmium [369].

### **2.5.3 miRNAs and Exercise**

As already noted, miRNAs are crucial for human health, by contributing to gene silencing and thus, maintaining healthy cellular physiology. Exercise is shown to be a key modulator for both miRNA biogenesis and export [378]. Although data according to the influence of exercise on miRNAs are preliminary, it has been shown that drosha, dicer and exportin-5 - discussed earlier and play a key role in miRNA formation and function- have been shown to increase after acute endurance exercise [378]. Correspondingly, exportin-5 expression was upregulating after resistance exercise as well [379]. Putting altogether underlines the influence of exercise on miRNA biogenesis. In the last few decades, it is shown that miRNAs contribute to a wide range of functions in muscle tissue biogenesis, metabolism, hypertrophy, plasticity and anti-inflammatory responses [380-382].

The role of miRNAs as a mediator to exercise-related responses has begun to be unveiled in animal studies, specifically concerning both skeletal and cardiac hypertrophy [383]. Particularly, various miRNAs may regulate directly and/or indirectly the signalling of the IGF1/PI3K/AKT/mTOR pathway [384,385]. This is of utmost importance since this biochemical pathway highly contributes towards muscle cell growth, differentiation, survival and skeletal protein synthesis [386]. The activation of the IGF1/PI3K/AKT/mTOR signalling pathway will result in a cascade of physiological responses, such as increased muscle protein synthesis due to the binding of IGF1 to IGF1R/insulin receptors [386].

In response to both endurance and resistance exercise training, specific miRNAs will be upregulated or downregulated. This is crucial for the individual to adapt successfully to the metabolic demands of the corresponding type of exercise [387]. For example, a four-month exercise intervention with moderate-intensity aerobic exercise upregulated miRNA-1 and miRNA-133a and downregulated PTEN and PI3K/Akt [388]. This is very interesting since miRNA-1 regulates muscle tissue cell regeneration and differentiation [389,390], while miRNA-133 regulates the myoblast proliferation [389,391]. On the other hand, downregulation of PTEN is shown to trigger the PI3K/akt pathway, which may lead to increased protein synthesis in

skeletal muscle cells. In the same line, different studies have shown that both resistance and endurance training upregulate miR-20a, underlying this hypertrophic response within the skeletal muscle tissue [392]. Nevertheless, reverse miRNAs regulations take place during periods of de-training or inactivity [393].

Except for increased protein synthesis in the skeletal muscle tissue, exercise improves cellular respiration by promoting increases in mitochondrial biogenesis. A key miRNA that is possible to be closely related to mitochondrial biogenesis in response to aerobic exercise is miR-133a [394]. Indeed, in animal studies in mice depleted of miR-133a, exercise performance was significantly reduced, but since investigations are in their very early stage, there is also conflicting evidence with regards to miRNA-133a [395]. Nevertheless, there is a significant number of miRNAs associated with mitochondrial biogenesis, such as miRNA -696, -761, -and 494, which suppression is related to increased mitochondrial biogenesis [396,397].

Beyond protein synthesis and mitochondrial biogenesis, exercise also regulates angiogenesis, and this effect is shown to be regulated by miRNA expression as well. Specifically, a six-week exercise intervention study showed that the downregulation of miRNA-29a/b/c related to angiogenesis in response to high-intensity intermittent exercise [398]. In accordance, five days of aerobic exercise reduced the expression of miRNA-29 in diabetic rats, improving the glucose homeostasis [399].

MicroRNAs are shown to mediate exercise-induced anti-inflammatory responses and antioxidant effects in muscle tissue as well. More specifically, a study that investigated the effect of a 12-week exercise intervention in patients with myositis, showed significant upregulation of the miRNA-196b [400]. In the group that received the exercise intervention, upregulation of MicroRNA-196b hindered the NF- $\kappa$ B cascade, highlighting as such the exercise-induced effects of exercise in the skeletal muscles. On the other hand, the SOD2 gene, regulating oxidative stress, seems to be upregulated by decreased expression of miRNA-7, a miRNA that is shown to be downregulated in athletes, as compared to sedentary counterparts [401].

In summary, it is suggested that circulating miRNAs (ci-miRNAs) could be used as a diagnostic biomarker to assess physiological responses to exercise. MicroRNAs have been shown to fluctuate differently according to exercise type and intensity [389,402,403]. This is of utmost importance since several vital responses to exercise, such as muscle hypertrophy, angiogenesis, anti-inflammatory and antioxidant responses be mediated by miRNA expression. Hence, differentiations in miRNA regulation are shown to be associated with both physiological adaptations to physical exertion and recovery.



#### 2.5.4 miRNAs and Sleep

Clock genes regulate circadian rhythm, which modulates rhythmically a wide range of physiological activities, including sleep. Recent evidence suggests that miRNAs modulate the circadian manner of gene expression, and the opposite [404]. For instance, there seems to be a diurnal pattern of Dicer activity in several tissues, which in turn can potentially affect the rhythm of mature miRNA [405]. On the other hand, miR-132 has been linked to the light-inducible clock entrainment [404]. Thus, beyond the significance of miRNAs in the regulation of several physiological systems in the body [406], miRNAs are under investigation concerning their association with the circadian rhythm.

One of the most crucial and well-studied circadian rhythms is the sleep-wake cycle. Sleep constitutes an essential element for health and well-being, being involved in various aspects of both mental and physical health. Sleep traits may result in a vicious circle between abnormal circadian miRNA expressions and sleep disorders. Several cerebral miRNAs, such as miR-125a, miR-132 and let-7, are associated with sleep deprivation [407]. Furthermore, partial sleep deprivation has been linked with the downregulation of several circulating microRNAs, such as of miR-125a, miR-126 and miR-146a [407,408]. It is very interesting that in animal studies, miR-125a is related to long-term sleep regulation [409]. Not only that but, loss of REM sleep, is also related to corresponding regulations of miR-132, miR-182, and miR-124 expression [410]. Thus, miRNA regulations may be indicative of relevant sleep disorders, including insomnia, excessive daytime sleepiness etc.

It is very interesting that in such a scenario of sleep disorder, such as insomnia, a single nucleotide polymorphism on the clock gene is associated with sleep problems, as well as depressive and bipolar disorder [411-413]. For example, a single nucleotide polymorphism in miR-146a is associated with insomnia, [414], and genetic variants of pre-miR-182 are present in depression patients with insomnia [415]. In the same line, alterations in circulating miRNAs have been found in narcolepsy patients, such as miR-182-3p, as well as miR-130a, miR-26a, miR-30c and let-7f [416]. Several miRNAs that are related to clock gene expression have also been identified in obstructive sleep apnea patients [417,418], such as miR-181a.

Even though research with regards to sleep and miRNAs is still in its infancy, it is clear that miRNAs mediate clock-gene expression, affecting circadian rhythm and being closely related to the sleep-wake cycle. As is prominent, specific miRNAs are common among patients with sleep disorders, underlying the bidirectional relationship of rhythmic expression of genes and miRNAs modulation [419]. Overall, miRNAs could be a useful tool for sleep quality assessment [420], being used as a more accurate complementary screening method of sleep disorders. Not only that, but sleep-related circulating miRNAs could potentially be used as sleep quality

indicators in order to explore novel lifestyle interventions, such as exercise and nutrition manipulations, in order to develop targeted therapeutic interventions for clinical populations.

### **2.5.5 miRNAs and the Microbiome interactions**

As already noted, miRNAs are apparent in several biological fluids such as urine, tears, breast milk, amniotic fluid, blood plasma, saliva, and semen [316-319]. Nevertheless, miRNAs were also found on stool samples as well. Towards this direction, there is growing evidence that miRNAs are interrelated to the formation of gut microbiota [421].

In particular, recent evidence shows that microbial metabolites can interact with epigenetic processes, promoting the host genome reprogramming [422]. This is very interesting since again miRNA can mediate this interaction as well, the interaction between the host and microbiome [423], with relevant implications for the health and disease [424]. More specifically, gut microbiota can regulate miRNAs, and actively affect the gut homeostasis of the host [425]. The microbiota in the gut interacts with the intestinal epithelial cells, forming specific miRNA profiles [426]. On the opposite, faecal miRNAs that are derived mainly from the intestinal epithelia cells of the host, as well as some hope-expressing cells, can regulate gut microbiota composition and gene expression [421].

These host-microbiome interactions require a complex communication network, with the research interest focusing on miRNAs as mediators for this network, specifically in immune and intestinal epithelial cells in the gut [427]. In this sense, specific miRNAs such as miR-1246 have been shown to interfere with inflammation in the gut, supporting tumour survival and as such are proposed as a potential biomarker for colorectal cancer [428]. Similarly, faecal miRNAs such as miR-515-5p and miR-1226- 5p that are produced by intestinal epithelial cells, have been found to increase *F. nucleatum* and *E. Coli* abundance, by modulating their gene expression [421].

Nevertheless, even though the evidence is scarce, the gut microbiome-miRNA interactions are proposed to extend beyond microbiome formation, to interactions in the central nervous system [429], immune system [425] and cardiovascular disease [430]. Hence, these interactions point towards a new field of research for the potential treatment of neurological, immune, and cardiovascular diseases.

## **CHAPTER 3: STATEMENT OF THE PROBLEM**



### 3.1 Statement of the problem

Sleep is a vital element for human health and wellbeing, being associated with numerous physiological systems, promoting cognition [135], immune function [431], tissue restoration [432] and regulating glucose metabolism [45]. However, over the years the prevalence of inadequate sleep is growing dramatically and there are very limited data with regards to non-pharmacological interventions to optimize sleep. Fortunately, there are indications that acute exercise [7] and nutrition manipulations can prevail a sleep-optimizing effect, with further benefits on the following morning's performance [8]. To our knowledge, no-long term nutrition intervention for sleep optimization has been studied yet. Therefore, it is still relatively unknown whether a long-term carbohydrate periodization protocol could optimize sleep-related parameters. Moreover, it would be of further interest to investigate if this intervention could induce alterations in gut microbiome function and miRNAs regulation, in a way that athletic performance and body composition will also be enhanced. This would allow to elucidate further potential interrelations and biological pathways underlying these adaptations.

### 3.2 Aims of the PhD Project

The purpose of this project is to examine the effect of long-term carbohydrate periodization protocols in combination with regular exercise training on sleep initiation, maintenance and architecture, physical performance, body composition, gut microbiome and miRNA in healthy trained individuals. More specifically:

- **Study 1** aimed to systematically review the effect of the i) quantity, ii) quality and iii) timing of CHO consumption in sleep quantity, continuity and architecture.
- **Study 2** aimed to examine the effect of the carbohydrate timing and quality manipulation on i) sleep initiation and duration, ii) sleep continuation, iii) sleep architecture and iv) self-perceived sleep-related measures, over a 4-week period.
- **Study 3** aimed to investigate the effect of carbohydrate periodization of quantity and quality over 4 weeks on body composition and physical fitness indices including i) whole and segmental body composition ii) aerobic capacity, iii) power and strength and iv) visual reaction time.
- **Study 4** aimed to explore the effect of a combined exercise and nutrition intervention, modulating carbohydrate periodization in both quantity and quality over a 4-week period on i) gut microbiome formation and ii) regulation of circulating miRNAs.
- **Study 5** aimed to i) to identify potential interrelationships between sleep, gut microbiome, miRNAs, exercise and body composition indices after the nutrition and exercise intervention and ii) to identify the intervention effect on poor vs good sleepers.

## **CHAPTER 4: GENERAL METHODOLOGY**



## 4.1 Participants

Based on primary outcomes of interest, an a priori sample size calculation was conducted to estimate adequate statistical power. Setting sleep as the primary outcome of interest, a total of 10 healthy physically active participants in each intervention group provided adequate statistical power to detect potential differentiations in sleep-related parameters [8]. An extra four subjects were recruited to accommodate for potential dropouts during the study, thus, resulting in a total of 42 participants. All volunteers were invited via advertisements in local sports clubs. Participants were healthy, physically active individuals ranging from 18-48 years of age, regularly participating in sports activities for at least 2 years. Exclusion criteria included any history of major diseases or medication, tobacco use, and excessive alcohol and caffeine consumption. All participants were informed of the purposes of the study and provided a written consent form. The study was approved by the Cyprus Bioethical Committee (EEBK/EII/2020/65) and registered in ClinicalTrials.gov (NCT05464342). All the procedures were conducted in accordance with the manual of the Declaration of Helsinki and its later amendments [433].

## 4.2 Research Overview

The present study was a randomized controlled trial with a parallel design. The intervention included lifestyle changes targeting exercise and nutrition. Specifically, the study was composed of three intervention groups investigating integrative physiological responses to modifications of pre-bed periodization of carbohydrate consumption quantity and quality in trained individuals. The intervention period lasted for 4 weeks for all intervention groups.

Initially, participants underwent preliminary testing consisting of the following five sessions in order to: i) interview them to evaluate their nutritional status, sleep-related habits, and provide them with a sleep diary, actigraph and stool collection kit, ii) measure their basic somatometric and anthropometric data, their  $VO_{2\text{peak}}$  as well as their visual reaction time (VRT) and countermovement jump (CMJ) performance, iii) conduct a sleep study with the gold-standard method of polysomnography, iv) Assess their 1 repetition maximum (1-RM) in hack squat, plate-loaded bench press, plate-loaded shoulder press and lat pull-down, v) Collect blood and stool samples, the sleep diary and actigraph. These procedures were followed in order to i) assess preliminary data to properly design personalized exercise and nutrition interventions for each participant, ii) collect baseline data for sleep-related traits, body composition, performance, gut microbiome profile and specific circulating miRNAs levels at baseline and iii) familiarize participants with study's procedures.

This randomized clinical trial used a parallel design combining an exercise intervention with three nutrition intervention conditions ( $1 \times 3$  design). Randomization was determined by a

computer-generated sequence of random numbers. The subjects were randomly allocated to each intervention group that undertook a specific combination of training and nutrition periodization programs for 4 consecutive weeks, as shown in Table 4.2.1. With regards to exercise, participants followed a combination of a strength-hypertrophy training program consisting of resistance training three days per week and two evening high-intensity interval training (HIIT) sessions on the treadmill.

With regards to nutrition intervention, trials were differentiated by the timing of carbohydrate (CHO) consumption, assigning participants either into “sleep low” (SL) or into “sleep high” (SH) condition, according to carbohydrate availability after the evening workout and pre-bed. The “sleep high” condition was further divided into two groups that received *-only in the evening-* either i) foods with a high glycemic index (HGI) or ii) foods with a low glycemic index (LGI). In all trials, carbohydrate quantity consumption was similar at approximately  $6\text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ , however, in the SL condition, participants consumed all the CHO amount until their evening training session; on the other hand, in the SH condition, CHO intake was spread out evenly throughout the day, following the same training session (60% of CHO were consumed until participants’ evening workout and 40% were consumed after). The intervention period was chosen based on previous results showing that 20 days are enough to induce relevant metabolic adaptations [434]. It has also been shown that gut microbiome diversity may rapidly respond to short-term (3-days) nutrition alterations [178].

At the end of the intervention period, the testing five sessions as described in the preliminary testing were repeated, to assess participants' compliance rate to the intervention, as well as to collect post-intervention data for sleep-related traits, body composition, performance, gut microbiome profile and specific circulating miRNAs levels, as described in chapters 5,6,7 and 8. An overview of the study design is depicted in Figure 4.2.

**Table 4.2.1.** Overview of Carbohydrate Manipulation Interventions.

Arm	Intervention/treatment
SH-LGI: Evening LGI Carbohydrate Consumption Group	Dietary Intervention: Carbohydrate Periodization (CHO was spread evenly throughout the day both prior -60% of total CHO intake- and after -40% of total CHO intake- evening training session. Type of Evening CHO intake: Low Glyceamic Index)
SH-HGI: Evening HGI Carbohydrate Consumption Group	Dietary Intervention: Carbohydrate Periodization (CHO was spread evenly throughout the day both prior -60% of total CHO intake- and after -40% of total CHO intake- evening training session. Type of Evening CHO intake: High Glyceamic Index)
SL-NCHO: Evening No-CHO Carbohydrate Consumption Group	Dietary Intervention: Carbohydrate Periodization (Participants consumed all their CHO amount at regular intervals prior to evening training session)

SL-NCHO, Sleep Low- No Carbohydrates; SH-LGI, Sleep High- Low Glycemic Index; SH-HGI, Sleep High- High Glycemic Index; CHO: Carbohydrates

At the end of the intervention period, the five-session testing, as described in the preliminary phase was repeated, in order to assess participants' compliance rate to the intervention, as well as to collect post-intervention data for sleep-related traits, body composition, performance, gut microbiome profile and specific circulating miRNAs levels, as described in chapters 5,6,7 and 8. An overview of the study design is depicted in Figure 4.2.1.



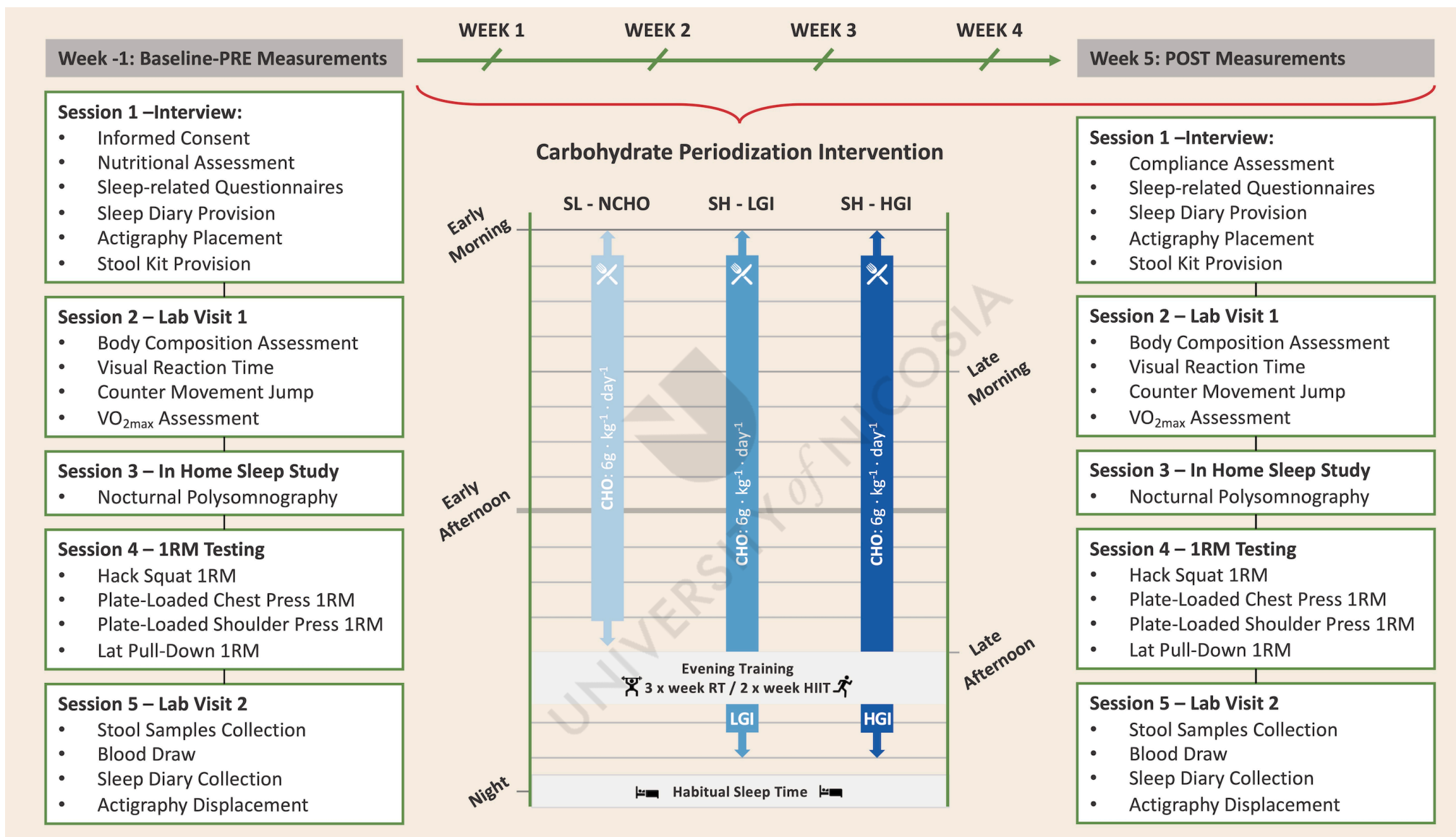


Figure 4.2.1. Schematic overview of study design.

## 4.3 Intervention Design

### *Nutrition Intervention*

All participants received a standardized dietary plan to follow, according to the trial they are assigned. The menus consisted of similar macronutrient percentages, differentiating in CHO timing/quality consumption. The typical nutrient intake for each intervention group is presented in Table 4.3.1.

Subjects were prescribed an isoenergetic nutritional plan, based on their calculated basal metabolic rate (BMR) multiplied with a physical activity level (PAL) to form their total daily energy expenditure (TDEE). Macronutrients distribution percentages were approximately 20% for protein, 60% for carbohydrates and 20% for fats, meeting all the guidelines given by the Food and Agriculture Organization of the United Nations (FAO) and the American College of Sports Medicine (ACSM), in order to avoid any nutrient imbalances and energy availability issues that were observed in previous studies investigating the SL strategy [435]. Total protein and CHO intake were the same for all groups ( $1.5\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$  and approximately  $5\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ , respectively), while the rest calories were derived from dietary fat. Micro-adjustments were possible to be made after the initial dietary plan prescription according to each participant's preferences in order to personalize the nutrition plan and hence increase subjects' adherence to the intervention.

In the SL trial, participants consumed all their CHO amount at regular intervals prior to the evening training session, while in the SH trials, CHO was spread evenly throughout the day both prior (60% of total CHO intake) and after (40% of total CHO intake) training session. The exact amount of both before evening training and post-workout carbohydrate intake is presented in Table 4.3.1. Furthermore, the SH-HGI group consumed HGI foods, while the LGI-SH group consumed LGI foods during the evening post-exercise period. For nutritional plans to be easily applicable and to increase nutritional adherence, participants for both SH-HGI and SH-LGI groups received specific nutritional recommendations manuals about potential substitutions and alternatives for HGI and LGI foods respectively.

In all scenarios, glycemic index and glycemic load were differentiated during post-workout/pre-bed. In the SH-LGI group, participants were prescribed with LGI ( $<55$ ) and LGL ( $\leq 10$ ) foods and potential alternatives. On the other hand, in the SH-HGI group, participants were instructed to consume foods with high ( $>70$ ) GI and GL ( $\geq 10$ ). In both scenarios, dietary protein intake, fat intake and vegetable consumption were similar in order not to impact the whole meal's GI and GL. Moreover, participants were asked to consume their last meal approximately 3h prior to usual bedtime. The focus on the glycemic index and meal timing manipulation was given since they have been shown to have promising sleep-optimization effects in acute preliminary

relevant studies [8,49,60]. As recommended, during the days of sleep studies, participants were not allowed to consume any caffeinated beverages or alcohol, at least 9 hours before usual bedtime [436].

**Table 4.3.1.** Interventions' Nutrition Plan Analysis.

	SL-NCHO	SH-LGI	SH-HGI
<b>TDEE</b>			
kcal	2513.5 ± 220.4	2477 ± 190.5	2481.8 ± 200.6
kcal/kg BW	30.7 ± 1.9	31 ± 1.9	32.2 ± 2.7
<b>Protein</b>			
g	123.6 ± 16.7	120.6 ± 15.7	116.9 ± 18.1
kcal	494.4 ± 66.7	482.5 ± 62.9	467.8 ± 72.4
% TDEE	19.6 ± 1.2	19.4 ± 1.2	18.8 ± 1.5
<b>CHO</b>			
g	392.8 ± 55.2	374 ± 34.5	371.4 ± 51
from which, consumed evenly until evening workout <sup>1</sup> (g)	-	224.4 ± 20.7	222.8 ± 30.6
from which, consumed after evening workout <sup>1</sup> (g)	-	149.6 ± 13.8	148.5 ± 20.4
kcal	1571.1 ± 220.8	1496 ± 138.1	1485.4 ± 204
% TDEE	62.4 ± 5.5	60.5 ± 4.3	59.7 ± 5.1
<b>Fat</b>			
g	49.8 ± 16.2	55.4 ± 13.8	58.7 ± 13.6
kcal	447.9 ± 145.9	498.5 ± 123.9	528.6 ± 122.5
% TDEE	18 ± 5.8	20.1 ± 4.8	21.6 ± 6.1

<sup>1</sup>Only in the SH group interventions. SL-NCHO, Sleep Low- No Carbohydrates; SH-LGI, Sleep High- Low Glycemic Index; SH-HGI, Sleep High- High Glycemic Index; TDEE: Total Daily Energy Expenditure; CHO: Carbohydrates, BW: Body Weight

### ***Exercise Intervention***

After preliminary testing, participants commenced a supervised 4-week/5-day training program combining both aerobic and strength training programs. All participants were asked to exercise in the evening, finishing their training plan approximately 4h prior to usual bedtime. On weekdays, the aerobic training program consisted of three full-body evening resistance training sessions separated by two HIIT sessions. The evening resistance training sessions were designed after the assessment of 1RM (described below) and are presented in Table 4.3.2. With

regards to HIIT sessions, after a 5-minute warm-up, the HIIT sessions consisted of  $10 \times 1$  minute running at speed corresponding to 90% of  $VO_{2peak}$ , followed by a 5-minute cool down [437].

**Table 4.3.2.** Resistance Training Overview.

<b>Day 1</b>	
Lat Pull Down	4 x 8 @ 80%1RM
Plate Loaded Chest Press	4 x 8 @ 80%1RM
Plate Loaded Shoulder Press	4 x 8 @ 80%1RM
Hack Squat	4 x 8 @ 80%1RM
Leg Extension	4 x 8 @ 80%1RM
Leg Curl	4 x 8 @ 80%1RM
<b>Day 2</b>	
Plate Loaded Row	4 x 8 @ 80%1RM
Bench Press	4 x 8 @ 80%1RM
Lateral Raises	4 x 8 @ 80%1RM
Leg Press	4 x 8 @ 80%1RM
Leg Extension	4 x 8 @ 80%1RM
Leg Curl	4 x 8 @ 80%1RM
<b>Day 3</b>	
Lat Pull Down	4 x 8 @ 80%1RM
Plate Loaded Chest Press	4 x 8 @ 80%1RM
Plate Loaded Shoulder Press	4 x 8 @ 80%1RM
Hack Squat	4 x 8 @ 80%1RM
Leg Extension	4 x 8 @ 80%1RM
Leg Curl	4 x 8 @ 80%1RM

RM: Repetition Maximum

### ***Compliance Rate***

At the end of the intervention period, participants were interviewed with regard to compliance rate on both nutritional intervention and exercise training. A modified questionnaire was used according to relevant studies in order to assess the compliance rate [438,439]. After the completion of the relevant interview, participants' compliance rate was calculated and participants were excluded from the analysis in case of compliance rate fell (below 80%). Overall, participants complied adequately with the present nutrition and exercise plan.

Collectively, participants self-reported average compliance rate was 89.7% for the nutrition plan and 91.2% for exercise plan. The 90.2% of participants met their energy requirement intake, and 92.6% met their goal CHO intake. Most importantly, the vast majority of participants self-reported a 97.6% compliance with post-workout/evening nutritional protocol.

## **4.4 Assessments**

### ***Anthropometry and body composition***

Anthropometry and body composition were assessed at both baseline and end of the intervention, as described in chapters 5,6,7 and 8. Weight and standing height were measured to the nearest 0.1kg and 0.5cm respectively, with Tanita WB-3000 digital beam scale (Tanita Corp, Tokyo, Japan). Individuals were barefooted and lightly dressed. Body composition was evaluated with both an estimation of body density via Harpenden skinfold calliper using Jackson's and Pollock's 7-site method [440] and bioelectrical impedance analysis (BIA). BIA was performed with a multi-frequency segmental analyzer device (Tanita MC-980 PLUS MA, Tokyo, Japan) with an accuracy of 0.1%. This specific device is equipped with 8 electrodes, four in the platform and two in each holder. Every BIA measurement was performed in accordance with the manufacturer's manual.

### ***Fitness and Exercise Performance***

Both at baseline and at the end of the intervention, individuals proceeded to Human Performance Laboratory of University of Nicosia to undertake a cascade of tests to measure their exercise performance, further described in chapters 6 and 8. Participants' countermovement jump, visual reaction time, and VO<sub>2</sub>peak were assessed at the abovementioned time points.

In the beginning, participants undertook a five-minute warm-up, and performed three CMJ and the highest one was recorded. Countermovement jump's height was estimated with an optoelectronic measurement system (Optojump Next, Version 1.3.20.0, Microgate, Bolzano, Italy). Optojump is composed of two bars. Led lights on the transmitting bar communicate continuously with those on the receiving bar and thus the device calculates the duration of any interruption between them with an accuracy of 1/1000 of a second. Flight height is calculated from flight time and data were recorded with an accuracy of 0,01cm.

Following, a visual reaction test (VRT) was performed using a computer and the Optojump measurement system. Subjects stayed in a semi-squat position with hands on hips. Immediately after receiving a visual stimulus on the computer's screen, subjects performed a squat jump with hands on hips as quickly as possible and as high as possible. Overall, the test consisted of

three visual stimuli. The reaction time after each stimulus was recorded at the nearest 0,001 second and the average VRT was calculated.

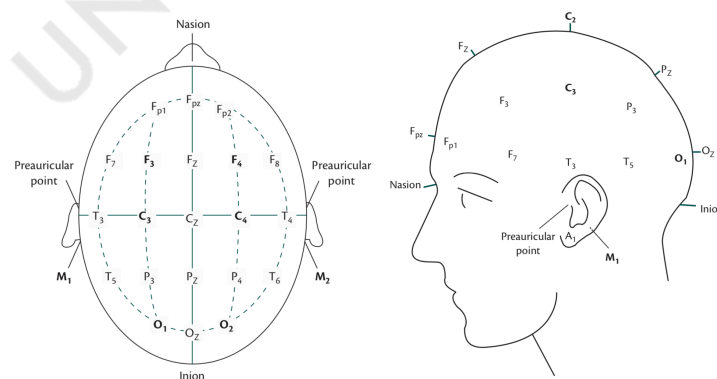
Afterwards,  $VO_{2peak}$  was assessed in a treadmill protocol ((h/p/cosmos pulsar 3p, 127 HP Cosmos, Nussdorf-Traunstein, Germany) using a breath-by-breath analyzer to record oxygen uptake (Quark CPET, Cosmed, Rome, Italy). The initial speed was set at  $5\text{km}\cdot\text{h}^{-1}$  for 3 minutes. Afterwards, the speed increased to  $8\text{km}\cdot\text{h}^{-1}$ , with further increases of  $1\text{km}\cdot\text{h}^{-1}$  every minute until volitional exhaustion. Heart rate was continuously monitored with a Polar heart rate monitor(Polar® H7, Polar Electro Oy®, Kempele, Finland). The maximum speed achieved during the test was later used to determine exercise intensity during the HIIT protocol.

Moreover, at the above-mentioned time points, participants' one repetition maximum (1RM) in specific exercises was assessed at least 72h after any exercise. Specifically, the 1RM testing was performed in hack squat, plate-loaded bench press, plate-loaded shoulder-press and lat-pull down. After a general warm-up, a specific warm-up in the given exercise was performed at 50% of 1RM, progressively increasing the load to 10% for each repetition, until a true 1RM was reached.

### ***Sleep-related parameters***

#### *Polysomnography*

Sleep architecture was assessed by overnight polysomnography (PSG) (Somnoscreen, Somnomedics GmbH, Randersacker, Germany) at baseline and at the end of the intervention as described in Chapters 5 and 8. Polysomnography is a multivariate construct to study sleep, recording respiratory and cardiac sensors combined with EEG, electrooculogram (EOG) and electromyogram (EMG). Gold-plated cup electrodes were placed at the head according to the international 10/20 system with EEG paste, to obtain data from EEG, EOG, EMG and ECG as shown in Figure 2.6.1.



**Figure 4.4.1.** Top and side view for international 10/20 system for electrode placement. Adopted from Chokroverty, Sudhansu, and Luigi Ferini-Strambi, eds. Oxford textbook of sleep disorders. Oxford University Press, 2017.

Specifically, the set-up of the examination included the application of the following: electroencephalogram (F3, F4, A1, A2, O1, O2, ground [at AFz] and a reference electrode at position FCz) right and left electrooculogram; submental and tibial electromyogram; electrocardiogram; heart rate; oxygen saturation; thoracic and abdominal efforts (piezoelectric transducers); nasal flow; chest and diaphragm breathing; snoring and body position. Sleep stages and arousals were determined using the standard criteria of the American Academy of Sleep Medicine (AASM) [441].

The PSG examination was performed at the participant's home in order to maintain as possible their sleep routine and environment. Home-based portable polysomnography was selected to assess sleep in the home environment, since changes in the sleeping environment may induce alterations in sleep patterns [442]. During the days of monitoring of sleep-related parameters, participants were asked not to take a nap during sleep-study days because of its effect on nocturnal sleep patterns [443]. In the evening, the researcher arrived at the participant's home and following the set-up of the device, the participants went to bed at their habitual time. Sleep duration was ad libitum in each trial. Polysomnograms (Somnoscreen, Somnomedics GmbH, Randersacker, Germany) were collected overnight. The PSG examination terminated the next morning. In case of failure to obtain sleep study-related data, participants were asked to repeat the study.

For each sleep period the sleep-related variables that were assessed are presented in table 4.4.1; for the polysomnography, the following variables were calculated: Total Sleep Time (i.e. the amount of sleep time whilst in bed); Sleep stages N1, N2, N3 and Rapid-Eye-Movement (REM) as a percentage of TST, (N1, N2 stages are often denoted as "light sleep" and N3 as "deep sleep", all N1, N2, N3 stages are denoted as Non-REM (NREM) sleep); % Sleep Efficiency (SE), (i.e.  $TST/Time\ in\ bed \times 100$ ); Sleep Onset Latency (SOL), (i.e. the time elapsed from laying down until N1 stage sleep onset); REM Onset Latency (i.e. the time elapsed from laying down until REM stage sleep onset); Deep Sleep Onset Latency (i.e. the time elapsed from laying down until N3 stage sleep onset); Total Wake Time (i.e. the total amount of wakefulness from N1 stage sleep onset until the final awakening); Arousal Index (i.e. the number of arousals per hour,) during REM stage, NREM stages and in total.

### *Actigraphy*

Sleep initiation and maintenance, as well as intra-individual standard deviations for each sleep measure for both baseline and end of the intervention described in chapter 5 and 8, was assessed with actigraphy. All participants wore the same monitor throughout the duration of their respective monitoring period (ActiGraph GT9X Link, ActiGraph, Pensacola, FL, USA). The

monitor has been validated for sleep with adult and adolescent populations against laboratory PSG [16,18,19]. Each subject was asked to wear the actigraph for seven days both at baseline and at the end of the intervention. Participants wore the activity monitor on their non-dominant wrist, and they were advised to wear the monitor after their last meal of the day. The accelerometer was set to record accelerations at a sampling rate of 30 Hz, and accelerations were converted into activity counts per 1<sup>-min</sup> epoch length during post-data processing. Following data conversion to 60-second epochs, they were processed in Actilife Software (version 6.5.3, ActiGraph, Pensacola, FL, USA). The Cole-Kripke sleep algorithm was applied to analyze the data with the Tudor-Locke default for sleep period detection. Furthermore, sleep onset and sleep offset were manually marked where appropriate, in accordance with each participant's sleep diary. Afterwards, data were converted into Excel files to proceed with statistical analysis. Data derived from the monitors were used to estimate Bed Time, Sleep onset Time, Wake Time, Time in Bed (TIB), Total Sleep Time (TST), Sleep Efficiency (SE) and Sleep Fragmentation Index, as shown in Table 4.4.1. For each of the sleep variables, the intra-individual standard deviations were calculated [444] and used in the statistical analysis.

#### *Sleep Diary*

In addition to actigraphy devices, for the studies described in chapters 5 and 8, participants recorded their time in bed, daytime naps, intake of caffeine and alcohol, and the time at which they turned lights out for sleep in sleep diaries. Specifically, the sleep diary consisted of two parts. Part A was filled out after morning wake up, and participants were asked to record sleep-related data, such as bedtime, wake time, sleep onset latency, sleep duration, sleep quality and refreshment. The second part was filled out before sleep, and consisted of questions about daily sleepiness, mood and relaxation before sleep, as well as additional information about daytime napping and caffeine consumption. Diary data were used to define the scoring interval for actigraphic sleep, according to the procedure outlined by Acebo and colleagues [27].

#### *Sleep quality, daily sleepiness and fatigue Questionnaires*

Moreover, at the same time points as described in chapters 5 and 8, participants were asked to regularly complete several sleep-wake scales including, the Pittsburgh Sleep Quality Index (PSQI), Epworth Sleepiness Scale (ESS), and Fatigue Severity Scale (FSS) respectively.

The PSQI questionnaire consists of 19 items that assess sleep quality during the previous month [445]. These items are separated into seven components: i) subjective sleep quality, ii) sleep latency, iii) sleep duration, iv) habitual sleep efficiency, v) sleep disturbances, vi) use of sleeping medication, vii) and daytime dysfunction. Each item is given a score from 0 to 3. The sum of the scores for these components ranges from 0-21, with the higher scores indicating



worse sleep quality. An overall score greater than 5 is yielded an adequate diagnostic sensitivity and specificity to identify impaired sleep quality [446].

The ESS is an eight-item scale used to evaluate daytime sleepiness [447]. Questions ranged from 0 to 3, according to the probability of dozing off while engaged in daily activities. Overall scores classify individuals with normal (0-9 points), mild to moderate (10-15 points), and severe excessive daytime sleepiness ( $\geq 16$  points).

The FSS is a standardized tool with nine items, used to rate the extent of fatigue symptoms and their impact on patient functioning for the past 2 weeks [448]. Each item is given a score between 1 (completely disagree) and 7 (completely agree). The total FSS score ranges from 1 (no fatigue) to 7 (very severe fatigue) and is calculated as the average score of each of the nine items. The proposed cut-off score for this scale was set at  $\geq 4$



**Table 4.4.1.** Sleep Variables per Sleep Assessment Method.

Domain	Sleep Variable	Definition	PSG	Actigraphy	Sleep Diary	PSQI	FSS	ESS
Sleep Initiation and Continuity	Bedtime	the self-reported clock time at which a participant went to bed to attempt to sleep	+	+	+	+		
	Sleep onset time	The clock time that a participant fell asleep at the start of a sleep period	+	+	+			
	Sleep offset time	the clock time that a participant woke at the end of a sleep period	+	+	+	+		
	Total Sleep Time	The total time spent asleep during the recording night	+	+	+	+		
	% Sleep Efficiency	The percentage of the Ratio of Total Sleep Time to Time in Bed	+	+	+			
	Sleep Onset Latency	Time from lights out until sleep onset (generally defined as first epoch of sleep Stage 1).	+	+	+	+		
	Wake After Sleep Onset	The duration of wake during the night after initial sleep onset.	+	+	+			
	Arousal Index/ Sleep Fragmentation Index	The number of arousals per hour of nocturnal sleep	+	+				

*(Continued on next page)*

Table 3.4.1. (Continued)

Domain	Sleep Variable	Definition	PSG	Actigraphy	Sleep Diary	PSQI	FSS	ESS
Sleep Architecture	REM Onset Latency	The interval between sleep onset and the onset of the first REM sleep period	+					
	Stage 1 (N1)	Duration of sleep Stage 1, presented as minutes or percentage of Total Sleep Time	+					
	Stage 2 (N2)	Duration of sleep Stage 2, presented as minutes or percentage of Total Sleep Time	+					
	Stage 3 (N3)	Duration of Slow Wave Sleep (SWS), presented as minutes or percentage of Total Sleep Time	+					
	REM Sleep	REM Sleep (REM): Duration of REM generally presented as minutes or percentage of Total Sleep Time	+					
Sleep habits and subjective feelings of sleep quality	Daytime Nap duration	The total amount of sleep obtained during a daytime nap			+			
	Subjective sleep quality	Participants' self-rating of sleep quality			+	+		
	Daytime Fatigue	Participants' self-rating of daytime fatigue					+	
	Daytime Sleepiness	Participants' self-rating of daytime sleepiness			+			+

### ***Gut Microbiome Analysis***

In order to study the participants' gut microbiome, the investigation focused on alterations of specific gut bacteria as described in chapter 7 and 8. Stool samples were collected from participants in designated fecal sample containers at baseline and at one-month period for each trial, and kept frozen at  $-80^{\circ}\text{C}$  until processed for analysis in the Human Biology laboratory of the University of Nicosia. Microbial DNA was extracted using the PureLink Microbiome DNA Purification Kit (Invitrogen, ThermoFisher Scientific, Carlsbad, California, USA) according to the manufacturer's instructions. Afterwards, the concentration and purity of the extracted DNA was assessed using spectrophotometry. This technique involves measuring the absorbance of the DNA sample at specific wavelengths, usually 260 nm and 280 nm. Absorbance at 260 nm is indicative of the DNA concentration, while absorbance at 280 nm primarily reflects the presence of protein contaminants. The ratio of absorbance values at these wavelengths ( $A_{260}/A_{280}$ ) is used as an indicator of DNA purity, with a value between 1.8 and 2.0 generally considered to be pure DNA. Any significant deviation from this range could suggest the presence of contaminants, such as proteins or RNA, in the sample. Conventional polymerase chain reaction (PCR) with specific primers was designed to amplify the 16S rRNA gene sequences specific to the *Bacteroides* and *Clostridium* genera. Finally, amplified DNA fragments were separated using agarose gel electrophoresis and the resulting band patterns were used to determine the relative abundance of the *Bacteroides* and *Clostridium* genera. This semi-quantitative analysis was conducted by measuring the band density with the aid of a publicly accessible software ImageJ (version 1.53k, 2021) (<https://imagej.nih.gov/ij/>)

### ***Circulating miRNA Identification***

Blood draw was performed in the last two studies described in chapters 7 and 8. After an overnight fast, blood samples (5mL) were collected by experienced personnel of the university of Nicosia on two occasions: at baseline and at the end of each trial, in order to identify potential alterations in circulating miRNA levels that are associated with hypertrophy or cardiovascular function in response to the exercise/nutrition intervention [449,450]. Samples were taken from a superficial forearm vein using standard techniques and collected in EDTA tubes to be processed immediately. In order to separate plasma from red blood cells, the samples were centrifuged at 3.000 rounds per minute for a total of 10 minutes at  $4^{\circ}\text{C}$ . Plasma samples were stored in Eppendorf tubes at  $-80^{\circ}\text{C}$  until analysis in the Human Biology laboratory, University of Nicosia. After the extraction of miRNAs from blood plasma, the concentration and purity determination of the extracted miRNAs was assessed with spectrophotometry. Afterwards, qRT-PCR was performed using the miScript Reverse Transcription and miScript SYBR Green PCR Kit, according to the manufacturer's protocol (Qiagen, Germany). The relative and

absolute quantification of microRNAs miRNA125a and miRNA132 was calculated using standard curves and the utilization of the small nuclear RNA (snRNAs) RNU6 as reference.

### ***Statistical Analysis***

Specific details regarding the statistical procedures used during each experimental trial are described in the methods section of each experimental chapter. Raw data are presented as mean  $\pm$  SD. Statistical analyses were conducted using IBM®SPSS® statistics for Windows, version 25.0 (IBM Corp, Armonk, NY). All data sets were checked for normative distribution using the Shapiro-Wilks test. A repeated measures analysis of variance was used to investigate the difference in the selected timestamps on sleep-related parameters, gut microbiome, exercise performance and body composition among the intervention trials. Potential significant effects were further explored using the Bonferroni post hoc procedures. Effect sizes were calculated using partial eta squared ( $\eta^2$ ). Values of 0.1 were considered as small, 0.3 as medium and 0.5 as large effect size. Potential correlations were identified by estimating Pearson's  $r$  or Spearman's correlation coefficient for normal and non-normal distributed variables, respectively. Alpha level for all statistical analyses was set at  $p < 0.05$ , two-tailed tests.

**CHAPTER 5: A SYSTEMATIC REVIEW, META-ANALYSIS  
AND META-REGRESSION ON THE EFFECTS OF  
CARBOHYDRATES ON SLEEP**



## 5.1 Introduction

Sleep constitutes a lifelong element of human existence. It is defined as a reversible state of decreased or absent consciousness that initiates from wakefulness and evolves to light, deep and rapid eye movement (REM) sleep stages [14]. Alterations in sleep-related parameters are often part of physiological responses that may be induced by nutrition interventions and might be translated into modifications in sleep architecture [53], quantity or continuity [8].

The required sleep quantity varies among individuals and its adequacy is reflected by the absence of sleep-induced or sleep-related health issues, daytime dysfunction or sleepiness [451]. Insufficient sleep traits have been increased over the last years [6,452]. Its association with numerous diseases [453] underlined the necessity to examine practical and effective approaches, including manipulation of nutritional aspects to optimize sleep [454]. Various nutrition interventions have been studied for their effects on sleep-related parameters. Interestingly, both dietary supplements (e.g. tryptophan [455]) and consumption of whole foods (e.g. kiwifruit [456], tart cherry juice [74]) have shown promising effects on improving sleep quality.

Animal models revealed that hormones [56,57] and peptides [457] entrain the body's circadian system and affect sleep. Since the circadian system facilitates most of human behavioral and physiological processes, observational studies in humans have tried to investigate the effect of macronutrient intake in sleep quality and stated that alterations in the distribution and periodization of macronutrients are associated with sleep optimization [37]. Towards this direction, the findings of nutrient-sleep interactions persist to positive but yet unverified and with unsettled biological mechanism explanation [458].

The acute manipulation of dietary carbohydrate (CHO) has been highlighted over the years with regards to its effect on sleep-related parameters. Carbohydrates are considered to be a critical macronutrient for sleep, not only because they serve as a primary source of energy for all human cells, but due to their relationship with the brain function and sleep-related hormonal regulation [459]. Glucose metabolism is highly interrelated to sleep [45] by modifying the plasma tryptophan concentration [60], a precursor of serotonin and melatonin, which, in turn, has a significant effect on sleep initiation and continuity [460]. Two recent reviews suggest that dietary melatonin intake (either from fruits and vegetables [461], or milk and cherry juice [462]) could have sleep-promoting effects. Research shows that carbohydrates are associated with alterations in sleep onset latency [49], sleep time [8], sleep continuity [8] and sleep stages [463]. Nonetheless, the effect of CHO intake in sleep has not been systematically reviewed yet. Furthermore, since dietary CHO intake from individual studies may vary in quantity, quality or timing/duration of intervention, it is of utmost importance for these factors to be taken into account and analyzed distinctively. Therefore, this review aimed to assess the effects of CHO

consumption in sleep through systematic review and meta-analysis. Specifically, the objectives of this review were to:

- Examine the effect of the quantity of CHO consumption in sleep quantity, continuity and architecture.
- Address potential effects of the quality of CHO (e.g. Glycaemic Index or Glycaemic Load) sleep quantity, continuity and architecture.
- Investigate the effect of the timing of CHO consumption in sleep quantity, continuity and architecture.

## **5.2 Materials and Methods**

### **5.2.1 Information sources and search strategy**

The review was conducted according to the PRISMA guidelines [464] and the research strategy performed using the PICO model (Population: healthy participants aged above 18years old, Intervention/ Comparison: groups with alterations in carbohydrate consumption, Outcome: Nocturnal sleep-related parameters obtained from polysomnography (PSG), sleep electroencephalography (EEG) or actigraphy). Three electronic databases (PubMed, SCOPUS and Cochrane Library) were systematically searched by two of the authors (AV, EA) for studies published from inception to October 10, 2020. For all data bases, search term included the following combination of keywords: “(((((((carbohydrate\*) OR CHO) OR glyceamic index) OR glyceamic index) OR glyceamic load) OR glyceamic load)) AND ((((((((((sleep) OR sleep patterns) OR sleep characteristics) OR sleep architecture) OR sleep habits) OR sleep quality) OR sleep quantity) OR sleep duration) OR sleep efficiency) OR sleep stage)”. Retrieved records from Pubmed search were limited according to species (Humans) and language (English). Accordingly, retrieved records from SCOPUS search were limited according to source type (Journal), document type (Article) and language (English). Retrieved records from Cochrane Library were limited to trials. A supplementary search for relevant studies was conducted from the reference list of the screened manuscripts.

### **5.2.2 Eligibility criteria**

The eligibility of retrieved records was screened according to the following criteria: 1) studies were written in English, 2) carbohydrate intervention was manipulated by the researchers and described adequately and 3) nocturnal sleep-related parameters were measured objectively via PSG, EEG, actigraphy or a combination of these methods 4) participants aged above 18 years old. Only clinical trials were considered for inclusion. Articles were excluded if: 1) participants had a history of major diseases, 2) nutrition intervention was affected by other intervention or



condition 3) sleep-related data were assessed after travel, other manipulated intervention (e.g. altitude or light exposure) or any other dietary or pharmacological intervention.

### **5.2.3 Study selection and data extraction**

With the inclusion criteria, the identified articles were first screened by their titles and abstracts, followed by a full-text screening. After the removal of duplicated records from the retrieved studies of the initial search, titles abstracts and full manuscripts were assessed with a hierarchical approach. The study selection flowchart is illustrated in Figure 1. The database search and study selection were performed by two reviewers (A.V and E.A.) independently. Any discrepancies in the selection process were discussed until a consensus was reached.

A standardized data extraction form was employed to obtain the following: 1) publication details: name of first author and year of publication, 2) study design and participants' characteristics (sample size and sex) 3) nutrition intervention data: i) CHO intervention classification (quantity, quality and timing) and ii) macronutrient analysis of the dietary intervention 4) sleep-related parameters (further described below).

### **5.2.4 Evidence quality appraisal**

All studies were assessed for their methodological quality using the 'QualSyst' tool (Supplementary Table 1) [465]. Two reviewers (AV, EA) used this 14-item checklist to assess the included studies. When a criterion was satisfied, a score of '2' (=yes) or '1' (=partial) was awarded. Otherwise, a score of '0' (=0) was awarded the corresponding item. The overall score was calculated as the total score, divided by the total possible score. Studies rating was classified as their evidence quality as weak ( $\leq 55\%$ ), moderate (55-75%) or strong ( $\geq 75\%$ ).

### **5.2.5 Definitions of nutrition-related and sleep-related parameters**

Nutrition interventions were categorized based on the variance of the quantity of CHO consumption, the quality of CHO consumption or the timing of CHO consumption during a short-term (one or more days) or an acute (less than two meals) intervention. The quantity of CHO was defined by the amount (g) of CHO intake and lower CHO intakes (LCI) were compared to higher CHO intakes (HCI). The quality of CHO consumption was based on meals' Glycaemic Index (GI) or Glycaemic Load (GL). The GI is a ranking of carbohydrates on a scale from 0 to 100 according to the extent to which they raise blood sugar (glucose) levels after eating [466]. Foods with  $GI \geq 70$  are classified as high, foods with GI 56-69 as moderate and foods with  $\leq 55$  as low GI. Glycaemic Load is a combination of the quantity and quality of carbohydrates. It is calculated as:  $GL = GI \times \text{Carbohydrate (g) content per portion} \div 100$ . Similar to the GI, the GL of a food can be classified as low ( $\leq 10$ ), medium (11-19), or high ( $\geq 20$ ). In

the case of acute CHO intervention, the timing was defined as the timing of meal consumption related to the usual bedtime.

The selected nutrition parameters were selected in order to systematically evaluate their effect on the following sleep variables:

- Total Sleep Time (TST): The total time spent asleep during the recording night.
- Sleep Efficiency % (SE): The percentage of the Ratio of Total Sleep Time (TST) to Time in Bed.
- Sleep Onset Latency (SOL): Time from lights out until sleep onset (generally defined as first epoch of sleep Stage 1).
- Wake After Sleep Onset (WASO): The duration of wake during the night after initial sleep onset. This term was used in parallel with “Total Wake Time” because of their similarity and the interchangeable use of them in the shortlisted studies.
- REM Onset Latency (ROL): the interval between sleep onset and the onset of the first REM sleep period.
- Stage 1 (N1): Duration of sleep Stage 1, presented as minutes or percentage of TST.
- Stage 2 (N2): Duration of sleep Stage 2, presented as minutes or percentage of TST.
- Stage 3 (N3): Duration of Slow Wave Sleep (SWS), presented as minutes or percentage of TST.
- REM Sleep (REM): Duration of REM generally presented as minutes or percentage of TST.

### **5.2.6 Data Synthesis**

Data were extracted in as many sets as possible, separating data sets for each type of intervention. Extracted data sets were categorized, analysed and interpreted according to CHO intervention quantity, quality and duration/timing of CHO intervention. The mean and standard deviations (SD) for all data were recorded. Where required, SD was calculated from reported Confidence Intervals (CIs) or imputed as the average SD of similar studies, as proposed by the Cochrane Handbook for Systematic Reviews of Interventions [467]. When data were reported in different units, they have transformed accordingly for purposes of uniformity. In the case of merged N1 and N2 stages of sleep (as light sleep), the results are presented but not included in the calculation of general or subgroup mean. In case of sleep scoring with 5 stages (REM, N1, N2, N3 and N4), the N3 and N4 stages were combined [14].

For all analyses the comparator group received CHO with either different quantity, quality or timing of CHO consumption or ingested a similar supplement or was placebo-controlled. If a study included two CHO intervention group and a placebo-control group that were all part of the nutrition intervention, all the intervention groups were retrieved and compared pairwise.

Where the change in SD ( $\Delta$ SD) was not reported, it was imputed from before and after intervention SD. The correlation coefficient (corr) was calculated according to the Cochrane Handbook for Systematic Reviews of Interventions [467]:

$$\text{Corr} = (\text{SD}_{\text{pre}}^2 + \text{SD}_{\text{post}}^2 - \text{SD}_{\text{change}}^2) / (2 \times \text{SD}_{\text{pre}} \times \text{SD}_{\text{post}})$$

In addition to the effect of CHO on sleep-related parameters, two main domains (discriminated accordingly by the reporting of sleep stages in minutes or % of TST) were created:

1. Sleep Depth (min): defined by shorter duration of N1 and N2 sleep stage, and longer duration of N3 sleep stage in minutes.
2. Sleep Depth (%): defined by lower TST percentage of N1 and N2 sleep stage, and higher percentage of N3 sleep stage.
3. REM attainment (min): defined by shorter ROL and increased REM duration in minutes.
4. REM attainment (%): defined by shorter ROL and increased % REM.

### 5.2.7 Statistical Analyses and Meta-Analytic Calculations

Hedges  $g'$  effect size (ES) with 95% CIs was calculated to reflect standardized differences in means for individual sleep-related parameters between two CHO interventions or before and after CHO intervention. Scores were rated as were rated small (ES <0.4), moderate (ES= 0.4–0.7), or large (ES>0.7). For interpretation purposes, effect sizes were further transformed in the corresponding units using pooled SD for each CHO domain (quantity or quality). For the sleep domains, single variables ES with SEs was inserted into one meta-analysis for each sleep domain, after adjustment for direction (For Sleep Depth: N1 and N2 ES were multiplied by -1 and for REM attainment: ROL ES was multiplied by -1). Thus, each study could contribute multiple times into the same domain, according to the number of variables the particular study reported in the domain. Only studies that reported all sleep variables for each sleep domain were included in this specific analysis.

Multiple sensitivity analyses such as Q and  $I^2$  statistics were performed to determine if any of the results were influenced by heterogeneity. An  $I^2$  value >50% was regarded as evidence of substantial heterogeneity and thus, a random-effect model was then preferred to a fixed-effect model. Furthermore, since heterogeneity statistic  $I^2$  is proposed that can be biased in small meta-analyses [468], funnel plots were assessed graphically for publication bias (supplemental material; Figure S1 and Figure S2) calculated by Meta-Essentials: Workbook for meta-analysis [469]. Meta-analyses were conducted when at least two data-sets were available. In an effort to further explore potential sources of heterogeneity, meta-regressions were performed for all the primary outcomes, using a random-effects model. For studies investigating the effect of carbohydrate quantity on sleep, the differences of the percentage of carbohydrate dietary intake between trials was set as moderator. For studies investigating the effect of carbohydrate quality

on sleep, the differences of glycemic load of the pre-bed meal was set as moderator. A minimum of 4 data sets was required to perform the regression analysis. Meta-regression was chosen instead of subgroup analysis, in order to further explore potential collinearity between the effectiveness of each intervention and the continuous covariate (moderator). A significance level of  $p < 0.05$  was set throughout the meta-analysis.

### 5.3 Results

#### 5.3.1 Studies' selection

The literature search resulted in 2316 studies (Figure 5.3.1.1.). After duplicate removal, 2136 studies were initially screened by title and abstract. Ninety-nine studies were short-listed and assessed for eligibility. Through full-text screening, 88 articles were excluded, resulting in 11 articles identified that included in the qualitative synthesis and quantitative analysis.

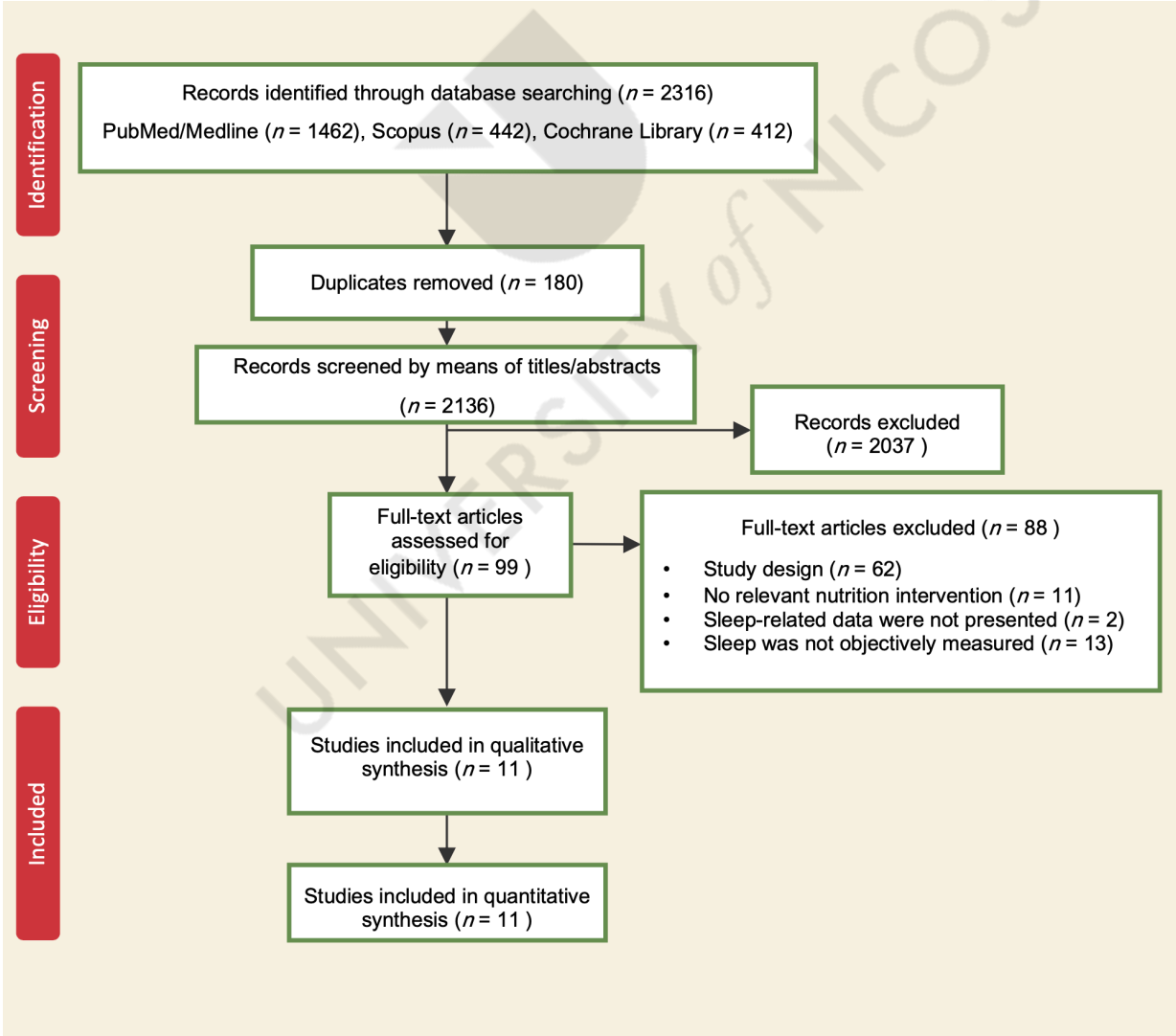


Figure 5.3.1.1. PRISMA Flow chart for study selection.

### 5.3.2 Studies' characteristics

The characteristics of the 11 studies are presented in Table 5.3.2.1.. Included studies were published from 1975 to 2019. Eight studies used a cross-over design [8,39,47,49,50,463,470,471] and three used a single group pre-post design [52,53,472]. Three studies were conducted in United Kingdom [47,52,463], three in Australia [49,50,53], three in United States [39,470,472], one in Cyprus [8] and one in Brazil [471]. According to the 'QualSyst' scale, evidence quality of five studies [52,53,463,471,472] were classified as moderate and 6 studies [8,39,47,49,50,470] were classified as strong quality. None of the studies was scored as weak evidence quality.

Table 5.3.2.1. Characteristics of included studies.

Author, year, reference	Country	Study Design	N	Sex	Age (y)	Quality Score
Phillips et al., 1975 [463]	UK	Crossover	8	M	NR	Moderate
Porter and Horne, 1981 [47]	UK	Crossover	6	M	NR	Strong
Kwan et al., 1986 [52]	UK	Single Group pre-post design	6	F	20-23	Moderate
Afaghi et al., 2007 [49]	Australia	Crossover	12	M	18-35	Strong
Afaghi et al., 2008 [53]	Australia	Single Group pre-post design	14	M	18-35	Moderate
Jalilolghadr et al., 2011 [50]	Australia	Crossover	8	M & F	8-12	Strong
Lindseth et al., 2013 [39]	USA	Crossover	44	NR	19-22	Strong
Lindseth and Murray, 2016 [470]	USA	Crossover	36	M & F	20.9 ± 1.9	Strong
St-Onge et al., 2016 [472]	USA	Single-Group pre-post design	26	M & F	30-45	Moderate
Vlahoyiannis et al., 2018 [8]	Cyprus	Crossover	10	M	18-26	Strong
Daniel et al., 2019 [471]	Brazil	Crossover	9	M	18.0 ± 0.7	Moderate

M: Males; F: Females; NR:Not Reported.

The included sample was 179 participants (males n=59; females n=6; combined -without clear sex segregation- n=114), with age ranging from 8 to 45 years. Overall, participants were tested in 27 trials of nutrition intervention, resulting in 16 comparison data sets (Table 5.3.2.2). Specifically, eleven comparisons investigated the effect of CHO quantity in sleep [39,47,52,53,463,470,472] and five investigated the effect of CHO quality in sleep [8,49,50,471]. Of these data sets, seven studied an acute CHO intervention [8,47,49,50,471], eight studied a short-term (over a period of few days) CHO intervention [39,52,53,463,470,472] and two data sets compared an acute response to a few days of CHO intervention [53]. In table 2, the consumption of the other two macronutrients, fats and proteins, is also presented, as well as the dietary energy content. It is noted that, in the majority of the studies, dietary fat was interchanged with carbohydrates between trials, with no significant modifications in protein intake. Sleep was monitored either with polysomnography or EEG recordings, except for three studies [39,470,471] that measured sleep with actigraphy. The extracted sleep-variables for each study are presented in the Table 5.3.2.3.

**Table 5.3.2.2.** Nutrition Intervention Data.

Author, year, reference	Type of intervention (A=CHO quantity; B=CHO quality; C=CHO timing)	Duration of intervention (A=Acute; B=Short-Term)	Timing of intervention (A=45-1h; B=4h)	Trial	Nutrition Intervention Macronutrient Analysis						
					Kcal	CHO (g)	CHO (%)	Fat (g)	Fat (%)	Protein (g)	Protein (%)
Phillips et al., 1975 [463]	A	B	NA	HCI	2997	600.0	80.1	33.0	9.9	75.0	10.0
		B	NA	LCI	2995	100.0	13.4	255.0	76.6	75.0	10.0
Porter and Horne, 1981 [47]	A	A	A	HCI	714	130.0	72.8	18.0	22.7	8.0	4.5
		A	A	ZCI	0	0.0	0.0	0.0	0.0	0.0	0.0
		A	A	LCI	401	47.0	46.9	21.0	47.1	6.0	6.0
Kwan et al., 1986 [52]	A	B	NA	HCI	1929	240.0	49.8	83.0	38.7	64.0	13.3
		B	NA	LCI	2066	49.0	9.5	164.0	71.4	103.0	19.9
Afaghi et al., 2007 [49]	B & C	A	A	HGI (1h)	764	173.0	90.6	1.3	1.5	15.0	7.9
		A	B	HGI (4h)	764	173.0	90.6	1.3	1.5	15.0	7.9
		A	B	LGI (4h)	764	173.0	90.6	1.3	1.5	15.0	7.9
Afaghi et al., 2008 [53]	A	A & B	B	HCI	1090	196.0	71.9	15.0	12.4	42.0	15.4
		A	B	LCI (acute)	1090	2.0	0.7	74.0	61.1	103.0	37.8
		A & B	B	LCI (2 days)	1090	2.0	0.7	74.0	61.1	103.0	37.8

*(Continued on next page)*

Table 5.3.2.2. (Continued)

Author, year, reference	Type of intervention (A=CHO quantity; B=CHO quality; C=CHO timing)	Duration of intervention (A=Acute; B=Short-Term)	Timing of intervention (A=45-1h; B=4h)	Trial	Nutrition Intervention Macronutrient Analysis						
					Kcal	CHO (g)	CHO (%)	Fat (g)	Fat (%)	Protein (g)	Protein (%)
Lindseth et al., 2013 [39]	A	B	NA	HCI	NR	NR	56.0	NR	22.0	NR	22.0
					NR	NR	50.0	NR	35.0	NR	15.0
					NR	NR	22.0	NR	56.0	NR	22.0
Jalilolghadr et al., 2011 [50]	B	A	A	HGI	238.4	45.1	75.6	0.6	2.3	13.2	22.1
					277	25.9	37.3	13.6	44.2	12.8	18.5
Lindseth and Murray, 2016 [470]	B	B	NA	HCI	NR	NR	80.0	NR	10.0	NR	10.0
					NR	NR	50.0	NR	35.0	NR	15.0
					NR	NR	25.0	NR	65.0	NR	10.0
St-Onge et al., 2016 [472]	A	B	NA	HCI	NR	NR	53.5	NR	31.0	NR	17.0
					NR	NR	54.6	NR	32.7	NR	14.0
Vlahoyiannis et al., 2018 [8]	B	A	B	HGI	801.2	178.0	88.9	2.4	2.7	16.9	8.4
					801.2	178.0	88.9	2.4	2.7	16.9	8.4
Daniel et al., 2019 [471]	B	A	A	HGI	1058	169.5	64.1	27.9	10.5	29.9	25.4
					1083	160.3	59.2	33.1	12.2	34.4	28.6

CHO=Carbohydrates; HCI= High Carbohydrate Intake; LCI= Low Carbohydrate Intake; ZCI= Zero Carbohydrate Intake; HGI= High Glycemic Index; LGI= Low Glycemic Index;.



**Table 5.3.2.3.** Sets of sleep-related derived variables.

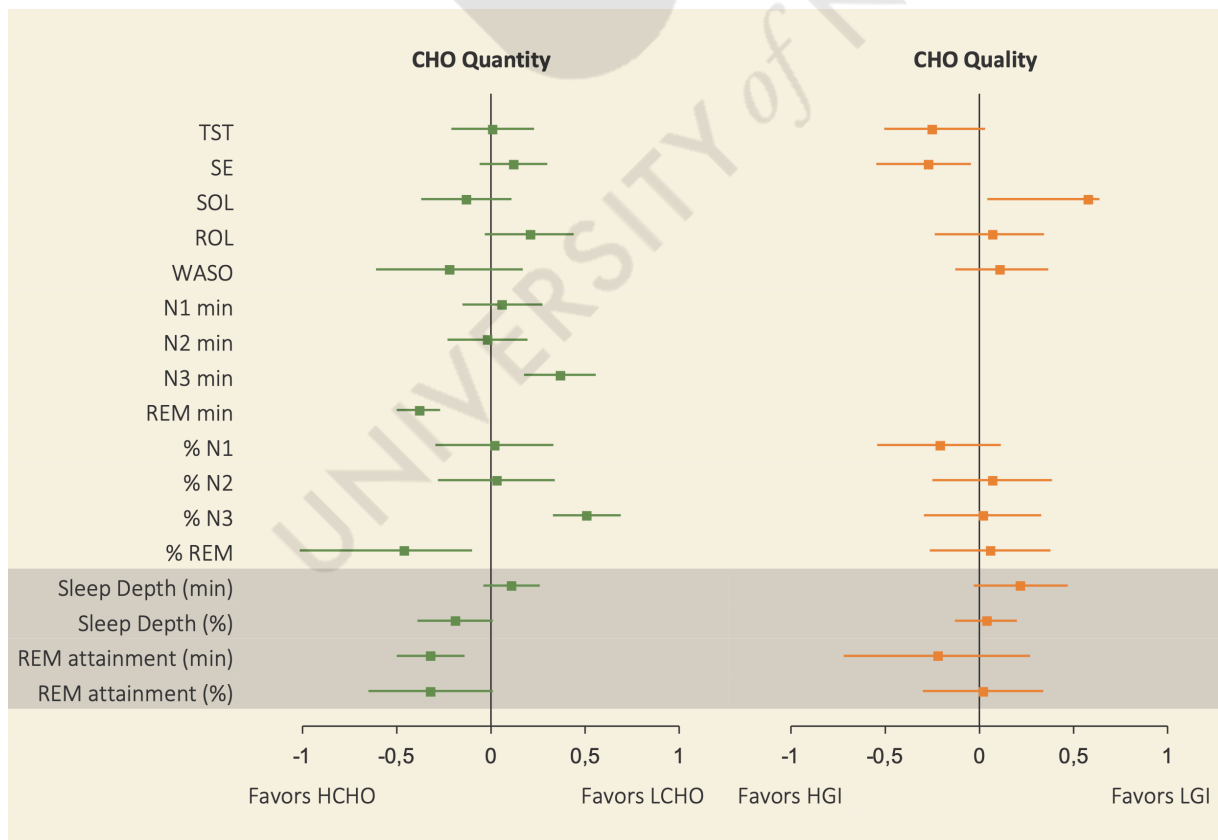
Author, year, reference	Monitoring Method	Familia- rization	Nights Recorded	TST (min)	SE (%)	SOL (min)	WASO (min)	ROL (min)	N1 (min)	N1 (%)	N2 (min)	N2 (%)	N3 (min)	N3 (%)	REM (min)	REM (%)
Phillips et al., 1975 [463]	EEG	Y	2	X		X		X	X	X	X	X	X	X	X	X
Porter and Horne, 1981 [47]	PSG	Y	3			X		X	X		X		X		X	
Kwan et al., 1986 [52]	EEG	Y	2	X		X		X	X	X	X	X	X	X	X	X
Afaghi et al., 2007 [49]	PSG	Y	1	X	X	X	X	X		X		X		X		X
Afaghi et al., 2008 [53]	PSG	Y	1	X	X	X	X	X	X	X	X	X	X	X	X	X
Jalilolghadr et al., 2011 [50]	PSG	Y	1	X	X	X	X	X	X		X		X		X	
Lindseth et al., 2013 [39]	PSG	Y	1	X		X			X		X		X		X	
Lindseth and Murray, 2016 [470]	Actigraphy	N	4		X	X										
St-Onge et al., 2016 [472]	Actigraphy	Y	4	X	X	X	X									
Vlahoyiannis et al., 2018 [8]	PSG	Y	1	X	X	X	X	X	X	X	X	X	X	X	X	X
Daniel et al., 2019 [471]	Actigraphy	N	1	X	X	X	X									

PSG: Polysomnography; Y: Yes; N: No; TST: Total Sleep Time; SE: Sleep Efficiency; SOL: Sleep Onset Latency; WASO: Wake After Sleep Onset; ROL: REM Onset Latency; REM: Rapid Eye Movement.

### 5.3.3 Effect of Carbohydrate quantity on Sleep

Meta-analytic calculations for all sleep variables are presented in Table 5.3.3.1 and Figure 5.3.3.1.. Total Sleep Time, SE, SOL, ROL, N1 (min and %) and N2 (min and %) and Sleep Depth (min) did not differentiated significantly between HCI or LCI. Compared with HCI, LCI intake moderately increased N3 stage both in duration (minutes) and proportion (ES=0.37; 95% CI= 0.18,0.56; I<sup>2</sup>=0%; p<0.001 and ES=0.51; 95% CI= 0.33,0.69; I<sup>2</sup>=0%; p<0.001, respectively). HCI intake prolonged moderately REM stage duration (ES=-0.38; 95% CI=0.05,-8.05; I<sup>2</sup>=0%; p<0.001) and its proportion (ES=-0.46; 95% CI=-0.83,-0.01; I<sup>2</sup>=0%; p<0.001), compared to LCI intake. REM attainment (both min and %) increased in HCI compared to LCI trial (ES=-0.31; 95% CI= -0.47,-0.15; I<sup>2</sup>=0%; p<0.001 and ES=-0.32; 95% CI= -0.57,-0.08; I<sup>2</sup>=21,5%; p=0.003, respectively), as well as Sleep Depth % (ES=-0.19; 95% CI= -0.57,-0.08; I<sup>2</sup>=0%; p<0.001), with a small effect size.

Large heterogeneity was observed for SOL. Funnel plot suggested that may the effects of CHO quantity on SOL may be affected by potential publication bias. Sensitivity analysis was performed excluding one study [472] that had the largest effect size and could be potentially an outlier. By excluding this data set, heterogeneity was reduced to 0%, without altering the results between the two conditions.



**Figure 5.3.3.1.** Summary representation of the combined effect sizes with 95% CI for all sleep domains.

**Table 5.3.3.1.** Metanalytic calculations for sleep-related variables according to CHO quantity and quality.

	CHO Quantity						CHO Quality					
	Hedges' g	SE	z-value	p	I <sup>2</sup> index	Results <sup>a</sup>	Hedges' g	SE	z-value	p	I <sup>2</sup> index	Results <sup>a</sup>
<b>TST</b>	0.01	0.09	0.08	0.936	12.33	NS	-0.25	0.16	-1.57	0.059	25.19	NS
<b>SE</b>	0.12	0.07	1.69	0.092	0	NS	-0.27	0.21	-1.27	0.203	63.09	NS
<b>SOL</b>	-0.13	0.11	-1.24	0.213	58.38	NS	0.58	0.47	1.24	0.213	83.54	NS
<b>ROL</b>	0.21	0.14	1.55	0.121	20.29	NS	0.07	0.20	0.34	0.731	46.08	NS
<b>WASO</b>	-0.22	0.12	-1.78	0.075	31.25	NS	0.11	0.20	0.57	0.569	58.09	NS
<b>N1 min</b>	0.06	0.1	0.60	0.551	0	NS						
<b>N2 min</b>	-0.02	0.09	-0.19	0.849	0	NS						
<b>N3 min</b>	0.37	0.07	5.13	<0.001	0	+						
<b>REM min</b>	-0.38	0.05	-8.05	<0.001	0	-						
<b>% N1</b>	0.02	0.12	0.16	0.872	0	NS	-0.21	0.16	-1.37	0.171	0.00	NS
<b>% N2</b>	0.03	0.08	0.39	0.698	0	NS	0.07	0.11	0.63	0.532	0.00	NS
<b>% N3</b>	0.51	0.06	8.90	<0.001	0	+	0.02	0.06	0.29	0.772	0.00	NS

<sup>a</sup>plus sign indicates that the specific sleep measure was significantly more in the LCHO/LGI trial than HCHO/HGI at  $p < 0.05$ ; N.S indicates no significant group differences. CHO: Carbohydrates; GI: Glycemic Index; TST: Total Sleep Time; SE: Sleep Efficiency; SOL: Sleep Onset Latency; WASO: Wake After Sleep Onset; ROL: REM Onset Latency; REM: Rapid Eye Movement; a: minus sign indicates that the specific sleep measure was significantly more in the HCHO/HGI trial than LCHO/LGI at  $p < 0.05$ ;

### 5.3.4 Effect of Carbohydrate quality on Sleep

Carbohydrate quality did not affect significantly TST, SE, SOL, ROL, WASO, N1 (%), N2(%) REM (%), or Sleep Depth (%) and REM attainment (both min and %). Data were not adequate to perform metanalytic calculations for all sleep stages that were reported in minutes. LGI pre-bed meals increased Sleep Depth (min) with a small effect size (ES=0.22; 95% CI= -0.03,0.47; I<sup>2</sup>=0%;  $p=0.022$  and ES=0.22; 95% CI= -0.03,0.47; I<sup>2</sup>=0%;  $p=0.022$ , respectively).

Because of the large heterogeneity in SE, SOL, and WASO, funnel plots were inspected graphically to evaluate potential publication bias. It was shown that except for SOL, there was no evidence of publication bias. Sensitivity analysis with exclusion of the data set with the larger effect size for SE, SOL, WASO, as a potential outlier, showed that results and I<sup>2</sup> did not substantially change

### 5.3.5 Carbohydrate Timing and Sleep

According to carbohydrate timing, no meta-analytical calculations could be performed due to the inadequate data sets. Specifically, from the seven data sets of comparisons of CHO quantity, 2 sets of one study [47] were classified as an acute nutrition intervention and seven as short term [39,52,53,463,470,472] with a maximum of 4 days of the adoption of a nutrition protocol [39,470,473]. From the 5 data sets of comparisons between CHO quality, all data studied acute nutrition interventions on sleep [8,49,50,471]. Thus, the only comparison that could be applied is between the short-term study of Porter and colleagues, and the rest trials that studied CHO quantity in sleep. All the included studies show that both a pre-bed meal LCI and a LCI diet over a period of maximum 4 days may decrease REM and increase N3 sleep stages, compared to a HCI meal or diet respectively. According to the acute effect of the pre-bed meal, Afaghi and colleagues showed that 4h pre-bed HGI meal could decrease SOL more than a similar meal consumed 1h before bedtime [49].

### 5.3.6 Meta-Regression Analyses

The large heterogeneity observed for SOL in the studies that investigated alterations in carbohydrate quantity consumption was further explored with meta-regression analysis. It was found that % energy alterations in CHO intake explained a part of the variance in the effectiveness of CHO quantity in SOL (coefficient=0.007, SE=0.003, 95% CI= 0.0003,0.014,  $z=2.365$ ,  $p=0.018$ , Adj.  $R^2=25.87$ ). Not any other variance, with regards to sleep continuity or architecture, was significantly explained by the alteration of the % energy from CHO intake between trials.

In an effort to understand the increased heterogeneity for SE, SOL and WASO in the studies that investigated the effects of carbohydrate quality in sleep, meta-regressions were performed according to the alterations of the glycemic load between trials. These analyses explained significantly part of the variance of the effectiveness of CHO quality in SE (coefficient=-0.018, SE=0.003, 95% CI= -0.028,0.009,  $z=-5.490$ ,  $p<0.001$ , Adj.  $R^2=89.2$ ), SOL (coefficient=0.028, SE=0.014, 95% CI= -0.011,0.067,  $z=1.971$ ,  $p=0.048$ , Adj.  $R^2=50.8$ ) and WASO (coefficient=0.014, SE=0.006, 95% CI= -0.002,0.030,  $z=2.35$ ,  $p=0.018$ , Adj.  $R^2=64.9$ ). No any other variance with regards to sleep continuity of architecture was significantly explained by the alteration of the glycemic load between trials.

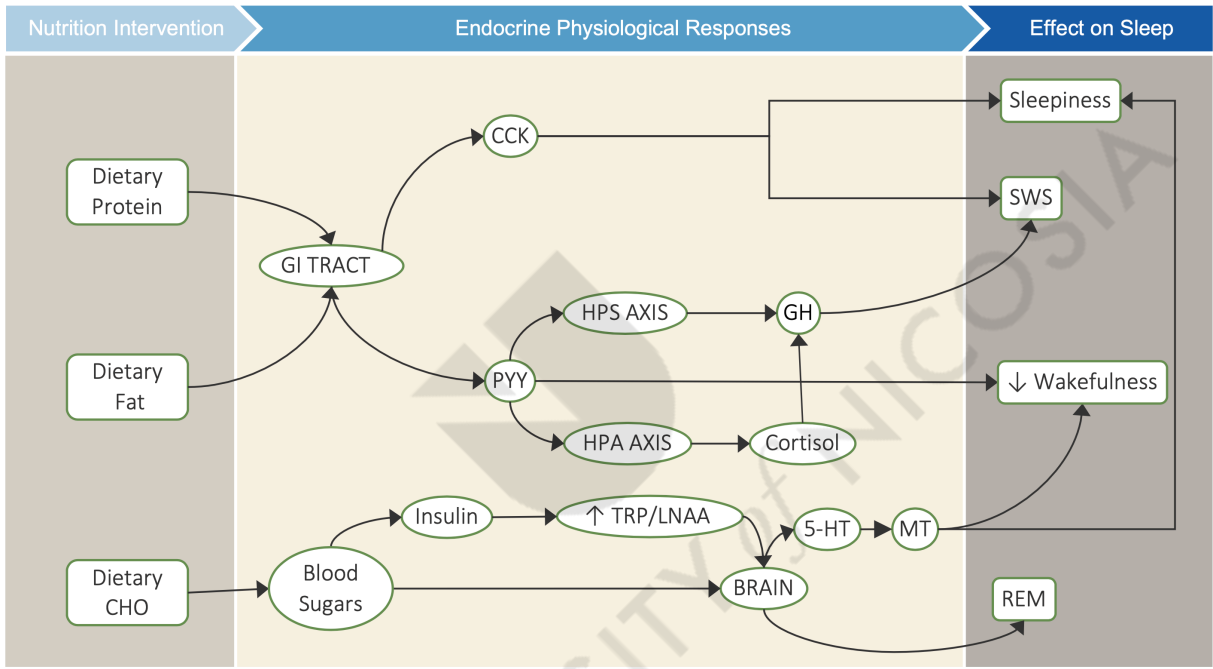
A similar meta-regression analysis was not possible to be performed on the remaining set of sleep-related data and carbohydrate periodization due to the lack of available data.

## 5.4 Discussion

Over the last decades, there has been a great interest in the relationship of diet on sleep. The current meta-analysis of clinical trials showed that CHO intake could significantly affect both sleep architecture, sleep initiation and continuity. In particular, a lower quantity of CHO intake significantly lengthens N3 stage sleep proportion and duration, compared to higher CHO consumption. Increased dietary CHO intake significantly prolonged REM stage sleep compared to lower CHO intake. Small effects were also observed for increased CHO quantity and REM attainment and sleep depth compared to lower CHO intake. The quality of CHO intake did not show any significant effect on sleep stages. Sleep onset latency showed to be affected by both carbohydrate quantity and quality. Alterations in the quality of carbohydrate intake showed a significant effect on measures of sleep continuity.

Diet-induced alternations in CHO quality and quantity have shown impressive results with regards to sleep-related parameters. Replacing a typical diet, that is mainly composed of CHO as main source of energy intake, with a LCI diet showed increases in both duration and proportion of deep sleep. From a molecular basis, sleep duration seems to be associated with rs2031573 and rs1037079 alleles [474]; however, it is not established yet whether diet modifications could affect the epigenetics of those genes and consequently the quality of sleep. The effect of CHO intake in deep sleep could be attributable to various biological mechanisms related to diet-dependent hormonal regulation (Figure 5.4.1). It is proposed that dietary fat and protein (or their digestion products) stimulate cholecystokinin (CCK) release in a greater extent than CHO [54,55]. CCK is produced in a number of tissues in humans, including enteroendocrine cells of duodenum, and is distributed in the brain as well [475]. In healthy male and female volunteers, a LCHO meal led to higher postprandial CCK concentrations and increased subjective feelings of sleepiness [55]. Nonetheless, the relationship between CCK and sleep was first demonstrated in animal experiments [56,57]. In both rats and rabbits, intraperitoneal injection of cholecystokinin showed a dose-response relationship between circulating CCK levels and N3 sleep stage (referred as SWS or “deep sleep”). Following the same line, both dietary fat and CCK may result in higher levels of Peptide-tyrosine-tyrosine (PYY) [54]. PYY is a peptide released in the gastrointestinal tract and it is usually studied for its role in appetite regulation and especially, its anorexigenic effect. In animal models, nocturnal intraperitoneal administration of PYY decreased wakefulness and enhanced NREM sleep [457]. In contrast to PYY, ghrelin is an appetite-stimulating hormone that is linked to sleep-wake behaviour [476]. The magnitude of the effect of ghrelin on sleep is differentiated according to sex, being greater in males than females [477]. It is proposed that ghrelin stimulates the activity of the hypothalamic-pituitary-adrenal and hypothalamic-somatotrophic axes, altering growth hormone (GH) and cortisol levels [476], both hormones that are involved in sleep regulation. N3

sleep stage coincides with approximately 70% of GH pulses and the amount of N3 sleep stage is positively related to the amount of GH secretion during these pulses [478]. Moreover, cortisol administration in both young [479] and elder adults [480] is positively linked with endogenous GH secretion and N3 sleep stage. In this meta-analysis, all individual studies that modified CHO quantity acutely or in short-term showed increases in SWS [47,52,53,463]. The pooled results suggest that a LCHO pre-bed meal ranging between 247g of CHO or a LCHO diet ranging between 2-100g of CHO increased N3 sleep stage by 8.5 minutes or by 3.2%. As N3 occupies approximately 20% of TST [14], an increase of 3,2% is translated to 16% of the time that is spent in this specific sleep stage.



**Figure 5.4.1.** Graphical illustration of potential biological pathways behind macronutrients-sleep interactions.

On the other hand, this meta-analysis revealed that higher CHO intake increased REM sleep as compared to lower CHO consumption. Frequently, the proposed model behind this effect is attributed to the insulin effect on tryptophan regulation. It is known that both the quantity [61] and the quality [60] of dietary CHO affect tryptophan (TRP) availability and the ratio of TRP to large neutral amino acids (LNAA) in plasma. Postprandial insulin secretion triggers peripheral uptake of LNAA and inhibits the release of peripheral amino acids. However, TRP is largely bounded to albumin and as a consequence, the concentration of the other competing LNAA's is reduced and TRP level in plasma increases [61,481]. As TRP/LNAA ratio and TRP availability increase, the traverse of TRP through the blood-brain barrier is favored. In the brain, increased TRT levels induce synthesis of serotonin, a precursor of melatonin. Free plasma-tryptophan

correlates positively with the amount of REM sleep among healthy individuals [482]. Parker and Rossman in 1971 showed that glucose infusion during the first 3h of sleep increased REM and decreased light sleep [483]. It could be hypothesized that in a specific time frame, increases in REM sleep result in a reciprocal reduction of NREM sleep. Furthermore, a possible explanation could be that given that the metabolic and glucose demands are higher during REM sleep compared to SWS [62,484], REM sleep is reduced when dietary CHO is restricted and vice versa. This positive relationship of CHO amount and REM sleep stage was consistent for the included studies' suggesting that increased CHO intake either in pre-bed meal (ranging from 130g to 196g) or in the daily dietary plan (ranging from 240g to -600g) prolonged REM sleep by 8,9 minutes or 2,6%. Taking into account the norm of 25% of TST spent in REM stage, the increase of 5,6% reflects a proportion of 22.4% of total REM sleep.

According to CHO quality, it has often been suggested that a HGI may be beneficial for improving sleep-related parameters. The included studies showed ambiguous results according to CHO quality and sleep. All experiments manipulated the GI of the last or two meals before bedtime. While some of the studies found significant improvements of HGI in SOL, SE or WASO [8,49], others found weak or no effect [50,471]. The studies that found that sleep-related parameters were optimized after a HGI pre-bed meal attributed these effects on potentially increased melatonin synthesis. In agreement, a meta-analysis showed that exogenous melatonin administration induced similar sleep-related alterations to a pre-bed HGI meal, such as a reduction in SOL, increases TST and SE [460]. A recent review suggested that food sources of melatonin such as milk and tart cherries could act as a safeguard for sleep-related issues, by improving sleep quality [462]. Nevertheless, none of the included studies in the current meta-analysis found any significant effect on sleep architecture. Pooled effect sizes showed a small effect on sleep depth in favour of LGI diets/meals that may be attributed to small decreases of the proportion of N1 and N2 sleep rather than increases in the N3 sleep stage. Because of the limited information, we were unable to conduct meta-analysis for sleep stages or domains as sleep depth or REM pressure, as only two studies reported the related data [8,50].

The apparent lack of causality for CHO quantity and quality with other sleep variables as sleep onset latency, sleep duration or continuity led to the hypothesis that the aforementioned studies included good sleepers and thus, the potential for sleep improvement was limited. Nevertheless, meta-regression analyses showed that the degree of differentiation of carbohydrate quantity showed a linear association with sleep onset latency and that alteration in GL explained a part of the variance of several sleep continuity parameters. Towards this direction, a previous investigation of the effect of CHO quality as a post-exercise meal in good-sleepers, showed several improvements in both sleep quality and quantity [8]. This optimization of sleep efficiency and continuity after a HGI meal intervention as a post-workout meal could be

attributed to the increased post-exercise insulin sensitivity [485], which may promote greater effects on plasma TRP availability. Thus, potential covariates are of further interest and should be treated with caution when investigating the effect of nutrition interventions in sleep.

Results of the present meta-analysis support the employ of CHO quantity as a reasonable non-pharmacological tool for modification of sleep architecture, if needed. As appropriate, high CHO quantity could be used to potentially increase REM, while lower CHO could be useful to increase N3 sleep stage. Since 1980, SWS is suggested to be associated with widespread bodily restorative functions, while REM sleep may be more associated with synthetic processes of brain reorganization and repair [486]. SWS is known to be a marker of sleep homeostasis [487] and its nocturnal appearance, with the longest episode usually in the first sleep cycle, is tied to growth hormone release [488], implying its restorative properties. In healthy adult volunteers, selective SWS deprivation led to decreases in behavioral performance and a significant rebound of SWS on the recovery night [489]. Contrarily, REM stage sleep deprivation seems not to be detrimental to behavior and psychomotor function in healthy subjects or those who are schizophrenic or depressed [490]. However, disturbed melatonin metabolism in combination with selective lesions of acetylcholine neurons in the pedunculopontine tegmental nucleus that modulate both REM and arousal, may be involved in sleep disorders in individuals with Cockayne syndrome [491].

Moreover, the practical application of this intervention may extend towards to the optimization of sleep quality in poor sleepers via the modification of the prescribed CHO quality. Scientific evidence indicated that three main indicators of poor sleeping (SOL, SE and WASO) were significantly improved in response to CHO quality manipulation. Hence, it could be hypothesized that both sleep initiation and sleep continuity could be also optimized in poor sleepers via other means. Since the prevalence of poor sleeping traits increases dramatically [452], these results are of further importance, and relevant studies should be conducted.

Several potential limitations of this meta-analysis need to be considered. A common constraint in meta-analyses is heterogeneity of the included studies [436]. In the current meta-analysis, the estimates for clinical trials that monitored sleep after a CHO-related intervention were relatively homogenous and efforts have been made to compare clinical trials with similar design. Extracted results did not affect from potential outliers and any sensitivity analysis did not contribute to the formation of altered results. Furthermore, nutrition timing seems to affect sleep potentially, but up to date, only one eligible trial was identified that compared the timing of dietary CHO in sleep [49]. Thus, the lack of studies investigating CHO timing do not allow to draw safe conclusions from these results. Furthermore, the included studies did not report an adequate number of data sets differentiating according to age or sex. Thus, more studies are needed to identify the mediating role of age in the link between carbohydrates and sleep during

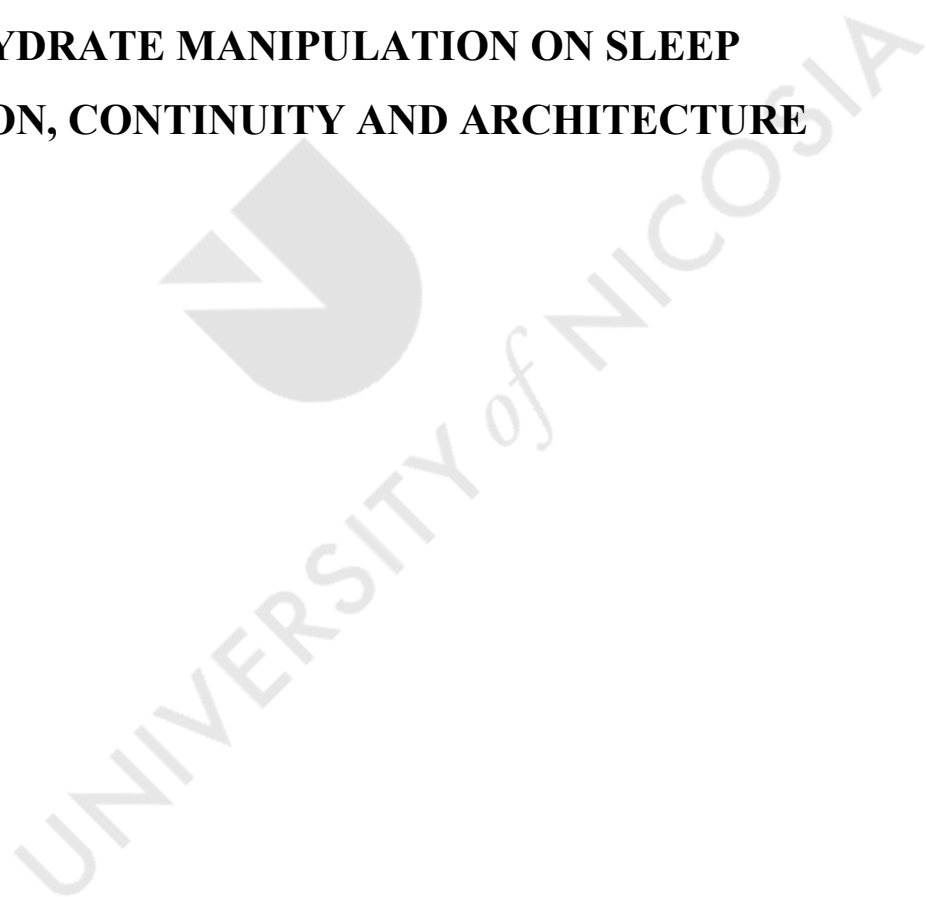


the lifespan. Additionally, more evidence from controlled clinical trials on the effects of long-term sleep optimizing interventions is desirable. In addition, with regard to CHO quality, only acute-effect interventions were studied and it was further observed that no long-term effects of CHO quantity and quality in sleep had been studied yet.

## **5.5 Conclusion**

The current results highlight the effect of nutrition, and especially carbohydrates on sleep. It was observed that a lower quantity of CHO intake significantly increases N3 stage sleep and higher dietary CHO intake significantly prolongs REM stage sleep. The quality of CHO intake did not show any significant effect on sleep stages. The effectiveness of carbohydrate quantity and quality in sleep onset latency was significantly explained by alterations of carbohydrate intake as a percentage of daily energy intake and alterations in the glycaemic load, respectively. Changes in glycaemic load partially explained the variance of the effectiveness of sleep quality in sleep efficiency and wake after sleep onset. Up to date, there is no clear interpretation of the relevant biological mechanisms. Results for CHO quantity and sleep stages are promising and need to be further addressed in future studies with long-term interventions in different age groups for both genders.

**CHAPTER 6: THE EFFECT OF PRE-SLEEP  
CARBOHYDRATE MANIPULATION ON SLEEP  
INITIATION, CONTINUITY AND ARCHITECTURE**



## 6.1 Introduction

Sleep research evolved dramatically over the last few decades, unveiling the innumerable vital physiological functions of sleep, including metabolic clearance in the central nervous system [492], cognition [135], immune function [431], overall tissue restoration [432] and glucose metabolism [45]. At the same time, the prevalence of inadequate sleep has been increased dramatically to more than the half of the general [452] and athletic populations [31]. On the one hand, inadequate sleep patterns are constantly associated with numerous diseases [453]. On the other hand, the prescription of common drugs for treating insomnia may result in adverse health side effects [493]. Hence, there is a fundamental need to explore alternative, non-pharmacological interventions to optimize sleep [494] and subsequently overall health [495].

Towards this direction, acute lifestyle interventions exploring novel nutrition or exercise interventions, in order to enhance natural sleep reveal promising results [494]. Sleep-wake cycle has been linked with a number of neurotransmitters and hormones, such as serotonin and melatonin, that can be actively modulated with lifestyle interventions and potentially improve sleep-related parameters [496]. These sleep-related hormones and peptides can optimize recovery by modulating the body's circadian rhythm and impacting sleep initiation and maintenance [38].

So far, only acute nutrition interventions have been investigated, but with promising results on sleep optimization either when combined with exercise [8] or not [47]. These sleep-promoting nutritional interventions include both dietary supplements, such as tryptophan administration [455] and whole foods consumption, such as kiwifruit [456] or tart cherry juice [74]. Moving from dietary supplements and specific foods to macronutrients, carbohydrates, as an integral part of human nutrition, are under extensive investigation for their effects on sleep, due to their relationship with brain function [44] and their consequential interrelationship between sleep and glucose homeostasis [45,46]. Acute studies on pre-bed carbohydrate quantity consumption have shown significant effects on sleep architecture [47], while recent data established the effect of both pre-bed carbohydrate quantity and quality on sleep-related parameters [496].

Sleep quality shares also a reciprocal relationship with exercise [80]. Acute interventions have constantly shown that exercise can improve sleep-related parameters [494] and reduce the gravity of symptoms in patients suffering from sleep disorders [81]. Towards this direction and given the high frequency of exercise training in athletes, one would expect that athletic populations exhibit exceptional sleep patterns. Paradoxically, there is growing evidence of inadequate sleep and sleep disorders in athletes [32,497], underlying the need to find novel and feasible approaches to optimize athletes' sleep duration and quality [59].

In this sense, it is very interesting that the relationship between exercise and sleep is potentially altered when exercise training is performed acutely or in the long term, as it happens with athletes. Thus, since there is a lack of long-term sleep-promoting nutrition interventions, two significant -and interrelated- questions are raised: i) Is the sleep-enhancing effect of an acute nutrition intervention altered when implemented in the long term? and ii) Could a long-term nutrition intervention optimize naturally sleep in a physically active population?

Based on previous promising effects of acute carbohydrate manipulations on sleep [496], the present study aims to investigate whether a long-term carbohydrate periodization protocol could optimize sleep in physically active populations. Specifically, the purpose of this study is to examine the effect of carbohydrate periodization of quantity and quality manipulation on sleep-related parameters including i) sleep initiation and duration, ii) sleep continuity, iii) sleep architecture and iv) self-perceived sleep-related measures, in trained individuals over a 4-week period.

## **6.2 Materials and Methods**

### **6.2.1 Participants**

Forty-two healthy, physically active male volunteers were recruited for this study. All volunteers were regularly participating in sports activities for at least two years and were accustomed to both resistance and high-intensity interval training (HIIT) (specific inclusion and exclusion criteria described in chapter 4). Participants had a mean ( $\pm$  SD) age of  $27 \pm 7$  years, height of  $177 \pm 6$  cm and body mass of  $80.3 \pm 11.1$  kg. All participants completed the intervention period with high compliance rate as described in the chapter 4.3. Volunteers had been informed about the purpose of the study and provided written consent. The study was approved by the Cyprus Bioethical Committee (EEBK/EII/2020/65) and with a clinical trial registration : NCT05464342. All procedures were conducted according to the manual of the Declaration of Helsinki in 1964 and its later amendments.

### **6.2.2 Study Design**

This is a randomized controlled trial with a parallel design. The study consisted of three nutrition intervention groups investigating sleep-related responses to pre-bed periodization of carbohydrate consumption quantity and quality. Specifically, the nutrition intervention groups were: i) Sleep Low- No Carbohydrates (SL-NCHO): participants consumed all their carbohydrate intake at regular intervals prior to the evening exercise training session, ii) Sleep High- Low Glycemic Index (SH-LGI) and iii) Sleep High- High Glycemic Index (SH-HGI): Carbohydrate intake was spread evenly throughout the day both prior (60% of total CHO intake)

and after (40% of total CHO intake). The SH-LGI and SH-HGI groups differentiated in the evening carbohydrate quality, consuming either LGI or HGI foods, respectively. Each subject was assigned to one of the arms for a 4-week period, by a computer-generated sequence of random numbers. Alongside, participants performed a supervised standardized exercise program combining resistance exercise and HIIT sessions. All nutrition and exercise intervention procedures are described in detail in chapters 4.2 and 4.3.

At the initial phase of the study, participants underwent preliminary testing in order to evaluate their nutritional status, their basic somatometric and anthropometric data, as well as their fitness level in order to design personalized exercise and nutrition interventions for each participant. Both at baseline and at the end of the intervention, a sleep study was conducted, with the gold-standard method of polysomnography. In addition, participants were asked to collect actigraphic data and sleep diary data for one week prior to the beginning of the intervention and at the end of the intervention. At these specific time points, participants were also interviewed for their sleep quality, daytime sleepiness and fatigue. The main phase of the study took place in the Human Performance Lab of the University of Nicosia, the fitness premises (UFIT) of the University of Nicosia, and in the participants' home environment.

### **6.2.3 Assessments**

#### ***Anthropometrics***

Anthropometrics were collected at the baseline of the intervention, as described in chapter 4.4. Weight and standing height were measured to the nearest 0.1kg and 0.5cm respectively, with Tanita WB-3000 digital beam scale (Tanita Corp, Tokyo, Japan). Individuals were barefooted and lightly dressed.

#### ***Sleep-related Data***

In-detail sleep evaluation was performed at both baseline and at the end of the intervention. Sleep assessments included polysomnography, actigraphy, sleep diary, PSQI, ESS and FSS, as described in detail in chapter 4.4.

Specifically, sleep architecture was assessed with Polysomnography using the guidelines of the American Academy of Sleep Medicine. The examination included the application of the following: electroencephalogram (F3, F4, A1, A2, O1, O2, ground [at AFz] and a reference electrode at position FCz) right and left electrooculogram; submental and tibial electromyogram; electrocardiogram; heart rate; oxygen saturation; thoracic and abdominal efforts (piezoelectric transducers); nasal flow; chest and diaphragm breathing; snoring and body

position. The PSG examination performed at the participant's home in order to maintain as possible their sleep routine and environment.

Sleep initiation and maintenance, as well as intra-individual standard deviations for each sleep measure, were assessed with actigraphy. Each subject was asked to wear the actigraph on their non-dominant wrist, for seven days both at baseline and at the end of the intervention. Full actigraphic records were available for 484 nights, with an average of 11 nights per participant (Pre-Intervention: 5.6 nights/participant Post-Intervention: 5.3 nights/participant).

In addition to actigraphy devices, participants recorded their sleep habits using sleep diaries. Diary data were used to define the scoring interval for actigraphic sleep, according to the procedure outlined by Acebo and colleagues [27].

Moreover, at the same time points, participants were asked to complete several self-perceived sleep-wake scales including, the Pittsburgh Sleep Quality Index (PSQI), Epworth Sleepiness Scale (ESS), and Fatigue Severity Scale (FSS) respectively.

#### **6.2.4 Statistical Analysis**

Data are presented as mean  $\pm$  standard deviation. Intra-individual Standard deviations (iSDs) or intra-individual variability (IIV) were calculated for each of the sleep variable that was measured with actigraphy [444]. Inferential statistical analyses were conducted using IBM®SPSS® statistics for Windows, version 25.0 (IBM Corp, Armonk, NY). Variables' distribution was identified using the Shapiro-Wilks test. Two-factor repeated measures ANOVA was used to evaluate differences between trials. Significant main effects and time\*trial interactions were further explored with post-hoc pairwise comparisons, using the Bonferroni correction. Effect sizes were calculated using partial eta squared ( $\eta^2_r$ ). Alpha level for all statistical analyses was set at  $p < 0.05$ , two-tailed tests.

### **6.3 Results**

#### **6.3.1 Sleep Initiation and Duration**

There was a significant main effect of time for both sleep initiation and duration measures (Table 6.3.1.1 & Figure 6.3.1.1.). Sleep onset was accelerated in all nutrition interventions by an average of 45.6% ( $F(1, 39) = 23.01, p < 0.001, \eta^2_r = 0.37$ ). In the same line, there was a significant main effect of time on SOL IIV ( $F(1, 39) = 13.10, p = 0.001, \eta^2_r = 0.25$ ). No statistically significant findings in TIB were observed. Nevertheless, TIB IIV showed both a main time effect ( $F(1, 39) = 8.85, p = 0.005, \eta^2_r = 0.16$ ) and a significant time x trial interaction ( $F(2, 39) = 3.51, p = 0.039, \eta^2_r = 0.15$ ). In particular, TIB IIV decreased significantly by 30.5% ( $p = 0.008$ ) and 27.5 % ( $p = 0.008$ ) in the SL-NCHO and SL-LGI group, respectively, in contrast

to HGI that did not show significant differences ( $p = 0.657$ ). Regarding sleep duration, a main effect of time was found. Specifically, all interventions prolonged TST by 7% or 24.9min ( $F(1, 39) = 8.46, p = 0.006, \eta^2_r = 0.18$ ), without a significant time x group interaction effect. Nevertheless, statistically significant time x group interaction was observed in TST IIV ( $F(2, 39) = 3.61, p = 0.036, \eta^2_r = 0.16$ ). It was shown that in both the SL-NCHO and SH-LGI group, TST IIV decreased significantly by 26.4% ( $p = 0.034$ ) and 26.6% ( $p = 0.028$ ) respectively, in contrast to the SH-HGI group ( $p = 0.298$ ). No main effect of group was found for both variables.



**Table 6.3.1.1.** Intervention effect on Sleep Initiation and Duration.<sup>1</sup>

	Group <sup>2</sup>	Pre-Intervention <sup>3</sup>	Post-Intervention	Δ	Δ (%)	<i>P</i> (group)	<i>P</i> (time)	<i>P</i> (I)
<b>Total Sleep Time (min)</b>								
PSG	SL-NCHO	349.8 ± 74.5	435.4 ± 106.3	85.6	24.5	0.696	<b>0.019*</b>	0.663
	SH-LGI	387 ± 98	432.8 ± 109.5	45.9	11.9			
	SH-HGI	359.2 ± 63.6	400.2 ± 96	40.9	11.4			
Actigraphy	SL-NCHO	357.8 ± 44.2	381.3 ± 62.1	23.5	6.6	0.292	<b>0.006*</b>	0.525
	SH-LGI	382.3 ± 58.4	395.9 ± 60.8	13.6	3.6			
	SH-HGI	342.2 ± 37.8	379.7 ± 63.4	37.5	11			
IIV	SL-NCHO	73.0 ± 39.0	50.1 ± 22.4	-22.9	-31.4	0.145	0.055	<b>0.036*</b>
	SH-LGI	92.2 ± 56.4	68.5 ± 25.9	-23.7	-25.7			
	SH-HGI	56.4 ± 28.9	67.4 ± 19.4	11	19.5			
<b>Time in Bed (min)</b>								
PSG	SL-NCHO	363.8 ± 72.6	452.6 ± 103.9	88.9	24.4	0.745	<b>0.020*</b>	0.424
	SH-LGI	405.4 ± 93.2	456.4 ± 101	51.1	12.6			
	SH-HGI	397.5 ± 89.7	416.5 ± 102.9	19.0	4.8			
Actigraphy	SL-NCHO	473.4 ± 43.5	468.9 ± 57.3	-4.6	-1	0.226	0.163	0.355
	SH-LGI	480.3 ± 50.0	495.9 ± 45.1	15.6	3.2			
	SH-HGI	446.7 ± 41.3	469.9 ± 69.3	23.2	5.2			
IIV	SL-NCHO	86.5 ± 35.9	60.1 ± 23.9	-26.4	-30.5	0.145	<b>0.005*</b>	<b>0.039*</b>

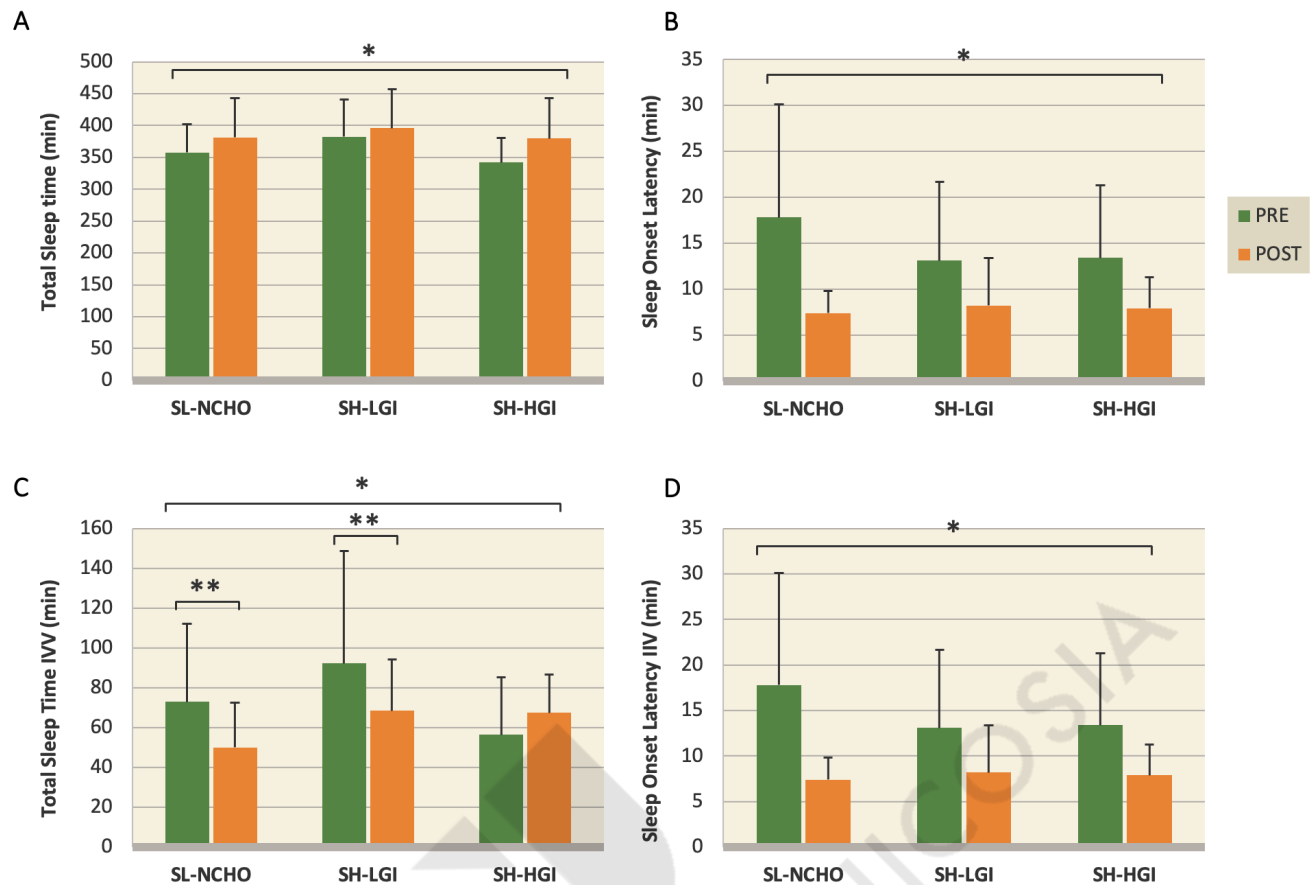
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Table 6.3.1.1. (Continued)

	Group <sup>2</sup>	Pre-Intervention <sup>3</sup>	Post-Intervention	Δ	Δ (%)	<i>P</i> (group)	<i>P</i> (time)	<i>P</i> (I)
	SH-LGI	96.8 ± 46.1	70.2 ± 33.0	-26.6	-27.5			
	SH-HGI	65.2 ± 35.5	69.5 ± 20.1	4.3	6.6			
<b>Sleep Onset Latency (min)</b>								
PSG	SL-NCHO	9.5 ± 11.8	5.6 ± 7.3	-3.9	-41.3	0.384	0.187	0.841
	SH-LGI	16.9 ± 18.5	11.2 ± 14.3	-5.6	-33.4			
	SH-HGI	10.3 ± 11.5	8.8 ± 9.3	-1.5	-14.7			
Actigraphy	SL-NCHO	17.8 ± 12.3	7.4 ± 2.4	-10.5	-58.7	0.597	<b>&lt;0.001*</b>	0.237
	SH-LGI	13.1 ± 8.6	8.2 ± 5.2	-4.9	-37.6			
	SH-HGI	13.4 ± 7.9	7.9 ± 3.4	-5.4	-40.7			
IIV	SL-NCHO	17.8 ± 18.6	5.4 ± 3.8	-12.4	-69.7	0.700	<b>0.001*</b>	0.137
	SH-LGI	12.8 ± 9.6	6.8 ± 6.2	-6	-46.9			
	SH-HGI	10.7 ± 7.5	7.9 ± 6.4	-2.8	-26.2			

SL-NCHO, Sleep Low- No Carbohydrates; SH-LGI, Sleep High- Low Glycemic Index; SH-HGI, Sleep High- High Glycemic Index; PSG, Polysomnography; IIV, Intra-Individual Variability; <sup>1</sup>Data are presented as mean ± SD. <sup>2</sup>For sleep diary and actigraphic measures n = 42 (SL-NCHO: n = 14; SH-LGI: n = 14; SH-hGI: n = 14); for polysomnographic measures n=28 ( SL-NCHO: n = 11; SH-LGI: n = 10; SH-HGI: n = 7). <sup>3</sup>No preintervention differences between groups were present. \* Denotes statistically significant differences at the 0.05 level (2-tailed).



### 6.3.2 Sleep Continuity

There was a significant main effect of time for sleep continuity measures (Table 6.3.1.1 & Figure 6.3.1.1.) Specifically, there was a significant main effect of time for SE, WASO, average awakening length and SFI (all  $p < 0.05$ ). SE was increased by 5.9% overtime ( $F(1, 39) = 23.64$ ,  $p < 0.001$ ,  $\eta^2_r = 0.38$ ), while WASO, average awakening length and SFI were decreased by 13.4% ( $F(1, 39) = 4.75$ ,  $p = 0.035$ ,  $\eta^2_r = 0.11$ ), 14.2% ( $F(1, 39) = 12.03$ ,  $p = 0.001$ ,  $\eta^2_r = 0.24$ ) and 10.8% ( $F(1, 39) = 6.81$ ,  $p = 0.013$ ,  $\eta^2_r = 0.15$ ), respectively. No main effect of group or time x group interaction was found for these variables. With regard to the IIV of SE and WASO, a significant group effect was found ( $p < 0.05$ ), without a main effect of time or time x group interaction.

**Table 6.3.2.1.** Intervention effect on Sleep Continuity.<sup>1</sup>

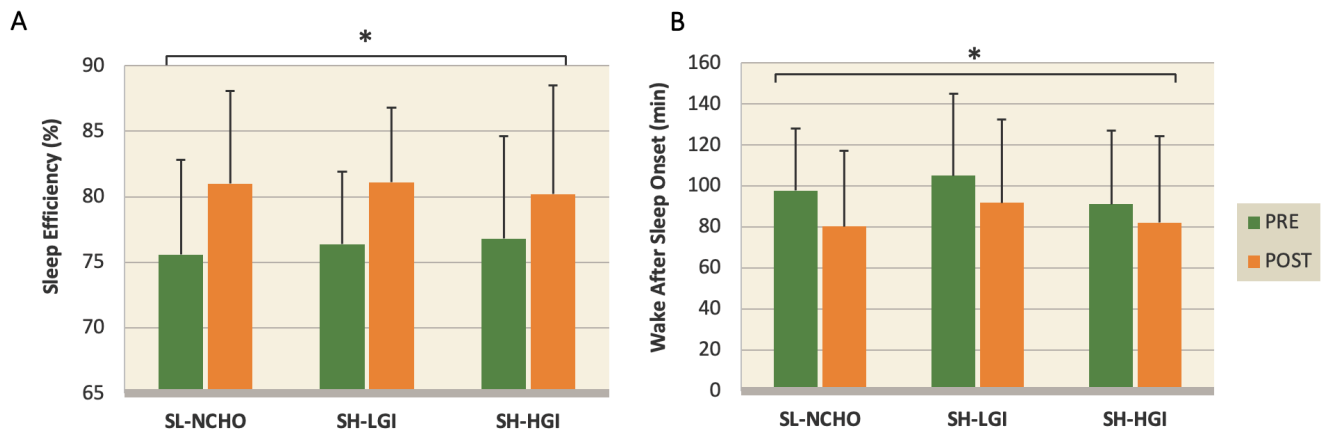
	Group <sup>2</sup>	Pre-Intervention <sup>3</sup>	Post-Intervention	Δ	Δ (%)	P (group)	P (time)	P (I)
<b>Sleep Efficiency (%)</b>								
PSG	SL-NCHO	93.3 ± 3.4	94.6 ± 3.2	1.2	1.3	0.327	<b>0.001*</b>	0.224
	SH-LGI	90.8 ± 5	94.4 ± 3.5	3.6	3.9			
	SH-HGI	88.9 ± 7	93.9 ± 2.9	5.1	5.7			
Actigraphy	SL-NCHO	75.6 ± 7.2	81 ± 7.1	5.4	7.2	0.980	<b>&lt;0.001*</b>	0.667
	SH-LGI	76.4 ± 5.5	81.1 ± 5.7	4.7	6.1			
	SH-HGI	76.8 ± 7.8	80.2 ± 8.3	3.4	4.4			
IIV	SL-NCHO	7.8 ± 4.2	6.8 ± 3.3	-1.0	-12.8	<b>0.003*</b>	0.475	0.373
	SH-LGI	8.2 ± 4.1	9.4 ± 4.8	1.2	14.6			
	SH-HGI	4.2 ± 2.3	5.7 ± 2.9	1.5	35.7			
<b>Wake After Sleep Onset (min)</b>								
PSG	SL-NCHO	24.2 ± 13.4	23.6 ± 11.1	-0.7	-2.7	0.274	<b>0.004*</b>	0.073
	SH-LGI	36 ± 17	24.2 ± 14.2	-11.8	-32.7			
	SH-HGI	48.9 ± 41.4	25.9 ± 14.5	-23.0	-47.0			
Actigraphy	SL-NCHO	97.8 ± 30.3	80.2 ± 36.9	-17.6	-17.9	0.603	<b>0.035*</b>	0.846
	SH-LGI	105 ± 40.2	91.8 ± 40.8	-13.2	-12.5			
	SH-HGI	91.2 ± 36	82.2 ± 42.2	-8.9	-9.8			
IIV	SL-NCHO	39.9 ± 19.0	30.3 ± 15.7	-9.6	-24.1	<b>0.005*</b>	0.942	0.206
	SH-LGI	40.4 ± 25.0	45.3 ± 21.9	4.9	12.1			
	SH-HGI	22.0 ± 9.0	27.5 ± 16.9	5.5	25.0			
<b>Awakenings</b>								
Number	SL-NCHO	24.8 ± 5.8	23.3 ± 8.1	-1.5	-6.1	0.879	0.205	0.968
	SH-LGI	26 ± 7.3	24.1 ± 7.9	-1.9	-7.3			

*(Continued on next page)*

Table 6.3.2.1. (Continued)

	Group <sup>2</sup>	Pre-Intervention <sup>3</sup>	Post-Intervention	Δ	Δ (%)	P (group)	P (time)	P (I)
Number IIV	SH-HGI	24.7 ± 6.3	23.5 ± 7.1	-1.1	-4.7	<b>0.032*</b>	0.768	0.957
	SL-NCHO	9.1 ± 5.2	8.7 ± 3.3	-0.4	-4.4			
	SH-LGI	9.3 ± 5.0	9.0 ± 5.5	-0.3	-3.2			
Length	SH-HGI	5.6 ± 1.8	5.7 ± 3.9	0.1	1.8	0.881	<b>0.001*</b>	0.459
	SL-NCHO	4 ± 1	3.3 ± 1.1	-0.7	-18.2			
	SH-LGI	4.1 ± 1.1	3.4 ± 1.1	-0.7	-17.1			
Length IIV	SH-HGI	3.7 ± 1	3.5 ± 1.4	-0.3	-7.5	0.739	0.791	0.084
	SL-NCHO	1.5 ± 0.9	1.3 ± 0.9	-0.2	-13.3			
	SH-LGI	1.6 ± 1.2	1.7 ± 1.8	0.1	6.2			
<b>Sleep Fragmentation Index</b>	SH-HGI	1.1 ± 0.6	0.9 ± 0.4	-0.2	-18.2	0.945	<b>0.013*</b>	0.988
	SL-NCHO	34.6 ± 8.4	30.8 ± 5.4	-3.8	-11.1			
	SH-LGI	33.9 ± 8.4	30 ± 9.7	-3.8	-11.3			
IIV	SH-HGI	33.6 ± 7.6	30.3 ± 8.1	-3.4	-10	0.561	0.950	0.104
	SL-NCHO	11.3 ± 5.7	12.2 ± 7.3	0.9	8.0			
	SH-LGI	12.1 ± 5.9	13.0 ± 7.6	0.9	7.4			
	SH-HGI	8.9 ± 4.1	9.0 ± 2.9	0.1	1.1			

SL-NCHO, Sleep Low- No Carbohydrates; SH-LGI, Sleep High- Low Glycemic Index; SH-HGI, Sleep High- High Glycemic Index; PSG, Polysomnography; IIV, Intra-Individual Variability; <sup>1</sup>Data are presented as mean ± SD. <sup>2</sup>For sleep diary and actigraphic measures n = 42 (SL-NCHO: n = 14; SH-LGI: n = 14; SH-hGI: n = 14); for polysomnographic measures n=28 ( SL-NCHO: n = 11; SH-LGI: n = 10; SH-HGI: n = 7). <sup>3</sup>No preintervention differences between groups were present. \* Denotes statistically significant differences at the 0.05 level (2-tailed).



**Figure 6.3.2.1.** Intervention effect on Sleep Continuity. \*Denotes statistically significant main effect of time at the 0.05 level (2-tailed). SL-NCHO, Sleep Low- No Carbohydrates; SH-LGI, Sleep High- Low Glycemic Index; SH-HGI, Sleep High- High Glycemic Index.

### 6.3.3 Sleep Architecture

The effects of nutrition intervention on sleep architecture are presented in Table 6.3.3.1 and Figure 6.3.1.1. No statistically significant differences were found for N1 sleep stage latency. On the other hand, there was a main effect of time on N1 sleep stage duration ( $F(1, 25) = 8.61, p = 0.007, \eta^2_r = 0.26$ ), but no for N1 % TST ( $p > 0.05$ ). No main effect of group or time x group interaction was found for N1 sleep stage variables.

No main effect of group, time, or group\*time interaction was found for N2 Sleep stage latency, duration or N2 % TST. In the same line, no main effect of group, time, or group\*time interaction was found for N3 Sleep stage latency, duration and N3 % TST.

**Table 6.3.3.1.** Intervention effect on Sleep Architecture.<sup>1</sup>

	Group <sup>2</sup>	Pre- Intervention <sup>3</sup>	Post-Intervention	Δ	Δ (%)	P (group)	P (time)	P (I)
<b>N1 Sleep Stage</b>								
Latency (min)	SL-NCHO	9.5 ± 11.8	5.6 ± 7.3	-3.9	-41.3	0.384	0.187	0.841
	SH-LGI	16.9 ± 18.5	11.2 ± 14.3	-5.6	-33.4			
	SH-HGI	10.3 ± 11.5	8.8 ± 9.3	-1.5	-14.7			
Duration (min)	SL-NCHO	41.2 ± 23.7	52 ± 27	10.7	26	0.325	<b>0.007*</b>	0.330
	SH-LGI	25.1 ± 11.9	46.2 ± 15.3	21.1	84			
	SH-HGI	36.5 ± 19.9	50.6 ± 31.7	14.1	38.6			
%TST	SL-NCHO	11.8 ± 6.3	12.2 ± 7.1	0.4	3.3	0.212	0.128	0.330
	SH-LGI	6.8 ± 3.4	10.9 ± 3.7	4.1	59.4			
	SH-HGI	12.4 ± 4.5	13.3 ± 8.3	0.9	7.3			
<b>N2 Sleep Stage</b>								
Latency (min)	SL-NCHO	19.9 ± 16	13.9 ± 12.1	-6.1	-30.5	0.571	0.755	0.161
	SH-LGI	26 ± 18.5	19.8 ± 17.0	-6.2	-23.8			
	SH-HGI	17.4 ± 13.0	26.4 ± 17.4	15.8	99.3			
Duration (min)	SL-NCHO	188.6 ± 70.1	222 ± 74.2	33.4	17.7	0.532	0.268	0.532
	SH-LGI	213.6 ± 77.8	208.9 ± 63.7	-4.6	-2.2			
	SH-HGI	180.9 ± 56.5	203.1 ± 50	22.2	12.3			
%TST	SL-NCHO	52.6 ± 11	50.2 ± 8.9	-2.3	-4.4	0.944	0.220	0.220

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Table 6.3.3.1. (Continued)

	Group <sup>2</sup>	Pre- Intervention <sup>3</sup>	Post-Intervention	Δ	Δ (%)	P (group)	P (time)	P (I)
	SH-LGI	55.6 ± 15.1	49.3 ± 13	-6.3	-11.4			
	SH-HGI	50.3 ± 13.2	51.1 ± 7.9	0.8	1.6			
<b>Light Sleep</b>								
Duration (min)	SL-NCHO	229.8 ± 80.3	273.9 ± 79.2	44.1	19.2	0.970	0.091	0.658
	SH-LGI	238.7 ± 79.5	255.1 ± 69.9	16.5	6.9			
	SH-HGI	236.4 ± 60	253.7 ± 52	17.3	7.3			
%TST	SL-NCHO	64.4 ± 12.1	62.5 ± 7.7	-1.9	-3.0	0.769	0.363	0.993
	SH-LGI	62.4 ± 15.4	60.2 ± 14.2	-2.2	-3.6			
	SH-HGI	66 ± 13.2	64.4 ± 10	-1.6	-2.5			
<b>N3 Sleep Stage</b>								
Latency (min)	SL-NCHO	49.6 ± 21.1	65.9 ± 42.7	16.4	33.0	0.847	0.316	0.718
	SH-LGI	56.3 ± 22.9	59.6.2 ± 25.5	3.5	6.2			
	SH-HGI	49.9 ± 42.6	53.9 ± 25.9	4.0	8.0			
Duration (min)	SL-NCHO	88.9 ± 31.9	100.8 ± 33.4	11.9	13.3	0.826	0.726	0.826
	SH-LGI	107.7 ± 73.5	105.6 ± 69.4	-2.1	-2			
	SH-HGI	87.9 ± 46.9	89.1 ± 23.4	1.2	1.4			
%TST	SL-NCHO	26.7 ± 11.6	23.9 ± 8.3	-2.8	-10.5	0.933	0.218	0.218
	SH-LGI	27.4 ± 14.6	23.1 ± 14.5	-4.3	-15.7			

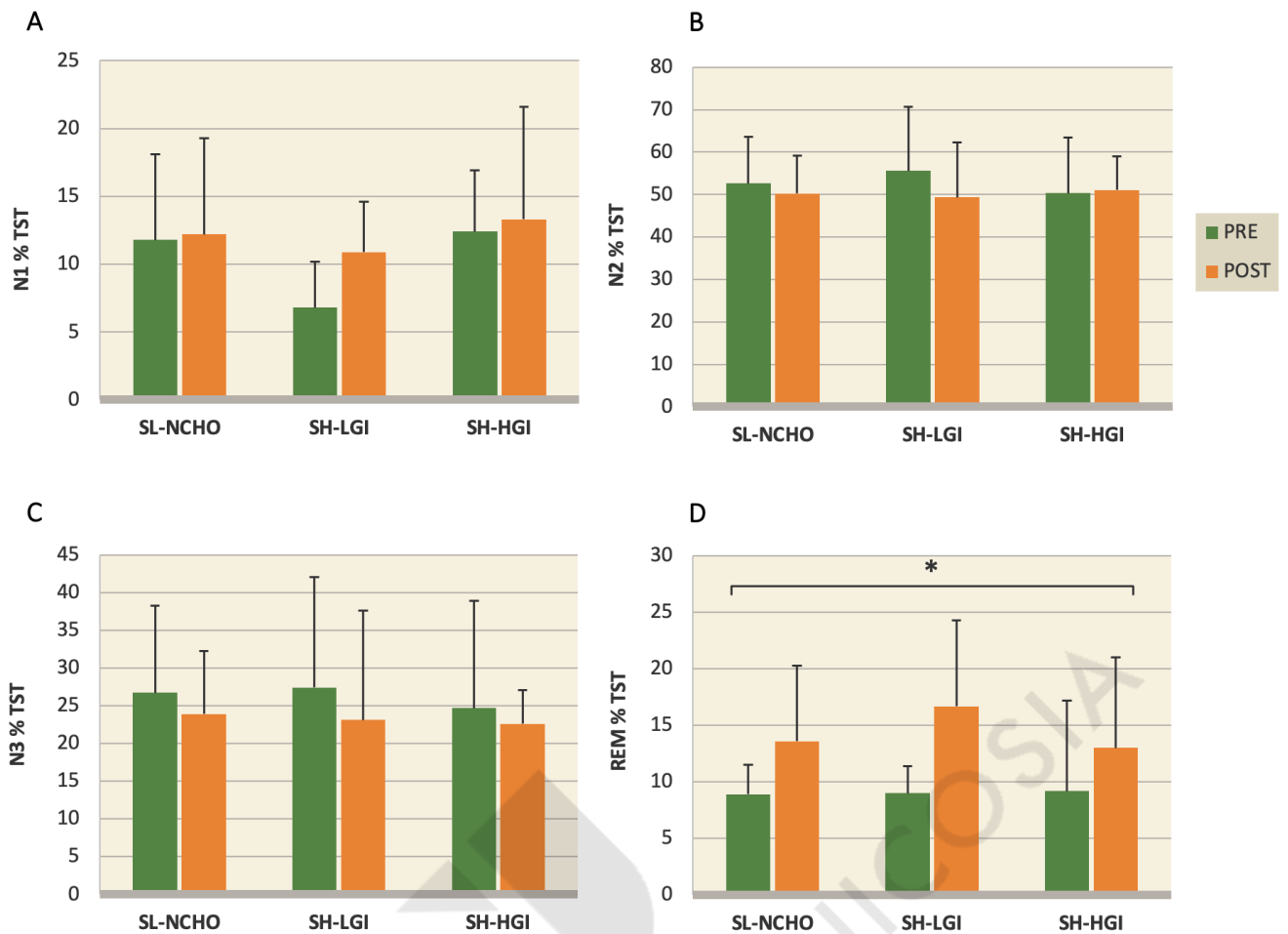
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Table 6.3.3.1. (Continued)

	Group <sup>2</sup>	Pre- Intervention <sup>3</sup>	Post-Intervention	Δ	Δ (%)	P (group)	P (time)	P (I)
	SH-HGI	24.7 ± 14.2	22.6 ± 4.5	-2.2	-8.7			
<b>REM Sleep Stage</b>								
Latency (min)	SL-NCHO	71.8 ± 73	58.5 ± 48.9	-13.2	-18.4	0.789	0.360	0.677
	SH-LGI	80.3 ± 64.7	83.3 ± 89.6	3.0	3.7			
	SH-HGI	90.5 ± 54	64.6 ± 48.7	-25.9	-28.7			
Duration (min)	SL-NCHO	31 ± 10.1	60.7 ± 39.7	29.6	95.5	0.805	<0.001*	0.665
	SH-LGI	33.3 ± 12.7	72.2 ± 38.8	38.9	116.8			
	SH-HGI	34.9 ± 32.8	57.4 ± 46	22.4	64.3			
%TST	SL-NCHO	8.9 ± 2.6	13.6 ± 6.7	4.7	52.6	0.698	0.001*	0.487
	SH-LGI	9.0 ± 2.4	16.7 ± 7.6	7.7	85.5			
	SH-HGI	9.2 ± 8	13 ± 8	3.8	40.8			

SL-NCHO, Sleep Low- No Carbohydrates; SH-LGI, Sleep High- Low Glycemic Index; SH-HGI, Sleep High- High Glycemic Index; IIV, Intra-Individual Variability; TST, Total Sleep. Time; REM, Rapid Eye Movement; <sup>1</sup>Data are presented as mean ± SD. <sup>2</sup>n = 42 (SL-NCHO: n = 11; SH-LGI: n = 10; SH-HGI: n = 7). <sup>3</sup>No pre-intervention differences between groups were present. \* Denotes statistically significant differences at the 0.05 level (2-tailed)





**Figure 6.3.3.1.** Intervention effect on Sleep Architecture. \*Denotes statistically significant main effect of time at the 0.05 level (2-tailed). SL-NCHO, Sleep Low- No Carbohydrates; SH-LGI, Sleep High- Low Glycemic Index; SH-HGI, Sleep High- High Glycemic Index; TST: Total Sleep Time.

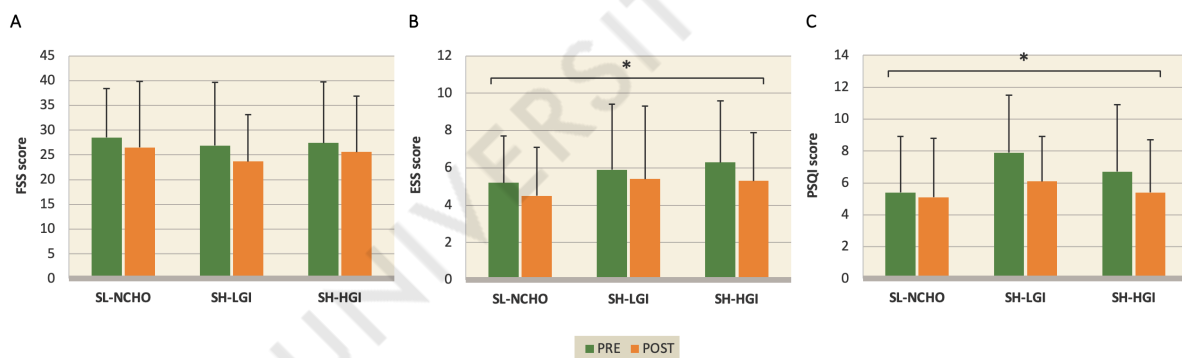
### 6.3.4 Self-Perceived Sleep Measures

Participants' self-perceived fatigue (FSS score), daytime sleepiness (ESS) and sleep quality (PSQI) are presented in Table 6.3.4.1 and Figure 6.3.4.1. No statistically significant findings were found for FSS score. On the other hand, there was a main effect of time on both ESS scores ( $F(1, 39) = 4.36, p = 0.043, \eta^2_r = 0.10$ ) and PSQI scores ( $F(1, 39) = 4.19, p = 0.047, \eta^2_r = 0.10$ ). On average, ESS scores decreased by 12.6% after the nutrition intervention, while PSQI scores decreased by 15.5%. For both variables, the decrease was higher in the SH-LGI group, however, without reaching statistical significance levels. No main effect of group or time x group interaction was found either for ESS scores or PSQI scores.

**Table 6.3.4.1.** Intervention effect on Self-Perceived Sleep Measures.<sup>1</sup>

	Group <sup>2</sup>	Pre- Intervention <sup>3</sup>	Post- Intervention	Δ	Δ (%)	P (group)	P (time)	P (I)
<b>FSS</b>	SL-NCHO	28.5 ± 9.9	26.5 ± 13.3	-2.0	-7.0	0.862	0.175	0.956
	SH-LGI	26.9 ± 12.7	23.7 ± 9.4	-3.1	-11.7			
	SH-HGI	27.4 ± 12.3	25.6 ± 11.3	-1.9	-6.8			
<b>ESS</b>	SL-NCHO	5.2 ± 2.5	4.5 ± 2.6	-0.7	-13.6	0.677	<b>0.043*</b>	0.855
	SH-LGI	5.9 ± 3.5	5.4 ± 3.9	-0.5	-8.4			
	SH-HGI	6.3 ± 3.3	5.3 ± 2.6	-1.0	-15.9			
<b>PSQI</b>	SL-NCHO	5.4 ± 3.5	5.1 ± 3.7	-0.2	-4.0	0.325	<b>0.047*</b>	0.467
	SH-LGI	7.9 ± 3.6	6.1 ± 2.8	-1.9	-23.4			
	SH-HGI	6.7 ± 4.2	5.4 ± 3.3	-1.3	-19.1			

SL-NCHO, Sleep Low- No Carbohydrates; SH-LGI, Sleep High- Low Glycemic Index; SH-HGI, Sleep High- High Glycemic Index; IIV, Intra-Individual Variability; FSS, Fatigue Severity Scale; ESS, Epworth Sleepiness Scale; PSQI, Pittsburgh Sleep Quality Index; <sup>1</sup>Data are presented as mean ± SD. <sup>2</sup>n = 42 (SL-NCHO: n = 14; SH-LGI: n = 14; SH-hGI: n = 14). <sup>3</sup>No pre-intervention differences between groups were present. \* Denotes statistically significant differences at the 0.05 level (2-tailed).



**Figure 6.3.4.1.** Intervention effect on Self-Perceived Sleep Measures.\*Denotes statistically significant main effect of time at the 0.05 level (2-tailed). SL-NCHO, Sleep Low- No Carbohydrates; SH-LGI, Sleep High- Low Glycemic Index; SH-HGI, Sleep High- High Glycemic Index; FSS, Fatigue Severity Scale; ESS, Epworth Sleepiness Scale; PSQI, Pittsburgh Sleep Quality Index.

## 6.4 Discussion

The present investigation is the first controlled trial that examined the effect of habitual evening carbohydrate modification combined with exercise training on sleep-related parameters. The purpose of the current study was to compare the effects of pre-bed carbohydrate quantity and quality manipulation in combination with exercise on sleep-related parameters over a 4-week period. The main findings were that i) adherence to a personalized dietary plan with sufficient nutrient intake promotes sleep initiation, sleep continuity and prolongs sleep duration, ii) both the quantity and quality of pre-bed carbohydrate intake may affect time to bed and sleep duration variability after a 4-week period, even when energy and macronutrient daily intake are equated, and iii) intervention-induced sleep extension led to subsequent increases mainly in N1 and REM sleep stages.

An increasing amount of research has reported the positive effects of several nutrition interventions, and especially carbohydrate manipulation, on sleep initiation and duration [496]. For example, a HGI pre-bed meal combined with [8], or without [49] evening training has been shown to improve sleep initiation and duration acutely, compared to a LGI meal. Notably, the results of the present study showed that SOL was decreased approximately in half, and sleep duration increased by 24.9 minutes after the implementation of the nutrition interventions over a 4-week period. Hence, it is shown that, in contrast to the acute effect of HGI on sleep initiation or sleep extension, HGI foods are not superior compared to LGI foods as a part of the pre-bed meal over a 4-week period. Moreover, it was revealed that when both energy and macronutrient intake are equated, carbohydrate timing throughout the day does not further benefit sleep initiation and duration. Collectively, these results confirm potential links between nutrition and sleep derived from epidemiological data, that point towards significant relationships between sleep initiation and duration with both energy or carbohydrate intake [498].

Furthermore, sleep continuity was significantly improved in all intervention groups. Specifically, SE increased by an average of 5.9%, while WASO decreased by 13.4%. In the literature, there are mixed findings with regards to carbohydrate manipulation and sleep continuity. Acute interventions in some studies have shown significant effects on both SE and WASO [8,49], while others prevailed with a weak or no effect [50,471]. Nevertheless, these acute sleep-related alterations are often attributed to the indirect link between carbohydrate manipulation and blood tryptophan elevation, with subsequent effects on melatonin levels [8,49]. Collectively, the present study indicates that the latter hypothesis may be ambiguous and that in the long-term overall sleep continuity is improved, especially when equating daily energy and macronutrient intake.

According to sleep architecture and carbohydrate intake, two different relationships are established, linking high carbohydrate intake to REM sleep stage and low carbohydrate intake

to N3 sleep stage [496]. In the present study, sleep extension was accompanied by the extension of both REM sleep duration and REM % TST. This transition to REM sleep stage, confirms the already established link between carbohydrates and REM sleep stage, since most of the daily energy intake in this investigation was derived from carbohydrates. Not only that but it could be hypothesized that both the carbohydrate intake and energy availability were increased in all intervention groups compared to habitual dietary intake of carbohydrates prior to intervention, since the majority of people who exercise do not meet the dietary recommendations for energy and carbohydrate intake [499].

Alongside these significant improvements in objectively measured sleep-related variables, participants reported significantly decreased daytime sleepiness and increased sleep quality. This aligns with the literature, which has shown that alterations in SE% and WASO and TST are closely related to sleep quality [500]. These results could be attributed to the restorative properties of quality sleep that have constantly associated sleep with brain reorganization and repair, [486] hormonal responses that promote recovery [488] and increased cognitive performance [489]. These findings bear high significance as daytime sleepiness increase dramatically the risk of car and work accidents [501], impaired productivity performance [502], increased fatigue [503] and as such, affecting the overall quality of life. A key difference that was observed between the groups was sleep duration variability. Even when energy and macronutrient daily intake were equated, both the quantity and quality of pre-bed carbohydrate intake affected TST IIV after the 4-week intervention period. Specifically, sleep duration variability was reduced by 26.4% and 26.6% in the SL-NCHO and SH-LGI trials respectively, without significant alterations in the SH-HGI group. Even though there are no comparative data in the literature, these results are very interesting because they indicate that specific parts of glucose metabolism can be altered throughout the day [504]. These responses of glucose metabolism to the circadian rhythm could ultimately result in sleep variability alterations, even when overall daily nutrient intake is equated.

Although identifying underlying biological mechanisms leading to the present results was beyond the scope of the present study, potential assumptions can be made. With regards to the improvements in sleep initiation, duration and continuity, it may be implied that both avoiding overeating -which could potentially result in heartburn and disrupt sleep [505]- and achieving nutrient adequacy resulted in improved sleep patterns. In literature, both sufficient energy intake and carbohydrate consumption have been constantly related to improved sleep patterns [498]. On the other hand, carbohydrates consumption could potentially indirectly trigger melatonin production [496] and that can possibly be translated into extension of total sleep period. On the same basis, the increase in the percentage of REM to total sleep time can also be attributed to both energy and carbohydrate availability [62,484]. From a metabolic perspective, this is justified

since REM is a more energy-demanding state compared to the N3 sleep stage [62,484]. Early studies showed that glucose infusion during the first 3 hours of sleep significantly increased REM sleep stage [483]. Towards this direction, REM sleep has been shown to increase after acute interventions with high carbohydrate consumption and vice versa [496]. Hence, since energy availability and carbohydrate quantity in all groups were sufficient and equated, this can explain the nutrition intervention effects on sleep architecture.

A significant difference among trials was found regarding sleep duration variability. This result could possibly be mediated by the different effects of nutrition interventions on blood glucose fluctuations during the evening. Based on this, it could be hypothesized that both in the SL-NCHO and SH-LGI groups, blood fluctuations would be relatively less, in contrast to the high glucose spike induced by the implementation of HGI foods in the SH-HGI group. Collectively, these may indicate that glucose metabolism is not only differentiated through the circadian clock [504] but is regulated by fluctuations in blood glucose and subsequently affect sleep patterns. Additionally, it could be hypothesized that insulin sensitivity could be altered after a long period of time due to the daily increased pre-bed glucose fluctuations. Consequently, relevant interventions' duration may differentiate the effect of pre-bed glycemic index to sleep on acute and chronic, as has been shown in previous studies [8]. Furthermore, it is possible that the SL-NCHO and SH-LGI groups consumed more dietary fibers than the HGI group. In this sense, fibers and glucose intake have been shown to affect gut microbiome composition to a great extent [217]. This bears high significance because the gut microbiome seems to have a promising effect on sleep regulation [294,295]. However, since the gut microbiome requires at least 24 hours of changed nutrition patterns to be altered [178,179], this could be another reason for the discrepancies in acute and long-term nutrition intervention effects on sleep-related parameters.

In the current study, there are strengths and weaknesses that need to be addressed. To start with, this is the first study to approach the effect of a long-term lifestyle change on sleep-related parameters, measured with a combination of gold-standard polysomnography methodology, continuous actigraphic measures alongside sleep diaries and self-report sleep quality assessment. However, technical problems such as non-compliance with wearing the actigraphy devices or filling out the sleep diary daily, resulted in 5.6 nights of recordings per participant per trial (pre-post), rather than the aim of 7 nights. On the other hand, such inconvenience is relatively less in the present study compared to previous investigations that showed up to 28% of weekly sleep recordings can be lost for several reasons [506]. Moreover, it would be of further interest to examine the effects of similar nutrition interventions on sleep-related parameters for even longer periods. Another limiting factor is that, due to financial constraints, it was unable to provide all-day's meals to participants for the trial period. On the other hand, this led to the

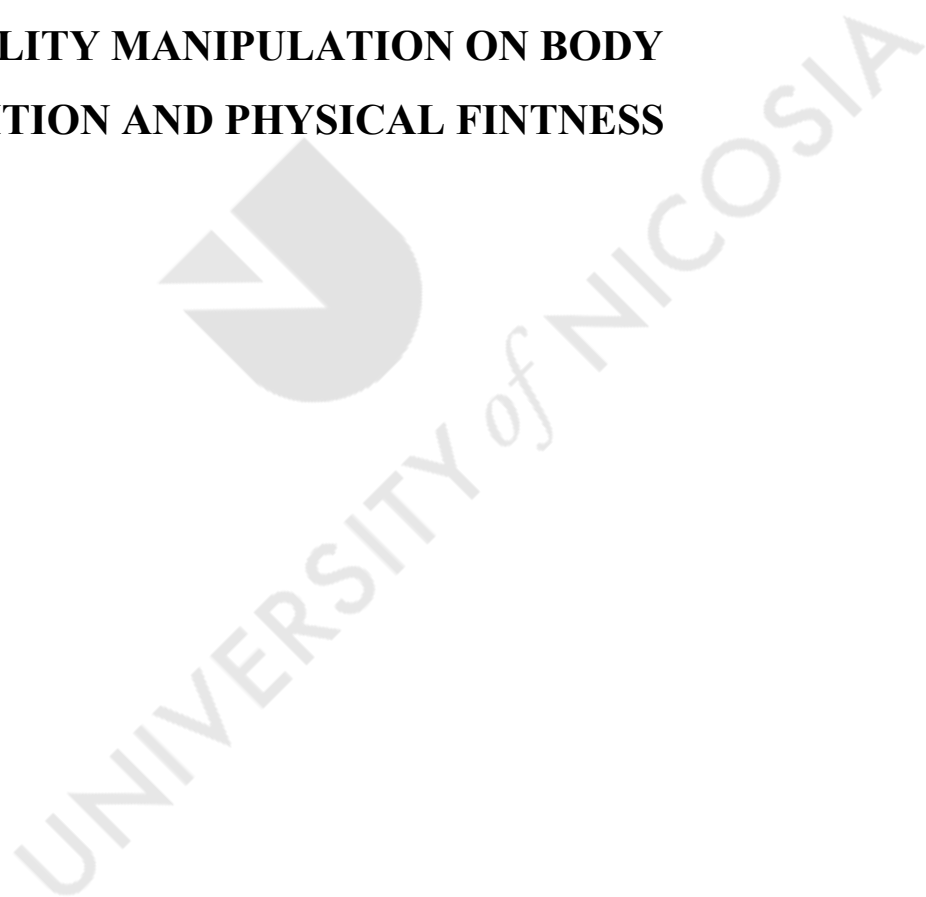
development of individualized, flexible and increased adherence to nutrition plans that are easy to implement in everyday clinical practice, as shown by the present study's high compliance rate.

Altogether, these practical implications of the study extend beyond the limits of the current investigations for several reasons. As it was shown, adequate nutrient intake contributes effectively to the optimization of the majority of sleep-related variables such as sleep initiation, continuity and duration. Notably, similar results on sleep initiation and maintenance have been shown after melatonin administration in both athletes [507] and patients with primary sleep disorders [508]. Based on this, targeted nutrition interventions are promising and point towards the further investigation of lifestyle medicine as a potential complementary treatment for sleep disorders. Furthermore, relevant interventions may act as a safeguard against inadequate sleep patterns in athletes and people who exercise, in order to promote restoration, recovery and overall health.

## **6.5 Conclusion**

To summarize, the present study shows that habitual adherence to a personalized dietary plan with sufficient nutrient intake can significantly improve the majority of sleep-related parameters and as such, optimise sleep. However, the quantity and quality of evening carbohydrate intake affect sleep duration variability, even when energy and nutrient intake are equated. Hence, further studies are needed to unveil the potential effects of relevant interventions on sleep variability in the long term. Taking into account those findings, it is important that healthy lifestyle and especially targeted nutrition interventions will be further investigated in order to improve individuals' sleep quality and potentially aid as a complementary treatment in sleep disorders.

**CHAPTER 7: THE EFFECT OF CARBOHYDRATE TIMING  
AND QUALITY MANIPULATION ON BODY  
COMPOSITION AND PHYSICAL FITNESS**



## 7.1 Introduction

Body re-composition is a term used to describe the concept of improving body composition, without losing weight, but by gaining muscle mass and losing fat tissue [509]. This bears high significance for two reasons; the first is that not only increased adiposity, but decreased muscle mass as well is closely related to higher morbidity and mortality rate [510-512]. Secondly, there is an increasing prevalence of a group of individuals that may be underweight but not under-fat [513,514]. Thus, body composition optimization is of utmost importance in order to improve both fat mass and fat-free mass ratio, potentially without any subsequent weight change.

To date, the majority of research with regard to fat loss interventions recommends the combination of nutrition and exercise to achieve optimal body composition outcomes [515]. A great amount of evidence supports a healthy nutrition plan combined with physical activity, which are key lifestyle medicine factors that modulate overall health, improving people's quality of life [516]. Moreover, since exercise-induced stress can stimulate muscle protein synthesis, it is indicated that there is an additional advantage of implementing physical activity during a period of body fat reduction, in order to promote muscle hypertrophy and body re-composition [517].

Dietary patterns are probably the greatest modifiable factor that shape body weight, aid to achieve a healthy body weight and have a great impact on body composition. In particular, adequate protein intake and diet-induced hypocaloric conditions are crucial factors for muscle hypertrophy and fat loss, respectively [518]. Nevertheless, evidence according to the quantity, quality and especially the timing of nutrient intake is still controversial. Based on nutrient timing, there is a relatively new field of nutrition science altogether that remains to be investigated, referred to as chrono-nutrition [504]. In particular, chrono-nutrition highlights the relevance and importance of how nutrients interact with circadian clocks, defining the shape of metabolic processes that could ultimately lead to subsequent body composition formation [504].

This focus on nutrient timing is not completely new in the sports nutrition field, since carbohydrate manipulations are usually recruited in athletes in order to optimize performance [519]. Towards this direction, a distinct carbohydrate periodization protocol rose and opened new horizons for athletes' health and performance improvement [1]. The key difference between the old carbohydrate periodization protocols and the new one, is that the latter manipulates carbohydrate timing "within the day" and not "between days". Thus, beyond several established physiological adaptations of carbohydrate periodization protocols in athletes [3], it remains to be unveiled if carbohydrate manipulation during the day could aid towards body re-



composition, and if potential alterations on fat-free mass to fat- mass ratio could be translated into improvements in physical fitness.

Overall, despite the fact that body fat reduction and body re-composition is a common aim and necessity from both personal and clinical perspectives, there is still substantial confusion with regard to evidence-based strategies to accomplish this goal [520]. Furthermore, data are limited with regards to the effect of carbohydrate manipulation during a day and its effect on body re-composition and performance of athletic populations during isocaloric dietary conditions [521]. Hence, the purpose of the present study is to examine the effect of carbohydrate periodization of quantity and quality over 4 weeks on body composition and physical fitness indices including i) whole and segmental body composition ii) aerobic capacity, iii) power and strength and iv) visual reaction time.

## **7.2 Materials and Methods**

### **7.2.1 Participants**

This study included 42 healthy, physically active volunteers, who regularly participating in sports activities for at least two years (specific inclusion and exclusion criteria described in chapter 4). Participants had a mean ( $\pm$  SD) age of  $27 \pm 7$  years, height of  $177 \pm 6$  cm and body mass of  $80.3 \pm 11.1$  kg. All participants had been informed about the purpose of the study and provided written consent. The study was approved by the Cyprus Bioethical Committee (EEBK/EII/2020/65) and with a clinical trial registration: NCT05464342. All of the procedures were conducted according to the Declaration of Helsinki in 1964 and its later amendments.

### **7.2.2 Study Design**

The present study was a randomized controlled trial with a parallel design. Specifically, the study consisted of three nutrition intervention groups investigating the effect of pre-bed periodization of carbohydrate consumption quantity and quality on body composition and exercise performance. Alongside nutrition intervention, participants performed a supervised standardized exercise program combining resistance exercise and HIIT sessions. All nutrition and exercise intervention procedures are described in detail in chapters 4.2 and 4.3. Each subject was assigned to one of the three nutritional interventions for a 4-week period, by a computer-generated sequence of random numbers.

In the first phase of the study, participants underwent preliminary testing in order to evaluate their nutritional status, their basic anthropometric data and their fitness level in order to design personalized exercise and nutrition interventions for each participant. Both at baseline and at the end of the intervention, physical fitness and exercise performance were evaluated through

the assessment of countermovement jump, visual reaction time,  $VO_{2peak}$  and 1RM in hack squat, plate-loaded bench press, plate-loaded shoulder press and lat-pulldown. The main phase of the study took place in the Human Performance Lab of the University of Nicosia and the fitness premises (UFIT) of the University of Nicosia.

### **7.2.3 Assessments**

#### ***Anthropometry and body composition***

Anthropometrics and Somatometrics were collected at the baseline of the intervention, as described in chapter 4.4. Individuals were barefooted and lightly dressed. Weight and standing height were measured to the nearest 0.1kg and 0.5cm respectively. Body composition was assessed with both an estimation of body density via Harpenden skinfold calliper using Jackson's and Pollock's 7-site method and by bioelectrical impedance analysis (BIA).

#### ***Physical Fitness and Exercise Performance***

Physical fitness and Exercise Performance evaluation was performed at both baseline and at the end of the intervention. Assessments included CMJ, VRT,  $VO_{2peak}$  and 1RM as described in detail in chapter 4.4. In the beginning, participants undertook a five-minutes warm-up, and performed three CMJ and the highest one was recorded with an accuracy of 1/1000 of a second. Flight height is calculated from flight time and data were recorded with an accuracy of 0,01cm. Following, a visual reaction test (VRT) was performed. Overall, the test consisted of three visual stimuli. The reaction time after each stimulus was recorded at the nearest 0,001 second and the average VRT was calculated. Afterwards,  $VO_{2peak}$  was assessed with a treadmill protocol. Heart rate was continuously monitored. Moreover, at the above-mentioned time points, participants' 1 repetition maximum in specific exercises was assessed in hack-squat, plate-loaded bench press, plate-loaded shoulder-press and lat-pulldown.

### **7.2.4 Statistical Analysis**

Continuous variables are presented as mean  $\pm$  standard deviation. Statistical analyses were conducted using IBM®SPSS® statistics for Windows, version 25.0 (IBM Corp, Armonk, NY). Variables' distribution was identified using the Shapiro-Wilks test. A repeated measures analysis of variance was used to investigate the potential effect of the intervention on exercise performance and body composition indices. Significant effects were further explored using the Bonferroni post hoc procedures. Effect sizes were calculated using partial eta squared ( $\eta^2$ ). Alpha level for all statistical analyses was set at  $p < 0.05$ , two-tailed tests.

## 7.3 Results

### 7.3.1 Body Composition

A main effect of time was found regarding body composition parameters (Table 7.3.1.1 and Figure 7.3.1.1.). Weight was decreased over time by an average of 0.6 kg with a significant time effect ( $F(1, 39) = 8.81, p = 0.005, \eta^2_r = 0.18$ ). In the same line, body fat percentage decreased by an average of 1.5% ( $F(1, 39) = 17.50, p < 0.001, \eta^2_r = 0.31$ ), FM by 1.4kg ( $F(1, 39) = 16.42, p < 0.001, \eta^2_r = 0.30$ ) while FFM increased by 0.9kg ( $F(1, 39) = 8.28, p = 0.006, \eta^2_r = 0.18$ ). No main effect of group or time x group interaction was observed for these variables. Moreover, no significant effects were found for trunk fat, TBW, ECW and ICW ( $p > 0.05$ ).



**Table 7.3.1.1.** Intervention effect on Body Composition.<sup>1</sup>

	Group <sup>2</sup>	Pre-Intervention <sup>3</sup>	Post-Intervention	Δ	Δ (%)	P (group)	P (time)	P (I)
<b>Weight (kg)</b>	SL-NCHO	82.9 ± 11.3	82 ± 10.9	-0.9	-1.1	0.525	<b>0.005*</b>	0.306
	SH-LGI	80.6 ± 10.1	80.4 ± 9.6	-0.2	-0.2			
	SH-HGI	78 ± 12.1	77.3 ± 12.5	-0.7	-0.9			
<b>Skinfold</b>								
BF (%)	SL-NCHO	16.4 ± 7.5	14.4 ± 5.6	-2.0	-12.5	0.066	<b>&lt;0.001*</b>	0.338
	SH-LGI	13.4 ± 4.2	12.6 ± 3.9	-0.8	-6.3			
	SH-HGI	11.8 ± 4.2	10 ± 3.6	-1.8	-15.6			
FM (kg)	SL-NCHO	14 ± 7.3	12 ± 5.4	-2.0	-14.3	0.111	<b>&lt;0.001*</b>	0.362
	SH-LGI	11.1 ± 5.2	10.3 ± 4.4	-0.8	-6.9			
	SH-HGI	9.6 ± 4.4	8.1 ± 4.1	-1.5	-15.8			
FFM (kg)	SL-NCHO	66.5 ± 10	67.6 ± 10.2	1.1	1.7	0.978	<b>0.006*</b>	0.816
	SH-LGI	67.1 ± 7.5	67.8 ± 7.9	0.7	1.0			
	SH-HGI	66.2 ± 9.2	67.3 ± 9.5	1.1	1.7			
Abdominal (mm)	SL-NCHO	28.9 ± 17.9	22.8 ± 11.3	-6.1	-21.1	0.053	<b>0.004*</b>	0.678
	SH-LGI	20.4 ± 9.8	17.3 ± 6.4	-3.1	-15.4			
	SH-HGI	19 ± 9.2	15.1 ± 4.8	-3.9	-20.5			
Suprailiac (mm)	SL-NCHO	17.9 ± 11.6	11.7 ± 6.2	-6.2	-34.5	0.314	<b>0.003*</b>	0.282
	SH-LGI	12.9 ± 5.5	11.6 ± 4.9	-1.3	-10.2			
	SH-HGI	13.6 ± 6.3	9.7 ± 4.6	-4.0	-29.1			

*(Continued on next page)*

Table 7.3.1.1. (Continued)

	Group <sup>2</sup>	Pre-Intervention <sup>3</sup>	Post-Intervention	Δ	Δ (%)	P (group)	P (time)	P (I)
Midxillary (mm)	SL-NCHO	14.3 ± 8.1	13.2 ± 7.2	-1.1	-8.0	0.83	0.182	0.736
	SH-LGI	10.9 ± 4.3	10.8 ± 5.1	-0.1	-1.3			
	SH-HGI	9.8 ± 3.7	8.7 ± 3.2	-1.0	-10.6			
Subscapular (mm)	SL-NCHO	17 ± 6.7	14.9 ± 5.3	-2.1	-12.4	0.095	0.076	0.474
	SH-LGI	14.2 ± 4.4	14 ± 5.1	-0.1	-1.0			
	SH-HGI	12.9 ± 4	11.6 ± 2.9	-1.3	-10.2			
Triceps (mm)	SL-NCHO	8.4 ± 3.1	8.5 ± 2.5	0	0.4	0.383	1.000	0.699
	SH-LGI	8.7 ± 3.4	9.2 ± 4.7	0.5	5.7			
	SH-HGI	7.8 ± 2.6	7.2 ± 2.8	-0.5	-6.9			
Thighs (mm)	SL-NCHO	17.3 ± 8.3	16.5 ± 6.8	-0.7	-4.1	0.207	0.131	0.592
	SH-LGI	16.3 ± 5.8	13.8 ± 5.1	-2.6	-15.8			
	SH-HGI	13.5 ± 4.9	12.9 ± 4.7	-0.7	-5.0			
Pectoralis (mm)	SL-NCHO	15.3 ± 8.8	14.4 ± 7.9	-0.9	-5.8	0.039	0.281	0.202
	SH-LGI	11.1 ± 5.8	11.7 ± 5.5	0.7	6.0			
	SH-HGI	9.6 ± 3.6	8.3 ± 3	-1.3	-13.4			
<b>Bioelectrical Impedence</b>								
Trunk Fat (%)	SL-NCHO	33 ± 2.9	33.1 ± 2.9	0.2	0.5	0.977	0.410	0.631
	SH-LGI	32.9 ± 2.6	33.2 ± 2.4	0.3	1.0			

*(Continued on next page)*

Table 7.3.1.1. (Continued)

	Group <sup>2</sup>	Pre-Intervention <sup>3</sup>	Post-Intervention	Δ	Δ (%)	P (group)	P (time)	P (I)
MM (%)	SH-HGI	32.8 ± 3	32.9 ± 3.2	0.1	0.4	0.999	<b>0.006*</b>	0.446
	SL-NCHO	61.3 ± 6	61.7 ± 6.4	0.4	0.7			
TBW (kg)	SH-LGI	61 ± 5.6	62 ± 5.2	1.0	1.6	0.436	0.264	0.378
	SH-HGI	61.2 ± 6.5	61.7 ± 6.9	0.4	0.7			
	SL-NCHO	46.3 ± 3.6	46.2 ± 3.7	-0.2	-0.3			
ECW (kg)	SH-LGI	45.5 ± 3.4	45.9 ± 3.2	0.4	0.8	0.613	0.407	0.453
	SH-HGI	43.2 ± 8.1	45.4 ± 4.3	2.2	5.1			
	SL-NCHO	19.4 ± 3.3	18.7 ± 1.5	-0.6	-3.2			
ICW (kg)	SH-LGI	18.6 ± 1.5	18.6 ± 1.5	0	0	0.585	0.459	0.074
	SH-HGI	18.4 ± 1.7	18.4 ± 1.7	0	0.2			
	SL-NCHO	28.4 ± 4.1	27.4 ± 2.2	-1.0	-3.4			
	SH-LGI	26.9 ± 2	27.3 ± 1.9	0.4	1.4			
	SH-HGI	27 ± 2.6	27 ± 2.7	0	0.2			

SL-NCHO, Sleep Low- No Carbohydrates; SH-LGI, Sleep High- Low Glycemic Index; SH-HGI, Sleep High- High Glycemic Index; IIV, Intra-Individual Variability; BF, Body Fat; FM, Fat Mass; FFM, Fat Free Mass; MM, Muscle Mass; TBW, Total Body Water; ECW, Extra Cellular Water; ICW, Intra Cellular Water; <sup>1</sup>Data are presented as mean ± SD. <sup>2</sup>n = 42 (SL-NCHO: n = 14; SH-LGI: n = 14; SH-hGI: n = 14). <sup>3</sup>No preintervention differences between groups were present. \* Denotes statistically significant differences at the 0.05 level (2-tailed).

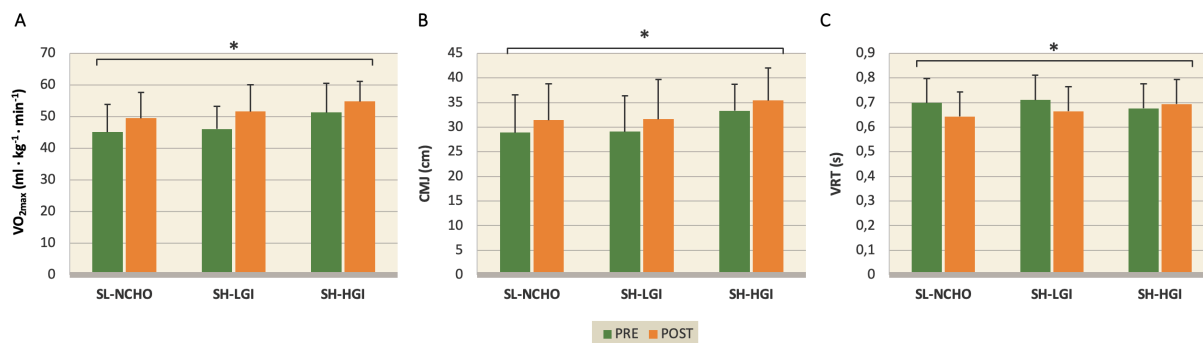
### 7.3.2 Cardiorespiratory Fitness

A main effect of time was observed with regard to maximum aerobic capacity (Table 7.3.2.1 and Figure 7.3.2.1). In particular, the intervention resulted in increased  $VO_{2peak}$  in all participants ( $F(1, 36) = 40.39, p < 0.001, \eta^2_r = 0.54$ ), with an average increase of  $4.5 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ . Accordingly, the maximum speed that participants reached during the  $VO_{2peak}$  increased over time ( $F(1, 36) = 6.42, p = 0.016, \eta^2_r = 0.15$ ). No statistically significant time x group interaction was observed for these variables. Interestingly, at the same time with the increases maximum aerobic capacity,  $HR_{max}$  during the  $VO_{2peak}$  test dropped slightly in all groups, with a significant time effect ( $F(1, 36) = 5.06, p = 0.031, \eta^2_r = 0.13$ ). No main effect of group, time or time x group interaction was observed for recovery of heart rate one minute and three minutes after the  $VO_{2peak}$  test.

**Table 7.3.2.1.** Intervention effect on cardiorespiratory fitness. <sup>1</sup>

	Group <sup>2</sup>	Pre- Intervention <sup>3</sup>	Post- Intervention	$\Delta$	$\Delta$ (%)	P (group)	P (time)	P (I)
<b><math>VO_{2peak}</math></b> ( $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ )	SL-NCHO	45.1 ± 8.8	49.5 ± 8.1	4.4	9.7	0.134	<0.001*	0.520
	SH-LGI	46.1 ± 7.1	51.6 ± 8.5	5.5	12			
	SH-HGI	51.3 ± 9.2	54.8 ± 6.3	3.6	7.0			
<b>Speed<sub>max</sub></b> ( $\text{km}\cdot\text{h}^{-1}$ )	SL-NCHO	14.1 ± 1.8	14.2 ± 1.8	0	0.1	0.041*	0.016*	0.596
	SH-LGI	14.8 ± 2	15.2 ± 2.3	0.5	3.1			
	SH-HGI	15.7 ± 1.6	16.2 ± 1.5	0.4	2.8			
<b><math>HR_{max}</math></b> (bpm)	SL-NCHO	183.5 ± 12.1	181.6 ± 7.7	-1.9	-1.0	0.135	0.031*	0.535
	SH-LGI	188.0 ± 10.7	183.8 ± 7.4	-4.2	-2.2			
	SH-HGI	191.3 ± 10.7	189.8 ± 10.0	-1.5	-0.7			
<b>1min Recovery HR (bpm)</b>	SL-NCHO	27.7 ± 10.0	28.8 ± 8.4	1.1	4.0	0.881	0.898	0.515
	SH-LGI	29.0 ± 9.1	30.0 ± 6.0	1.0	3.4			
	SH-HGI	29.6 ± 7.4	26.9 ± 7.6	-2.7	-10.0			
<b>3min Recovery HR (bpm)</b>	SL-NCHO	51.5 ± 14.7	56.5 ± 14.8	5.0	9.7	0.612	0.276	0.263
	SH-LGI	54.1 ± 10.5	58.0 ± 8.2	3.9	7.2			
	SH-HGI	59.0 ± 9.9	56.6 ± 7.1	-2.4	-4.0			

SL-NCHO, Sleep Low- No Carbohydrates; SH-LGI, Sleep High- Low Glycemic Index; SH-HGI, Sleep High- High Glycemic Index;  $VO_{2peak}$ , Maximal Oxygen Consumption; HR, Heart Rate; BPM, Beat Per Minute; <sup>1</sup>Data are presented as mean ± SD. <sup>2</sup>n = 42 (SL-NCHO: n = 14; SH-LGI: n = 14; SH-hGI: n = 14). <sup>3</sup>No pre-intervention differences between groups were present. \* Denotes statistically significant differences at the 0.05 level (2-tailed).



**Figure 7.3.2.1.** Intervention effect on Sports Performance.\*Denotes statistically significant main effect of time at the 0.05 level (2-tailed). SL-NCHO, Sleep Low- No Carbohydrates; SH-LGI, Sleep High- Low Glycemic Index; SH-HGI, Sleep High- High Glycemic Index; VO<sub>2peak</sub>, Maximal Oxygen Consumption; CMJ, CounterMovement Jump; VRT, Visual Reaction Time.

### 7.3.3 Strength and Explosive power

The combination of exercise and nutrition intervention led to significant improvements in both CMJ and 1RM testing (Table 7.3.4.1 and Figure 7.3.2.1). Specifically, there was a significant main effect of time on CMJ ( $F(1, 39) = 25.33, p < 0.001, \eta^2_r = 0.40$ ), with an average increase of 7.9% over time. In the same line, 1RM on hack squat ( $F(1, 39) = 11.40, p = 0.002, \eta^2_r = 0.23$ ), plate loaded chest press ( $F(1, 39) = 7.21, p = 0.011, \eta^2_r = 0.16$ ), shoulder press ( $F(1, 39) = 17.47, p < 0.001, \eta^2_r = 0.30$ ), and Lat Pulldown ( $F(1, 39) = 4.34, p = 0.044, \eta^2_r = 0.10$ ), increased significantly by 7.4kg, 4.2kg, 7.2kg and 1.6kg respectively. No main effect of group or time x group interaction was observed for these variables.



**Table 7.3.3.1.** Intervention effect on Strength and Power.<sup>1</sup>

	Group <sup>2</sup>	Pre-Intervention <sup>3</sup>	Post-Intervention	Δ	Δ (%)	P (group)	P (time)	P (I)
<b>CMJ (cm)</b>	SL-NCHO	28.9 ± 7.7	31.4 ± 7.4	2.5	8.7	0.228	<b>0.000*</b>	0.823
	SH-LGI	29.1 ± 7.3	31.6 ± 8.1	2.5	8.6			
	SH-HGI	33.3 ± 5.4	35.4 ± 6.6	2.1	6.3			
<b>Hack Squat 1RM (kg)</b>	SL-NCHO	115.2 ± 46.7	121.8 ± 44.4	6.6	5.7	0.702	<b>0.002*</b>	0.827
	SH-LGI	106.6 ± 39	112.9 ± 38.7	6.3	5.9			
	SH-HGI	101.1 ± 38.8	110.4 ± 40.8	9.3	9.2			
<b>Chest Press 1RM (kg)</b>	SL-NCHO	105.7 ± 36.1	109.3 ± 34.2	3.6	3.4	0.950	<b>0.011*</b>	0.951
	SH-LGI	109.3 ± 44.2	113.9 ± 47	4.6	4.2			
	SH-HGI	109.1 ± 33.3	113.8 ± 35.1	4.6	4.3			
<b>Shoulder Press 1RM (kg)</b>	SL-NCHO	100.4 ± 28	106.4 ± 28.6	6.1	6.0	0.778	<b>0.000*</b>	0.474
	SH-LGI	94.1 ± 30.9	104.3 ± 33.2	10.2	10.8			
	SH-HGI	93 ± 25.1	98.4 ± 28.5	5.4	5.8			
<b>Lat Pulldown 1RM (kg)</b>	SL-NCHO	75.7 ± 15.8	76.9 ± 13.9	1.1	1.5	0.760	<b>0.044*</b>	0.886
	SH-LGI	78.9 ± 14.2	80.5 ± 15.3	1.6	2.0			
	SH-HGI	74.8 ± 16.7	76.8 ± 15.5	2.1	2.8			

SL-NCHO, Sleep Low- No Carbohydrates; SH-LGI, Sleep High- Low Glycemic Index; SH-HGI, Sleep High- High Glycemic Index; CMJ, CounterMovement Jump; RM, Repetition Maximum; <sup>1</sup>Data are presented as mean ± SD. <sup>2</sup>n = 42 (SL-NCHO: n = 14; SH-LGI: n = 14; SH-hGI: n = 14). \* Denotes statistically significant differences at the 0.05 level (2-tailed)

### 7.3.4 Visual Reaction Performance

There was a significant main time effect on visual reaction time (VRT) (Table 7.3.5.1). Participants VRT decreased by an average of 4.5% ( $F(1, 39) = 10.09, p = 0.003, \eta^2_r = 0.21$ ). However, no significant group or time x group interaction was observed for this variable.

**Table 7.3.4.1.** Intervention effect on Visual Reaction Time Performance.<sup>1</sup>

	Group <sup>2</sup>	Pre- Intervention <sup>3</sup>	Post- Intervention	$\Delta$	$\Delta$ (%)	P (group)	P (time)	P (I)
VRT (sec)	SL- NCHO	0.698 ± 0.1	0.643 ± 0.1	-0.055	-7.8	0.903	<b>0.003*</b>	0.168
	SH-LGI	0.710 ± 0.1	0.664 ± 0.1	0.046	-5			
	SH-HGI	0.676 ± 0.1	0.694 ± 0.1	0.018	-0.8			

SL-NCHO, Sleep Low- No Carbohydrates; SH-LGI, Sleep High- Low Glycemic Index; SH-HGI, Sleep High- High Glycemic Index; VRT, Visual Reaction Time; <sup>1</sup>Data are presented as mean ± SD. <sup>2</sup>n = 42 (SL-NCHO: n = 14; SH-LGI: n = 14; SH-hGI: n = 14). <sup>3</sup>No preintervention differences between groups were present. \* Denotes statistically significant differences at the 0.05 level (2-tailed). \*\*Derived from Sleep Diary, rated 1-4.

## 7.4 Discussion

The present study investigated for the first time the long-term effect of the within-day periodization of carbohydrate quantity and quality consumption on both body re-composition and exercise performance. It was revealed that all nutrition interventions resulted in body composition optimization and improved a wide range of exercise performance indices, such as aerobic capacity, strength and reaction time. Hence, it is highlighted that when both energy and nutrient intake are equated within the day, carbohydrate intake timing and pre-bed carbohydrate quality do not hinder any beneficial adaptations of exercise training on body re-composition and physical fitness.

In the present study, body mass decreased slightly by 0.6kg over the period of one month, but FM decreased by 1.4kg and FFM increased by 0.9kg, without any significant differences among the trials. This fact indicates that neither carbohydrate intake timing nor the glyceamic index of the pre-bed meal significantly affect body re-composition when there is nutrient and energy adequacy. This is in contrast to the common notion that “carbohydrate consumption during the evening can make you fat” or that body recomposition cannot be achieved in well-trained individuals. However, these findings are in line with the theory of calories in/calories out, which describes the idea that weight management is mainly determined by the energy balance between energy consumption and energy expenditure [518]. Beyond these findings, the present results

support that -as with protein intake [522]- the overall daily consumption of carbohydrates is more important than timing when it comes to body composition; without this to overlook the importance of carbohydrate timing for exercise performance, especially in athletes [523].

As for exercise performance, the current results showed a significant time effect on all trials on participants'  $VO_{2peak}$  test. In particular, cardiorespiratory fitness was significantly improved regardless of trial, indicating that both pre-bed carbohydrate quality and timing didn't intervene with participants' progress. Notable, improvements in  $VO_{2peak}$  were accompanied by both increases in the maximum speed that participants reached during the  $VO_{2peak}$  test, as well as slightly lower maximum heart rate. This is in line with several studies reporting that  $HR_{max}$  may be reduced following regular aerobic exercise [524]. Indeed, there are plenty of data in the literature, showing that one month of consistent HIIT training can improve both  $VO_{2peak}$  and heart rate output [525]. Overall, these effects are considered to be of utmost importance, since increases in  $VO_{2peak}$  are constantly related to reduced mortality rate [526].

Another aspect of exercise performance that was significantly improved was CMJ and 1 repetition maximum on hack squat, chest press, shoulder press and lat pulldown. This is in line with the current body of evidence and could be attributed to both regular exercise training and adequate nutrient intake [527]. Specifically for exercise training, it has been previously shown that combining multi-joint exercises in the regular exercise program can improve 1RM [21]. It goes without saying that adequate strength levels are directly linked to increased quality of life [528], while decreasing all-cause mortality rates [529].

Again, since no differences were observed between groups, it could be hypothesized that post-evening workout carbohydrate intake, both in terms of quantity and quality, does not hinder strength improvements in athletes. These results challenge the common notion that peri-workout nutrient and carbohydrate intake is essential for body composition improvements [530-532]. The effect of the timing of post-workout carbohydrate intake in glycogen replenishment is indeed significant, but only when glycogen-depleting events are closer than 8h one from each other [533]. In this study, as well as in a common real-world scenario, participants' training was approximately one day apart, hence post-workout carbohydrate timing did not show any significant effect on strength since daily carbohydrate quantity was equated. In the same line, there is evidence that the glycemic index of the post-workout meal as well does not significantly alter glycogen synthesis either at 8h or 24-h post-exercise [534]. Collectively the present results with regards to exercise performance indicate that, when macronutrients and energy are equated throughout the day, both carbohydrate timing and pre-bed carbohydrate quality intake may not significantly alter responses to resistance exercise training.

Visual reaction performance was another exercise performance parameter that was improved in all trials. However, this time to react may be more related to potential improvements in other

specific domains of participants' lifestyle, rather than to improvements in body composition or power-to-weight ratio. For example, it has been previously shown that increased sleep duration improves visual reaction time [8]. Indeed, when sleep is extended, concentration and reaction time may increase significantly [33,535], but when sleep is disturbed, there are significant decreases in vigilance [536]. Since adequate carbohydrate intake is closely related to improved sleep patterns [496], potential sleep improvements could potentially mediate this effect on VRT performance [33,536,537]. This finding is of utmost importance, since visual reaction performance, despite its relationship with sports and exercise performance, it is considered to be related also with cognitive function [538].

Collectively, these findings raise several questions according to potential physiological responses that could have occurred. On the one hand, there are epidemiological data that correlate late evening energy and carbohydrate intake with decreases in glucose tolerance and obesity in sedentary populations [539,540]. However, this relationship did not appear in the present study. In fact, the combination of regular exercise training with adequate nutrient intake led to significant improvements in both body composition and exercise performance, regardless of carbohydrate timing. This may indicate that overall energy consumption, protein intake and the combination of nutrition intervention with regular exercise training may be more critical for body re-composition, physical fitness and exercise performance. There is a consensus on the importance of energy intake for athletes' health and performance [523]. This is of great significance since inadequate energy availability has been reported in relevant studies with similar carbohydrate periodization protocols [435]. Moreover, protein intake is an essential dietary factor for muscle protein synthesis [522]. Since the combination of adequate energy and protein intake with resistance exercise training are crucial to maximize muscle protein accretion [522], it can be assumed that these factors are more critical than carbohydrate timing and glycemic index of the pre-bed meal in order to decrease adiposity, increase fat-free mass and improve performance.

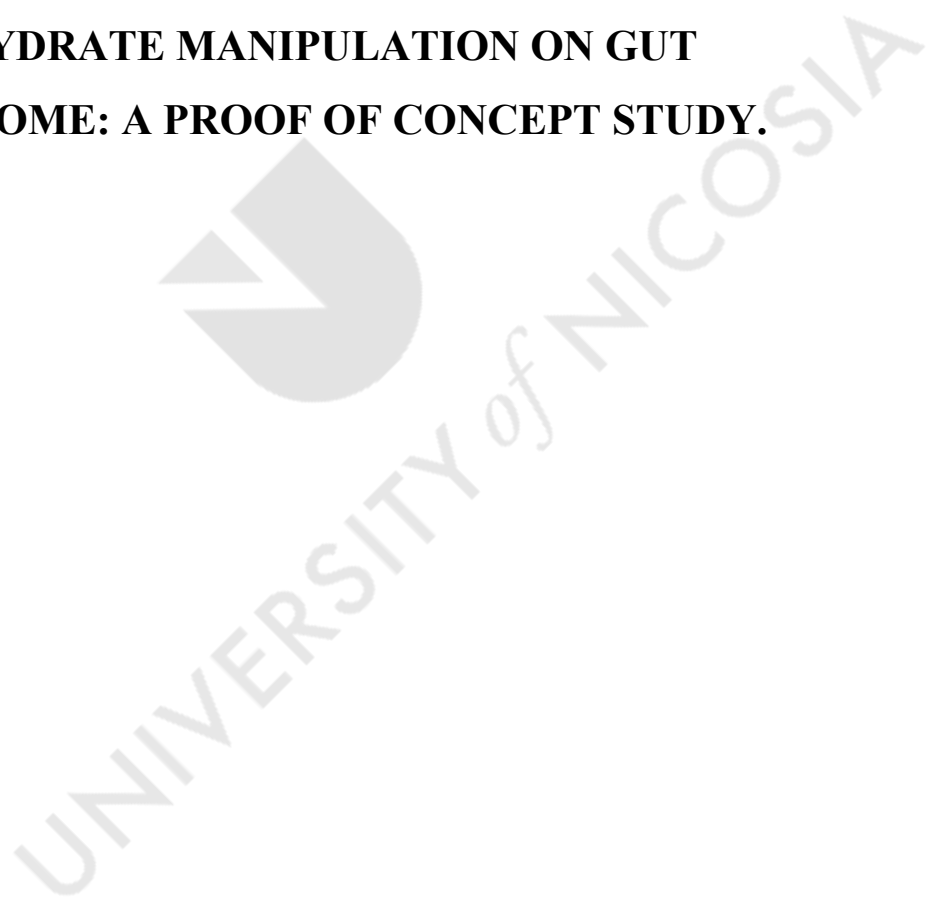
As in every research, there are specific limited factors that should be addressed. First, due to financial constraints, providing participants with their daily meal plan was impossible. As a result, flexible and individualized nutrition plans were developed to increase the compliance rate. Moreover, despite the fact that both skinfold measurements and segmental BIA impedance have constantly showed adequacy in measuring adiposity alterations [518], it would be very interesting to assess body composition with dual-energy X-ray absorptiometry, in order to extract more data with regards to muscle mass alterations between trials. Moreover, it would be of utmost importance to study similar interventions for even longer periods of time, to further explore the effect of chrononutrition on body composition and exercise performance.

Taking into account that the current approaches to improve body composition may be inadequate in the long term [541], the application of the results of the present study in a real-world scenario, may build the bridge between research and practice. Overall, the present study showed that exercise-induced improvements in body composition and exercise performance are not hindered by carbohydrate timing or pre-bed meal's carbohydrate quality, when energy and nutrient intake is equated. This points towards the development and implementation of flexible and easy-to-follow nutrition interventions, which in turn may end the vicious circle between thinking in black-and-white terms and weight regain [542], promoting long-term results in body re-composition.

## **7.5 Conclusion**

To summarize, the present study showed that exercise-induced improvements in body composition and exercise performance are not hindered by carbohydrate timing or pre-bed meal's carbohydrate quality, when energy and nutrient intake is equated. Towards this direction, more research is needed in order to unveil integral physiological responses to similar interventions. Novel and flexible nutrition interventions should be designed in order to aid body re-composition and exercise performance, while promoting overall health in the long term.

**CHAPTER 8: THE EFFECT OF PRE-SLEEP  
CARBOHYDRATE MANIPULATION ON GUT  
MICROBIOME: A PROOF OF CONCEPT STUDY.**



## 8.1 Introduction

Health is a multidimensional concept that includes both mental and physical wellness. It is well established that there are three main cornerstones that biologically preserve health and well-being: nutrition, exercise and sleep [31,496]. Recent findings underline the complex and interactive relationships among them, mediated by several biological factors that remain to be explored, such as the gut microbiome [424].

In fact, it is estimated that humans host as many microbial cells as human cells [159]. Weighting only about 0.3% of the human body, microbes interact with their micro-environment, having a great impact on human biological processes [158,180]. Gut microbiota can indirectly affect a plethora of vital functions through the digestion of specific nutrients such as SCFAs and tryptophan metabolites [156,158]. The latter can cross the gastro-intestinal barrier, enter the bloodstream and pass through the blood-brain barrier, affecting host behaviour [168,182].

Over the last few years, it has been recognized that gut microbiome is susceptible to a number of lifestyle alterations. Diet is probably the most established modulator that shapes gut microbiome in both positive and negative ways [178], with significant alterations that can occur within 24h of dietary change [178]. There are two distinct routes by that nutrition affects microbiota: i) by increasing or decreasing the population of specific species of bacteria or ii) by regulating the metabolites produced in the intestine [158]. Not only that but physical activity is another important modifying factor for the intestinal microbiome. Exercise affects bacterial diversity, both in terms of the number and the taxa that colonize the human gastrointestinal tract [158,275].

Taken altogether, it is profound that the effect of gut microbiome on human health is tremendous. However, there is no available research exploring gut microbiome responses to integrative lifestyle changes, such as exercise and nutrition. Since gut microbiome formation is closely related to carbohydrate metabolism [218-220,331], and exercise [158,275,389,402,403], it would be of utmost importance to elucidate potential alterations in response to relevant interventions. Hence the purpose of the present study was to examine the effect of a combined exercise and nutrition intervention, modulating carbohydrate periodization in both quantity and quality over a 4-week period on gut microbiome formation.

Considering the significance of gut microbiome in human health, it is crucial to investigate the responses to comprehensive lifestyle modifications, such as exercise and nutrition. In this study, we employed quantitative conventional PCR to examine the impact of a 4-week combined exercise and nutrition intervention, involving carbohydrate periodization in terms of quantity and quality, on the gut microbiome composition (focusing on the *Bifidobacterium* and *Clostridium* genera). We concentrated on individuals in our cohort for whom the dietary intervention has affected Total Sleep Time (TST). Upon clustering our data, this targeted

approach will serve as a proof of principle, paving the way for more extensive microbiome profiling in future research.

## **8.2 Materials and Methods**

### **8.2.1 Participants**

Forty-two healthy, physically active males were recruited for this study. All volunteers were accustomed to both resistance and HIIT exercise training (specific inclusion and exclusion criteria described in chapter 4). Participants had a mean ( $\pm$  SD) age of  $27 \pm 7$  years, height of  $177 \pm 6$  cm and body mass of  $80.3 \pm 11.1$  kg. All participants had been informed about the purpose of the study and provided written consent. The study was approved by the Cyprus Bioethical Committee (EEBK/EII/2020/65) and with a NCT number: NCT05464342. All of the procedures were conducted according to the manual of the Declaration of Helsinki in 1964 and its later amendments.

### **8.2.2 Study Design**

This was a randomized controlled trial study, with a parallel design. Specifically, the investigation consisted of three nutrition intervention groups focusing on the effect of pre-bed periodization of carbohydrate consumption quantity and quality on the gut microbiome. Alongside nutrition intervention, participants performed a supervised standardized exercise program combining resistance exercise and HIIT sessions. All nutrition and exercise intervention procedures are described in detail in chapters 4.2 and .3.

At baseline, participants underwent preliminary testing in order to evaluate their nutritional status, their basic somatometric and anthropometric data, as well as their fitness level in order to design personalized exercise and nutrition interventions for each participant. Both at baseline and at the end of the intervention, participants were asked to collect actigraphic data and sleep diary data for one week prior to the beginning of the intervention and at the end of the intervention in order to assess sleep duration. Furthermore, at the same time points, stool samples were collected in order to investigate alterations in specific gut bacteria. The  $\Delta$ Changes of sleep duration were used as an indicator of “good responders” and “poor responders”, discriminating the sample into two distinct groups. These groups consisted of the upper 12% and lower 12% of the sample with the greatest sleep duration differentiation. Following the determination of these groups, the clustered data were used as the new cohort for further investigation. The Bifidobacterium and Clostridium genera within the gut microbiome of these participants was investigated, in a semi-quantitative way, using convention PCR to assess the impact of the lifestyle interventions on microbiome composition. The main phase of the current



study took place in the Human Biology laboratory of the Department of Life Sciences of the University of Nicosia and the fitness premises (UFIT gym) of the University of Nicosia.

### **8.2.3 Assessments**

#### ***Anthropometry and Body Composition***

Anthropometrics and body composition data were collected at the baseline of the intervention, as described in chapter 4.4. Individuals were barefooted and lightly dressed. Weight and standing height were measured to the nearest 0.1kg and 0.5cm, respectively.

#### ***Sleep-related Data***

Sleep evaluation was performed at both baseline and at the end of the intervention. Total Sleep Time was assessed with actigraphy, as described in detail in chapter 4.4. Each subject was asked to wear the actigraph on their non-dominant wrist, for seven days both at baseline and at the end of the intervention. In addition to the actigraphy devices, participants recorded their sleep habits using sleep diaries used to define the scoring interval for the actigraphic sleep. The average sleep duration of the seven days at the baseline and at the end of the intervention were used to calculate the  $\Delta$ Changes (POST-PRE) for TST, in order to cluster the sample on “good responders” vs “poor responders”.

#### ***Gut Microbiome Analysis***

In order to study the participants’ gut microbiome, stool samples were collected from participants at baseline and at a one-month period for each trial, as described in detail in chapter 4.4. Afterwards, DNA was extracted, and the presence of Bifidobacterium and Clostridium genera was examined semi-quantitatively using specific primers and conventional PCR.

### **8.2.4 Statistical Analysis**

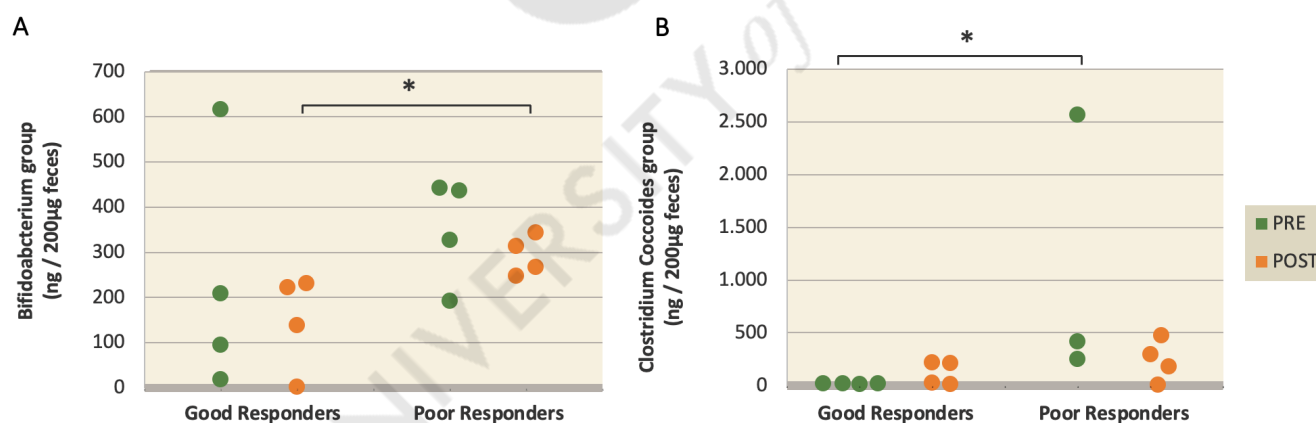
Continuous variables are presented as mean  $\pm$  standard deviation. Inferential statistical analyses were conducted using IBM®SPSS® statistics for Windows, version 25.0 (IBM Corp, Armonk, NY). Variables’ distribution was identified using the Shapiro-Wilks test. In order to compare different levels of Bifidoabcterium and Clostridium genera between poor responders and good responders, Mann-Whitney U test was used with a correlational effect size. Alpha level for all statistical analyses was set at  $p < 0.05$ , two-tailed tests.

## **8.3 Results**

### 8.3.1 Gut Microbiome

At baseline, there was a trend towards higher levels of both Bifidobacterium and Clostridium genera levels in individuals with increased sleep duration compared to individuals with lower sleep duration. Specifically, individuals who slept more than 360min had higher levels of Bifidobacterium ( $394.9 \pm 179.8$  ng/200 $\mu$ g feces vs  $190.0 \pm 182.3$  ng/200 $\mu$ g feces,  $p > 0.05$ ) and Clostridium genera ( $1000.0 \pm 1372.9$  ng/200 $\mu$ g feces vs  $75.3 \pm 113.9$  ng/200 $\mu$ g feces,  $p > 0.05$ ) compared to individuals with less than 360min of total sleep time.

Moreover, significant variations were revealed for both the Bifidobacterium and Clostridium genera between good and poor responders (Figure 8.3.1.1). According to Bifidobacterium genus there were no significant differences at baseline between groups ( $235.0 \pm 266.3$  ng/200 $\mu$ g feces vs  $349.9 \pm 117.5$  ng/200 $\mu$ g feces,  $p > 0.05$ ). However, at the end of the intervention the Bifidobacterium group levels were significantly less in good responders compared to poor responders ( $148.4 \pm 106.4$  ng/200 $\mu$ g feces vs  $292.9 \pm 43.6$  ng/200 $\mu$ g feces;  $U = 0.0$ ,  $p = 0.021$ ,  $r = 0.81$ ). On the other hand, regarding Clostridium group, poor responders had significantly higher levels of clostridium genus at baseline, compared to good responders ( $18.4 \pm 2.7$  ng/200 $\mu$ g feces vs  $1076.0 \pm 1295.7$  ng/200 $\mu$ g feces;  $U = 0.0$ ,  $p = 0.034$ ,  $r = 0.79$ ). At the end of the intervention, no significant difference were found for the Clostridium group between groups ( $115.9 \pm 114.5$  ng/200 $\mu$ g feces vs  $238.1 \pm 195.1$  ng/200 $\mu$ g feces,  $p > 0.05$ ).



**Figure 8.3.1.1.** Intervention effect on Bacteroides and Clostridium genera between good and poor responders. \*Denotes statistically significant difference at the 0.05 level (2-tailed).

### 8.4 Discussion

The present study investigated for the first time both gut microbiome responses to a comprehensive lifestyle modification. Specifically, we focused on the effect of a 4-week combined exercise and nutrition intervention on Bifidobacterium and Clostridium genera, in individuals with the greatest sleep duration differentiation. Notably, it was revealed that in baseline the poor responders had significantly higher levels of Clostridium coccoides group than

the good responders, while at the end of the intervention the good responders had lower levels of bifidobacterium group compared to poor responders.

Clostridium and Bifidobacterium are anaerobic bacteria that are commonly found in the gut microbiome. Nevertheless the research on the relationship between Bifidobacterium and Clostridium with sleep is limited, there is some evidence pointing towards a positive relationship between gut microbiome diversity and sleep patterns [294,296]. In the present study it was found that there was a trend at baseline towards higher levels of both Bifidobacterium and Clostridium genera levels in individuals with increased sleep duration compared to individuals with lower sleep duration. In line with these results, there is evidence showing that the Firmicutes phylum (to which the Clostridium genus belongs) is positively correlated with both sleep duration and sleep efficiency [294]. Furthermore, the phylum Actinobacteria (to which the Bifidoabcterium genus belongs) is negative correlated with sleep fragmentation [294]. Moreover, in the present investigation, the specific gut microbiome genera were further explored beyond their relationship with baseline sleep duration, in order to identify potential alterations in response to the 4-week lifestyle intervention. It was showed for the first time that the baseline level of Clostridium Cocoides group was significantly higher in poor responders compared to good responders. On the other hand, good responders had lower levels of Bifidobacterium group compared to poor responders at the end of the intervention period. Even though there is a considerable amount of data indicating that both nutrition [217] and exercise interventions [158,275] are important modifying factors for the intestinal microbiome, there are no relevant data in the literature addressing the potential alterations after a nutrition and exercise intervention in gut microbiome discriminating poor and good responders.

Taken altogether, the findings of the present study highlight the impact of lifestyle interventions on gut microbiome formation and vice versa; the potential impact of certain type of bacteria on physiological responses that take place after lifestyle interventions. However, in the current study there are strengths and weaknesses that need to be addressed. Since there are no similar tagerted studies on the alterations in gut microbiome after a lifestyle intervention -with further focus on good responders and poor responders- it would be of further interest for a gut microbiome and miRNA profiling in all participants to be performed. However this could not be possible due to time and budget limitations. Hence, the results of the present study should be interepreted with caution. For example, the present study showed that the baseline level of Clostridium Cocoides was significantly higher in poor responders and at the end of intervention the levels of Bifidobacterium group were higher in poor responders, compared to good responders. Notably, these results are genus-specific and do not represent potential responses at phylum level.

Taking into account the rapid evolution of the gut microbiome field, the current approach is of utmost importance for several reasons. First, since the Bifidobacterium genus levels was different at the baseline on good vs poor responders, it could be used as a predictor marker for individualization of future nutrition and exercise interventions. In the same line, since Clostridium cocoides level were altered significantly at the end of the intervention between good and poor responders, it could be used as a marker of the efficiency of relevant lifestyle interventions in sleep-related parameters, and especially sleep duration.

Collectively, these results point towards the study of the gut microbiome composition regulation of poor and good responders to lifestyle interventions, in order to personalize future exercise and nutrition interventions and assess their efficiency. Moreover, based on these results, targeted nutrition and exercise interventions should be personalized and be implemented into lifestyle medicine as a potential complementary treatment for sleep disorders. Overall, the present study points towards the investigation of the unexplored field of gut microbiome regulation with regards to sleep, in order to elucidate potential biological mechanisms, assess the efficiency of lifestyle interventions and aid to design personalized interventions in order to promote promote restoration, recovery and overall health.

## **8.5 Conclusion**

To conclude, the results of the present study shows that the combined nutrition and exercise-intervention promotes significant alterations in gut microbiome formation paving the way for more extensive microbiome profiling in future research. Moreover, gut-microbiome alterations should be studied as potential biomarkers to design and assess the efficiency of nutrition and exercise interventions. Further studies are needed to explore this promising field and elucidate potential biological mechanisms.

**CHAPTER 9: INTEGRATIVE PHYSIOLOGICAL  
RESPONSES TO PRE-SLEEP CARBOHYDRATE  
MANIPULATION ON SLEEP, BODY COMPOSITION,  
EXERCISE PERFORMANCE AND GUT MICROBIOME:  
POOR VS GOOD SLEEPERS**

## 9.1 Introduction

Over the last few years, nutrition, exercise and sleep have been recognized as fundamental elements of health that promote and preserve mental and physical well-being [543,544]. Recent data reveal a complex and interactive association among them [424]. This relationship may affect each another directly or indirectly, through several physiological responses that remain to be explored, such as gut microbiome formation [424]. Based on this potential, lifestyle interventions are a hot topic in the literature, exploring the impact of exercise and nutrition interventions as therapeutic options to improve life quality in both general and clinical populations [545].

Nutrition is a critical part of a lifestyle that acts as a safeguard for health and well-being. Dietary behaviour affects a wide range of human physiological systems, having a profound effect on body composition [518]. This, of course, is of high significance since both increased adiposity and decreased muscle mass are closely related to higher morbidity and mortality rate [510-512]. Usually, nutrition modifications are examined as an integral part of lifestyle medicine, including both foods [74] or nutrient-based supplements [455]. However, there are also specific nutritional patterns, such as carbohydrate intake, that affect directly a plethora of biological systems, such as gut microbiome [178].

An additional pillar of lifestyle medicine is exercise. Growing evidence suggests that a healthy nutrition plan combined with regular physical activity, are key lifestyle factors that modulate overall health, improving people's quality of life [516]. Furthermore, exercise-induced stress stimulates muscle protein synthesis, promoting muscle hypertrophy and body re-composition [517]. As with nutrition, responses to exercise extend to other physiological systems. For example, exercise is related to gut bacterial diversity, and specific circulating miRNAs, regulation [389,402,403]. This is crucial, since the latest data show that several responses to exercise, such as muscle hypertrophy, angiogenesis, anti-inflammatory and antioxidant responses may be mediated by miRNA expression [380-382].

Another component of lifestyle medicine that has gained a lot of attention over the last few decades is sleep. Sleep research evolution unveiled the innumerable vital physiological functions of sleep, including metabolic clearance in the central nervous system [492], cognition [135], immune function [431] and overall tissue restoration [432]. However, sleep is often studied as an outcome of nutrition [496] and exercise interventions [31], and not as an intervention by itself. This is because sleep patterns cannot be allotted in specific increments, as opposed to the prescription of specific meal portions or exercise duration.

Nevertheless, exercise and nutrition are significantly related to sleep [31,498]. On the other hand, alterations in sleep-related parameters could potentially trigger gut microbiome [288,301]. For

example, inadequate sleep has been constantly related to gut dysbiosis [288,301]. Hence, it is implied that “poor sleepers” develop a specific physiological “profile” that is different compared to “good sleepers”. This physiological profile, extends further than gut microbiome formation, affecting directly the response to drug prescription among poor and good sleepers [546].

Taking into account that the prevalence of inadequate sleep has dramatically increased [452] and this phenomenon is associated with numerous diseases [453], there is a fundamental need to explore alternative, non-pharmacological lifestyle interventions to optimize sleep. Moreover, the lack of relevant data in the literature highlights the need to study responses to lifestyle changes comprehensively, in order to unveil potential physiological mechanisms. As such, the aims of the present study are two-fold: i) to identify potential interrelationships between sleep, gut microbiome, exercise and body composition indices after a long-term nutrition and exercise intervention and ii) to identify the effect magnitude of the intervention on poor and good sleepers.

## **9.2 Materials and Methods**

### **9.2.1 Participants**

Forty-two healthy, physically active male volunteers were recruited for this study. Specific inclusion and exclusion criteria are described in Chapter 4. Participants had a mean ( $\pm$  SD) age of  $27 \pm 7$  years, height of  $177 \pm 6$  cm and body mass of  $80.3 \pm 11.1$  kg. All participants had been informed about the purpose of the study and provided written consent. The study was approved by the Cyprus Bioethical Committee (EEBK/EII/2020/65) and with a NCT number: NCT05464342. All of the procedures were conducted according to the manual of the Declaration of Helsinki in 1964 and its later amendments.

### **9.2.2 Study Design**

The present study was a randomized controlled trial with a parallel design. Three nutrition intervention groups were investigated with regard to integrative physiological responses to pre-bed periodization of carbohydrate consumption quantity and quality and discriminate these responses on poor vs good sleepers. Specifically, the nutrition intervention groups were: i) Sleep Low- No Carbohydrates (SL-NCHO): participants consumed all their carbohydrate intake at regular intervals prior to the evening exercise training session, ii) Sleep High- Low Glycemic Index (SH-LGI) and iii) Sleep High- High Glycemic Index (SH-HGI): Carbohydrate intake was spread evenly throughout the day both prior (60% of total CHO intake) and after (40% of total CHO intake). The SH-LGI and SH-HGI groups differentiated in the evening

carbohydrate quality, consuming either LGI or HGI foods, respectively. Alongside nutrition intervention, participants performed a supervised standardized exercise program combining resistance exercise and HIIT sessions. All nutrition and exercise intervention procedures are described in detail in chapters 4.2 and 4.3.

At baseline, participants underwent preliminary testing in order to evaluate their nutritional status, their basic somatometric and anthropometric data, and their fitness level in order to design personalized exercise and nutrition interventions for each participant.

Both at baseline and at the end of the intervention, a sleep study was conducted, with the gold-standard method of polysomnography. In addition, participants were asked to collect actigraphic data and sleep diary data for one week prior to the beginning of the intervention and at the end of the intervention. At these specific time points, participants were also interviewed for their sleep quality, daytime sleepiness and fatigue.

Fitness and exercise performance was evaluated in the same time points, by assessing countermovement jump, visual reaction time,  $VO_{2peak}$  and 1RM in hack squat, plate-loaded bench press, plate-loaded shoulder-press and lat-pulldown.

Furthermore, both at baseline and at the end of the intervention, stool samples were collected in order to investigate alterations in specific gut bacteria. The main phase of the study took place in the Human Performance Lab of the University of Nicosia, the laboratory of the department of Human Biology of the University of Nicosia and the fitness premises (UFIT) of the University of Nicosia.

### **9.2.3 Assessments**

#### ***Anthropometry and body composition***

Anthropometrics and Somatometrics were collected at the baseline of the intervention, as described in chapter 4.4. Individuals were barefooted and lightly dressed. Weight and standing height were measured to the nearest 0.1kg and 0.5cm respectively. Body composition was assessed with both an estimation of body density via Harpenden skinfold caliper using Jackson's and Pollock's 7-site method and by bioelectrical impedance analysis (BIA).

#### ***Physical Fitness and Exercise Performance***

Physical fitness and exercise performance evaluation was performed at both baseline and at the end of the intervention. Assessments included CMJ, VRT,  $VO_{2peak}$  and 1RM as described in detail in chapter 4.4. Participants performed three CMJ and the highest one was recorded with an accuracy of 0,01cm. Following, a visual reaction test (VRT) was performed, consisting of three visual stimuli. The reaction time after each stimulus was recorded at the nearest 0.001



second and the average VRT was calculated. Afterwards,  $VO_{2peak}$  was assessed with a treadmill protocol. Heart rate was continuously monitored. Moreover, at the above-mentioned time points, participants' 1 repetition maximum in specific exercises was assessed in hack-squat, plate-loaded bench press, plate-loaded shoulder-press and lat-pulldown.

### ***Sleep-related Data***

In-detail sleep evaluation was performed at both baseline and at the end of the intervention. Sleep assessments included polysomnography, actigraphy, sleep diary, PSQI, ESS and FSS, as described in detail in chapter 4.4. Sleep architecture was assessed with Polysomnography. The PSG examination was performed at the participant's home in order to maintain as possible their sleep routine and environment. Polysomnograms were collected overnight. Sleep stages and arousals were determined using the standard criteria of the American Academy of Sleep Medicine (AASM).

Sleep initiation and maintenance, as well as intra-individual standard deviations for each sleep measure were assessed with actigraphy. Each subject was asked to wear the actigraph on their non-dominant wrist, for seven days both at baseline and at the end of the intervention. In addition to actigraphy devices, participants recorded their sleep habits using sleep diaries used to define the scoring interval for actigraphic sleep. Moreover, at the same time points, participants were asked to complete PSQI, ESS, and FSS respectively in order to assess their sleep quality, daytime sleepiness and fatigue, respectively.

The  $\Delta$ Changes of the average total sleep time derived from actigraphic recordings were used as an indicator of "good responders" and "poor responders", discriminating the sample into two distinct groups. These groups consisted of the upper 12% and lower 12% of the sample with the greatest sleep duration differentiation. Following the determination of these groups, the clustered data were used as the new cohort for further investigation, in order to analyze gut microbiome.

Furthermore, in this study subjects were also classified as "poor" or "good" sleepers according to their baseline TST, SE and PSQI scores. In particular, the sample was clustered in tertiles for these particular variables, and statistical analyses were performed comparing good sleepers to poor sleepers. For TST the two cut-offs were 331.2min and 383.2 min, for %SE were 74% and 79.8%, and for PSQI scores were 4 and 9. For TST and %SE, subjects in the first tertile were classified as poor sleepers, while for PSQI, subjects in the third tertile were classified as poor sleepers. Contrarily, for TST and %SE, subjects in the third tertile were classified as good sleepers, while for PSQI, subjects in the first tertile were classified as good sleepers.

### ***Gut Microbiome Analysis***

In order to study the participants' gut microbiome, stool samples were collected at baseline and at a one-month period for each trial as described in detail in chapter 4.4. Stool samples were analyzed from specific participants as described in the chapter 9.2.2. Microbial DNA was extracted using the PureLink Microbiome DNA Purification Kit (Invitrogen, ThermoFisher Scientific, Carlsbad, California, USA) according to the manufacturer's instructions. Afterwards, the concentration and purity of the extracted DNA was assessed using spectrophotometry. Conventional polymerase chain reaction (PCR) with specific primers was designed to amplify the 16S rRNA gene sequences specific to the Bacteroides and Clostridium genera. Finally, amplified DNA fragments were separated using agarose gel electrophoresis and the resulting band patterns were used to determine the relative abundance of the Bacteroides and Clostridium genera.

#### **9.2.4 Statistical Analysis**

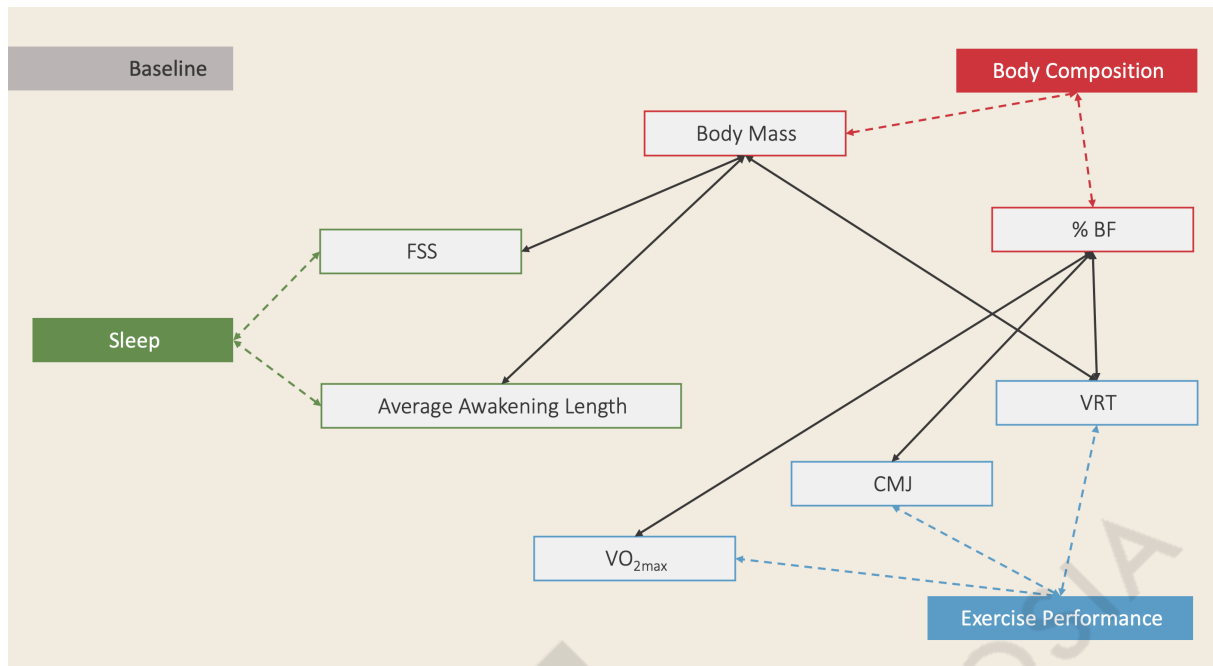
Continuous variables are presented as mean  $\pm$  standard deviation and non-continuous variables as median (interquartile range). Inferential statistical analyses were conducted using IBM®SPSS® statistics for Windows, version 25.0 (IBM Corp, Armonk, NY). Potential correlations were identified by estimating Pearson's  $r$  or Spearman's correlation coefficient for normal and non-normal distributed variables, respectively. In order to compare  $\Delta$ changes between poor sleepers and good sleepers in the same group, Mann-Whitney U test was used with a correlational effect size. Differences of  $\Delta$ -changes among poor or good sleepers between interventions were explored using the Kruskal Wallis test, using the  $\eta^2$  to illustrate the effect size. Alpha level for all statistical analyses was set at  $p < 0.05$ , two-tailed tests.

### **9.3 Results**

#### **9.3.1 Integrative responses on Sleep, Body Composition and Exercise Performance**

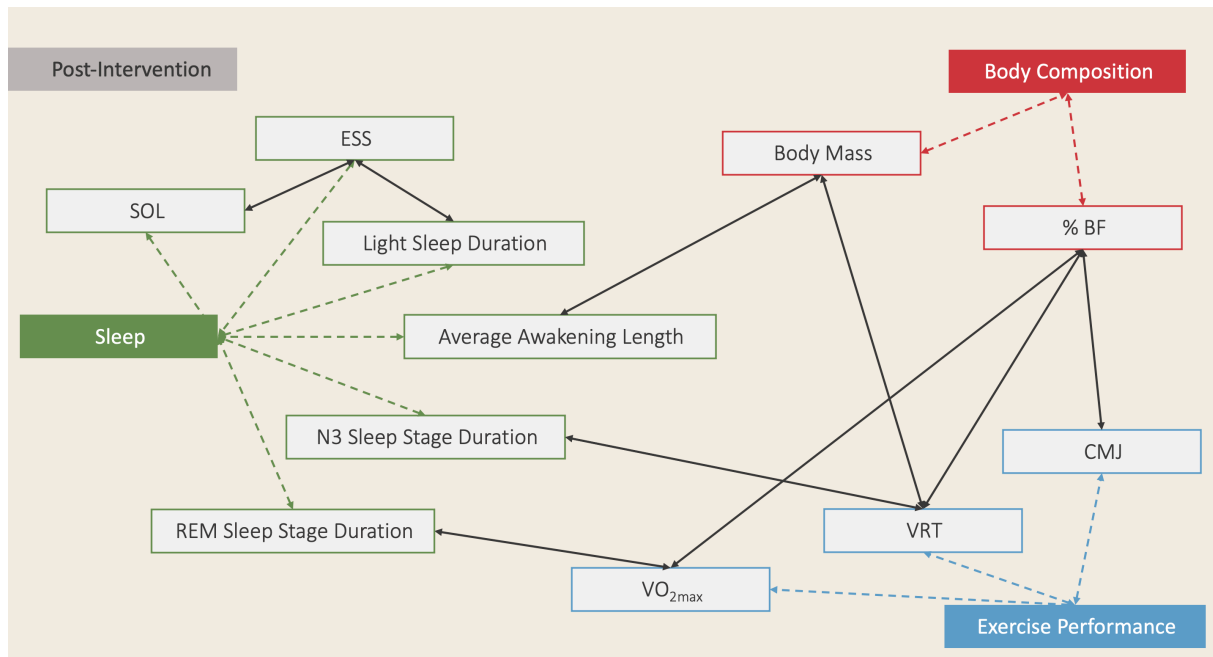
At the baseline, there were no significant relationships between objectively and subjectively measured sleep-related variables (Figure 9.3.1.1). In the same line, no significant association were observed between sleep-related parameters and exercise performance indices. With regards to sleep and body composition, increased average awakening length during nocturnal sleep was related to increased body weight ( $r=0.441$ ,  $p=0.003$ ), and increased body weight was also related to increased FSS scores ( $r = 0.433$ ,  $p = 0.004$ ). Furthermore, body composition measures were related to exercise performance indices. For example, increased body weight was significantly related to increased VRT time ( $r=0.538$ ,  $p<0.001$ ). On the other hand,

increased %BF was related to decreased CMJ performance ( $r=-0.500$ ,  $p=0.001$ ), VRT performance ( $r=0.557$ ,  $p<0.001$ ) and  $VO_{2peak}$  ( $r=-0.431$ ,  $p=0.006$ ).



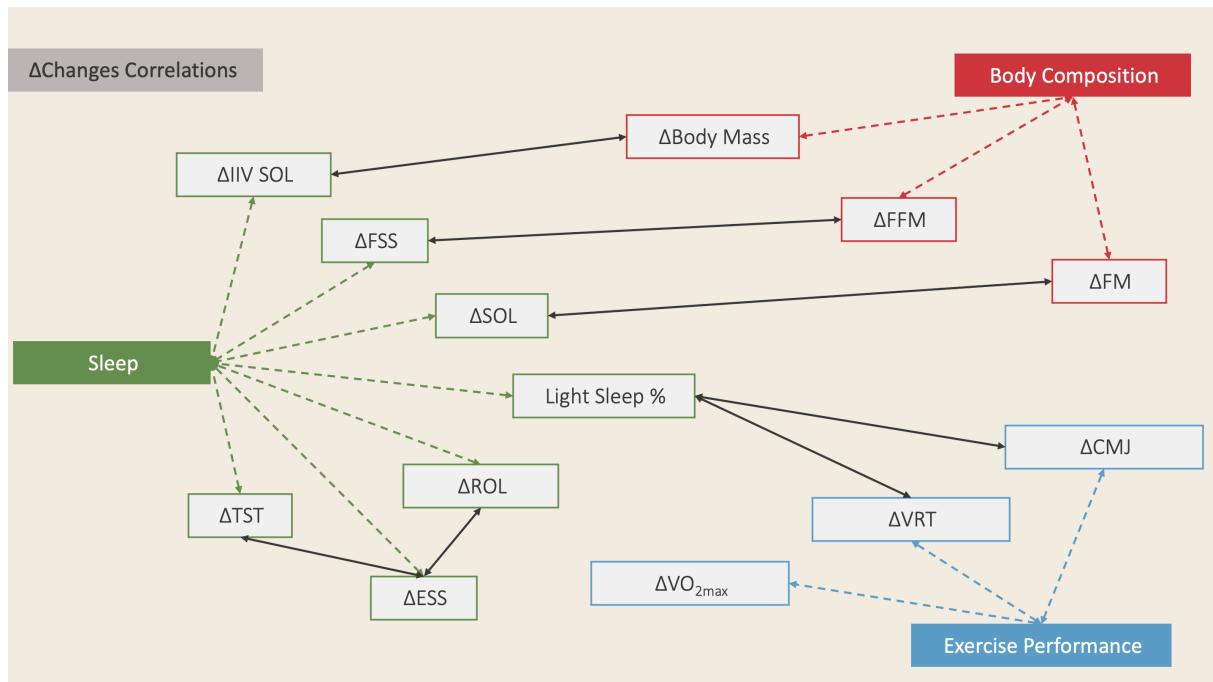
**Figure 9.3.1.1.** Significant Correlations between sleep, body composition and exercise performance at baseline.

Several of these associations were altered at the end of the intervention period (Figure 9.3.1.2). There were significant correlations between objectively and subjectively assessed sleep-related measures. For example, post-intervention ESS scores were inversely related to both Light Sleep Duration -derived from the sum of N1 and  $\Delta N2$  sleep stage duration- ( $r=-0.457$ ,  $p=0.014$ ) and SOL ( $r=0.322$ ,  $p=0.037$ ). Moreover, there were significant associations between sleep-related parameters and exercise performance indices. Specifically, REM duration was positively related to  $VO_{2peak}$  ( $r=0.453$ ,  $p=0.023$ ) and increased N3 duration was related to decreases in VRT, indicating improved performance ( $r=-0.421$ ,  $p=0.023$ ). In line with the pre-intervention data, sleep was related to body composition as well. In particular, increased average awakening length was significantly related to increased body weight ( $r=0.373$ ,  $p=0.015$ ). On the other hand, increased weight was significantly related to increased VRT time, indicating lower performance ( $r=0.340$ ,  $p=0.030$ ), while %BF was again inversely related to both CMJ performance ( $r=-0.536$ ,  $p<0.001$ ), VRT performance ( $r=0.369$ ,  $p=0.017$ ) and  $VO_{2peak}$  ( $r=0.581$ ,  $p<0.001$ ).



**Figure 9.3.1.2.** Significant Correlations between sleep, body composition and exercise performance at post-intervention.

Additionally, potential associations between the  $\Delta$ -Changes of the assessed variables in response to the intervention were further explored (Figure 9.3.1.3). It was found that there were significant associations between objectively and subjectively measured sleep-related variables. In this sense,  $\Delta$ ESS scores were inversely related to  $\Delta$ TST ( $r=-0.325$ ,  $p=0.036$ ) and positively related to  $\Delta$ REM Onset Latency ( $r=-0.325$ ,  $p=0.036$ ). However,  $\Delta$ ESS scores were not related to N3 Onset Latency ( $p > 0.05$ ). With regards to sleep and body composition, increased  $\Delta$ SOL IVV was significantly associated with increased  $\Delta$ Weight ( $r=0.348$ ,  $p=0.024$ ). Moreover,  $\Delta$ SOL was significantly associated with FM ( $r=0.400$ ,  $p=0.009$ ) but not with FFM ( $p > 0.05$ ). However,  $\Delta$ FFM was inversely related to  $\Delta$ FSS scores ( $r=-0.325$ ,  $p=0.036$ ). With regards to sleep and exercise performance, Delta changes in sleep-related variables were translated to improvements in CMJ and VRT performance. Specifically, changes in Light Sleep % TST was positively related to both CMJ ( $r=0.486$ ,  $p=0.010$ ) and VRT performance ( $r=0.484$ ,  $p=0.011$ ). On the other hand, there was no significant association between  $\Delta$ Deep Sleep (N3 sleep stage) or REM sleep stage and CMJ or VRT ( $p > 0.05$ ).



**Figure 9.3.1.3.** Significant Correlations between sleep, body composition and exercise performance delta changes.

### 9.3.2 Relationship between Gut Microbiome Formation and with Sleep-related Parameters

Gut microbiome formation was apparent mediating factors of the effect of the intervention on sleep-related parameters. With regards to gut microbiome composition, it was shown that the post-intervention levels of the Bifidobacterium genus were inversely related to sleep efficiency ( $r=-0.846, p=0.008$ ) and sleep duration ( $r=-0.754, p=0.031$ ). In the same line, increased levels of the Bifidobacterium group was related to increased awakening numbers ( $r=0.766, p=0.027$ ). On the other hand, the Clostridium genus prevailed a strong relationship with sleep architecture. Specifically, the  $\Delta$ change of the Clostridium group fecal levels were inversely related to N3 sleep stage duration ( $r=-0.901, p=0.037$ ) and positively correlated with Light sleep % TST ( $r=0.902, p=0.036$ ). In the same line, the Clostridium genus was positively related with ESS scores ( $r=0.839, p=0.009$ ).

### 9.3.3 Integrative physiological adaptations in poor sleepers vs good sleepers

Exploring further the physiological adaptations in poor vs good sleepers in each trial, significant differences were revealed (Table 9.3.3.1). According to baseline sleep duration classification, it was found that in the SL-NCHO trial, deep sleep was increased more in individuals with shorter sleep duration than in those with increased sleep duration ( $6.2 \pm 5.8\%$  vs  $-13.5 \pm 12.9\%$ ,  $p=0.002$ ;  $U=0.5, p=0.036, r=0.74$ ). In the same line, individuals with shorter sleep duration in the SL-NCHO individuals increased more their  $VO_{2peak}$  ( $6 \pm 2.8$  vs  $1 \pm 1.6$ ,  $p=0.039$ ;  $U=1.0$ ,

$p=0.049$ ,  $r=0.68$ ) than those with increased sleep duration. Importantly though, individuals with inadequate sleep duration increased more their TST ( $73.9 \pm 31.9$  vs  $-3.5 \pm 65$ ,  $p=0.031$ ;  $U=2.0$ ,  $p=0.041$ ,  $r=0.61$ ) when following the LGI than individuals who had higher sleep duration at the baseline. When the sample was categorized according to baseline SE, it was shown that individuals that slept with low efficiency at baseline, increased even more their percentage of N3 sleep stage ( $11.3 \pm 8.9\%$  vs  $-10.1 \pm 12.5\%$ ,  $p=0.029$ ,  $r=0.77$ ) after following the NCHO, than those who slept with higher efficiency at baseline. Accordingly, individuals who slept with low efficiency at baseline, improved more their VRT performance, than individuals that sleep higher efficiency in the SH-HGI group ( $-0.047 \pm 0.04\text{sec}$  vs  $0.009 \pm 0.03\text{sec}$ ,  $p=0.047$ ;  $U=7.0$ ,  $p=0.046$ ,  $r=0.55$ ). With regards to PSQI categorization, individuals with low sleep quality at baseline, increased even more their performance at VRT ( $-0.051 \pm 0.33\text{sec}$  vs  $0.040 \pm 0.06\text{sec}$ ,  $p=0.036$ ;  $U=0.0$ ,  $p=0.014$ ,  $r=0.80$ ) than individuals with higher sleep quality index, after following the HGI Nevertheless, no significant differences were found when exploring the different responses on poor sleepers between trials ( $p>0.05$ ).

**Table 9.3.3.1.** Intervention effects on poor vs good sleepers, according to TST (min), % SE and PSQI scores.

Variable	Classification based on TST (min)			Classification based on % SE			Classification based on PSQI scores		
	SL-	SH-	SH-	SL-	SH-	SH-	SL-	SH-	SH-
	NCHO	LGI	HGI	NCHO	LGI	HGI	NCHO	LGI	HGI
$\Delta$ TST (min)		+							
$\Delta$ SE (%)									
$\Delta$ SOL (min)									
$\Delta$ WASO (min)									
$\Delta$ N1 % TST									
$\Delta$ N2 % TST									
$\Delta$ N3 % TST	+			+					
$\Delta$ REM % TST									
$\Delta$ VO <sub>2peak</sub> (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )	+								
$\Delta$ CMJ (cm)									
$\Delta$ VRT (sec)						+			+

Variable	Classification based on TST (min)			Classification based on % SE			Classification based on PSQI scores		
	SL-	SH-	SH-	SL-	SH-	SH-	SL-	SH-	SH-
	NCHO	LGI	HGI	NCHO	LGI	HGI	NCHO	LGI	HGI
$\Delta$ IRM (ALL)									
$\Delta$ BF (%)									

SL-NCHO, Sleep Low- No Carbohydrates; SH-LGI, Sleep High- Low Glycemic Index; SH-HGI, Sleep High- High Glycemic Index; IIV, Intra-Individual Variability; TST, Total Sleep Time; REM, Rapid Eye Movement;  $VO_{2peak}$ , Maximal Oxygen Consumption; CMJ, Counter Movement Jump; RM, Repetition Maximum; “+” Denotes statistically significant differences at the 0.05 level (2-tailed)

## 9.4 Discussion

The purpose of the present study was to investigate physiological responses to carbohydrate timing and quantity manipulation on sleep, body composition, exercise performance and gut microbiome collectively. Several associations between these domains were found both at baseline and at the end of the intervention; notably, the intervention-induced improvements in sleep-related parameters were translated to improvements in body composition and exercise performance indices. These sleep-related alterations were also associated with specific gut microbiome formation. Furthermore, this is the first study to explore the response of each nutrition intervention on poor sleepers vs good sleepers, depending on individuals’ baseline sleep habits. It was revealed that the SL-NCHO increased more the deep sleep stage in individuals with inadequate sleep duration and low sleep efficiency, compared to good sleepers, while the SL-LGI trial prolonged more total sleep time in these individuals, compared to good sleepers. On the other hand, the SH-HGI trial improved more VRT performance in both individuals with low sleep efficiency and low self-reported sleep quality, compared to good sleepers.

Overall, relationships between nutrition, exercise and sleep variables altered from pre to post intervention period. At baseline, the main sleep domains were related to body composition but not exercise performance, while at the end of the intervention period, sleep, body composition and exercise performance were interrelated altogether. This could be indicative not only of the intervention effect on these variables, but highlight the complex relationships among all these variables. In line with these results, ambiguous associations between these variables are present in the literature as well. For example, sleep quality is positively related to higher  $VO_{2max}$  in

healthy individuals [105]. However, no similar association appears in athletic populations between sleep quality and  $VO_{2max}$  [106,107].

On the other hand, intervention-induced improvements in sleep-related variables were related to both body composition optimization and exercise performance. Specifically, decreased average sleep onset latency and its variability, were related to decreases in fat mass and body mass respectively, while sleep architecture was related to both CMJ and VRT. These associations are consistent with previous findings that showed a positive impact of sleep extension on body composition optimization [547,548]. This relationship is shown to be reciprocal, since there are negative effects on body composition when sleep is disturbed [547,548].

With regard to exercise performance, light sleep was related to both CMJ and VRT performance. Overall, as shown by the present findings, it could be hypothesized these exercise performance improvements could be attributed to the N2 sleep stage restorative properties [549]. Previous studies have shown that sleep extension may improve reaction time performance [33,535], and, as in body composition, sleep disturbances may negatively impact alertness [536]. On the one hand, since there are indications that acute carbohydrate interventions may aid sleep [496], these potential sleep improvements could potentially mediate this effect on VRT performance [33,536,537]. On the other hand, with regard to CMJ performance, other studies didn't show any significant relationship between CMJ and sleep extension acutely [8]. However, this could be attributed to the duration of the present intervention, lasting for 4-weeks in order for potential adaptations to take place.

In the present study it was revealed that there was a significant association between sleep, gut microbiome formation in both poor and good responders. Specifically, it was showed that increased levels of Bifidoabcterium genus at the end of the intervention period, were related to decreased sleep duration and efficiency, and increased number of awakenings. Another key finding was that high levels of Clostridium Cocoides were proportionally related to light sleep and were inversely related to subsequent increases in deep sleep stage. In literature, bacterial diversity and the presence of the Firmicutes phylum (to which the Clostridium genus belongs) are positively correlated with sleep efficiency and total sleep time [294]. In the same line, the Actinobacteria phylum (to which the Bifidoabcterium genus belongs) is negatively associated with the number of awakenings, hence sleep fragmentation [294]. The phenomenical discrepancy of the results of this investigation with the currently available literature could be attributed to the fact that in the present study only specific genera were targeted. In this sense, the present results are genus-specific and may not represent potential responses at phylum level. The effect of carbohydrate manipulation extended beyond, to specific responses on sleep optimization and athletic performance as well. Particularly, deep sleep was increased more in



people with inadequate sleep duration or low sleep efficiency after the SL-NCHO trial, compared to good sleepers. In line with these results, there are studies that showed similar acute effects of decreased evening carbohydrate intake on N3 sleep stage [47,52,53,463]. Moreover, individuals with short sleep duration increased their total sleep time more than good sleepers in the SH-LGI trial. This is very interesting, taking into account that, as showed in previous study in chapter 4, the SH-LGI not only increased TST significantly, but reduced the intraindividual variability of sleep duration, compared to pre-intervention. However, research on long-term sleep optimizing long-term nutrition interventions is scarce, and thus more studies are needed to comparatively investigate these results.

With regards to exercise performance, subjects with low sleep duration increased more their maximum aerobic capacity after the SL-NCHO. Literature is inconclusive with regards to these findings since post-exercise carbohydrate intake may affect directly glycogen resynthesis and recovery [523]. However, data are limited and there is no evidence to date that suggests a direct relationship between post-workout carbohydrate consumption and  $VO_{2max}$ , especially when daily carbohydrate intake is equated. In this sense, the data of the study in chapter 5, showed that when adequacy in nutrients is met, there are no alterations in exercise performance over a period of four weeks. However, there may be an indirect role of N3 on self-perceived sleep quality and  $V_{2Omax}$ . Typically, in the literature, tissue restoration after extreme endurance activities is linked to N3 sleep [35,36]. Hence, since poor sleepers achieved a better prolongation of N3 sleep stage, and its subsequent restorative properties, it may aid in improving the  $VO_{2max}$  in the long term. According to the VRT test, it was significantly improved more after the SH-HGI trial in people with lower sleep efficiency and decreased self-reported sleep quality, compared to good sleepers. Even though the available data are scarce, there are indications that the VRT is increased acutely after a post-workout HGI meal and this is often attributed to sleep-related improvements [8].

A number of biological mechanisms have been reported in the literature that could partially explain the results of the present study. For example, several individual studies that modify CHO quantity acutely show alterations in sleep architecture, promoting deep sleep [47,52,53,463]. This effect of CHO intake in sleep architecture is often attributable to various biological mechanisms related to diet-dependent hormonal regulation and specifically cholecystokinin (CCK) regulation [496]. Both in rats and rabbits, intraperitoneal injection of cholecystokinin shows a dose-response relationship between circulating CCK levels and N3 sleep stage. This bears high significance because CHO intake stimulates cholecystokinin (CCK) release to a less extent than dietary fat and protein [54,55]. Thus, it has been shown that a meal low in CHO and high in protein and fat led to higher postprandial CCK concentrations and increased subjective feelings of sleepiness [55].

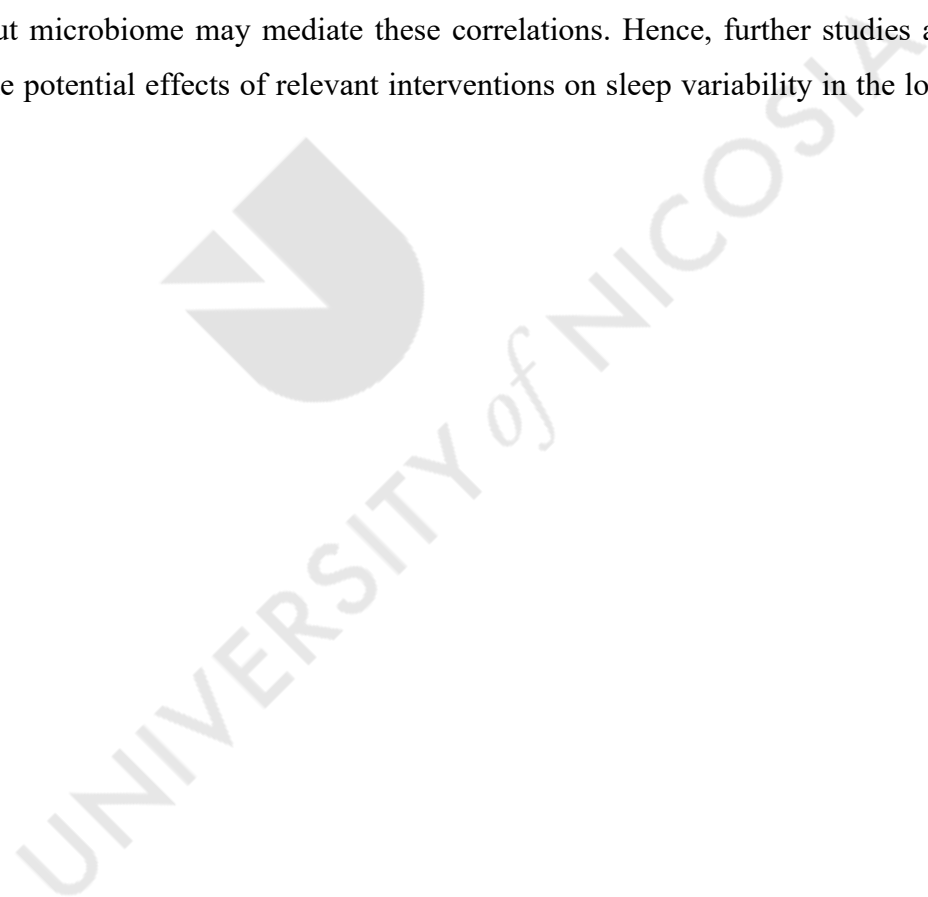
With regard to the effect of the manipulation of carbohydrate quality in relevant physiological systems, there are inconclusive data in the literature. While some of the studies found significant improvements of a pre-bed HGI meal in SOL, SE or WASO [8,49], others found weak or no effect [50,471]. Hence, even though acute studies point towards the potential superiority of pre-bed HGI meal in sleep-related parameters, in the present study, the SL-NCHO trial showed significant improvements in deep sleep in individuals with decreased sleep duration or low efficiency, while TST was improved more in the SL-LGI group. Even though there are very limited relevant investigations, in the present study it was shown that gut microbiome mediate this relationship. This could be attributed to several neurotransmitters and hormones distributed in the intestine have both effects on the brain and gut [288-290], since gut microbiome formation are closely related to carbohydrate metabolism [218-220,331], and exercise [158,275,389,402,403]. In the current study there are several strengths but weaknesses that need to be addressed. First, due to financial constraints, it was unable to provide all day's meals to participants for the whole trial period. On the other hand, this led to the development of individualized, flexible and easily to adhere dietary plan, as shown by the present study's high compliance rate. In the same line, it would be of additional interest if full microbiome and miRNAs profiling could be conducted. Moreover, it would be of further interest to examine the effects of similar nutrition interventions on sleep-related parameters for even longer periods. Notably, even though this is the first study to examine comprehensively the responses to carbohydrate manipulation over a period of 4 weeks, it would be of utmost importance for further studies with a larger sample size to be conducted. In this sense, although categorization according to the baseline sleeping habits is crucial to further understand responses to a most vulnerable part of the population, the poor sleepers, who are largely understudied with regards to potential lifestyle interventions to promote better quality of life.

The findings of the present study are very promising, exploring both the integrative physiological responses to carbohydrate manipulation and its responses on poor vs good sleepers. Since there is a growing prevalence of poor sleepers [452], relevant interventions could be implemented in order to discover safe, non-pharmacological and efficient approach for sleep optimization. Not only that but, increases in VRT performance, indicate improvement in cognitive function [538], with further benefits in the overall quality of life. Altogether, these practical implications of the study extend beyond the limits of the current investigations for several reasons. This investigation points towards the comprehensive study of these complex relationships between exercise, nutrition and sleep. Towards this approach, this is the first study to approach the effect of a long-term lifestyle change on sleep-related parameters and propose the employ of CHO manipulation as a reasonable non-pharmacological tool for the modification of sleep in poor sleepers, if needed. Based on this, targeted nutrition interventions are promising

and point towards the further investigation of lifestyle medicine as a potential complementary treatment for sleep disorders. Furthermore, relevant interventions may act as a safeguard against inadequate sleep patterns in athletes, in order to promote restoration, recovery and overall health.

## **9.5 Conclusion**

To summarize, the present study shows that, carbohydrate intake timing can directly increase deep sleep in individuals with inadequate sleep duration and low sleep efficiency, compared to good sleepers, while pre-bed LGI meal may aid in increasing total sleep time in individuals who experience the same issue. On the other hand, pre-bed HGI seems to improve visual reaction time in both individuals with low sleep efficiency and low self-reported sleep quality, compared to good sleepers. Overall, complex relationships appear between exercise nutrition and sleep, while gut microbiome may mediate these correlations. Hence, further studies are needed to unveil the potential effects of relevant interventions on sleep variability in the long term.



## **CHAPTER 10 GENERAL DISCUSSION**



## 10.1 Discussion of major findings

Overall, the purpose of this investigation was to examine for the first time the effect of long-term carbohydrate periodization protocols on sleep initiation, maintenance and architecture, physical performance, body composition and gut microbiome in healthy, physically active individuals. The primary objective was to examine the effect of long-term carbohydrate quantity and quality manipulation on sleep-related parameters. As secondary objectives, were the effect of this intervention on exercise performance indices, body composition and gut microbiome was studied. Moreover, the integrated physiological response in these domains was elucidated, as well as targeted responses to the intervention in poor and good sleepers.

Collectively, the main findings of the present project are presented in Table 10.1.1. The meta-analysis and meta-regression of previous studies showed that acute interventions with low CHO quantity favored N3 sleep stage, whilst increased CHO intake prolonged REM sleep stage. Moreover, the acute manipulation of CHO quality affected measures of sleep continuity. The main results of the present intervention studies showed that sleep initiation, continuity and duration were improved in all trials, but sleep duration variability reduced significantly only in SL-NCHO and SH-LGI trials. Deep sleep was increased more in individuals with inadequate sleep duration and low sleep efficiency in the SL-NCHO trial, compared to good sleepers and sleep duration increased more in poor sleepers in the SH-LGI trial, than in good sleepers. Body fat mass, fat-free mass, as well as athletic performance, were significantly improved in all trials. In contrast, visual reaction time performance improved more in individuals with low sleep efficiency and low self-reported sleep quality in the SH-HGI trial, compared to good sleepers. Overall, complex relationships appeared between exercise nutrition and sleep, while gut microbiome mediated these correlations.

The acute manipulation of dietary carbohydrates has been highlighted over the years with regard to its effect on sleep-related parameters [470]. Glucose metabolism is proposed to be highly interrelated to sleep [45] by modifying the plasma tryptophan concentration [60], a precursor of serotonin and melatonin, which, in turn, is associated with acute alterations in sleep onset latency [49], sleep time [8] and sleep continuity [8]. In the present meta-analysis of clinical trials, described in Chapter 5, it was shown that CHO intake can significantly impact sleep architecture, sleep initiation and continuity. The main findings were that lower CHO intake is related to N3 sleep stage extension acutely, compared to higher dietary CHO intake. On the other hand, higher dietary CHO intake significantly prolonged REM stage sleep compared to lower CHO intake. Furthermore, it was revealed that the pre-bed quality of CHO intake did not show any significant effect on sleep architecture. On the other hand, alterations in the quality of carbohydrate intake showed a significant effect on measures of sleep continuity.

**Table 10.1.1.** Major Project Findings.

## Key Findings

- Study 1**
- Acute interventions with decreased intake of carbohydrate (CHO) quantity favors N3 stage sleep proportion and duration, whilst increased dietary CHO intake prolongs REM stage sleep.
  - The acute manipulation of the CHO quality does not show any significant effect on sleep stages, however affects measures of sleep continuity.
- Study 2**
- Habitual adherence to a personalized dietary plan with sufficient nutrient intake can significantly improve the majority of sleep-related parameters and as such, optimise sleep.
  - However, the quantity and quality of evening carbohydrate intake affect sleep duration variability, even when energy and nutrient intake are equated.
- Study 3**
- Exercise-induced improvements in body composition and exercise performance are not hindered by carbohydrate timing or pre-bed meal's carbohydrate quality, when energy and nutrient intake is equated.
- Study 4**
- The baseline level of Clostridium Cocoides genus was significantly higher in poor responders compared to good responders.
  - Good responders had lower level of Bifidobacterium group compared to poor responders at the end of the intervention period.
- Study 5**
- Decreased pre-bed carbohydrate intake may increase deep sleep in individuals with inadequate sleep duration and low sleep efficiency, compared to good sleepers
  - Pre-bed LGI meal may aid in increasing total sleep time in individuals who experience the same issue.
  - Pre-bed HGI seems to improve visual reaction time in both individuals with low sleep efficiency and low self-reported sleep quality, compared to good sleepers.
  - Complex relationships appear between exercise nutrition and sleep, while gut microbiome may mediate these correlations.

Beyond the underlying biological mechanisms discussed in detail in Chapter 5, the results of the present metanalysis raised two main points: i) Throughout the analysis of the available data in the literature collectively, it was revealed that there is a need to employ non-pharmacological lifestyle interventions in order to compensate for the increased phenomenon of inadequate sleep [6,452] and its catastrophic consequences [453]. Towards this direction, the implementation of acute CHO quantity manipulations was highlighted as a reasonable non-pharmacological tool

for the modification of sleep architecture, if needed. As appropriate, high CHO quantity could be used to potentially increase REM, while lower CHO could be useful to increase N3 sleep stage promoting a widespread bodily restorative functions and synthetic processes of brain reorganization and repair, respectively [486]. ii) The second point that was raised throughout the meta-analysis was that there were no interventions exploring the effects of nutrition on sleep-related parameters in the long term.

On this basis, the study in Chapter 6 investigated for the first time the effect of evening carbohydrate modification in people who exercise on sleep-related parameters in the long-term. In particular, the purpose was to compare the effects of pre-bed carbohydrate quantity and quality manipulation on sleep-related parameters over a 4-week period. Overall, it was found that adherence to a personalized dietary plan with sufficient nutrient intake accelerated sleep initiation, optimized sleep continuity and prologued sleep duration. Even though, both the timing of carbohydrate intake and the quality of pre-bed carbohydrate intake may affect time in bed period and sleep duration variability after a 4-week period, even when energy and macronutrient daily intake are equated.

There is growing evidence of the beneficial, however, acute effect of nutrition on sleep initiation and duration [496]. For instance, a HGI pre-bed meal combined with [8], or without [49] evening exercise has been shown to improve significantly sleep initiation and duration acutely, compared to a LGI meal. Nonetheless, the study of chapter 6 it was revealed that HGI pre-bed foods did not improve further sleep-related parameters, compared to LGI over a 4-week period. Moreover, it was revealed that when both energy and macronutrient intake are equated, carbohydrate timing throughout the day does not show any further benefit on sleep initiation and duration in the long term. Since there are no relevant long-term interventions in the literature, there is not adequate evidence to comparatively investigate these novel results. However, similar results can be derived from epidemiological data, that point towards significant associations between sleep initiation and duration with both energy or carbohydrate intake [498].

The impact of this intervention was remarkable, since sleep optimization evaluated by objective sleep-related methodology, was accompanied by subjective improvements in sleep quality and daytime sleepiness. This is in line with the literature, that has shown that alterations in SE% and WASO and TST are closely related to sleep quality [500]. These results could be attributed to the restorative properties of quality sleep that have constantly associated sleep with brain reorganization and repair, [486] hormonal responses that promote recovery [488] and increased cognitive performance [489].

A key difference that was elucidated between the groups was sleep duration variability. Even when energy and macronutrient daily intake were equated, both the quantity and quality of pre-

bed carbohydrate intake affected TST IIV after a 4-week period. Specifically, sleep duration variability was reduced by 26.4% and 26.6% in the SL-NCHO and SH-LGI trials respectively, without any significant alterations in SH-HGI group. Again, even though there are no comparative data in the literature, these results were very interesting, as they indicate that there are specific parts of glucose metabolism that could be altered throughout the day [504]. These responses of glucose metabolism to the circadian rhythm could ultimately mediate sleep variability alterations, even when overall daily nutrient intake is equated.

Since there is growing evidence about the reciprocal relationship between nutrition, exercise and sleep [543,544], the next study further described in Chapter 7, aimed to examine the impact of this intervention on both body composition and exercise performance indices. The findings showed that all nutrition interventions resulted in body composition optimization and improved a wide range of exercise performance indices, such as aerobic capacity, strength and reaction time. Hence, it was highlighted that when both energy and nutrient intake are equated within the day, carbohydrate intake timing and pre-bed carbohydrate quality do not hinder any beneficial adaptations of exercise training on body re-composition and exercise performance.

Primarily, body re-composition in that study was achieved by decreasing FM by 1.4kg, while simultaneously increasing FFM by 0.9kg, with negligible differences in body mass. Since no significant differences among trials were found, it is implied that when there is nutrient and energy adequacy, the timing of carbohydrate consumption or the glycemic index of the pre-bed meal does not significantly affect the progress on fat mass reduction or fat-free mass increase. These findings are in line with the theory of calories in/calories out, determined from the energy balance between energy consumption and energy expenditure, and mainly used to describe weight management [518]. Overall these findings support that -as with protein intake [522]- the total daily consumption of carbohydrates is more important than timing when it comes to body composition; without this to overlook the importance of carbohydrate timing for exercise performance, especially in athletes [523].

In the same investigation, it was shown that several exercise performance indices, such as cardiorespiratory fitness, power and visual reaction test, all improved after the intervention. Cardiorespiratory fitness was significantly improved regardless of trial, indicating that both pre-bed carbohydrate quality and timing didn't intervene with participants' progress. In line with the current body of evidence, CMJ and 1 repetition maximum on hack squat, chest press, shoulder press and lat pulldown was significantly improved as well, and could be attributed to both regular exercise training and adequate nutrient intake [527]. Moreover, significant benefits were detected for visual reaction performance as well. However, this effect may be mediated by potential improvements on other specific domains of participants' lifestyle, rather than improvements in body composition or power-to-weight ratio. For example, it has been



previously shown that increase sleep duration improves visual reaction time [8]. Indeed, when sleep is extended, concentration and reaction time may significantly [33,535], but when sleep is disturbed there are significant decreases in vigilance [536]. Since adequate carbohydrate intake is closely related to improved sleep patterns [496], potential sleep improvements as noted in Chapter 6 could potentially mediate this effect on VRT performance [33,536,537].

These findings are considered to be of outmost importance, since increases in  $VO_{2peak}$  are constantly related to reduced mortality rate [526] and strength optimization is directly linked to increased quality of life [528], while decreases in all-cause mortality rates [529]. However, since no significant differences were observed between groups, it could be hypothesized that post-evening workout carbohydrate intake, both in terms of quantity and quality, does not hinder exercise performance improvements in athletes. This challenges the concept that peri-workout nutrient and carbohydrate intake is essential for body composition and exercise performance improvements [530-532]. Indeed, the effect of the timing of post-workout carbohydrate intake crucial for glycogen replenishment, but only when glycogen-depleting events are closer than 8h the one from each other [533]. In the study of Chapter 7, as well as in a common real-world scenario, participants' training was approximately one day apart, hence post-workout carbohydrate timing did not interfere with exercise-related improvements since daily carbohydrate quantity was equated. In the same line, there is evidence that the quality of the carbohydrates in post-workout meal does not significantly affect glycogen synthesis either at 8h or 24-h post-exercise [534]. Collectively the present results with regards to athletic performance indicate that, when macronutrients and energy are equated throughout the day, both carbohydrate timing and pre-bed carbohydrate quality intake may not significantly alter responses to resistance exercise training.

Taken altogether, the findings of the investigations of Chapters 6 and 7, revealed that carbohydrate modifications, as well as lifestyle interventions collectively, can actively impact several aspects of human physiology. Since gut microbiome formation is closely related to carbohydrate metabolism [218-220,331], and exercise [158,275,389,402,403], it was of outmost importance to elucidate potential alterations and interrelationships between gut microbiome formation and miRNAs regulation in response to this intervention. Hence, to elucidate the underlying mechanisms behind these profound effects on sleep, body composition and exercise performance, the study of Chapter 8 explored the effect of the previously investigated nutrition intervention on gut microbiome formation.

Upon clustering the sample into "good responders" and "poor responders" according to individuals' sleep duration differentiation during the intervention, a quantitative conventional PCR was implemented to examine the impact of a 4-week combined exercise and nutrition intervention, on the gut microbiome composition (focusing on the Bifidobacterium and

Clostridium genera). Targeted microbiome analyses revealed for the first time that the baseline level of Clostridium Cocoides group was significantly higher in poor responders compared to good responders. Moreover, it was showed that good responders had lower levels of Bifidobacterium group compared to poor responders at the end of the intervention period. However, even if there is a growing amount of literature showing that both nutrition [217] and exercise interventions [158,275] are important modifying factors for the intestinal microbiome, there are no relevant data in the literature addressing the potential alterations after a nutrition and exercise intervention in gut microbiome discriminating poor and good responders.

Collectively, the results from all the investigations in Chapters 6, 7 and 8, strongly suggested that the intervention had a great impact on all physiological systems that were explored. Hence, it was essential to unveil these responses comprehensively, in order to understand potential physiological mechanisms. Moreover, since inadequate sleep has been constantly related to gut dysbiosis [288,301], it was implied that “poor sleepers” form a specific physiological “profile” that is different compared to “good sleepers”. Towards this direction, the investigation described in the Chapter 9 examined potential interrelationships between sleep, gut microbiome, exercise and body composition indices after a long-term nutrition and exercise intervention. Furthermore, a second analysis was conducted in order to identify the effect magnitude of each intervention on poor and good sleepers.

In this last study of the present investigation, several associations between these domains were found, underlying a main relationship between the intervention-induced optimization in sleep-related parameters with the improvements in body composition and exercise performance indices. These improvements were specifically linked to specific gut microbiome genera. According to the intervention response on poor vs good sleepers, it was revealed that individuals with inadequate sleep duration and low sleep efficiency, increased more their deep sleep stage after following the SL-NCHO compared to good sleepers. Along the same line, the SL-LGI trial prolonged more total sleep time in individuals with inadequate sleep duration, compared to good sleepers. On the other hand, the SH-HGI trial improved more VRT performance in both individuals with low sleep efficiency and low self-reported sleep quality, compared to good sleepers.

Previous findings have shown a positive relationship between sleep extension and body composition optimization [547,548]. This relationship is shown to be reciprocal, since when sleep is disturbed, there are negative effects on body composition [547,548]. In line with these results, decreased average sleep onset latency and its variability, were related to decreases in fat mass and body mass respectively. Moreover, sleep architecture was related to both CMJ and VRT. Overall though, it could be hypothesized these exercise improvements could be attributed to the N2 sleep stage restorative properties [549]. On the other hand, a growing amount of evidence

suggests that sleep extension may improve reaction time performance [33,535]. Since there are indications that acute carbohydrate interventions may aid sleep [496], these potential sleep improvements could potentially mediate this effect on VRT performance [33,536,537]. On the other hand, with regards to CMJ performance, other similar studies didn't show any significant relationship between CMJ and sleep extension acutely [8]. However, this could be attributed to the duration of the present intervention, lasting for 4-weeks in order for potential adaptations to take place.

Despite the fact that there is a great number of novelties in the present investigation, the analyses based on good and poor sleepers, are of utmost importance in order to both elucidate different responses on this sleep-optimization intervention based on individuals' baseline sleep-related characteristics and provide valuable results in order to guide further studies in the specific field. The main results of this analysis showed that deep sleep was increased more in people with inadequate sleep duration or low sleep efficiency after the SL-NCHO trial, compared to good sleepers. In line with these results, there are acute studies that showed a similar effect of decreased evening carbohydrate intake on N3 sleep stage [47,52,53,463]. Moreover, individuals with short sleep duration increased their total sleep time more than good sleepers in the SH-LGI trial. This is very interesting, taking into account that, as showed in the previous study in chapter 5, the SH-LGI not only increased TST significantly, but reduced the intraindividual variability of sleep duration, compared to pre-intervention. However, research on long-term sleep optimizing long-term nutrition interventions is scarce, and thus more studies are needed to comparatively investigate these results.

Another key finding was that subjects with low sleep duration increased more their maximum aerobic capacity after the SL-NCHO compared to good sleepers. As with Chapter 7 with regards to carbohydrate manipulation on exercise performance, post-workout carbohydrate intake, did not interfere with cardiorespiratory fitness improvements. Nevertheless, the present analysis revealed sleep as a key mediating factor that may affect this relationship. It is established that tissue restoration after extreme endurance activities is linked to N3 sleep [35,36]. Hence, since poor sleepers in the SL-NCHO group prolonged more the N3 sleep stage -and potentially its subsequent restorative properties- than good sleepers, it could possibly boost the  $VO_{2max}$  improvements in the long term. Thus, relevant interventions in the future should not only investigate their effect in the long term but in a number of physiological systems, in order to better understand their potential on restoration, recovery and overall health.

## **10.2 Limitations and strengths of the PhD project**

In the current number of studies, there are strengths and weaknesses that need to be addressed. To start with, this is the first investigation to approach the effect of a long-term lifestyle change

intervention on sleep-related parameters, body composition, exercise performance, gut microbiome and miRNAs regulation, collectively. Not only that, but in the vast majority of these domains, the gold standard methodology was recruited, for example in sleep, assessed with for a combination of gold-standard polysomnography, continuous actigraphic measures alongside sleep diaries and self-report sleep quality assessment. However, technical problems such as non-compliance with wearing the actigraphy devices or filling out the sleep diary daily, resulted in 5.6 nights of recordings per participant per trial (pre-post), rather than the aim of 7 nights. Moreover, it would be of further interest to examine the effects of similar nutrition interventions on these physiological systems collectively for even longer periods of time with an increased sample size of poor sleepers, to draw safer conclusions. In addition, while high-throughput sequencing of miRNAs or miRNA profiling is the gold standard for assessing these changes, time constraints and budget limitations necessitate a more targeted approach at this stage. In the same line, potential alterations on blood melatonin, glucose and insulin responses should be further explored. Another limiting factor is that, due to financial constraints, it was unable to provide all-day meals to participants for the whole trial period. On the other hand, this led to the development of individualized, flexible and increased adherence to nutrition plans that are easy to implement in everyday clinical practice, as shown by the present study's high compliance rate. Furthermore, despite the fact that both skinfold measurements and segmental BIA impedance have constantly showed adequacy in order to measure adiposity alterations [518], it would be very interesting to assess body composition with dual-energy X-ray absorptiometry, in order extract more data with regards to muscle mass alterations between trials.

### **10.3 Practical Applications and Future Research Directions**

Altogether, the practical implications of these studies extend beyond the limits of the current investigation for several reasons. As it was shown, adequate nutrient intake contributes effectively to the optimization of the majority of sleep-related variables such as sleep initiation, continuity and duration. Notably, similar results on sleep initiation and maintenance have been shown after melatonin administration in both athletes [507] and patients with primary sleep disorders [508]. Based on this, targeted nutrition interventions are promising and point towards the further investigation of healthier lifestyle as a potential complementary treatment for sleep disorders. Furthermore, relevant interventions may act as a safeguard against inadequate sleep patterns in athletes, in order to promote restoration, recovery and overall health.

Furthermore, taking into account that the current approaches to improve body composition may be inadequate in the long term [541], the application of relevant interventions in a real-world scenario, may build the bridge between research and practice. Overall, the present investigation

showed that exercise-induced improvements in body composition and exercise performance over a four-week period, are not hindered by carbohydrate timing or pre-bed meal's carbohydrate quality, when energy and nutrient intake is equated. This points towards the development and implementation of flexible and easy-to-follow nutrition interventions, which in turn may end the vicious circle between thinking in black-and-white terms and weight regain [542], promoting long-term results in body re-composition.

Finally, this investigation revealed complex relationships between exercise, nutrition and sleep and the responses of the present intervention showed dependence on the preliminary sleep characteristics of the participants. Based on this is the first study to integrally approach the effect of a long-term lifestyle change on sleep-related parameters and propose the employ of CHO manipulation as a reasonable non-pharmacological tool for the modification of sleep in poor sleepers, if needed. Notably, gut-microbiome alterations should be studied as potential biomarkers to both design and assess the efficiency of nutrition and exercise interventions. Collectively, targeted nutrition interventions are promising and suggest further investigation of lifestyle medicine as a potential complementary treatment for sleep disorders. Hence, relevant interventions may act as a safeguard against inadequate sleep patterns in athletes, in order to promote restoration, recovery and overall health.

#### **10.4 Conclusions**

To summarize, the present study shows that habitual adherence to a personalized dietary plan with sufficient nutrient intake can significantly improve the majority of sleep-related parameters and as such, optimise sleep. However, the quantity and quality of evening carbohydrate intake seem to affect sleep duration variability, even when energy and nutrient intake are equated. Moreover, exercise-induced improvements in body composition and exercise performance are not hindered by carbohydrate timing or pre-bed meal's carbohydrate quality, when energy and nutrient intake is equated. Collectively though, carbohydrate intake timing can directly increase deep sleep in individuals with inadequate sleep duration and low sleep efficiency, compared to good sleepers, while pre-bed LGI meal may aid in increasing total sleep time in individuals experiencing the same issue. On the other hand, pre-bed HGI seems to improve visual reaction time in both individuals with low sleep efficiency and low self-reported sleep quality, compared to good sleepers. Overall, complex relationships appear between exercise nutrition and sleep, while gut microbiome may mediate these correlations. Taking into account those findings, it is important that lifestyle medicine to be implemented in clinical practice and targeted interventions to be further investigated in order to improve individuals' quality of life and unveil relevant underlying mechanisms.

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## Appendices

### Appendix I: Consent Form

<b>ΕΝΤΥΠΑ ΣΥΓΚΑΤΑΘΕΣΗΣ</b> <b>για συμμετοχή σε ερευνητικό πρόγραμμα</b> (Τα έντυπα αποτελούνται συνολικά από .....8.. σελίδες)
<b>Τίτλος του Προγράμματος στο οποίο καλείστε να συμμετάσχετε</b>
Φυσιολογικές προσαρμογές στον διατροφικό περιορισμό σε υγιείς ασκούμενους: Από τον ύπνο και την μικροχλωρίδα του εντέρου έως την αθλητική απόδοση

Στο έντυπο αυτό δίνονται εξηγήσεις σε απλή και κατανοητή γλώσσα σχετικά με το τι ζητείται από εσάς ή/και τι θα συμβεί σε εσάς, εάν συμφωνήσετε να συμμετάσχετε στο πρόγραμμα:

1. Περιγράφονται οποιοδήποτε κίνδυνοι μπορεί να υπάρξουν ή ταλαιπωρία που τυχόν θα υποστείτε από την συμμετοχή σας στο πρόγραμμα.
2. Επεξηγείται με κάθε λεπτομέρεια ποιος ή ποιοι θα έχουν πρόσβαση στα δεδομένα που σας αφορούν και θα προκύψουν από το πρόγραμμα που θα συμμετάσχετε ή/και άλλο υλικό/δεδομένα που εθελοντικά θα δώσετε για το πρόγραμμα.
3. Δίνεται η χρονική περίοδος για την οποία οι υπεύθυνοι του προγράμματος θα έχουν πρόσβαση στις πληροφορίες ή/και υλικό σας αφορά.
4. Επεξηγείται το τί ευελπιστούν να μάθουν οι υπεύθυνοι του προγράμματος σαν αποτέλεσμα και της δικής σας συμμετοχής.
5. Δίνεται μία εκτίμηση για το όφελος που μπορεί να υπάρξει για τους ερευνητές ή/και χρηματοδότες αυτού του προγράμματος.
6. **Δεν πρέπει να συμμετάσχετε, εάν δεν επιθυμείτε ή εάν έχετε οποιουδήποτε ενδιαρισμούς που αφορούν τη συμμετοχή σας στο πρόγραμμα.**
7. Εάν αποφασίσετε να συμμετάσχετε, πρέπει να αναφέρετε εάν είχατε συμμετάσχει σε οποιοδήποτε άλλο πρόγραμμα έρευνας μέσα στους τελευταίους 12 μήνες.
8. Εάν αποφασίσετε να μην συμμετάσχετε και είστε ασθενής, η θεραπεία σας δεν θα επηρεαστεί από την απόφασή σας.
9. **Είστε ελεύθεροι να αποσύρετε οποιαδήποτε στιγμή εσείς επιθυμείτε τη συγκατάθεση για την συμμετοχή σας στο πρόγραμμα.**
10. Εάν είστε ασθενής, η απόφασή σας να αποσύρετε την συγκατάθεση σας, δεν θα έχει οποιεσδήποτε επιπτώσεις στη θεραπεία σας.
11. Πρέπει όλες οι σελίδες των εντύπων συγκατάθεσης να φέρουν το ονοματεπώνυμο και την υπογραφή σας.

Επιστημονικός υπεύθυνος του Προγράμματος στο οποίο καλείστε να συμμετάσχετε
Δρ. Χριστόφορος Γιαννάκη

Επίθετο:	..... .....	Όνομα:	..... .....
Υπογραφή :		Ημερομηνία :	

<b>ΕΝΤΥΠΑ ΣΥΓΚΑΤΑΘΕΣΗΣ</b> <b>για συμμετοχή σε ερευνητικό πρόγραμμα</b> (Τα έντυπα αποτελούνται συνολικά από .....8.. σελίδες)
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Χρονική διάρκεια του Προγράμματος:
24 μήνες

Δείτε συγκατάθεση για τον εαυτό σας ή για κάποιο άλλο άτομο;	
Εάν πιο πάνω απαντήσατε για κάποιον άλλο, τότε δώσετε λεπτομέρειες και το όνομα του.	

Ερώτηση	ΝΑΙ ή ΟΧΙ
Συμπληρώσατε τα έντυπα συγκατάθεσης εσείς προσωπικά;	
Τους τελευταίους 12 μήνες έχετε συμμετάσχει σε οποιοδήποτε άλλο ερευνητικό πρόγραμμα;	
Διαβάσατε και καταλάβατε τις πληροφορίες για ασθενείς ή/και εθελοντές;	
Είχατε την ευκαιρία να ρωτήσετε ερωτήσεις και να συζητήσετε το Πρόγραμμα;	
Δόθηκαν ικανοποιητικές απαντήσεις και εξηγήσεις στα τυχόν ερωτήματά σας;	
Καταλαβαίνετε ότι μπορείτε να αποσυρθείτε από το πρόγραμμα, όποτε θέλετε;	
Καταλαβαίνετε ότι, εάν αποσυρθείτε, δεν είναι αναγκαίο να δώσετε οποιεσδήποτε εξηγήσεις για την απόφαση που πήρατε;	
(Για ασθενείς) καταλαβαίνετε ότι, εάν αποσυρθείτε, δεν θα υπάρξουν επιπτώσεις στην τυχόν θεραπεία που παίρνετε ή που μπορεί να πάρετε μελλοντικά;	
<b>Συμφωνείτε να συμμετάσχετε στο πρόγραμμα;</b>	
Με ποιόν υπεύθυνο μιλήσατε;	
Επίθετο:	Όνομα:
Υπογραφή:	Ημερομηνία:

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<b>ΕΝΤΥΠΑ ΣΥΓΚΑΤΑΘΕΣΗΣ</b> <b>για συμμετοχή σε ερευνητικό πρόγραμμα</b> (Τα έντυπα αποτελούνται συνολικά από .....8.. σελίδες)
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<b>Σύντομη περιγραφή του προγράμματος (διαδικασίες και σκοπός).</b>
<p>Ο ύπνος, η διατροφή και η άσκηση θεωρούνται οι ακρογωνιαίοι λίθοι της ανθρώπινης υγείας και ευρωστίας. Ερευνητικά δεδομένα τονίζουν την σημασία του ύπνου για την υγεία, την σύσταση σώματος αλλά και την αθλητική απόδοση. Επίσης, η βιβλιογραφία τονίζει την μείζονα σημασία της διατροφής στον ύπνο, με στόχο την βελτιστοποίησή του. Παρ' όλα αυτά, φαίνεται πως ο ύπνος των ασκούμενων είναι μικρής διάρκειας, χαμηλής αποδοτικότητας και πιθανώς ανεπαρκής, σύμφωνα με τις διεθνείς συστάσεις. Σε μία πρόσφατη μελέτη από την ερευνητική μας ομάδα φάνηκε πως μία βραχυπρόθεσμη διατροφική παρέμβαση μετά την απογευματινή προπόνηση ατόμων που ασκούσαν συστηματικά, βοήθησε στην βελτιστοποίηση του ύπνου τους και αύξησε συγκεκριμένες παραμέτρους αθλητικής απόδοσης την επόμενη μέρα. Παρ' ότι η διερεύνηση του βιολογικού μηχανισμού ήταν πέραν του στόχου της μελέτης, η αρχική υπόθεση ήταν πως μία απλή διατροφική παρέμβαση, βελτιστοποίησε το ορμονικό προφίλ των ασκούμενων -αυξάνοντας τα επίπεδα σεροτονίνης και μελατονίνης-, οδηγώντας στα αντίστοιχα ευεργετικά αποτελέσματα. Νέα δεδομένα δείχνουν ότι ένα μεγάλο ποσοστό σεροτονίνης βρίσκεται στο εντερικό σύστημα του ανθρώπου, αλλά η σχέση του με τον ύπνο παραμένει ακόμη αδιευκρίνιστη. Λαμβάνοντας υπόψιν την αύξηση του επιπολασμού του ανεπαρκούς ύπνου στους αθλητές, και των αρνητικών σε αυτούς συνεπειών του, το τμήμα Επιστημών Υγείας του Πανεπιστημίου Λευκωσίας διεξάγει την παρούσα μελέτη με στόχο την βελτιστοποίηση του ύπνου των ασκούμενων μέσω του διατροφικού περιορισμού, και την περαιτέρω διερεύνηση της μικροχλωρίδας του εντέρου, της σύστασης σώματος και απόδοσης των ασκούμενων αυτών.</p>

<b>Λεπτομέρειες του τι θα ζητηθεί ή/και τι θα συμβεί στους συμμετέχοντες στο πρόγραμμα</b>								
<p>Για τη διεξαγωγή της μελέτης θα σας ζητηθεί να επισκεφθείτε το εργαστήριο Ανθρώπινης Απόδοσης του Πανεπιστημίου Λευκωσίας τέσσερις φορές (μία πριν την έναρξη, την δεύτερη εβδομάδα, την τέταρτη εβδομάδα και την όγδοη εβδομάδα). Εκεί, κατά την πρώτη επίσκεψη θα γίνει εκτίμηση του της σύστασης σώματός σας, μέτρηση του βασικού μεταβολισμού ηρεμίας και της μέγιστης πρόσληψης οξυγόνου ηρεμίας σας και των διατροφικών σας συνηθειών. Έπειτα θα δημιουργηθεί ένα εξατομικευμένο πρόγραμμα διατροφής που θα κληθείτε να ακολουθήσετε για 8 εβδομάδες, και κατά την διάρκεια αυτών των εβδομάδων θα γυμνάζεστε πέντε φορές την εβδομάδα, υπό επίβλεψη στο UFIT, το γυμναστήριο του Πανεπιστημίου Λευκωσίας. Οι παρακάτω μετρήσεις θα πραγματοποιηθούν κατά την διάρκεια της μελέτης:</p>								
<table border="1"> <tr> <td>Επίθετο:</td> <td></td> <td>Όνομα:</td> <td></td> </tr> <tr> <td>Υπογραφή:</td> <td></td> <td>Ημερομηνία:</td> <td></td> </tr> </table>	Επίθετο:		Όνομα:		Υπογραφή:		Ημερομηνία:	
Επίθετο:		Όνομα:						
Υπογραφή:		Ημερομηνία:						

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<p>-Εκτίμηση του ύπνου στον χώρο σας, πριν την παρέμβαση, στην δεύτερη, τέταρτη και όγδοη εβδομάδα. Από αυτές, στην πρώτη και τελευταία μελέτη ύπνου θα χρειαστεί να τοποθετηθούν ειδικά καλώδια στο κεφάλι (εγκεφαλογραφήμα), ηλεκτρομυογράφημα στον πρόσθιο κνημιαίο μυ των κάτω άκρων, ζώνες στην μέση και στο στήθος και δύο καλώδια στο στήθος για αξιολόγηση της καρδιακής συχνότητας. Της υπόλοιπες φορές ο ύπνος σας θα εκτιμάται με μια απλή συσκευή ακτιγραφίας και σχετικά ερωτηματολόγια.</p> <p>- Μέτρηση αθλητικής απόδοσης και σύστασης σώματος πριν την παρέμβαση, στο τέλος του πρώτου μήνα και στο τέλος της παρέμβασης στο εργαστήριο ανθρώπινης απόδοσης του Πανεπιστημίου Λευκωσίας και στο UFIT, το γυμναστήριο του Πανεπιστημίου Λευκωσίας. Εκεί, ανάλογα με την μέθοδο προπόνησής σας (αερόβια ή με αντιστάσεις), θα μετρηθεί αντίστοιχα είτε ο χρόνος αντίδρασης, η μέγιστη πρόσληψη οξυγόνου και η αντοχή σας στο εργοποδήλατο, είτε το κατακόρυφο άλμα, ο χρόνος αντίδρασης, η ισοκινητική δύναμη και η μέγιστη προσπάθεια στις έκτοτε ασκήσεις δύναμης που θα πραγματοποιείτε στην προπόνησή σας.</p> <p>-Διερεύνηση της μικροχλωρίδας του εντέρου μετά της συλλογής κοπράνων πριν την παρέμβαση, στην δεύτερη, τέταρτη και όγδοη εβδομάδα, στις Εγκαταστάσεις του Πανεπιστημίου Λευκωσίας. Η συλλογή των δειγμάτων θα πραγματοποιηθεί σε δοχείο κοπράνων όπου θα σας δοθεί και η φύλαξη θα γίνει στα ψυγεία (-80°C). Η ανάλυση τους θα γίνει στο εργαστήριο Βιολογίας του ανθρώπου του Πανεπιστημίου Λευκωσίας και τα δείγματα θα πεταχτούν μετά το πέρας των 5 ετών.</p> <p>-Λήψη αίματος πριν την παρέμβαση, στην δεύτερη, τέταρτη και όγδοη εβδομάδα, στις εγκαταστάσεις του Πανεπιστημίου Λευκωσίας. Η αιμοληψία θα πραγματοποιηθεί από εξειδικευμένο νοσηλευτικό προσωπικό του Πανεπιστημίου Λευκωσίας. Τα δείγματα θα φυλαχθούν και θα αναλυθούν εργαστήριο Βιολογίας του ανθρώπου του Πανεπιστημίου Λευκωσίας και τα δείγματα θα πεταχτούν μετά το πέρας των 5 ετών.</p> <p>Όλες οι αξιολογήσεις που θα πραγματοποιηθούν είναι ανώδυνες και ασφαλείς και θα πραγματοποιηθούν από εξειδικευμένο προσωπικό.</p>			
Επίθετο:		Όνομα:	
Υπογραφή:		Ημερομηνία:	

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Λεπτομέρειες της χρηματοδότησης του ερευνητικού προγράμματος	
Το εν λόγω πρόγραμμα δεν έχει λάβει χρηματοδότηση.	
Λεπτομέρειες οποιονδήποτε κινδύνων που πιθανόν να υπάρξουν ή ταλαιπωρία που τυχόν θα υποστούν οι συμμετέχοντες στο πρόγραμμα.	
<p>Κατά την αιμοληψία υπάρχει η αίσθηση ελαφρού πόνου, "τσιμπήματος", κατά την είσοδο της βελόνας. Υπάρχει η πιθανότητα δημιουργίας μώλωπα. Το έμπειρο προσωπικό αιμοληψίας (έμπειρη νοσηλεύτρια-καθηγήτρια νοσηλευτικής) θα λάβει κάθε μέτρο για μείωση οποιασδήποτε δυσφορίας κατά τη λήψη αίματος, και θα είναι στη διάθεση σου για οποιαδήποτε βοήθεια τυχόν χρειαστείτε κατά την αιμοληψία.</p> <p>Κατά την εργομετρική αξιολόγηση δεν αναμένονται κάποιες παρενέργειες ή σοβαρές ενοχλήσεις πέραν της φυσιολογικής κόπωσης που συνοδεύεται με την άσκηση.</p> <p>Κατά τις μελέτες ύπνου, πιθανόν να επέλθει κάποια δυσφορία ή ενοχλήσεις ή δυσκολία στην έλευση ύπνου λόγω των καλωδίων, η οποία όμως είναι σπάνια.</p>	
Λεπτομέρειες για το ποιες πληροφορίες ή/και τι υλικό θα συλλεγεί στα πλαίσια του προγράμματος, ποιος/ποιοι θα έχουν πρόσβαση σε αυτά και για πόσο χρονικό διάστημα.	
Στα πλαίσια του προγράμματος θα συλλέξουμε πληροφορίες σχετικά με τη φυσική σας κατάσταση, τη σωματική σας σύσταση, τις διατροφικές σας συνήθειες, την αρχιτεκτονική του ύπνου σας, διαφόρων αιματολογικών δεικτών και της μικροχλωρίδας του εντέρου σας. Πρόσβαση σε αυτές τις πληροφορίες θα έχει ο Δρ. Χριστόφορος Γιαννάκης μέχρι και πέντε χρόνια από το τέλος της μελέτης.	
Επίθετο:	Όνομα:
Υπογραφή:	Ημερομηνία:

(Έντυπο ΕΕΒΚ03)

5/8



<b>ΕΝΤΥΠΑ ΣΥΓΚΑΤΑΘΕΣΗΣ</b> <b>για συμμετοχή σε ερευνητικό πρόγραμμα</b> (Τα έντυπα αποτελούνται συνολικά από .....8.. σελίδες)
<b>Τίτλος του Προγράμματος στο οποίο καλείστε να συμμετάσχετε</b>
Φυσιολογικές προσαρμογές στον διατροφικό περιορισμό σε υγιείς ασκούμενους: Από τον ύπνο και την μικροχλωρίδα του εντέρου έως την αθλητική απόδοση

<b>ΟΠΟΥ ΙΣΧΥΕΙ, ΜΕΛΛΟΝΤΙΚΗ ΑΠΟΘΗΚΕΥΣΗ ΚΑΙ ΧΡΗΣΗ ΒΙΟΛΟΓΙΚΩΝ ΔΕΙΓΜΑΤΩΝ ΚΑΙ ΠΡΟΣΩΠΙΚΩΝ ΔΕΔΟΜΕΝΩΝ:</b>	
Παρακαλούμε σημειώστε και υπογράψτε είτε αριστερά είτε δεξιά	
Εκτός από τους σκοπούς του παρόντος προγράμματος που θα διαρκέσει 5 χρόνια  <b>Αποδέχομαι</b> <input type="checkbox"/> όπως:	Εκτός από τους σκοπούς της παρούσας μελέτης που θα διαρκέσει 5 χρόνια  <b>Δεν αποδέχομαι</b> <input type="checkbox"/> όπως:
<b>Υπογραφή:</b>	<b>Υπογραφή:</b>
τα βιολογικά μου δείγματα (παρειαικά επιχρίσματα ή σάλιο ή DNA) και γενετικά δεδομένα μου που θα φυλάσσονται στο εργαστήριο Βιολογίας του ανθρώπου του Πανεπιστημίου Λευκωσίας <b>να μπορούν να κρατηθούν πέραν των 5 χρόνων και να χρησιμοποιηθούν σε μελλοντικές μελέτες</b> αφού πρώτα εγκριθεί κάτι τέτοιο από την Εθνική Επιτροπή Βιοηθικής Κύπρου (ΕΕΒΚ) μετά από σχετικό αίτημα ανανέωσης προς την ΕΕΒΚ από τον υπεύθυνο ερευνητή του παρόντος προγράμματος. Καταλαβαίνω ότι θέματα εμπιστευτικότητας θα ισχύουν πάντοτε.	

Σε περίπτωση που ανακαλυφθούν νέες πληροφορίες που επηρεάζουν άμεσα την υγεία σας θα θέλατε να πληροφορηθείτε;		
ΝΑΙ  <input type="checkbox"/>	ΟΧΙ  <input type="checkbox"/>	ΔΕΝ ΜΠΟΡΩ ΝΑ ΑΠΟΦΑΣΙΣΩ ΤΩΡΑ, ΝΑ ΕΡΩΤΗΘΩ ΕΚ ΝΕΟΥ ΕΦΟΣΟΝ ΥΠΑΡΞΕΙ ΑΝΑΓΚΗ  <input type="checkbox"/>
Λεπτομέρειες για το ποια δεδομένα θα προκύψουν για σας στα πλαίσια του προγράμματος και ποιος/ποιοι θα έχουν πρόσβαση σε αυτά και για πόσο χρονικό διάστημα.		
Στα πλαίσια του προγράμματος θα συλλέξουμε πληροφορίες σχετικά με τη φυσική σας κατάσταση, τη σωματική σας σύσταση, τις διατροφικές σας συνήθειες, την αρχιτεκτονική του ύπνου σας, διαφόρων αιματολογικών δεικτών και της μικροχλωρίδας του εντέρου σας. Πρόσβαση σε αυτές τις πληροφορίες θα έχει ο Δρ. Χριστόφορος Γιαννάκης μέχρι και 5 χρόνια από το τέλος της μελέτης.		
Επίθετο:	..... ....	Όνομα:
Υπογραφή:		Ημερομηνία:

(Έντυπο ΕΕΒΚ03)

6/8

**ΕΝΤΥΠΑ ΣΥΓΚΑΤΑΘΕΣΗΣ**  
**για συμμετοχή σε ερευνητικό πρόγραμμα**  
(Τα έντυπα αποτελούνται συνολικά από .....8.. σελίδες)

**Τίτλος του Προγράμματος στο οποίο καλείστε να συμμετάσχετε**

Φυσιολογικές προσαρμογές στον διατροφικό περιορισμό σε υγιείς ασκούμενους: Από τον ύπνο και την μικροχλωρίδα του εντέρου έως την αθλητική απόδοση

Αναμενόμενο όφελος για τους συμμετέχοντες

Στο πλαίσιο της παρούσας μελέτης θα σας δοθεί η ευκαιρία να βελτιώσετε τις διατροφικές σας συνήθειες. Επίσης θα ενημερωθείτε για τα επίπεδα της φυσικής κατάστασης, της σύστασης σώματος, του μεταβολισμού και της αθλητικής σας απόδοσης. Πέρα από αυτά αναμένουμε πως με το τέλος της παρούσας μελέτης θα έχετε βελτιώσει τη φυσική σας κατάσταση, τη σύσταση του σώματος σας καθώς και της ποιότητας και αρχιτεκτονικής του ύπνου σας.

Αναμενόμενο όφελος για ερευνητές ή/και χρηματοδότες

Όλα τα αποτελέσματα από τη μελέτη θα χρησιμοποιηθούν εμπιστευτικά από τους ερευνητές, και αποκλειστικά για τους σκοπούς της έρευνας και δεν θα δημοσιοποιηθούν σε καμιά περίπτωση τα ονόματα των συμμετεχόντων. Από τη μελέτη αναμένετε να προκύψει δημοσίευση σε επιστημονικό περιοδικό.

Λεπτομέρειες συνθηκών τερματισμού ή πρόωρης διακοπής του ερευνητικού προγράμματος.

Σημειώνουμε ότι διατηρείτε το δικαίωμα του να αποσυρθείτε από την έρευνα αν το θελήσετε σε οποιοδήποτε σημείο της έρευνας, χωρίς να χρειάζεται να δώσετε οποιαδήποτε δικαιολογία ή εξήγηση. Σε περίπτωση που κάποιος συμμετέχοντας αποφασίσει να αποσυρθεί από τη μελέτη, τα συλλεχθέντα δεδομένα θα αποσυρθούν και αυτά

Χώρος και χρονική διάρκεια φύλαξης δεδομένων ή/και βιολογικών δειγμάτων που θα ληφθούν στο πλαίσιο του προγράμματος

Οι πληροφορίες που θα συλλέξουμε κατά τη διάρκεια αυτής της έρευνας θα παραμείνουν εμπιστευτικές. Οι πληροφορίες που θα συγκεντρωθούν (θα περαστούν σε αρχείο 'excel' ή 'spss') κατά τη διάρκεια της έρευνας θα αφαιρεθούν και κανένας, εκτός από τον κύριο ερευνητή και τον κο Άγγελο Βλαχογιάννη δεν θα μπορέσει να τις δει. Οποιαδήποτε πληροφορία για εσάς θα έχει έναν αριθμό πάνω σε αυτή αντί για το όνομά σας. Μόνο ο κύριος ερευνητής θα γνωρίζει ποιος είναι ο αριθμός σας και θα κλειδώσουμε τις πληροφορίες αυτές με κλειδαριά και κλειδί. Τα δεδομένα θα φυλαχθούν μέχρι και 5 χρόνια μετά το τέλος της μελέτης στο γραφείο του Δρ. Γιαννάκη στο Πανεπιστήμιο Λευκωσίας σε χώρο που δεν έχει κανένα άλλο άτομο πρόσβαση, και μετά θα καταστραφούν.

Επίθετο:		Όνομα:	
Υπογραφή:		Ημερομηνία:	

(Έντυπο ΕΕΒΚ03)

<b>ΕΝΤΥΠΑ ΣΥΓΚΑΤΑΘΕΣΗΣ</b> <b>για συμμετοχή σε ερευνητικό πρόγραμμα</b> (Τα έντυπα αποτελούνται συνολικά από .....8.. σελίδες)
<b>Τίτλος του Προγράμματος στο οποίο καλείστε να συμμετάσχετε</b>
Φυσιολογικές προσαρμογές στον διατροφικό περιορισμό σε υγιείς ασκούμενους: Από τον ύπνο και την μικροχλωρίδα του εντέρου έως την αθλητική απόδοση

Περιγραφή διαδικασιών χειρισμού δεδομένων ή/και βιολογικών δειγμάτων συμμετεχόντων που θα αποσυρθούν από τη μελέτη πριν την ολοκλήρωση της.

Σημειώνουμε ότι διατηρείτε το δικαίωμα του να αποσυρθείτε από την έρευνα αν το θελήσετε σε οποιοδήποτε σημείο της έρευνας, χωρίς να χρειάζεται να δώσετε οποιαδήποτε δικαιολογία ή εξήγηση. Σε περίπτωση που κάποιος συμμετέχοντας αποφασίσει να αποσυρθεί από τη μελέτη, τα συλλεχθέντα δεδομένα θα αποσυρθούν και θα καταστραφούν και αυτά.

Πλήρη στοιχεία επικοινωνίας και θέση ατόμου στο οποίο οι συμμετέχοντες μπορούν να υποβάλλουν παράπονα ή καταγγελίες που αφορούν το πρόγραμμα στο οποίο συμμετέχουν.

Σε περίπτωση που για οποιοδήποτε λόγο επιθυμείτε να διαμαρτυρηθείτε για οποιοδήποτε λόγο σχετικό με την ερευνητική διαδικασία ή να ζητήσετε την ανεξάρτητη γνώμη κάποιου λειτουργού του Πανεπιστημίου μας σε σχέση με την έρευνα που λαμβάνετε μέρος, σας παρακαλούμε να μην διστάσετε να επικοινωνήσετε με το γραφείο έρευνας του Πανεπιστημίου Λευκωσίας και συγκεκριμένα με τον κ. Θεόδωρο Μιλιδώνη, τηλ: 22841656 email: millidonis.t@unic.ac.cy.

Πλήρη στοιχεία επικοινωνίας και θέση ατόμου στο οποίο οι συμμετέχοντες μπορούν να απευθυνθούν για περισσότερες πληροφορίες ή διευκρινήσεις για το ερευνητικό πρόγραμμα.

Για οποιεσδήποτε απορίες, επεξηγήσεις ή ότι άλλο θέλετε να ρωτήσετε, μπορείτε να επικοινωνήσετε με τους Δρ. Χριστόφορο Γιαννάκη (τηλ. 22842325), Δρ. Ελένη Ανδρέου (τηλ.99464040) και κ. Άγγελο Βλαχογιάννη(τηλ. 996001354)

Επίθετο:		Όνομα:	
Υπογραφή:		Ημερομηνία:	

## Appendix II: Dietary Screening Questionnaire

### Dietary History

Name: \_\_\_\_\_ Contact Details: \_\_\_\_\_

Age: \_\_\_\_\_ Height: \_\_\_\_\_ Weight: \_\_\_\_\_ Usual Weight: \_\_\_\_\_ Comments: \_\_\_\_\_

Medical History:
Comments:

Number of Meals: \_\_\_\_\_

### Meal Description and Timing

BREAKFAST:

MORNING SNACK:

LUNCH:

AFTERNOON SNACK:

DINNER:

PRE-BED:

Foods that avoid: \_\_\_\_\_

Foods that prefer: \_\_\_\_\_

- |                     |   |   |       |
|---------------------|---|---|-------|
| 1. Legumes          | ✓ | x | _____ |
| 2. Dairy            | ✓ | x | _____ |
| 3. Eggs             | ✓ | x | _____ |
| 4. Meat - Poultry   | ✓ | x | _____ |
| 5. Fish             | ✓ | x | _____ |
| 6. Spaghetti/Starch | ✓ | x | _____ |
| 7. Fruit            | ✓ | x | _____ |
| 8. Vegetables       | ✓ | x | _____ |
| 9. Olive oil        | ✓ | x | _____ |
| 10. Alcohol         | ✓ | x | _____ |
| 11. Coffee          | ✓ | x | _____ |

P.A.: \_\_\_\_\_

Dietary Supplements /  
Comments \_\_\_\_\_

Angelos Vlahoyiannis MSc, PhD (s), Sports Nutritionist/Dietitian



## Epworth Sleepiness Scale

Name: \_\_\_\_\_

Date: \_\_\_\_\_

Your age: (Yr) \_\_\_\_\_ Your sex:  Male  Female

How likely are you to doze off or fall asleep in the situations described below, in contrast to feeling just tired?

This refers to your usual way of life in recent times.

Even if you haven't done some of these things recently try to work out how they would have affected you.

Use the following scale to choose the most appropriate number for each situation:-

- 0 = would never doze
- 1 = Slight chance of dozing
- 2 = Moderate chance of dozing
- 3 = High chance of dozing

Situation	Chance of dozing
Sitting and reading .....	<input type="text"/>
Watching TV .....	<input type="text"/>
Sitting, inactive in a public place (e.g. a theatre or a meeting) .....	<input type="text"/>
As a passenger in a car for an hour without a break .....	<input type="text"/>
Lying down to rest in the afternoon when circumstances permit .....	<input type="text"/>
Sitting and talking to someone .....	<input type="text"/>
Sitting quietly after a lunch without alcohol .....	<input type="text"/>
In a car, while stopped for a few minutes in the traffic .....	<input type="text"/>
Total .....	<input type="text"/>

Score:
0-10 Normal range
10-12 Borderline
12-24 Abnormal

## Appendix IV: Fatigue Severity Scale Questionnaire

### Fatigue Severity Scale (FSS) of Sleep Disorders

The Fatigue Severity Scale (FSS) is a method of evaluating the impact of fatigue on you. The FSS is a short questionnaire that requires you to rate your level of fatigue.

The FSS questionnaire contains nine statements that rate the severity of your fatigue symptoms. Read each statement and circle a number from 1 to 7, based on how accurately it reflects your condition during the past week and the extent to which you agree or disagree that the statement applies to you.

- A low value (e.g., 1) indicates strong disagreement with the statement, whereas a high value (e.g., 7) indicates strong agreement.
- It is important that you circle a number (1 to 7) for every question.

FSS Questionnaire							
During the past week, I have found that:	Disagree <-----> Agree						
My motivation is lower when I am fatigued.	1	2	3	4	5	6	7
Exercise brings on my fatigue.	1	2	3	4	5	6	7
I am easily fatigued.	1	2	3	4	5	6	7
Fatigue interferes with my physical functioning.	1	2	3	4	5	6	7
Fatigue causes frequent problems for me.	1	2	3	4	5	6	7
My fatigue prevents sustained physical functioning.	1	2	3	4	5	6	7
Fatigue interferes with carrying out certain duties and responsibilities.	1	2	3	4	5	6	7
Fatigue is among my three most disabling symptoms.	1	2	3	4	5	6	7
Fatigue interferes with my work, family, or social life.	1	2	3	4	5	6	7
	<b>Total Score:</b>						

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#### Scoring your results

Now that you have completed the questionnaire, it is time to score your results and evaluate your level of fatigue. It's simple: Add all the numbers you circled to get your total score.

#### The Fatigue Severity Scale Key

A total score of less than 36 suggests that you may not be suffering from fatigue.

A total score of 36 or more suggests that you may need further evaluation by a physician.

Subject's Initials \_\_\_\_\_ ID# \_\_\_\_\_ Date \_\_\_\_\_ Time \_\_\_\_\_ AM  
PM

**PITTSBURGH SLEEP QUALITY INDEX**

**INSTRUCTIONS:**

The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

1. During the past month, what time have you usually gone to bed at night?  
BED TIME \_\_\_\_\_
2. During the past month, how long (in minutes) has it usually taken you to fall asleep each night?  
NUMBER OF MINUTES \_\_\_\_\_
3. During the past month, what time have you usually gotten up in the morning?  
GETTING UP TIME \_\_\_\_\_
4. During the past month, how many hours of actual sleep did you get at night? (This may be different than the number of hours you spent in bed.)  
HOURS OF SLEEP PER NIGHT \_\_\_\_\_

**For each of the remaining questions, check the one best response. Please answer all questions.**

5. During the past month, how often have you had trouble sleeping because you . . .
  - a) Cannot get to sleep within 30 minutes  
Not during the past month \_\_\_\_\_ Less than once a week \_\_\_\_\_ Once or twice a week \_\_\_\_\_ Three or more times a week \_\_\_\_\_
  - b) Wake up in the middle of the night or early morning  
Not during the past month \_\_\_\_\_ Less than once a week \_\_\_\_\_ Once or twice a week \_\_\_\_\_ Three or more times a week \_\_\_\_\_
  - c) Have to get up to use the bathroom  
Not during the past month \_\_\_\_\_ Less than once a week \_\_\_\_\_ Once or twice a week \_\_\_\_\_ Three or more times a week \_\_\_\_\_

d) Cannot breathe comfortably

Not during the past month \_\_\_\_\_ Less than once a week \_\_\_\_\_ Once or twice a week \_\_\_\_\_ Three or more times a week \_\_\_\_\_

e) Cough or snore loudly

Not during the past month \_\_\_\_\_ Less than once a week \_\_\_\_\_ Once or twice a week \_\_\_\_\_ Three or more times a week \_\_\_\_\_

f) Feel too cold

Not during the past month \_\_\_\_\_ Less than once a week \_\_\_\_\_ Once or twice a week \_\_\_\_\_ Three or more times a week \_\_\_\_\_

g) Feel too hot

Not during the past month \_\_\_\_\_ Less than once a week \_\_\_\_\_ Once or twice a week \_\_\_\_\_ Three or more times a week \_\_\_\_\_

h) Had bad dreams

Not during the past month \_\_\_\_\_ Less than once a week \_\_\_\_\_ Once or twice a week \_\_\_\_\_ Three or more times a week \_\_\_\_\_

i) Have pain

Not during the past month \_\_\_\_\_ Less than once a week \_\_\_\_\_ Once or twice a week \_\_\_\_\_ Three or more times a week \_\_\_\_\_

j) Other reason(s), please describe \_\_\_\_\_

How often during the past month have you had trouble sleeping because of this?

Not during the past month \_\_\_\_\_ Less than once a week \_\_\_\_\_ Once or twice a week \_\_\_\_\_ Three or more times a week \_\_\_\_\_

6. During the past month, how would you rate your sleep quality overall?

Very good \_\_\_\_\_

Fairly good \_\_\_\_\_

Fairly bad \_\_\_\_\_

Very bad \_\_\_\_\_



7. During the past month, how often have you taken medicine to help you sleep (prescribed or "over the counter")?

Not during the past month \_\_\_\_\_ Less than once a week \_\_\_\_\_ Once or twice a week \_\_\_\_\_ Three or more times a week \_\_\_\_\_

8. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?

Not during the past month \_\_\_\_\_ Less than once a week \_\_\_\_\_ Once or twice a week \_\_\_\_\_ Three or more times a week \_\_\_\_\_

9. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?

No problem at all \_\_\_\_\_  
 Only a very slight problem \_\_\_\_\_  
 Somewhat of a problem \_\_\_\_\_  
 A very big problem \_\_\_\_\_

10. Do you have a bed partner or room mate?

No bed partner or room mate \_\_\_\_\_  
 Partner/room mate in other room \_\_\_\_\_  
 Partner in same room, but not same bed \_\_\_\_\_  
 Partner in same bed \_\_\_\_\_

If you have a room mate or bed partner, ask him/her how often in the past month you have had . . .

- a) Loud snoring

Not during the past month \_\_\_\_\_ Less than once a week \_\_\_\_\_ Once or twice a week \_\_\_\_\_ Three or more times a week \_\_\_\_\_

- b) Long pauses between breaths while asleep

Not during the past month \_\_\_\_\_ Less than once a week \_\_\_\_\_ Once or twice a week \_\_\_\_\_ Three or more times a week \_\_\_\_\_

- c) Legs twitching or jerking while you sleep

Not during the past month \_\_\_\_\_ Less than once a week \_\_\_\_\_ Once or twice a week \_\_\_\_\_ Three or more times a week \_\_\_\_\_

**Appendix VI: Sleep Diary**

Name, Surname: \_\_\_\_\_ Date \_\_\_\_\_

Dear participant, below you will find a subjective questionnaire aimed to evaluate your sleep experience. **You don't have to wake up during the night in order to evaluate your sleep.** Please only evaluate your sleep in the morning when you have woken up (NIGHT EVALUATION). Additionally please evaluate you day prior to going to sleep (DAY EVALUATION).

NIGHT EVALUATION							
Date:	Monday Morning	Tuesday Morning	Wednesday Morning	Thursday Morning	Friday Morning	Saturday Morning	Sunday Morning
<b>How do you rate your quality of sleep?</b> 1 = Very Good 2 = Good 3 = Bad 4 = Very Bad	1 - 2 - 3 - 4	1 - 2 - 3 - 4	1 - 2 - 3 - 4	1 - 2 - 3 - 4	1 - 2 - 3 - 4	1 - 2 - 3 - 4	1 - 2 - 3 - 4
<b>Do you feel refreshed after your sleep?</b> 1 = Very Refreshed 2 = Refreshed 3 = Neutral 4 = Not at all	1 - 2 - 3 - 4	1 - 2 - 3 - 4	1 - 2 - 3 - 4	1 - 2 - 3 - 4	1 - 2 - 3 - 4	1 - 2 - 3 - 4	1 - 2 - 3 - 4
<b>What time did you go to bed?</b>							
<b>How long did it take you to fall asleep?</b>							
<b>What time did you wake up</b>							
<b>What time did you get out of bed</b> <b>How many hours do you think you have slept?</b>							

<b>DAY EVALUATION</b>							
Date:	Monday Evening	Tuesday Evening	Wednesday Evening	Thursday Evening	Friday Evening	Saturday Evening	Sunday Evening
<b>Evaluate the maximum degree of daytime sleepiness.</b> 1 = Not Sleepy 2 = Slightly Sleepy 3 = Very Sleepy 4 = Extremely Sleepy	1 - 2 - 3 - 4	1 - 2 - 3 - 4	1 - 2 - 3 - 4	1 - 2 - 3 - 4	1 - 2 - 3 - 4	1 - 2 - 3 - 4	1 - 2 - 3 - 4
<b>How elevated was your mood during the day?</b> 1 = Very Good 2 = Good 3 = Bad 4 = Very Bad	1 - 2 - 3 - 4	1 - 2 - 3 - 4	1 - 2 - 3 - 4	1 - 2 - 3 - 4	1 - 2 - 3 - 4	1 - 2 - 3 - 4	1 - 2 - 3 - 4
<b>How relaxed do you feel before you go to bed?</b> 1 = Very relaxed 2 = Relaxed 3 = Neutral 4 = Not at all	1 - 2 - 3 - 4	1 - 2 - 3 - 4	1 - 2 - 3 - 4	1 - 2 - 3 - 4	1 - 2 - 3 - 4	1 - 2 - 3 - 4	1 - 2 - 3 - 4
<b>Did you nap during the day? If yes, for how long?</b>							
<b>Write the time that you drank coffee, black tea or other caffeinated beverages.</b>							

Notes: \_\_\_\_\_

**Nutritional Intervention and Training Plan** for Optimizing  
Human Well-being and Sleep

Dear

In this document you will find your nutrition and training plan that we would like you to follow for the next 4 weeks.

**In the first 5 pages**, you will find instructions regarding nutrition, you will also find alternative choices for your **post-workout and evening meals** (do not forget that this is the most important part of your nutritional intervention, in order to see its impact on the quality of your sleep) alongside your nutritional plan, which we have designed for you! Additionally, on page 5 you will find a collection of general nutritional guidelines that will make following your nutrition plan easy and simple.

**In the last 3 pages** of the document, you can find your workout plan for the next 4 weeks and instructions regarding your plan. Your workout plan has been personalized based on the total score of your physical evaluation and tests that were conducted last week.

Thank you for taking part in our study and thank you for being part of this team. Good Luck!

Kind Regards,  
Angelos

This is your **Nutritional Plan**, based on the nutritional intervention group you previously picked!

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
<b>Breakfast</b>	2 toasts with 2 slices of bread, 1 slice of cheese and 1 slice of turkey	300g yogurt 41g cereals 1 fruit or 1 tsp honey	300g yogurt 41g cereals 1 fruit or 1 tsp honey	2 toasts with 2 slices of bread, 1 slice of cheese and 1 slice of turkey	300g yogurt 41g cereals 1 fruit or 1 tsp honey	300g yogurt 41g cereals 1 fruit or 1 tsp honey	2 toasts with 2 slices of bread, 1 slice of cheese and 1 slice of turkey
<b>Morning Snack</b>				15 almonds or 8 walnuts			
<b>Lunch</b>	170g minced beef 3 cups spaghetti (350g) (bolognese) 2 cups vegetables 2 tbs olive oil	2 cups legumes 4 slices of bread 2 cups vegetables 2 tbs olive oil	200g chicken 3 potatoes (500g) 2 slices of bread 2 cups vegetables 2 tbs olive oil	170g minced beef 3 cups spaghetti (350g) (bolognese) 2 cups vegetables 2 tbs olive oil	2 cups legumes 4 slices of bread 2 cups vegetables 2 tbs olive oil	200g chicken or 170g steak 2 slices of bread 3 potatoes (500g) 2 cups vegetables 2 tbs olive oil	180g fish 2 cups rice (300g) 2 slices of bread 2 cups vegetables 2 tbs olive oil
<b>Evening snack</b>							
<b>Post-Exercise Snack</b>							
<b>Dinner</b>	140g minced beef 1,5 cups whole-wheat pasta (250g) 2 cups vegetables	160g fish 1,5 cups brown rice (250g) 2 cups vegetables	160g chicken 1,5 cups brown rice (250g) 2 cups vegetables	140g minced beef 1,5 cups whole-wheat pasta (250g) 2 cups vegetables	1,5 cups legumes 4 slices of whole wheat bread 2 cups vegetables	160g chicken or 140g steak 11,5 cups brown rice (250g) 2 cups vegetables	180g fish 1,5 cups brown rice (250g) 2 cups vegetables

1 fruit (one serving) **low glycemic index** (refer to the table on page 5)  
2 fruits (two servings) **low glycemic index** (refer to the table on page 5)

## What are we focusing on the most?

Aside your workout-plan and your nutritional plan that will help you improve your performance and your body composition, **the meals you consumer after your workout are of high interest in this study and thus, we will emphasize their importance a bit more.**

In a recent study conducted by our research team, results indicated that a short-term dietary intervention, following an afternoon workout, assisted in optimizing sleep and increasing specific parameters of athletic performance the subsequent day, in a group of people who exercised regularly.

Therefore, in this research study we are focusing on the **type** of carbohydrate consumed after your afternoon workout. Depending on the nutritional intervention group you choose, you will also have to do choose one of the following options: 1) **Do not consume carbohydrates** upon completion of your afternoon workout and until you fall sleep. 2) **Consume foods that have a low glycemic index** upon completion of your afternoon workout and until you fall sleep. 3) **Consume foods that have a high glycemic index** upon completion of your afternoon workout and until you fall sleep.

Examples for foods that have a low or high glycaemic index can be found on page 5.

**Important:** Throughout the rest of your day, regardless of which nutritional intervention team you choose, you will have to eat carbohydrates, according to the dietary plan we have provided.

Do not forget, that during your nutritional intervention we will be in touch, so if you require any changes to your nutritional plan we are here to assist you, ensuring in this way that 1) *you do not feel restricted* 2) *that we are investigating the true impact of carbohydrates on sleep but also the true impact of carbohydrates on overall fitness and performance.*

## Categorization of foods based on their Glycemic Index

Low Glycemic Index		High Glycemic Index	
Food	Portion Size	Food	Portion Size
Wholegrain Bread	30g	White Baguette	30g
Rice Parboiled	55g (boiled)	White Bread	30g
Brown Rice	55g (boiled)	White Pitta	30g
Wholegrain Pasta	50g (boiled)	Jasmine Rice	60g (boiled)
Lentils	60g (cooked)	Basmati Rice	60g (boiled)
Beans	70g (cooked)	Pasta	50g (boiled)
Peas	200g (cooked)	Boiled Potato	100g
Orange	120g	Sweet Potato	80g (roasted)
Pear	120g	Banana	120g
Apple	120g	Raisins	20g (dried)
Oats	25g	Cranberry Juice	115ml
Muesli	25g	Coco Pops	20g

**Note:** I have included the recommended portion size, so you can alternate between foods that are within the same category, depending on the intervention group you have chosen!

*Example:* Let's say that the recommended portion size for pasta is 50g and the recommended portion size for a boiled potato is 150g. If your dinner includes 100g of pasta (2 recommended portion sizes), you can alternate this with 300g of potatoes (which also equals to 2 recommended portion sizes)

**Important:** You can only alternate between foods that are **within the same glycemic index group**.

Advice **regarding your diet plan** so you can be more flexible:

- You need to follow the 7-day diet plan for 2 weeks and until we complete our follow-ups. Your 7-day diet plan can be done in **any order you please** (e.g., Wednesday, Tuesday, Saturday etc.).
- **Be as flexible as possible.** Even though I have given you, meal suggestions based on the tests you have completed and your nutritional evaluation, if you wish to move meals around that is okay! Just make sure that you only move meals that are prior to your afternoon workout (so the meals following your afternoon workout will have the exact amount of carbohydrates needed both in portion size and quality).
- 1 fruit is equal to a cup of peeled fruit (e.g., 1 cup cherries, 1 cup apples, 1 cup banana etc.). Same applies for vegetables.
- Your vegetables can be consumed raw, boiled, or roasted.
- Any **dairy products** consumed must be **light!**
- You are free to consume any foods that have **zero calories!** For example, Light Jell-O, vinegar, lemon juice and any other spices you please!
- If you feel the need to add or subtract anything from your diet plan, please do so, but make sure that you note it down and that you inform us. This helps us modify your future diet plans appropriately.

**Alongside** your diet plan, we advise you to:

- To stay hydrated. There are no set guidelines regarding water consumption, since each human body needs a different amount of water to stay healthy and keep up with its environmental conditions. Your urine is the best indicator about how hydrated your body is. You should consume water until your urine is clear!



## It's time for your **workout plan!**

Here you will find your evidence-based workout plan that we specifically designed for you based on your ergometric tests. Please remember that you will have to train in the afternoon and, alternate between resistance/weight training and a cardio program. More specifically your workout plan looks like this:

Monday: Resistance/weight training

Tuesday: Cardio

Wednesday: Resistance/weight training

Thursday: Cardio

Friday: Resistance/weight training

Saturday: Rest

Sunday: Rest

In the last two pages you will find your workout plan regarding resistance/weight training and your cardio program! Do not forget to complete a warmup prior to your workout and a cooldown post completing your workout (e.g., 10min treadmill). Lastly, do not forget to perform each exercise correctly, whilst avoiding overload and hurting yourself.

If you have any inquiries or questions, I am always at your disposal,

Angelos

## Resistance/Weight training Workout Plan

### Day 1

- Lat Pull Down 4 x 8 @ 75kg
- Plate Loaded Chest Press 4 x 8 @ 70kg
- Plate Loaded Shoulder Press 4 x 8 @65kg
- Hack Squat 4 x 8 @ 80g
- Leg Extension 4 x 8
- Leg Curl 4 x 8

### Day 3

- Plate Loaded Row 4 x 8
- Bench Press 4 x 8
- Lateral Raises 4 x 8
- Leg Press 4 x 8
- Leg Extension 4 x 8
- Leg Curl 4 x 8

### Day 5

- Lat Pull Down 4 x 8 @ 75kg
- Plate Loaded Chest Press 4 x 8 @ 70kg
- Plate Loaded Shoulder Press 4 x 8 @65kg
- Hack Squat 4 x 8 @ 80g
- Leg Extension 4 x 8
- Leg Curl 4 x 8

## Cardio Workout Plan

### Day 2 & 4

- Warm-up: 5-min at 7.0km.
- 10 reps x 60 seconds at 12.6km with a break in between for 60 seconds. Meaning:
  - Set 1: 60 sec @ 12.6km + 60sec rest
  - Set 2: 60 sec @ 12.6km + 60sec rest
  - Set 3: 60 sec @ 12.6km + 60sec rest
  - Set 4: 60 sec @ 12.6km + 60sec rest
  - Set 5: 60 sec @ 12.6km + 60sec rest
  - Set 6: 60 sec @ 12.6km + 60sec rest
  - Set 7: 60 sec @ 12.6km + 60sec rest
  - Set 8: 60 sec @ 12.6km + 60sec rest
  - Set 9: 60 sec @ 12.6km + 60sec rest
  - Set 10: 60 sec @ 12.6km + 60sec rest
- Cool-down: 5-min at 7.0km.