

Special Issue Reprint

The Effect of the Mediterranean Diet on Metabolic Health

Edited by
Alessandro Leone and Ramona Silvana De Amicis

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The Effect of the Mediterranean Diet on Metabolic Health

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Guest Editors

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About the Editors

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Preface

The Mediterranean diet continues to represent one of the most widely studied and clinically relevant dietary patterns for the promotion of health and the prevention of chronic diseases. This Reprint was prepared with the aim of bringing together recent evidence on its relationship with metabolic health, highlighting both consolidated knowledge and emerging research perspectives.

The articles included in this Reprint reflect the broad scope of the Special Issue, addressing the Mediterranean diet in relation to cardiometabolic risk, glycemic control, inflammation, kidney health, neurological conditions, women's health, diet quality, and personalized nutrition. Together, they illustrate how Mediterranean diet-based approaches may be adapted to different populations and clinical contexts.

As Guest Editors, we hope that this Reprint will be useful to researchers, clinicians, nutrition professionals, students, and all those interested in understanding how dietary patterns can contribute to health maintenance and disease prevention. We also hope that it will stimulate further interdisciplinary research aimed at translating scientific evidence into practical nutritional strategies.

Alessandro Leone and Ramona Silvana De Amicis

Guest Editors

The Effect of the Mediterranean Diet on Metabolic Health

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The Mediterranean Diet (MedDiet) is one of the healthiest and most balanced dietary patterns worldwide. The MedDiet refers to the traditional eating pattern that became widespread in the early 1950–1960s throughout the Mediterranean. At that time, the MedDiet was considered to be a plant-based dietary pattern, which largely consisted of locally grown food, i.e., fruit and vegetable, nuts, seed, pulses, unprocessed cereals, olive oil as a source of fat and, only in small quantities, dairy products, and meat, or meat products.

This dietary pattern contains a high amount of lipids, particularly olive oil- and nut-derived, healthy unsaturated fats; fiber from pulses, fruits, and vegetables; low levels of non-starchy carbohydrates; and a minimal amount of animal protein that is preferentially obtained from fish and seafood [1].

At the end of 1950s, Ancel Keys and his colleagues were the first to identify the protective effects of the MedDiet against coronary heart disease (CHD) [2]. Since then, the MedDiet has been widely studied, and numerous positive health effects have been reported. Nowadays, the MedDiet has been associated with a better metabolic status [3] and cognitive function [4,5] and a lower risk of major chronic non-communicable diseases, i.e., cardiovascular diseases [6], diabetes [7], cancer [8], metabolic syndrome [9], and mood and eating disorders [10–12]. However, the results should be interpreted with caution because of the heterogeneity among these studies, and more high-quality studies are needed to provide robust evidence about the effect of the Mediterranean diet on the incidence of metabolic syndromes and their related comorbidities and the use of pharmacotherapy, as well as to delineate the biological mechanisms responsible for any global health benefits. Therefore, we have organized a Special Issue containing new scientific evidence regarding the effect of the Mediterranean diet on metabolic health. This topic has become even more relevant after the COVID-19 pandemic because of the increased prevalence of obesity [13]. This Special Issue is composed of six articles and two narrative reviews that investigated the relationship between the MedDiet, lifestyles, and metabolic health.

Caldirola et al. [14] reviewed evidence about the association between the MedDiet and kidney health. This narrative review suggested that the MedDiet could be beneficial both in the prevention and progression of chronic kidney disease, slowing its evolution. Similarly, the MedDiet has been associated with a lower risk of premenstrual syndrome (PMS). Kwon et al. [15] reported that Korean women with a higher adherence to the MedDiet were at lower risk of PMS. On the other hand, the consumption of more bread and snacks is associated with an up to 2.59 times higher risk of PMS. The MedDiet has also been shown to improve the metabolic health of patients with the APOA-5 genetic variant rs662799, which is involved in triglycerides metabolism and linked to metabolic syndrome and CVD. De Luis Roman et al. [16] tested the short (3 months)- and long (9 months)-term effects of the MedDiet on cardiometabolic risk factors among patients with 2 rs662799 genotypes (TT and CT+CC). They observed that the adiposity parameters, systolic pressure,

total cholesterol, LDL cholesterol, leptin, adiponectin, and leptin/adiponectin ratio were improved in both genotypes. Moreover, the insulin, HOMA-IR, and triglyceride levels were decreased in non-C allele carriers. The MedDiet seems to also be beneficial for those with chronic inflammatory diseases, including primary Sjögren's syndrome. Alunno et al. [17] reports that a MedDiet with a low purine content could significantly reduce the serum levels of uric acid (SUA), which, in Sjögren's syndrome, appears to raise the risk of interstitial lung disease (ILD) and cardiovascular disease (CVD). In a narrative review, Di Majo et al. [18] reviewed the effects of both the Mediterranean and ketogenic diets on people's immune systems, neuroinflammation, and redox balance. They also developed a ketogenic dietary protocol including low-carbohydrates foods from the Mediterranean diet to reduce the level of neuroinflammation in patients with multiple sclerosis (MS), slowing the progression of the disease. Also Bergia et al. [19] highlighted the importance of considering the glycemic index (GI) in the context of the MedDiet. They conducted a 12-week-long randomized controlled trial among adults at risk of developing diabetes to test the effect of a low-GI or high-GI MedDiet on glucose metabolism. They assessed the postprandial glucose and insulin responses to high- or low-GI meals and the glycemic variability at the baseline and post-intervention. They observed stronger postprandial insulin responses after high-GI meals only at the baseline, but stronger glucose responses both at the baseline and post-intervention. Moreover, despite the average daily glucose concentration decreasing in both groups post-intervention, the indices of 24 h glycemic variability improved only among the adults treated with a low-GI MedDiet. The adherence to the Mediterranean lifestyle (encompassing the MedDiet and other aspects of healthy living, such as food preparation, physical activity, and socializing) has been associated with better metabolic health in patients after a myocardial infarction. Novaković et al. [20] observed that patients who adhere to the Mediterranean lifestyle present with better glucose and lipid profiles both before and after cardiac rehabilitation than the patients who do not adhere to it. Finally, Turki et al. [21] evaluated the changes in diet and lifestyle during the national isolation period among the Tunisian adult population. Despite the proven benefits of the Mediterranean diet, they found that the pandemic affected people's dietary and lifestyle habits, negatively impacting their adherence to a healthy MedDiet. Thus, sleep quality and physical activity could be additional risk factors for the development of non-communicable diseases.

In summary, the beneficial effects of the MedDiet on metabolic health have been proven. Hence, it would be important to encourage changes in diets that aim to restore the healthy dietary pattern that, in the past, had characterized our diet.

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Article

The Interplay between Cardiovascular Risk, Cardiovascular Events, and Disease Activity in Primary Sjögren's Syndrome: Is Uric Acid the Missing Link?

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Abstract: (1) Background: Uric acid is a well-known cardiovascular (CV) risk factor in the general population but its role in the setting of rheumatic diseases other than gout is unclear. (2) Methods: This is a retrospective study investigating a cohort of 105 pSS patients recording clinical, serological, and CV-related variables including adherence to the Mediterranean diet. (3) Results: We observed a strong relationship between disease activity, interstitial lung disease (ILD), and CV events. The association between ILD and CV events was dependent on higher SUA levels but independent of other traditional CV risk factors. All three cases of previous non-fatal stroke were reported by females aged <65 years, with higher SUA levels, and two of them also had pSS-ILD. Forty (38%) patients had a 10-year risk of fatal and non-fatal CV disease events beyond the cut-off recommended for their age, and using the correction factor of 1.5 currently applied only to rheumatoid arthritis, we could better identify patient subsets characterized by different CV risk profiles including different SUA levels. (4) Conclusions: This study is the first to investigate in depth the role of SUA in the CV scenario of pSS. Our findings underpin the importance of assessing SUA levels in pSS in addition to the other traditional CV risk factors and to consider applying the correction factor for CV risk assessment tools to achieve a better stratification of CV risk.

Keywords: cardiovascular risk; cerebrovascular disease; Sjögren's syndrome

1. Introduction

An unhealthy diet, with or without impaired renal urate excretion, is the most frequent cause of hyperuricemia [1]. Despite its pivotal role in the pathogenesis of gout, the clinical relevance of serum uric acid (SUA) levels goes well beyond the simple association with gout and/or nephrolithiasis [2,3]. Previous studies have pointed to hyperuricemia as a cardiovascular (CV) risk factor in the general population; therefore, the 2018 guidelines of the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH) included the assessment of uricemia among the screening tests to be performed in hypertensive patients [4]. Furthermore, in view of the association between hyperuricemia and mortality (both CV and all-cause) and CV events, it is conceivable that CV damage begins with levels of uricemia lower than 6 mg/dL. In this context, the first results of the URRAH (Uric Acid Right for Heart Health) study identified an SUA threshold value of 4.7 mg/dL for all-cause mortality and 5.6 mg/dL for CV mortality [5]. In addition, in the same study, SUA was an independent risk factor for cerebrovascular (CBV) events after adjusting for potential confounding variables, including arterial hypertension (AH), and it identified a valid prognostic cut-off value (>4.79 mg/dL). Concordantly, SUA levels >5.34 mg/dL (confidence interval, CI 4.37–5.6, sensitivity 52.32, specificity 63.96, $p < 0.0001$) were the univariate prognostic cut-off value for all heart failure, whereas SUA levels >4.89 mg/dL

(CI 4.78–5.78, sensitivity 68.29, specificity 49.11, $p < 0.0001$) were the univariate prognostic cut-off value for fatal heart failure [6].

Primary Sjögren's syndrome (pSS) is a chronic inflammatory disease with a 9:1 female-to-male ratio and a prevalence ranging between 0.5 and 1% in the general population. Despite showing a tropism for exocrine glands, pSS can virtually involve any organ and system and cause extra-glandular clinical manifestations in up to 75% of patients [7–9]. Therefore, although the clinical picture is dominated by signs and symptoms of mucosal dryness, extra-glandular involvement may lead to a more severe disease course with additional manifestations such as arthritis, neuropathies, and purpura to cite a few. B-cell lymphoma is the most severe complication of pSS occurring in about 5% of patients.

In addition, pSS is burdened by higher CV morbidity and mortality compared to the general population like other chronic inflammatory diseases [10]. Previous studies have demonstrated that patients with pSS display traditional CV risk factors such as hypercholesterolemia, AH, and obesity [11]. However, the excess CV risk they have is in part attributable to underlying chronic inflammatory diseases as well the possible effect of drugs such as glucocorticoids.

The only available study that has explored SUA levels in pSS is a retrospective analysis showing that SUA levels may be associated with the onset of AH in a Chinese population [12]. However, they did not look into the role of SUA on overall CV risk or the development of CV events, nor did they investigate any relationship with disease activity.

Based on the existing data about SUA in the general population, we postulated that SUA may be of particular relevance in the pSS scenario. Therefore, since data on pSS in this area are lacking, we aimed to explore how SUA levels correlate with other CV risk factors, previous CV events, and disease-related features in patients with pSS and without gout and/or nephrolithiasis.

2. Materials and Methods

2.1. Population Study and Inclusion Criteria

A cross-sectional study was conducted recruiting consecutive patients with pSS according to the 2016 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria of aged 18 years or above [13]. The exclusion criteria were gout and/or nephrolithiasis, ongoing treatment with urate-lowering agents, one or more systemic/organ-specific autoimmune diseases other than pSS, and a current or recent history of neoplasm. The sample size calculation estimated that approximately 130 subjects had to be enrolled to achieve a power of 80% with alpha (probability of type I error) = 0.05. This value was obtained using reference data on the prevalence of CV risk factors and CV diseases in the general population and in the population of patients with various rheumatological diseases. Due to the above-mentioned exclusion criteria, the number of eligible patients we enrolled was slightly lower than the target estimated by the sample size calculation. The study was approved by the local Institutional Review Board.

2.2. Clinical and Serological Features

2.2.1. Demographic and Disease-Specific Features

Disease activity was calculated with the EULAR Sjögren's syndrome disease activity index (ESSDAI) [14] while the patient perception of symptoms (overall dryness, ocular dryness, oral dryness, pain, and fatigue) was reported on 0–10 visual analog scales (VAS). The overall dryness, pain, and fatigue VAS were also used to calculate the EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI) [15]. Anthropometric parameters recorded were height and weight and used to calculate the body mass index (BMI) [weight (kg) divided by height squared (m^2), kg/m^2].

2.2.2. Cardiovascular Risk Factors

The following CV disease risk factors were considered: smoking (defined as previous/current/no use of at least one cigarette/day), AH (physician diagnosis and/or

prior/ongoing antihypertensive therapy), hypercholesterolemia (total serum cholesterol level >200 mg/dL in at least three assays and/or prior/ongoing lipid-lowering therapy), hypertriglyceridemia (serum triglyceride level >150 mg/dL in at least three assays and/or prior/ongoing lipid-lowering therapy), high-density lipoprotein cholesterol (HDL-c) level (reduced <40 mg/dL, normal 40–60 mg/dL, increased >60 mg/dL in at least three assays), low-density lipoprotein cholesterol (LDL-c) level (increased >115 mg/dL in at least three assays), type 2 diabetes mellitus (DM) (ongoing treatment with insulin or oral hypoglycemic agents and/or glucose level >126 mg/dL in at least two fasting glycaemia tests), and obesity (according to BMI). The degree of obesity was established as follows: 25–30 kg/m² (overweight), 30–34.9 kg/m² (grade I obesity), 35–39.9 kg/m² (grade II obesity), and ≥ 40 kg/m² (grade III obesity or severe obesity). Metabolic syndrome was defined according to the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) criteria [16]. SUA was measured upon recruitment. In eligible patients, the 10-year risk of fatal and non-fatal CV disease events was calculated using the HeartScore[®] online tool of the European Society of Preventive Cardiology that incorporates the SCORE2 and SCORE2-OP risk prediction algorithms [17]. These algorithms estimate 10-year fatal and non-fatal CV disease risk in individuals in Europe without previous CV events or diabetes aged 40–69 years (SCORE2) and aged over 70 years (SCORE2-OP). The HeartScore[®] online tool is also calibrated to four European risk regions, based on age- and sex-standardized CV disease mortality rates.

2.2.3. Cardiovascular Events

CV events, namely myocardial infarction, angina, heart failure, cerebrovascular events, and arteriosclerotic vascular disease were recorded. CV events were recorded only if the diagnosis was confirmed by hospital discharge records and/or available specific laboratory and diagnostic examinations.

2.2.4. Adherence to the Mediterranean Diet

Adherence to the Mediterranean Diet over the previous 12 months was assessed with the 14-item PREvencion con DIeta MEDiterranea' (PREDIMED) tool [18]. A total score ≤ 5 indicates poor adherence, a score between 6 and 9 indicates medium adherence, and a score ≥ 10 indicates good adherence. The 28-item Mediterranean Lifestyle (MEDLIFE) Index was also used [19]. The MEDLIFE is structured as follows: block 1 includes 15 questions on Mediterranean food consumption (e.g., How many servings of fish or seafood portions do you consume per week?); block 2 includes 7 questions on Mediterranean dietary habits (e.g., Do you usually choose whole grain products?); and block 3 includes 6 questions on physical activity, rest, social habits, and conviviality (e.g., Do you engage in physical activity (>150 min/week or 30 min/day)?).

2.3. Statistical Analysis

The data were analyzed with IBM SPSS v.28.0. The normality of the data was assessed with the Kolmogorov–Smirnov test. As detailed throughout, the χ^2 test, Mann-Whitney U test, or Kruskal-Wallis test with Dunn's test for multiple comparison post hoc was used to compare the groups. Univariate regression and multivariate binary logistic regression were employed to identify any association between the variables. Bivariate correlation (Spearman's ρ) was also performed. The significance level was two sided and set at p -values less than 0.05.

3. Results

3.1. Analysis of the Full Cohort

One hundred and five patients with pSS (100 females and 5 males) were eligible for enrollment. Demographic and clinical and laboratory features, including CV risk factors and CV events, are shown in Table 1. The mean (standard deviation, SD) value of SUA was 4.4 ± 1.1 in the whole cohort. According to the prognostic cut-off value for CBV diseases of

the URRAH study, 24 (23%) patients had SUA levels ≥ 4.79 mg/dL. In addition, seven (7%) patients had SUA levels ≥ 5.6 mg/dL, the cut-off value for CV mortality identified by the URRAH study [5].

Table 1. Characteristics of the study cohort ($N = 105$).

Variable	Mean	SD
Age, years	61.9	11.5
Age at diagnosis, years	56.3	12.5
Disease duration, years	5.7	5.0
ESSDAI	6.3	4.8
ClinESSDAI	6.0	4.6
ESSPRI	6.3	2.3
VAS dryness	6.0	2.4
VAS xerostomia	6.3	2.7
VAS xerophthalmia	5.8	2.9
VAS pain	5.8	3.2
VAS fatigue	7.1	2.7
Total cholesterol (mg/dL)	202.5	35.7
Triglycerides (mg/dL)	109.3	49.5
HDL-c (mg/dL)	58.0	14.4
LDL-c (mg/dL)	126.0	34.6
SUA (mg/dL)	4.4	1.1
Variable	N	%
Female gender	99	94
Autoantibodies		
Neither anti-Ro nor anti-La	62	59
Anti-Ro only	26	25
Both anti-Ro and anti-La	17	16
Rheumatoid factor	25	24
Never smoked	75	71
Former smoker	19	18
Current smoker	11	11
Obesity	17	16
Arterial hypertension	46	44
Hypercholesterolemia	41	39
LDL-c > 115 mg/dL	41	39
HDL-c < 40 mg/dL	18	17
Hypertriglyceridemia	12	11
Type 2 diabetes	3	3
BMI (kg/m ²)	25.2	4.4
Obesity	17	16

Table 1. Cont.

Variable	Mean	SD
Myocardial infarction	4	4
Angina	6	6
Heart failure	3	3
Stroke	3	3
TIA	14	13
PAD	5	5
CV Risk Factors		
0	31	30
1–2	48	46
>2	26	25
CV events		
0	80	76
1	17	16
>1	8	8

SD, standard deviation; BMI, body mass index; ESSDAI, EULAR Sjögren's syndrome disease activity index; ESSPRI, EULAR Sjögren's Syndrome Patient Reported Index; VAS, visual analog scale; TIA, transient ischemic attack; PAD, peripheral arterial disease; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; CV, cardiovascular; SUA, serum uric acid.

As shown in Table 2, patients with SUA levels ≥ 4.79 mg/dL had a significantly higher BMI (27.2 vs. 24.6, $p = 0.009$), higher triglycerides levels (134.7 vs. 98.9, $p = 0.009$), a higher number of CV risk factors (>2 risk factors in 42 vs. 20% of patients), and were more frequently current or former smokers (51 vs. 22%) compared to patients with SUA levels < 4.79 mg/dL.

Table 2. Characteristics of the study cohort ($N = 105$) according to serum uric acid levels.

Variable	SUA < 4.79 mg/dL ($N = 81$)		SUA ≥ 4.79 mg/dL ($N = 24$)		p Value
	Mean	SD	Mean	SD	
Age, years	62.6	12.1	59.7	9.0	0.167
Age at diagnosis, years	56.9	13.2	54.1	10.1	0.254
Disease duration, years	5.9	5.2	4.8	4.0	0.971
ESSDAI	5.5	4.2	9.0	5.6	0.005
ClinESSDAI	5.2	4.1	8.8	5.4	0.003
ESSPRI	6.3	2.3	6.5	2.3	0.662
VAS dryness	6.1	2.4	6.0	2.4	0.903
VAS xerostomia	6.3	2.6	6.2	3.1	0.984
VAS xerophthalmia	5.8	3.0	5.7	2.6	0.677
VAS vaginal dryness	5.1	3.3	6.8	2.9	0.044
VAS pain	5.7	3.3	6.2	2.9	0.663
VAS fatigue	7.1	2.7	7.3	2.9	0.628
Total cholesterol (mg/dL)	204.5	35.7	197.6	36.3	0.561
Triglycerides (mg/dL)	98.9	38.2	134.7	64.0	0.009
HDL-c (mg/dL)	59.2	15.1	54.2	11.7	0.275

Table 2. Cont.

	SUA < 4.79 mg/dL (N = 81)		SUA ≥ 4.79 mg/dL (N = 24)		
Variable	Mean	SD	Mean	SD	p Value
LDL-c (mg/dL)	127.6	34.6	122.4	35.4	0.6
BMI (kg/m ²)	24.6	4.3	27.2	3.9	0.009
Variable	N	%	N	%	p Value
Female gender	80	99	19	79	<0.001
Autoantibodies					0.408
Neither anti-Ro nor anti-La	50	62	12	50	
Anti-Ro only	17	21	9	38	
Both anti-Ro and anti-La	14	17	3	13	
Rheumatoid factor	19	23	6	25	0.876
Smoking habit					0.014
Never	63	78	12	50	
Former	10	12	9	38	
Current	8	10	3	13	
Obesity	9	11	8	33	0.023
Arterial hypertension	32	40	14	58	0.159
Hypercholesterolemia	31	38	10	42	0.814
LDL-c > 115 mg/dL	31	38	10	42	0.814
HDL-c < 40 mg/dL	13	16	5	21	
Hypertriglyceridemia	6	7	6	25	0.028
Type 2 diabetes	2	2	1	4	0.545
Myocardial infarction	3	4	1	4	0.917
Stroke	0	0	3	13	0.011
TIA	11	14	3	13	0.891
Heart failure	2	2	1	4	0.661
Angina	4	5	2	8	0.618
PAD	3	4	2	8	0.321
Hydroxychloroquine	39	48	14	58	0.49
Glucocorticoids	13	16	6	25	0.37
Other immunosuppressant	20	25	4	17	
CV Risk Factors					0.024
0	27	33	4	17	
1–2	38	47	10	42	
>2	16	20	10	42	
CV events					0.07
No	65	80	15	63	
Yes	16	20	9	38	

SD, standard deviation; BMI, body mass index; ESSDAI, EULAR Sjögren's syndrome disease activity index; ESSPRI, EULAR Sjögren's Syndrome Patient Reported Index; VAS, visual analog scale; TIA, transient ischemic attack; PAD, peripheral arterial disease; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; CV, cardiovascular; SUA, serum uric acid.

With regard to previous CV events, all cases of stroke occurred in patients with SUA levels ≥ 4.79 mg/dL while no difference was observed regarding the other types of events or the total number of events. The prevalence of stroke in our population (2.8%, age range 26–86 years) was higher than that of the Italian general population publicly available on the Progetto Cuore website (0.8% females–1% males, age range 35–79 years, period 2008–2012) [20]. Of interest, all the three patients who had experienced a stroke were aged 65 or below at study recruitment.

In addition, patients with SUA levels ≥ 4.79 mg/dL had higher disease activity compared to patients with SUA levels < 4.79 mg/dL (mean ESSDAI value 9 vs. 5.5, $p = 0.005$), and when assessing the individual ESSDAI domains, glandular, pulmonary, and hematological manifestations were more frequently observed in patients with SUA levels ≥ 4.79 mg/dL (Supplementary Table S1) compared to patients with SUA levels < 4.79 mg/dL.

We then sought to investigate the features of the seven patients with SUA levels ≥ 5.6 mg/dL, the cut-off value for CV mortality identified by the URRAH study. As shown in Supplementary Table S2, when comparing patients with SUA levels < 4.79 mg/dL, 4.79 mg/dL $\leq$ SUA < 5.6 mg/dL or ≥ 5.6 mg/dL, we observed that increasing SUA levels paralleled an increase in disease activity (mean ESSDAI value 5.5 vs. 8.7 vs. 9.9, $p = 0.019$) while patient-reported outcomes were comparable across the three groups. When assessing the ESSDAI domains, a progressively higher prevalence of glandular (13 vs. 23 vs. 57%, 0.01) and pulmonary (0 vs. 18 vs. 28%, $p < 0.001$) manifestations was observed in groups with higher SUA levels (Supplementary Table S3).

With regard to the Mediterranean diet, the majority of patients had a medium or good adherence ($N = 98/105$; 93%); however, no differences in the PREDIMED or any of the MEDLIFE scores were observed across groups with different SUA levels.

3.2. Analysis of pSS Patients with Arterial Hypertension

As mentioned above, SUA has been included by ESC/ESH among the screening tests to be performed in hypertensive patients [4]; therefore, we analyzed in more detail pSS hypertensive patients (Table 3).

Table 3. Characteristics of the study cohort ($N = 105$) according to the presence or absence of arterial hypertension (AH).

	Without Arterial Hypertension		With Arterial Hypertension		
	Number of Patients = 59		Number of Patients = 46		
Variable	Mean	SD	Mean	SD	<i>p</i> Value
Age, years	59.0	13.0	65.7	8.0	0.009
Age at diagnosis, years	53.9	13.6	59.3	10.3	0.036
Disease duration, years	5.2	5.4	6.4	4.3	0.014
ESSDAI	5.8	4.4	6.9	5.3	0.317
ClinESSDAI	5.5	4.2	6.6	5.1	0.3
ESSPRI	6.0	2.2	6.6	2.3	0.147
VAS dryness	5.8	2.4	6.3	2.4	0.335
VAS xerostomia	6.0	2.8	6.6	2.5	0.301
VAS xerophthalmia	5.6	2.9	6.0	3.0	0.512
VAS vaginal dryness	5.0	3.2	5.8	3.4	0.221
VAS pain	5.6	3.2	6.1	3.2	0.479
VAS fatigue	6.9	2.7	7.5	2.8	0.137

Table 3. Cont.

	Without Arterial Hypertension		With Arterial Hypertension		
	Number of Patients = 59		Number of Patients = 46		
Variable	Mean	SD	Mean	SD	<i>p</i> Value
Total cholesterol (mg/dL)	194.7	29.4	209.6	39.6	0.111
Triglycerides (mg/dL)	98.2	44.0	119.0	52.6	0.035
HDL-c (mg/dL)	62.1	15.3	53.9	12.5	0.043
LDL-c (mg/dL)	117.9	31.2	132.2	36.2	0.085
BMI (kg/m ²)	24.4	4.4	26.1	4.3	0.020
Variable	<i>N</i>	%	<i>N</i>	%	<i>p</i> Value
Female gender	56	95	43	93	0.753
Autoantibodies					0.676
Neither anti-Ro nor anti-La	37	63	25	54	
Anti-Ro only	13	22	13	28	
Both anti-Ro and anti-La	9	15	8	17	
Rheumatoid factor	13	22	12	26	0.651
Smoking habit					0.04
Never	45	76	30	65	
Former	6	10	13	28	
Current	8	14	3	7	
Obesity	8	14	9	20	0.435
Hypercholesterolemia	14	24	27	56	<0.001
LDL-c > 115 mg/dL	12	22	29	63	<0.001
HDL-c < 40 mg/dL	8	14	10	22	0.341
Hypertriglyceridemia	4	7	8	17	0.124
Type 2 diabetes	0	0	3	7	0.08
Myocardial infarction	1	2	3	6	0.317
Angina	1	2	5	11	0.08
Heart failure	1	2	2	4	0.580
Any cardiac event	2	3	8	17	0.02
Stroke	1	2	2	4	0.418
TIA	7	12	7	15	0.774
PAD	2	3	3	6	
CV Risk Factors other than hypertension					<0.0001
0	31	53	11	24	
1–2	23	39	26	56	
>2	5	8	9	20	
Previous CV event(s)	9	17	16	33	0.02

SD, standard deviation; BMI, body mass index; ESSDAI, EULAR Sjögren's syndrome disease activity index; ESSPRI, EULAR Sjögren's Syndrome Patient Reported Index; VAS, visual analog scale; TIA, transient ischemic attack; PAD, peripheral arterial disease; LDL-c, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; CV, cardiovascular.

The prevalence of AH in our pSS population was 44% and when compared to non-hypertensive patients, the 46 pSS patients with AH resulted older and with a longer disease duration. They had a higher BMI, albeit with a comparable prevalence of obesity, a worse lipid profile with higher total cholesterol and LDL-c, higher triglycerides and lower HDL-c. They were more frequently current or former smokers, had a higher number of CV risk factors in addition to AH, and were more likely to have a history of previous CV events. Of the three patients with a history of stroke, only two were hypertensive.

When evaluating the dietary habits, pSS patients with AH had significantly lower mean (SD) levels of MEDLIFE total scores compared to non-hypertensive patients (14.8 (3.3) vs. 16.4 (2.9) $p = 0.02$) and this was due to significantly lower levels of block 3 which explores healthy lifestyle habits (2.05 (1.1) vs. 2.9 (1.2) $p = 0.003$). Regarding individual domains of block 3, it emerged that hypertensive patients had more sedentary habits and did not engage in at least 150 min/week or 30 min/day of physical activity. Conversely, blocks 1 and 2 of the MEDLIFE tool and the total PREDIMED score, exploring Mediterranean food and dietary habits, were comparable in hypertensive and non-hypertensive patients. However, a more detailed analysis of individual nutrient intake confirmed the association between AH and lower fish consumption which has been described in the general population [21,22] and which we observed in an independent pSS cohort [23]. In particular, only 15/46 hypertensive patients (32%) consumed at least two servings of fish/seafood per week compared to 35/59 (59%) non-hypertensive patients ($p = 0.006$). Binary logistic regression identified an odds ratio of 2.4 (95% confidence interval 1.1–5.5, $p = 0.04$) of having AH in those with lower fish intake.

When exploring the differences among hypertensive patients based on SUA levels (Table 4), we noticed that hypertensive patients with SUA levels ≥ 4.79 mg/dL were more likely obese (43% vs. 9%, $p = 0.015$), current or former smokers, and with a higher disease activity (mean ESSDAI value 10.1 vs. 5.5 $p = 0.02$). As in the analysis of the full cohort, also within hypertensive patients were glandular, pulmonary, and hematological ESSDAI domains more frequently involved in patients with SUA levels ≥ 4.79 mg/dL.

Table 4. Characteristics of hypertensive patients ($N = 46$) according to serum uric acid levels.

Variable	SUA < 4.79 mg/dL ($N = 32$)		SUA ≥ 4.79 mg/dL ($N = 14$)		p Value
	Mean	SD	Mean	SD	
Age, years	66.9	8.8	63.1	5.0	0.12
Age at diagnosis, years	60.5	10.8	56.6	8.9	0.19
Disease duration, years	6.5	4.5	6.1	3.9	0.97
ESSDAI	5.5	4.1	10.1	6.4	0.02
ClinESSDAI	5.2	4.0	9.8	6.1	0.02
ESSPRI	6.6	2.2	6.6	2.6	1.00
VAS dryness	6.6	2.2	5.7	2.8	0.32
VAS xerostomia	6.9	2.2	5.9	3.1	0.25
VAS xerophthalmia	6.2	2.9	5.6	3.1	0.48
VAS vaginal dryness	5.3	3.6	7.1	2.6	0.20
VAS pain	5.9	3.2	6.6	3.0	0.50
VAS fatigue	7.5	2.8	7.4	2.9	0.98
Total cholesterol (mg/dL)	213.1	41.1	202.5	37.1	0.57
Triglycerides (mg/dL)	113.5	40.7	129.1	70.4	0.65
HDL-c (mg/dL)	53.3	14.2	55.4	6.3	0.61

Table 4. Cont.

Variable	SUA < 4.79 mg/dL (N = 32)		SUA ≥ 4.79 mg/dL (N = 14)		p Value
	Mean	SD	Mean	SD	
LDL-c (mg/dL)	135.3	37.6	126.0	34.2	0.63
BMI (kg/m ²)	25.0	3.9	28.7	4.2	0.03
Variable	N	%	N	%	p Value
Female gender	31	97	12	86	0.216
Autoantibodies					0.246
Neither anti-Ro nor anti-La	18	56	7	50	
Anti-Ro only	7	22	6	43	
Both anti-Ro and anti-La	7	22	1	7	
Rheumatoid factor	10	31	2	14	0.294
Smoking habit					0.02
Never	25	78	5	36	
Former	6	19	7	50	
Current	1	3	2	14	
Obesity	3	9	6	43	0.015
Hypercholesterolemia	20	62	7	50	0.522
LDL-c > 115 mg/dL	21	66	8	57	0.742
HDL-c < 40 mg/dL	8	25	2	14	
Hypertriglyceridemia	6	19	2	14	0.54
Type 2 diabetes	2	6	1	7	
Myocardial infarction	2	6	1	7	0.673
Stroke	0	0	2	14	0.088
TIA	4	12	3	21	0.658
Heart failure	1	3	1	7	0.521
Angina	3	9	2	14	0.633
PAD	2	6	1	7	0.910
CV Risk Factors other than hypertension					0.18
0	10	31	1	7	
1–2	18	56	8	57	
2–3	13	41	7	50	
>3	0	0	1	7	
CV events	8	25	7	50	0.18

SD, standard deviation; BMI, body mass index; ESSDAI, EULAR Sjögren's syndrome disease activity index; ESSPRI, EULAR Sjögren's Syndrome Patient Reported Index; VAS, visual analog scale; TIA, transient ischemic attack; PAD, peripheral arterial disease; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; CV, cardiovascular; SUA, serum uric acid.

3.3. Analysis of pSS Patients without Previous CV Events

Finally, we focused on pSS patients fulfilling the criteria for the application of the SCORE2 and SCORE2-OP risk prediction algorithms (individuals in Europe without previous CV events or diabetes aged 40–69 years (SCORE2) and aged over 70 years (SCORE2-OP)) (Table 5).

Table 5. Characteristics of patients eligible for SCORE calculation ($N = 79$).

Variable	Eligible for SCORE ($N = 79$)		Not Eligible for SCORE ($N = 26$)		<i>p</i> Value
	Mean	SD	Mean	SD	
Age, years	61.3	11.7	63.8	11.0	0.389
Age at diagnosis, years	55.8	12.7	57.7	12.1	0.506
Disease duration, years	5.8	5.2	5.5	4.3	0.946
ESSDAI	6.2	4.7	6.5	5.2	0.884
ClinESSDAI	5.9	4.6	6.2	4.9	0.875
ESSPRI	6.0	2.2	7.3	2.1	0.018
VAS dryness	5.8	2.3	6.8	2.7	0.086
VAS xerostomia	6.1	2.6	6.7	2.8	0.362
VAS xerophthalmia	5.5	2.9	6.8	2.8	0.037
VAS vaginal dryness	5.2	3.5	6.2	2.6	0.283
VAS pain	5.4	3.2	7.0	2.7	0.03
VAS fatigue	6.9	2.9	8.0	2.0	0.106
Total cholesterol (mg/dL)	194.9	31.4	228.3	38.3	0.002
Triglycerides (mg/dL)	97.7	32.6	147.6	73.5	0.005
HDL-c (mg/dL)	58.7	13.8	55.5	16.7	0.709
LDL-c (mg/dL)	119.6	30.7	150.8	38.9	0.014
SUA (mg/dL)	4.3	1.0	4.9	1.2	0.05
BMI (kg/m ²)	24.5	4.0	27.5	4.9	0.005
Variable	<i>N</i>	%	<i>N</i>	%	<i>p</i> Value
Female gender	76	96	23.0	88	0.160
Autoantibodies					0.234
Neither anti-Ro nor anti-La	47	60	15	58	
Anti-Ro only	17	21	9	34	
Both anti-Ro and anti-La	15	19	2	8	
Rheumatoid factor	19	24	6	23	0.574
Smoking habit					0.42
Never	59	75	16	62	
Former	13	16	6	23	
Current	7	9	4	15	
Obesity	9	11	8	31	0.031
Hypertension	30	38	16	62	0.04
Hypercholesterolemia	23	29	18	69	<0.001
LDL-c > 115 mg/dL	28	35			0.241
HDL-c < 40 mg/dL	9	11	9	35	
Hypertriglyceridemia	5	6	7	27	0.009
CV Risk Factors					
0	28	35	3	11	0.0001
1–2	38	48	10	38	
2–3	26	33	12	46	
>3	2	3	8	31	

SD, standard deviation; BMI, body mass index; ESSDAI, EULAR Sjögren's syndrome disease activity index; ESSPRI, EULAR Sjögren's Syndrome Patient Reported Index; VAS, visual analog scale; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; CV, cardiovascular; SUA, serum uric acid.

By using the online HeartScore[®] tool, we assessed 79 patients whose characteristics are shown in Table 5. Since we identified that patients eligible for SCORE have lower values of ESSPRI, we analyzed the possible correlation of this variable and CV risk observing a direct correlation between the ESSPRI score and the number of CV risk factors (Spearman's $\rho = 0.22$, $p = 0.026$). A total of 40 (38%) patients had an estimated risk above the cut-off level recommended by existing guidelines for individuals of the same age, while 6 (7%) had the same value as the cut-off level (Figure 1A). When focusing on SUA levels, we observed that among the patients with a risk below the cut-off level recommended by the guidelines ($N = 33$), six of them (18%) had SUA values ≥ 4.79 mg/dL, and one of these six patients reached values ≥ 5.6 mg/dL.

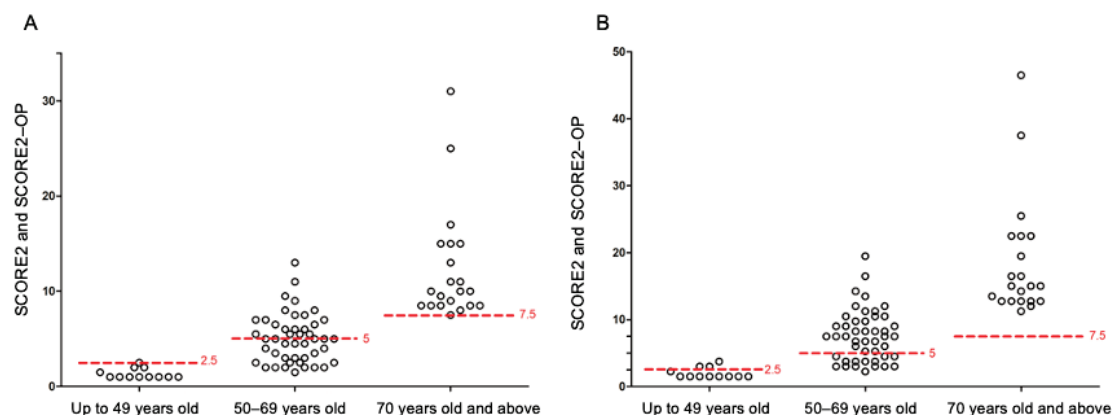


Figure 1. 10-year risk of fatal and non-fatal CV disease events calculated using the SCORE2 and SCORE2-OP risk prediction algorithms in the 79 eligible patients with primary Sjögren's syndrome. Panel (A) shows the normal calculation as in the general population; panel (B) shows the values using a 1.5 correction factor as currently applied in rheumatoid arthritis. Dotted lines and adjacent numbers show the corresponding cut-off value by age.

The 2021 update of the ESC guidelines for CV prevention, reinforced the importance that in patients with chronic inflammatory conditions such as rheumatoid arthritis (RA), multiplication of calculated total CV disease risk by a factor of 1.5 should be considered. Although evidence to use the 1.5 correction factor in connective tissue diseases is not yet solid enough to inform recommendations, the experts commented that it seems prudent to take into account the presence of such conditions when there is doubt regarding initiation of preventive interventions since the cumulative disease burden and recent degree of inflammation are important determinants of the risk-enhancing effect [24,25]. Therefore, for speculation purposes, we applied it to the risk scores of our cohort and observed that 10 out of 33 patients were reclassified above the cut-off level recommended by the guidelines for their age (Figure 1B).

We therefore compared the populations whose SCORE remained either within ($N = 23$) or beyond ($N = 46$) the cut-off regardless of the correction factor and the population was reclassified thanks to the correction factor. It emerged that the patients whose SCORE remained within the cut-off regardless of the correction factor are the youngest and show the lowest levels of total cholesterol, LDL-c, and triglycerides as well as the highest HDL-c values (all $p < 0.05$). In addition, only 10/23 (43%) patients had at least one CV risk factor (vs. 6/10 (60%) and 35/46 (76%) in the other populations, $p = 0.027$). None of the 23 patients whose SCORE remained within the cut-off regardless of the correction factor showed more than 2 CV risk factors (vs. 5/10 (50%) and 35/46 (76%) in the other populations, $p = 0.04$). As mentioned above, among the patients with a risk below the cut-off level recommended by the guidelines ($N = 33$), six of them (18%) had SUA values ≥ 4.79 mg/dL, and one of these six patients reached values ≥ 5.6 mg/dL. Of interest, these six patients were reclassified above the cut-off level following the application of the 1.5 correction factor.

3.4. Domains of Disease Activity, SUA Levels and CV Events

The analysis that we performed in the full cohort and in the different subgroups, showed that disease activity as well as the prevalence of involvement of three ESSDAI domains, glandular, pulmonary, and to a certain extent hematological, paralleled the levels of SUA. Therefore, we explored in more detail the relationship between disease activity, disease domains, SUA, and CV risk/CV events. Binary logistic regression demonstrated that pulmonary domain, but not glandular and hematological domains, is strongly associated with the outcome ‘previous CV event’ (univariate analysis: odds ratio (OR) = 15.1, 95% CI = 1.6–141.8, $p = 0.018$). Although the association between SUA levels ≥ 4.79 mg/dL and the outcome ‘previous CV event’ does not reach statistical significance at univariate analysis, when adding ‘SUA levels ≥ 4.79 mg/dL’ as a covariate, the association between the ESSDAI pulmonary domain and the outcome ‘previous CV event’ was no longer significant. In addition, the higher the number of CV events, the higher the odds of also having pulmonary manifestations (OR = 3.9, 95% CI = 1.5–10.0, $p = 0.004$).

Since in our cohort other variables resulted in being significantly associated with the outcome ‘previous CV event’ at univariate analysis, such as hypercholesterolemia (OR = 10.5 $p = 0.001$) and obesity (OR = 3.7, $p = 0.018$), we included them as covariates in the analysis for the association of the ESSDAI pulmonary domain and the outcome ‘previous CV event’. It emerged that both the ESSDAI pulmonary domain (OR = 18.5, $p = 0.02$), hypercholesterolemia (OR = 5.1, $p = 0.005$), and obesity (OR = 4.2, $p = 0.02$) are independently associated with the occurrence of CV events but the highest OR (namely, strongest association) was that with the ESSDAI pulmonary domain.

Finally, since two out of three patients who developed a stroke had pulmonary manifestations, we computed a logistic regression analysis, albeit recognizing the limitation of the analysis due to the small number of cases, and observed an association between ILD and stroke (OR = 66; $p = 0.02$).

Based on the data about the relationship between ESSPRI and the number of CV risk factors, we sought to investigate whether this was also related to CV events, and in fact, this score is also directly correlated with the number of CV events (Spearman’s rho = 0.24, $p = 0.015$).

4. Discussion

Over the last decade, observational studies have provided solid evidence of the association between high SUA levels and high CV risk in the general population [25]. This encompasses both clinical and subclinical CV manifestations and points to SUA as a risk factor worth assessing in the setting of CV prevention [4]. The only available study that has explored SUA levels in pSS is a retrospective analysis showing that SUA levels may be associated with the onset of AH in a Chinese population [12]. However, they did not look into the role of SUA on overall CV risk or the development of CV events, nor did they investigate any relationship with disease activity. We investigated in depth the role of SUA in the scenario of pSS and demonstrated a relationship between SUA levels, CV risk/diseases, and disease activity in pSS.

In line with existing evidence, the pSS patients in our cohort display traditional CV risk factors such as hypercholesterolemia, AH, and obesity [11]. Therefore, it is not surprising that in our cohort 24% of the subjects had already experienced a non-fatal CV event at the time of recruitment in the study. When classifying patients according to SUA levels, we could identify specific subgroups characterized not only by a different metabolic profile but also by different disease activity and disease features.

In our cohort, patients with SUA levels ≥ 4.79 mg/dL displayed higher disease activity than those with lower SUA levels and since both ESSDAI and ClinESSDAI were higher, this suggests that clinical rather than laboratory features (as ClinESSDAI excludes the biological domain) may be more relevant in this setting [26]. In more detail, patients with higher SUA levels have a higher prevalence of pulmonary manifestations, namely interstitial lung disease (ILD) and a relationship between pSS-ILD and the occurrence of previous

CV events has also emerged. In line with our findings, a recent study demonstrated that patients with ILD associated with RA display higher levels of SUA and also higher levels of bronchoalveolar lavage fluid (BALF)-UA [27]. A study using an experimental model of ILD demonstrated that bleomycin-induced lung injury triggers local production of UA, thereby activating the NALP3 inflammasome in the lung and perpetuating lung damage [28]. In addition, higher SUA and BALF-UA levels have been associated with worse pulmonary function at spirometry not only in RA-ILD but also in healthy subjects [27,29].

These data may suggest that similar events as described in RA occur in pSS thereby explaining our findings on the relationship between SUA and pSS-ILD.

In fact, the relationship between pSS-ILD CV events was dependent on having SUA levels ≥ 4.79 mg/dL but independent of other traditional CV risk factors such as hypercholesterolemia and obesity at multivariate analysis. Since there is evidence that damaged lungs may be a source of UA [28] and based on the dietary habit questionnaires, we could rule-out excessive intake of purine-rich food in our cohort and it is reasonable to speculate that in patients with pSS-ILD, at least part of circulating SUA may derive from the inflamed lungs and therefore contribute to increased CV risk on top of other risk factors.

When focusing on the type of CV event, we reported a higher prevalence of stroke in pSS compared to the general population. This is in line with a previous Italian study, demonstrating a higher prevalence of stroke in pSS [30]. However, a nationwide study demonstrated that Taiwanese patients with pSS had the same prevalence of stroke compared to the general population. Since the increased CV risk we observe in pSS results from the interplay of traditional and disease-specific risk factors, we believe that different ancestry, different dietary and voluptuary habits, as well as different environmental factors may account for this discrepancy [31]. In our cohort, all three cases of previous non-fatal stroke were reported by female patients with SUA levels ≥ 4.79 mg/dL, and two of them also had pSS-ILD.

A recent meta-analysis showed that SUA is a risk factor for stroke development particularly in healthy females [32] and this fits with our findings. However, the molecular mechanisms underlying this association in the general population are still unclear. To the best of our knowledge, there are no data about an association between ILD and stroke.

With regard to the effect of diet on SUA levels, a recent systematic review highlighted that low-calorie, purine-low, and Mediterranean-style diets induce a low, albeit significant reduction in SUA levels in patients with hyperuricemia/gout. Conversely, studies investigating the effect of CV risk factors were limited and inconclusive [33]. Since in our cohort the proportion of hyperuricemic (>6 mg/dL) patients was $<5\%$, this could explain the fact that no effect on SUA levels was observed according to the degree of adherence to the Mediterranean diet.

Finally, when focusing on pSS patients without previous CV events, we realized that 38% of them had values beyond the cut-off recommended for their age, and if using the correction factor of 1.5 currently applied only to RA, we could identify patient subsets characterized by different CV risk profiles. In fact, patients whose SCORE remained below the cut-off regardless of the correction factor were those with the most favorable metabolic profile. On the contrary, all six patients below the cut-off who were reclassified above the cut-off using the correction factor displayed higher SUA levels. Therefore, our findings underpin the importance of assessing SUA levels in addition to all the other traditional CV risk factors in all patients with pSS and to consider applying the correction factor for CV risk assessment tools not only in RA but also in pSS. As outlined by existing recommendations on CV prevention (REF), it is acceptable to possibly overestimate CV risk in light of the benefit of better stratifying patients and identifying those requiring more aggressive intervention and thorough follow-up.

Our study displays some limitations, including its retrospective nature, the sample size and consequent small number of CV events, and the lack of subclinical atherosclerosis assessments such as carotid intima media thickness. We recognize that our results need to be validated in larger multicenter studies to ensure generalizability; however, we were the

first to explore in detail the role of SUA in pSS and to demonstrate an additional facet of the complex CV scenario orchestrated by inflammation, traditional risk factors, and disease specific features.

5. Conclusions

In conclusion, our findings pave the way to more personalized CV preventive strategies in pSS [34] and, if their generalizability is confirmed by larger prospective studies, they will allow for the development of specific recommendations to be applied in clinical practice and ultimately reduce CV morbidity and mortality in this disease.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/nu15071563/s1>. Table S1: Prevalence of disease manifestations according to serum uric acid level (SUA) in the full cohort ($N = 105$); Table S2: Clinical characteristics of the full cohort ($N = 105$) stratified by different serum uric acid cut-off values; Table S3: Prevalence of disease manifestations according to various cut-off values of serum uric acid level (SUA) in the full cohort ($N = 105$).

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Article

Assessment of Mediterranean Diet Adherence and Lifestyle Change during COVID-19 National Lockdown in Tunisian Adult Population

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Abstract: The Mediterranean diet (MD) is a plant-based diet associated with a reduction in the risk of developing COVID-19 comorbidities. Lockdown instigation during the COVID-19 pandemic has affected eating habits and lifestyles, highlighting the need to analyze the healthiness of new consumption patterns. We conducted a survey to assess lifestyle change in Tunisian adults and their MD adherence. A total of 1082 respondents completed a self-administered online survey designed to assess their food and lifestyle habits. Poor overall adherence to MD was observed (mean MEDAS score 6.6, SD 1.07) in a preponderance of the mid-MD adherent subgroup (71.2% of the participants). Location, age, profession, and household welfare proxy were the main determinants of high MD adherence. When adjusting for sociodemographic variables, location and income remained statistically significant. Positive health outcomes were noticed in respondents with high MEDAS scores. Most importantly, binary logistic regression showed that risk of COVID-19 infection decreased as MEDAS score increased for unvaccinated obese participants (OR = 0.63; confidence interval (CI) 0.4–0.98; $p = 0.045$). Regarding lifestyle changes, confinement had contributed to an overall reduction in cigarette consumption, sleeping hours, and physical activity. Long-term consequences of these changes on health outcomes must be further explored.

Keywords: COVID-19; lockdown; MD adherence; lifestyle change; Tunisian adults

1. Introduction

In December 2019, a new infectious respiratory disease was first reported in Wuhan, Hubei Province, China. Caused by a new class of virus (SARS-CoV-2), it was recognized by the World Health Organization (WHO) as COVID-19 [1]. At the time of writing, this paper (3 August 2022), more than 575 million confirmed cases and 6 million deaths have been reported worldwide [2]. Globally, Tunisia experienced five waves of coronavirus. Breaking a record number of daily contaminations, the country was ranked on 6 July 2021 as the first in the Arab world and Africa in terms of number of contaminations and deaths [3]. To mitigate the spread of the disease, a variety of measures have been implemented, such as the banning of all social activities and cancellation of public events, up to the establishment of curfews and partial and complete lockdowns. These restrictive measures have influenced people's lifestyle and dietary habits [4]. In a recent cross-sectional study, we showed that of 1082 Tunisian adults, 57.8% reported a change in their eating habits [5].

In the territories around the Mediterranean basin, “Mediterranean diet” (MD) is a term that describes the traditional eating habits of people living in olive-growing areas [5]. UNESCO recognized the MD as a Cultural Heritage of Humanity in 2010 [6,7].

The MD is commonly accepted as a likely dietary model for the prevention and control of chronic noncommunicable diseases throughout life, and the protective effect of the MD against these diseases has been accurately reported by many studies [8–11]. The association between adherence to the Mediterranean diet and physical health function, controlling for confounding effects of age, smoking, BMI, alcohol consumption, and education, has been suggested to contribute to its beneficial effects [12]. In a recent ecological study, a negative association between MD adherence and COVID-19 cases and related deaths was documented in Spain and across 23 OECD (Organization of Economic Co-operation and Development) countries [13].

Despite its promotional health effects, the literature suggests that adherence to the MD has declined in the last few years in most regions of the world [14,15]. Globalization and cultural and social changes have caused a progressive abandonment of the MD and a simultaneous shift towards the Western diet [15]. This trend may have reversed or at least slowed during the initial phases of the current pandemic, as described in a recent systematic review of 12 observational studies [16].

Since the MD is generally associated with overall well-being and MD adherence is associated with reduction in risk of common comorbidities observed in COVID-19 patients, the aim of this study was to assess adherence to the MD among a large sample of the general Tunisian adult population along with their lifestyle changes, namely, physical activity, smoking status, and sleeping hours, during the COVID-19 lockdown period.

2. Subjects and Methods

2.1. Selection of Participants and Study Design

The study was carried out by the SURVEN research team (Nutrition Surveillance and Epidemiology in Tunisia) from the National Institute of Nutrition and Food Technology in Tunisia. It was a cross-sectional study based on a self-administered questionnaire with a nonprobabilistic sampling method. A Google Forms questionnaire was disseminated to the Tunisian population aged between 20 and 74 years through social networks (Facebook and Instagram) as well as institutional and private mailing lists. To calculate the sample size, the data published by the INS in 2021 were taken as reference. The Tunisian population was estimated at 12,075,950 million inhabitants [17]. Accordingly, the calculation of the sample size was carried out with 95% confidence level and 3% precision, and since the expected proportion of the change in population was unknown, 0.5 proportion was selected. The theoretical sample size was 1067 subjects [18]. Participation in the study was completely free, voluntary, and anonymous with the informed consent of the participants on data sharing and confidentiality policy. No personal data were requested, in accordance with the laws on the protection of personal data and the guarantee of digital rights. Therefore, this online survey did not require ethics committee approval.

2.2. Questionnaire Design

The questionnaire was established using Google Forms and disseminated in French and Arabic languages to meet the acceptance and understanding of the Tunisian population. The first section was dedicated to sociodemographic and economic data collection, and the second section included anthropometric and medical data. Weight and height were reported by the participants and used for the calculation of the body mass index (BMI), expressed as kg/m². For BMI below 18.5, the participant was considered underweight. For BMI between 18.5 and 24.9 the subject was classified as normal weight. For BMI between 25 and 29.9 the subject was considered overweight, and if BMI exceeded 30.0, the subject was considered obese. Data describing the general health status were recorded as well. Subjects suffering from multiple chronic diseases (more than 3 disease types) were considered of morbid health status.

Adherence to MD was assessed in the third section using the 14-item MEDAS screener [19]. According to the MEDAS screener responses, a MEDAS score was calculated, which ranges from 0 to 14. Based on this score, respondents were divided into three classes: (1) low MD

adherence for scores ranging from 0 to 5, (2) medium MD adherence for scores from 6 to 9, and (3) high MD adherence for scores ≥ 10 .

A fourth section included a structured questionnaire packet of 6 questions about daily consumption of certain foods not included in the MEDAS screener but important for MD pattern. The final section comprised 7 questions on lifestyle habits regarding smoking and sleeping habits, as well as physical activity. The full online version of the questionnaire is available in Appendix A. A pilot study on 20 women and 20 men of all age categories was launched and feedback collected to assess questionnaire quality.

2.3. Data Collection

Participants completed the forms directly connected to the Google platform. Once completed, each response was transmitted to this platform and the final database was downloaded as a Microsoft Excel spreadsheet. The questionnaire was disseminated over two months (between 17 May and 20 July 2021), coinciding with the fourth wave of COVID-19 in Tunisia. No general lockdown measure had been instigated during that period, but intercity movements were prohibited, and a curfew was imposed from 10 p.m. to 5 a.m.

2.4. Statistical Analyses

Descriptive results are expressed as proportions (%) for categorical variables and means with standard deviation and medians with interquartile range between square brackets [IQR] for continuous variables. Shapiro–Wilk and skewness–kurtosis tests were performed to assess variable distribution. Chi-squared and Fisher exact tests were used to evaluate equality distributions between groups. Mann–Whitney U and Kruskal–Wallis tests were applied to compare continuous variables among two or more groups whenever normality was not confirmed. Whenever relevant, post hoc tests were applied for more accurate analyses. Spearman’s correlation coefficient was calculated for associated continuous variables. McNemar tests were performed to compare between categorical variables (smoking status, sleeping habits, and sports practice) before and during COVID-19. Finally, associations between independent variable coded as 2 category response variables (virus infection or exposure) and sociodemographic variables (age, sex, region, education, professional activity, household welfare proxy) were assessed using binary logistic regression models (odds ratio, OR). For independent variables coded as more than 2 category response variables (MD adherence) we used multinomial logistic regression models (relative risk ratio, RRR) to estimate the association with sociodemographic covariates. The type I error risk was set at 0.05 for all analyses. Statistical analyses were performed using Stata 14.0 (StataCorp, College Station, TX, USA) [20]. For statistical analyses, risk of COVID-19 infection, MEDAS score, smoking status, and sleeping habits were considered outcome variables, while sociodemographic factors, i.e., sex, age, location, education, occupational status, household size, household welfare proxy, and health status (BMI, incidence of chronic disease, COVID-19 infection, and MD adherence status) were considered exposure variables.

3. Results

3.1. Participant Characteristics

A total of 1121 participants completed the questionnaire and 1082 responses were validated, representing a response rate of 101%. Tunisian nationals aged between 20 and 74 years were included in the study. As shown in Table 1, the study sample covered all Tunisian regions. Women represented 74.3% of the study population and subjects aged between 25 and 60 years were the most prevalent group for both sexes. Other sociodemographic data are summarized in Table 1.

Table 1. Sociodemographic characteristics of the participants.

	Total Population <i>n</i> (%) ¹	Women <i>n</i> (%)	Men <i>n</i> (%)
	1082 (100.0)	804 (74.3)	278 (25.7)
Location	<i>p</i> = 0.192 ²		
Greater Tunis	482 (44.6)	350 (43.5)	132 (47.5)
Northeast	154 (14.2)	120 (14.9)	34 (12.2)
Northwest	93 (8.6)	72 (9.0)	21 (7.5)
Center-east	195 (18.0)	151 (18.8)	44 (15.8)
Center-west	44 (4.1)	31 (3.9)	13 (4.7)
Southeast	90 (8.3)	67 (8.3)	23 (8.3)
Southwest	24 (2.2)	13 (1.6)	11 (4.0)
Age category	<i>p</i> < 0.0001 ²		
Young adult (20–25 years)	339 (31.3)	277 (34.4)	62 (22.3)
Adults (25–60 years)	691 (63.9)	503 (62.6)	188 (67.6)
Elderly (60 years and over)	52 (4.8)	24 (2.9)	28 (10.1)
Education	<i>p</i> < 0.0001 ²		
Not graduated	4 (0.4)	1 (0.1)	3 (1.1)
Primary school graduation	9 (0.8)	6 (0.7)	3 (1.1)
Secondary school graduation	76 (7.0)	37 (4.6)	39 (14.0)
University graduation	993 (91.8)	760 (94.5)	233 (83.8)
Occupational status	<i>p</i> < 0.0001 ²		
Unemployed or housewife	145 (13.4)	131 (16.3)	14 (0.5)
Student	423 (39.1)	346 (43.0)	77 (27.7)
Worker	71 (6.6)	40 (5.0)	31 (11.1)
Intermediate executive	132 (12.2)	84 (10.4)	48 (17.3)
Upper executive	272 (25.1)	186 (23.1)	86 (30.9)
Retiree	39 (3.6)	17 (2.1)	39 (7.9)
Household size (<i>n</i> = 1059) ³	<i>p</i> = 0.108 ²		
Living alone	29 (2.7)	17 (2.1)	12 (4.3)
Small family (≤4 members)	798 (73.8)	604 (75.1)	194 (69.8)
Large family (>4 members)	232 (21.4)	171 (21.3)	61 (21.9)
Unspecified	23 (2.1)	12 (1.5)	11 (4.0)
Household welfare proxy (<i>n</i> = 1059) ³	<i>p</i> = 0.14 ²		
Lower tertile	351 (32.5)	267 (33.2)	84 (30.2)
Middle tertile	353 (32.6)	251 (31.2)	102 (36.7)
Upper tertile	355 (32.8)	274 (34.0)	81 (29.1)
Not declared	23 (2.1)	12 (1.4)	11 (3.9)

¹ Data are expressed as total number of respondents *n* with percentages between brackets (%). ² *p*-value: null hypothesis of same distribution between both sexes (chi-squared and Fisher exact test); null hypothesis rejected at *p* < 0.05. ³ Number of respondents specifying their household size and welfare proxy.

Anthropometrics of the population are reported in Table 2. Mean age was 32.5 ± 12.0 years. A normal BMI was found for 48.8% of the study population, 5.5% were classified as underweight, 31.1% as overweight, and 14.5% as obese, with no sex difference. With regard to general health status, 78.7% of the respondents declared not suffering from any chronic diseases. As shown in Table 2, endocrine, nutritional, or metabolic diseases (24.3%), hematological diseases (11.3%) and cardiovascular diseases (10.9%) were the most mentioned chronic diseases with no sex difference. The great majority of the respondents declared eating spontaneously and not following a diet.

With regard to COVID-19 incidence, 67.9% of the surveyed subjects confirmed not being infected by SARS-CoV-2 and 81.9% had not experienced a family death due to COVID-19. Only 21.7% had been vaccinated against the virus at the period of the survey.

Table 2. Anthropometrics and medical data of the participants.

	Total Population <i>n</i> (%) ²		Women <i>n</i> (%)		Men <i>n</i> (%)	
	1082 (100)		804 (74.3)		278 (25.7)	
Age (years) ¹	32.5	12.0	31.1	10.8	36.5	14.2
Weight (kg) ¹	71.1	15.2	67.8	13.9	80.5	14.8
Height (m) ¹	1.68	0.08	1.64	0.06	1.77	0.06
BMI (kg/m ²) ¹	25.1	4.9	25.0	5.0	25.4	4.4
Nutritional status ²	<i>p</i> = 0.20 ³					
Underweight	60 (5.5)		50 (6.2)		10 (3.6)	
Normal weight	528 (48.8)		396 (49.2)		132 (47.5)	
Preobese	337 (31.1)		241 (30.0)		96 (34.5)	
Obese	157 (14.5)		117 (14.6)		40 (14.4)	
History of chronic diseases (number of diseases) ²	<i>p</i> = 0.20 ³					
No chronic disease	852 (78.7)		632 (78.6)		220 (79.1)	
One disease	195 (18.0)		143 (17.8)		52 (18.7)	
Multiple diseases	35 (3.2)		29 (3.6)		6 (2.1)	
History of chronic diseases (type of diseases) ²	<i>p</i> = 0.002 ³					
Cardiovascular diseases	25 (10.9)		14 (8.1)		11 (19.0)	
Endocrine, nutritional, or metabolic diseases	56 (24.3)		37 (21.5)		19 (32.7)	
Respiratory and ENT diseases	14 (6.1)		8 (4.6)		6 (10.3)	
Digestive diseases	13 (5.7)		12 (7.0)		1 (1.7)	
Hematological diseases	26 (11.3)		26 (15.1)		0 (0.0)	
Genecology, urinary and kidney diseases	9 (3.9)		5 (2.9)		4 (6.8)	
Cancer	2 (0.9)		2 (1.2)		0 (0.0)	
Other diseases	50 (21.7)		39 (22.7)		11 (19.0)	
Morbid health status (more than 3 diseases)	35 (15.2)		29 (16.9)		6 (10.3)	
Diet ²	<i>p</i> = 0.004 ³					
Spontaneous feeding with no diet	960 (88.7)		713 (88.7)		247 (88.8)	
Healthy diet	23 (2.1)		15 (3.2)		8 (1.4)	
Weight-loss diet	34 (3.1)		29 (3.6)		5 (1.8)	
Reduced sugar/salt diet	20 (1.8)		8 (1.0)		12 (4.3)	
Therapeutic diet	13 (1.2)		11 (1.4)		2 (0.7)	
Vegetarian diet	2 (0.2)		2 (0.2)		0 (0.0)	
Not specified	30 (2.8)		26 (3.2)		4 (1.4)	
Incidence of COVID-19 ²	<i>p</i> = 0.55 ³					
Not exposed	734 (67.8)		539 (67.0)		195 (70.1)	
Suspected infection	168 (15.5)		132 (16.4)		36 (12.9)	
Mid-form infection	167 (15.4)		124 (15.4)		43 (15.5)	
Severe form infection	13 (1.2)		9 (1.1)		4 (1.4)	
Occurrence of family death due to COVID-19 ²	<i>p</i> = 0.90 ³					
No	886 (81.9)		659 (82.0)		227 (81.6)	
Yes	196 (18.1)		145 (18.0)		51 (18.4)	
Vaccination against COVID-19 ²	<i>p</i> = 0.14 ³					
No	847 (78.3)		638 (79.3)		209 (75.2)	
Yes	235 (21.7)		166 (20.7)		69 (24.8)	

¹ Data are expressed as means with standard deviation in separate columns. ² Data are expressed as total number of respondents with proportions between brackets for categorical variables. ³ *p*-value: null hypothesis of same distribution between both sexes (chi-squared and Fisher exact test); null hypothesis rejected at *p* < 0.05.

Taking risk of COVID-19 infection as outcome variable and age, health status, sex and BMI as exposure variables, binary logistic regression showed that risk of COVID-19 infection significantly increased with the elderly group compared to young adults (OR = 5.8;

confidence interval (CI) = 1.6–20.5; $p = 0.006$). Subjects with more chronic diseases also had a higher risk of infection than healthy subjects (OR = 1.49; CI = 1.07–2.07; $p = 0.017$). For our study sample, no relationship was found between COVID-19 infection and sex or BMI ($p = 0.71$ and $p = 0.83$, respectively).

3.2. MD Adherence

After stratification of the study population in three classes based on calculated MEDAS score, MD adherence (outcome variable) was first analyzed according to sociodemographic factors (exposure variables). As shown in Table 3, the mean MEDAS score was 6.6 with standard deviation of 1.07 for the whole population with a preponderance of medium adherents (71.2%) compared to low adherents (23.7%) and high adherents (5.2%).

For the next analysis, we excluded subjects who reported following weight loss, therapeutic or vegetarian diets along with those who did not specify the diet. Hence, the sample size decreased to 806 individuals. The Mann–Whitney test showed no sex difference in MEDAS score ($p = 0.24$). An equal distribution of MD adherence classes among men and women was noticed as well. However, the Kruskal–Wallis test showed a significant difference in MEDAS score among geographical areas ($p = 0.028$), with the highest mean recorded in the center-east compared to Greater Tunis (post hoc analysis $p = 0.021$). It should also be stressed that the center-east presented the highest percentage (10.2%) of high MD adherents compared to other regions, whereas no high MD adherents were observed in the center-west, and a low percentage of this subpopulation was noticed in northwest Tunisia (1.5%).

Table 3. Adherence to Mediterranean diet over sociodemographic data.

Item	MEDAS Score ¹	Proportion of MD Adherents n (%) ²		
		Low Adherence	Medium Adherence	High Adherence
Total population ($n = 1082$)	6.6 ± 1.07 7 [6–8]	256 (23.7)	770 (71.2)	56 (5.2)
Sex ($n = 806$) ⁴	Mann–Whitney test $p = 0.24$ ³	Chi-squared test $p = 0.433$ ³		
Women	6.6 ± 1.7 7 [6–8]	145 (24.7)	409 (69.8)	32 (5.5)
Men	6.5 ± 1.6 6 [6–7]	50 (22.7)	162 (73.6)	8 (3.6)
Location ($n = 806$) ⁴	Kruskal–Wallis test $p = 0.028$ ³	Chi-squared test $p = 0.098$ ³		
Greater Tunis	6.5 ± 1.7 6 [5–8]	89 (25.2)	249 (70.5)	15 (4.2)
Northeast	6.5 ± 1.9 6 [5–8]	36 (28.8)	83 (66.4)	6 (4.8)
Northwest	6.41 ± 1.4 6 [6–7]	13 (19.4)	53 (79.1)	1 (1.5)
Center-east	7.1 ± 1.8 7 [6–8]	24 (17.5)	99 (72.3)	14 (10.2)
Center-west	6.3 ± 1.5 7 [5–7]	10 (27.8)	26 (72.2)	0 (0)
Southeast	6.6 ± 1.5 7 [5–8]	17 (25.7)	47 (71.2)	2 (3)

Table 3. Cont.

Item	MEDAS Score ¹	Proportion of MD Adherents <i>n</i> (%) ²		
		Low Adherence	Medium Adherence	High Adherence
Southwest	6.5 ± 1.8 6 [5-8]	6 (27.3)	14 (63.6)	2 (9)
Age category (<i>n</i> = 806) ⁴	Kruskal–Wallis test <i>p</i> < 0.001 ³ Spearman test (<i>r</i> = 0.15, <i>p</i> < 0.001) ³	Chi-squared test <i>p</i> < 0.001 ³		
Young adults (20-25 years)	6.3 ± 1.7 6 [5-7]	81 (30.6%)	176 (66.4)	8 (3)
Adults (25-60 years)	6.7 ± 1.7 7 [6-8]	113 (22.4%)	366 (72.5)	26 (5.1)
Elderly (over 60 years)	7.7 ± 1.8 7 [7-9]	1 (2.8%)	29 (80.5)	6 (16.6)
Education (<i>n</i> = 806) ⁴	Kruskal–Wallis test <i>p</i> = 0.22 ³	Chi-squared test <i>p</i> = 0.768 ³		
Not graduated	7.7 ± 0.9 7.5 [7-8.5]	0 (0)	4 (100)	0 (0)
Primary school graduation	7.5 [5.5-8]	2 (25)	6 (75)	0 (0)
Secondary school graduation	6.3 ± 1.4 6 [6-7]	12 (24.4)	36 (73.5)	1 (2)
University graduation	6.6 ± 1.7 7 [6-8]	181 (24.2)	525 (70.5)	39 (5.2)
Occupational status (<i>n</i> = 806) ⁴	Kruskal–Wallis test (<i>p</i> = 0.015) ³ Spearman test (<i>r</i> = 0.11, <i>p</i> = 0.017) ³	Chi-squared test <i>p</i> = 0.798 ³		
Unemployed/housewife	6.5 ± 1.5 6.5 [5-8]	26 (26.5)	70 (71.4)	2 (2)
Student	6.4 ± 1.8 6 [5-8]	94 (29.3)	213 (66.3)	14 (4.3)
Worker	6.3 ± 1.3 6 [6-7]	14 (24.6)	43 (75.4)	0 (0)
Intermediate executive	6.4 ± 1.5 6 [6-7]	24 (24.7)	71 (73.1)	2 (2)
Upper executive	7 ± 1.8 [6-8]	36 (17.1)	154 (73.3)	20 (9.5)
Retiree	7.4 ± 1.5 7 [7-8]	1 (4.3)	20 (86.9)	2 (8.7)
Household size (<i>n</i> = 788) ⁵	Kruskal–Wallis test <i>p</i> = 0.2 ³	Chi-squared test <i>p</i> = 0.079 ³		
Living alone	6.3 ± 1.1 6 [6-7]	4 (21)	15 (79)	0 (0)
Small family (≤4 members)	6.7 ± 1.7 6 [6-8]	128 (21.6)	433 (73)	32 (5.4)
Large family (>4 members)	6.4 ± 1.8 6.5 [6-8]	55 (31.2)	32 (64.8)	7 (4)
Household welfare proxy (<i>n</i> = 788) ⁵	Kruskal–Wallis test <i>p</i> < 0.001 ³ Spearman test (<i>r</i> = 0.214, <i>p</i> < 0.001) ³	Chi-squared test <i>p</i> < 0.001 ³		

Table 3. Cont.

Item	MEDAS Score ¹	Proportion of MD Adherents <i>n</i> (%) ²		
		Low Adherence	Medium Adherence	High Adherence
Lower tertile	6.17 ± 1.64 6 [5-7]	87 (33.1)	168 (63.9)	8 (3)
Middle tertile	6.55 ± 1.7 6 [6-8]	66 (23.6)	201 (71.8)	13 (4.6)
Upper tertile	7.11 ± 1.62 7 [6-8]	34 (13.9)	193 (78.8)	18 (7.3)

¹ Data are expressed as means with standard deviation and medians with interquartile range between square brackets [IQR]. ² Data are expressed as total number of respondents with proportions between brackets for categorical variables. ³ *p*-value: null hypothesis of same distribution between both sexes (chi-squared and Fisher exact test); null hypothesis rejected at *p* < 0.05. ⁴ Number of respondents under spontaneous feeding, healthy diet and sugar/salt-reduced diet. ⁵ Number of respondents specifying their household size and welfare proxy.

A significant difference (*p* < 0.001) in MEDAS score was found among different age-groups (Figure 1) with positive and significant Spearman correlation coefficients (*r* = 0.15, *p* < 0.001). Elderly subjects presented significantly higher MEDAS scores than younger groups (post hoc analysis *p* < 0.001, *p* < 0.001 for adults and young adults, respectively). In agreement with this result, the elderly group had a significantly (*p* < 0.001) higher proportion of high MD adherents (16.6%) than the other groups (3% and 5.1% for young adults and adults, respectively).

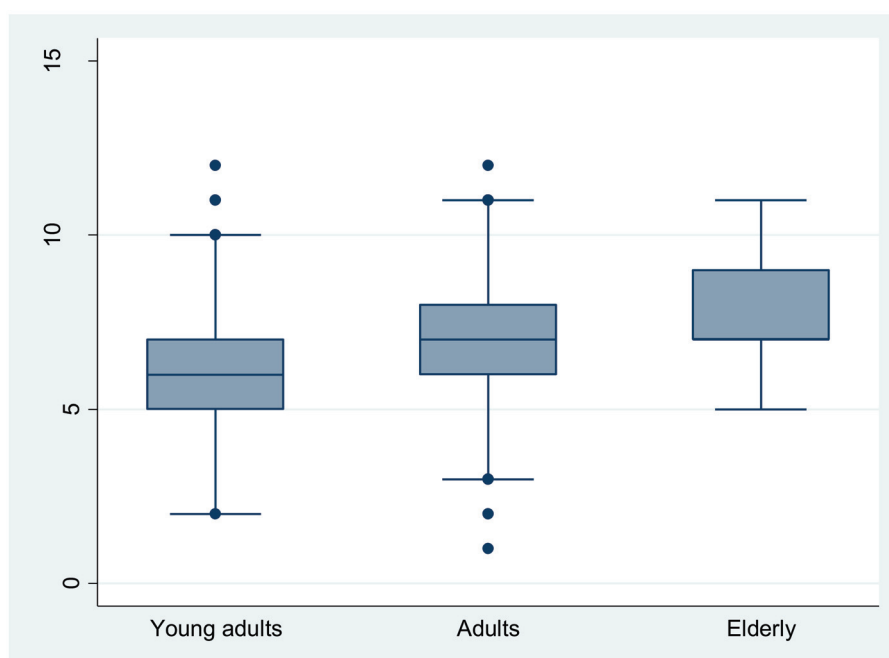


Figure 1. Distribution of MEDAS score over age categories. Elderly subjects had significantly higher MEDAS scores than younger groups (post hoc analysis *p* < 0.001, *p* < 0.001 for adults and young adults, respectively).

Unlike education, occupation made a significant difference in MEDAS score (*p* = 0.021). When post hoc analysis was performed, a significantly higher score for upper executives was found compared to students (*p* = 0.003) and middle executives (*p* = 0.05). Spearman's test demonstrated a positive significant correlation between MEDAS score and employment (*r* = 0.15, *p* < 0.001).

No significant difference ($p = 0.2$) between single, small, and large households was found for MEDAS score or distribution of MD adherents' classes. By contrast, with regard to welfare proxy parameter, our results showed a significant difference for the mean MEDAS score between the three economic classes ($p < 0.001$). A post hoc analysis showed that a significantly higher mean score is noticed for high economic level (upper tertile) compared to medium ($p = 0.025$) and low economic classes ($p < 0.001$) (Figure 2). Spearman's test confirmed the positive and statistically significant correlation between the participant's economic level and their MEDAS score ($r = 0.214$, $p < 0.001$). The proportion of high-MD adherents (7.3%) was significantly preponderant ($p < 0.001$) among high economic class compared to medium (4.6%) and low economic classes (3.4%).

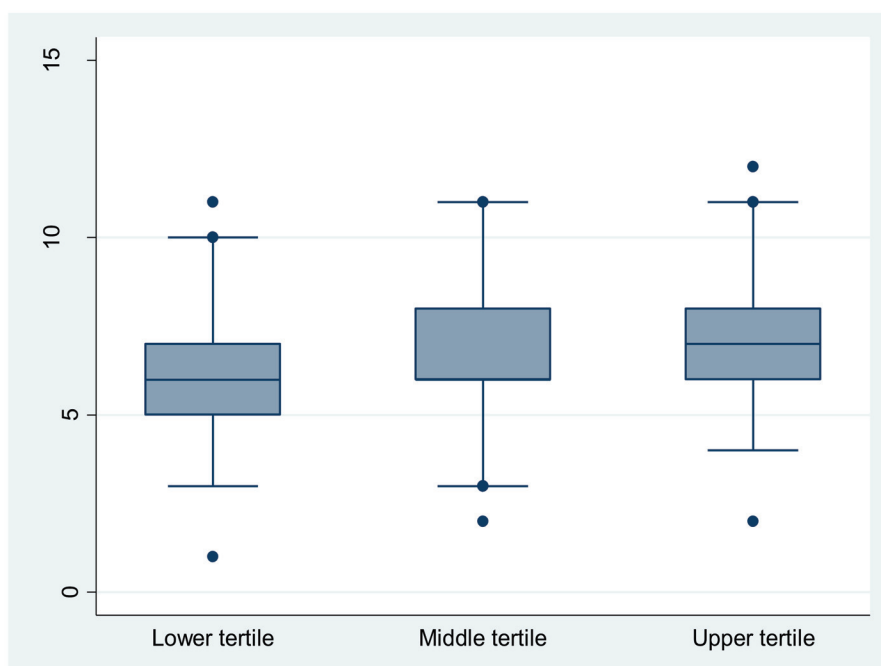


Figure 2. Distribution of MEDAS score over household welfare proxy. Significant difference for the mean MEDAS score between the three economic classes ($p < 0.001$). A post hoc analysis showed that a significantly higher mean score is noticed for high economic level (upper tertile) compared to medium ($p = 0.025$) and low economic classes ($p < 0.001$).

Multivariate logistic regression revealed that compared to Greater Tunis, respondents living in central east Tunisia were more likely to be high MD adherent (RRR = 4.51; 95% IC: 1.54–13.2; $p = 0.006$) along with elderly participants (RRR = 49.8; 95% (IC): 4.7–522.8; $p < 0.001$). Participants in the upper tertile of household welfare proxy were predicted to be highly MD-adherent in comparison with the lower tertile (RRR = 5.32; 95% (IC): 1.66–17; $p = 0.005$). To minimize the effect of selection bias, a classified analysis based on sex, age, education, occupation, region, household size, and economic level was conducted. Results showed that after adjustment on these variables, MD adherence remained significantly impacted by location and household welfare proxy. Participants living in central Tunisia were prone to be highly MD-adherent compared to those living in Greater Tunis (OR = 1.75; 95% (IC): 1.16–2.65; $p = 0.007$). Compared to the lower tertile, participants in the middle tertile of household welfare proxy were predicted to be highly MD-adherent (OR = 1.96; 95% (IC): 1.38–2.79; $p < 0.001$) along with those in the upper tertile (OR = 2.64; 95% (IC): 1.76–3.96; $p < 0.001$).

Kruskal–Wallis tests showed a significant difference in MEDAS score (outcome variable) related to health status considered as exposure variable ($p = 0.047$). As shown in Figure 3, the highest MEDAS score was recorded by healthy adults while the lowest score was observed among adults presenting a morbid health status with multiple chronic dis-

eases. For the elderly group, cardiovascular, endocrine, nutritional and metabolic diseases were the only diseases reported by high MD adherents (Figure 3).

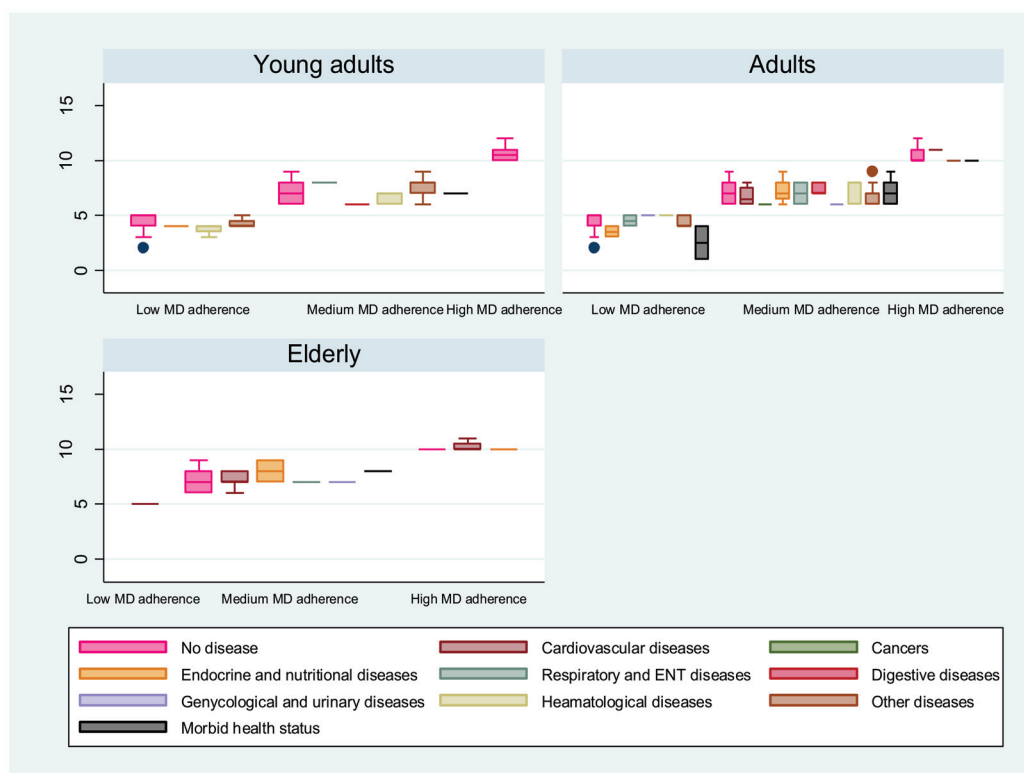


Figure 3. Impact of MD adherence on health status for the three MD-adherence profiles. Distribution of MEDAS score over MD adherence by age category and chronic diseases declared by participants. Kruskal–Wallis tests showed a significant difference in MEDAS score related to health status considered ($p = 0.047$). For all age categories, healthy participants recorded the highest MEDAS scores. Adults with morbid health status with multiple chronic diseases recorded the lowest MEDAS scores.

In agreement with those results, for adults and young adults, high MD adherents reported good health status with no relevant chronic diseases (unshown data). For elderly, high MD adherents reported less affection by chronic diseases than medium adherents. Cardiovascular and endocrine diseases were the only diseases declared by this group (Figure 3).

An analysis of the degree of compliance to the MD with each recommendation of the PREDIMED questionnaire for the three categories of MD adherence was carried out. As shown in Figure 4, a drastic difference in compliance was observed for almost all food groups except frequency of weekly preparation of Mediterranean dishes, which was comparable between the three adherence profiles (Figure 4). In addition, no difference was observed for the consumption of red meat and wine. This latter remains unadopted in the Tunisian culinary culture.

It is worth noting that high MD adherents were mostly noncompliant with the recommendations for the consumption of fish and seafood, legumes and nuts, with compliance below 60% (Figure 4). Comparison was carried out between the three adherence profiles for the consumption of foods belonging to the MD but not included in the PREDIMED questionnaire. For the three adherence profiles, no statistically significant difference was found for the consumption of cereals, wholemeal bread, cracked wheat, cooked wheat and corn, nor for milk, yoghurt, cheese, dairy products, or eggs (unshown data). However, a statistically significant and positive correlation was noted between the amount of water consumed and the degree of adherence to the MD ($r = 0.13$, $p < 0.001$).

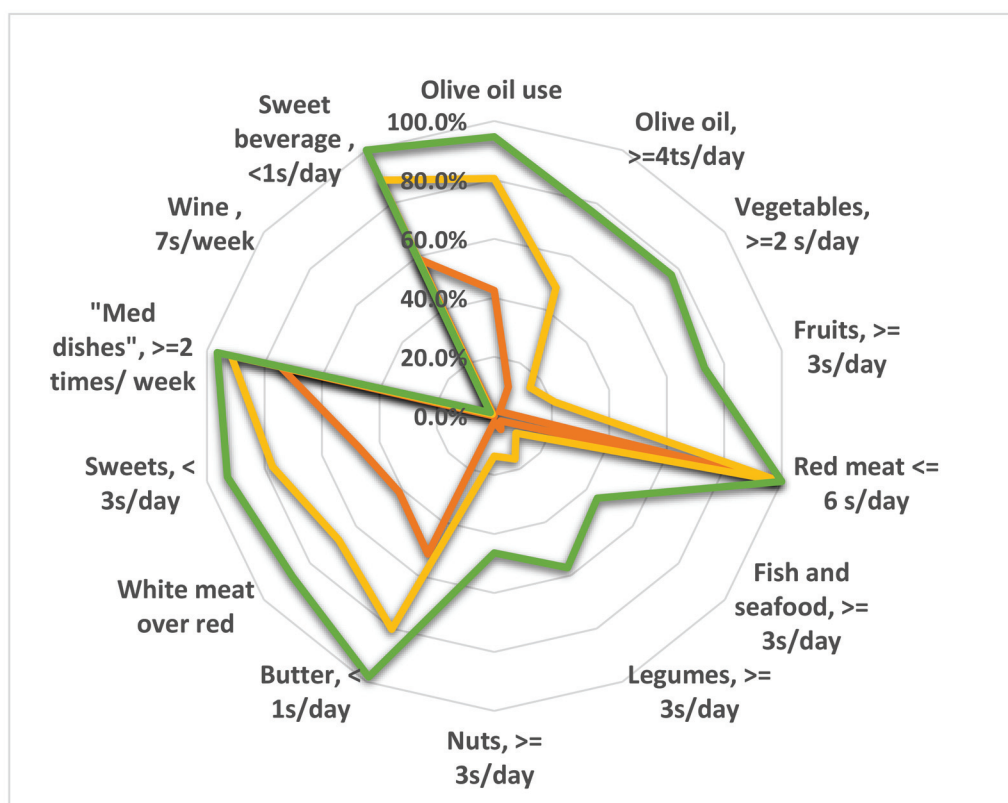


Figure 4. Compliance with items from PREDIMED questionnaire according to adherence profile: high (green), medium (yellow) and low adherence (red) to the Mediterranean diet (MD). The radar chart plots the values of each item of the questionnaire along a separate axis. Each axis refers to one of the 14 recommendations of PREDIMED questionnaire. Axis starts in the center of the chart (0% compliance) and ends at the outer ring (100% compliance). The values are the percentage of respondents compliant with each recommendation. The figure shows that consumption of fish and seafood, legumes, nuts and wine are commonly inappropriate for the three MD-adherence profiles.

For analyses related to COVID-19 infection, apart from subjects who declared following a specific diet, vaccinated subjects as well as those who suspected infection with no COVID test confirmation were also excluded from the subsequent analysis. To investigate correlation between COVID-19 infection (outcome variable) and MEDAS score (exposure variable), binary logistic regression showed that particularly for unvaccinated obese subjects with no specific diet ($n = 87$), as MEDAS score increased, the risk of being infected by COVID-19 significantly decreased (OR = 0.63; CI: 0.4–0.98; $p = 0.045$). The risk of infection significantly increased for obese subjects aged above 60 years (OR = 26.75; CI: 1.4–510.08; $p = 0.029$).

3.3. Lifestyle Habits

Regarding lifestyle habits, our study focused on changes in smoking, sleeping habits and physical activity before and after the occurrence of the COVID-19 pandemic.

Smoking: Overall, the study population reduced cigarette consumption during the confinement periods. Indeed, the number of nonsmokers increased slightly from 81.4% before the confinement to 83.27% during the confinement. It should also be noted that subjects smoking fewer than five cigarettes per day decreased from 4.5% to 3.4% and those smoking more than 10 cigarettes per day decreased from 10.3% to 8.9% (Table 4). This was confirmed by the McNemar test (McNemar value = 2.64, $p = 0.0018$).

Table 4. Changes in smoking status and sleeping habits before and during the COVID-19 pandemic.

	Smoking Pre-COVID-19	Smoking during COVID-19
Nonsmoking	878 (81.2) ¹	901 (83.2)
<5 cigarettes/day	49 (4.5)	37 (3.4)
5–10 cigarettes/day	44 (4.0)	48 (4.4)
>10 cigarettes/day	111 (10.2)	96 (8.8)
	Sleeping habits pre-COVID-19	Sleeping habits during COVID-19
<7 h/night	338 (31.2) ¹	421 (38.9)
7–9 h/night	573 (53)	499 (46.1)
>9 h/night	171 (15.8)	162 (15)

¹ Values are expressed as numbers and percentage (n (%)).

The Mann–Whitney test showed that sex (exposure variable) determines smoking status (outcome variable) in a statistically significant way before and after confinement ($p < 0.001$). Indeed, there were more nonsmoking women than men for both the pre- and post-COVID-19 periods. Changes in smoking habits had mostly affected men. Proportion of men consuming more than 10 cigarettes per day decreased from 27.6% to 21.9% during lockdown. Before the pandemic, the majority of nonsmokers were young adults (86.4%) and elderly (84.6%). During the containment periods, adults reduced their cigarette consumption, increasing the percentage of nonsmokers of this age category from 78.3% to 81.9%. Prior to the pandemic, a negative association ($r = -0.14$, $p < 0.001$) between cigarette consumption and instruction level had been ruled and remained unchanged after the pandemic ($r = -0.13$, $p < 0.001$). The highest percentage of nonsmokers was observed among housewives (92%). This percentage remained unchanged during the confinement periods. Post-COVID showed for all occupational classes, except students and retirees, a decrease in the percentage of heavy smokers. Finally, our results showed that those who live alone tend to consume more cigarettes before and during the confinement. The proportion of lone heavy smokers was 24.1% (before lockdown) and 20.7% (during lockdown) higher than those living in small (9.9% and 8.5%) and large (9% and 8.6%) households.

Sleeping: Our results showed that confinement contributed to an overall reduction in sleep hours (Table 4). Those who slept less than 7 h per night increased after the pandemic from 31.2% to 38.9%. On the other hand, those who slept between 7 and 9 h/night decreased from 52.9% to 46.1% in a similar way to those who sleep more than 9 h/night, who decreased from 15.8% to 14.9% (McNemar value = 1.9, $p < 0.001$).

During confinement, women seemed to decrease their sleep hours. Those who slept less than 7 h during lockdown reached 39.2%. This is comparable to that of men (38.1%) whose sleep habits did not seem to be affected by confinement (unshown data).

A significant negative association between sleeping hours and age was shown before ($r = -0.14$, $p < 0.001$) and during confinement ($r = -0.10$, $p = 0.0006$). It should be noted that unlike the elderly, confinement led youth and adults to reduce their sleep hours.

A statistically significant difference was demonstrated for sleep patterns related to occupational status before and during the pandemic ($p = 0.0001$). Before the pandemic, the highest numbers sleeping less than 7 h per night were retirees (53.8%) and housewives (40%), while the lowest were students (23.4%) and workers (23.9%). Those sleeping more than 9 h were more likely to be unemployed (28.4%) compared to upper executives (8%). During confinement, many housewives slept less than 7 h (46%) along with middle executives (52.2%). Unemployed people also kept the habit of sleeping more than 9 h/night (32.6%) versus little among upper executives (7.3%).

Physical activity: Obviously, the pandemic significantly decreased physical activity (McNemar value = 5.29, $p < 0.0001$). Those not engaged in physical activity increased from 46.7% pre-pandemic to 74.9% within the pandemic (Table 5). Physical activity seemed to be sex-associated ($p < 0.001$). Prior to the pandemic, sedentariness among women was 48.6%

and for men 41%. This trend does not seem to change after the pandemic, with an increase in sedentariness for both sexes, more for women (76.4%) than men (70.5%).

Table 5. Physical activities before and during COVID-19 pandemic.

Sport Practiced Pre-COVID-19	
None	505 (46.7) ¹
Gym, yoga, dance, or aerobics	105 (9.7)
Walking	293 (27.1)
Swimming	7 (0.6)
Football, basketball, volleyball, or tennis	50 (4.6)
Martial arts	14 (1.3)
Two activities	95 (8.8)
Three activities or more	13 (1.2)
Sport practiced during COVID-19	
None	810 (74.9) ¹
Gym, yoga, dance or aerobics	22 (2)
Weightless training	111 (10)
Weight training at home	33 (3)
Treadmill	16 (1.5)
Others	52 (4.8)
Two activities	32 (3)
Three activities or more	6 (0.5)

¹ Values are expressed as numbers and percentage (n (%)).

Cited by 27.1% of the participants, walking was the most popular activity before the pandemic. However, during the pandemic, as shown in Table 5, the top cited physical activity was training without weights (10.3%).

4. Discussion

This descriptive cross-sectional study was carried out at a critical period, considered to be the fourth wave of COVID-19 in Tunisia. During that period, the country recorded the highest rate of positive cases and deaths in Africa and the government limited freedom of citizens' travel and movement [3]. Thus, the survey was based on a self-administered questionnaire disseminated through social networks (Facebook and Instagram) as well as institutional and private mailing lists. A high response rate was obtained, and the sample population had an accepted distribution in terms of age stratification and territorial coverage over the Tunisian regions compared to internet-user distribution in Tunisia [21]. A prevalence of women (74.3%) and respondents with higher education (91.8%) was noticed. Similar trends were reported in many surveys [22–25], explained by the fact that women and university-educated respondents are more likely to manifest interest in food and health studies [25].

Changes in diet and physical activity consequent to the COVID-19 lockdown can lead to an increase in the prevalence of several chronic diseases, such as obesity and diabetes, which are considered risk factors for mortality in patients with COVID-19 [23]. Effect of restrictive measures on healthy eating was operationalized in this study as adherence to the Mediterranean diet using the MEDAS screener. The MEDAS questionnaire has been recently validated as an effective tool to assess MD adherence over different countries in the Mediterranean region [26].

Our results showed that location, age, occupational status, and household welfare proxy were the main sociodemographic determinants of high adherence to the MD. Adjustment of results to minimize effect of selection bias showed that respondents living in central Tunisia and those in the upper tertile of household welfare proxy were predicted to have higher MD adherence. It should be stressed that in Tunisia, employment and investment are afflicted by regional disparities, especially between the well-developed coastal northern and eastern areas and the poorer, marginalized interior southern and western

side of the country. Indeed, the coastal regions (central Tunisia) account for 90% of overall employment and 85% of the businesses operating in all sectors [27]. Moreover, like many countries all over the world, COVID-19 has stressed all segments of food supply chains, from farm production, food processing, transport and logistics, to final demand [28]. For example, the supply of fruit was adversely affected by the confinement and social distancing measures, as fruit farming is labor-intensive. Restrictions on movement of fruit farm workers sharply reduced fruit supply to local markets [29]. For similar reasons, the supply of fish and seafood to local markets might be sharply reduced especially for populations that are geographically distant from the coast [30]. This would explain the noncompliance with the recommendations for the consumption of fish and seafood observed in our study among the three classes of MD adherents. In addition to the health crisis, a deterioration in the social situation has signaled worsening economic regional disparities. Assuming a 5% decline in GDP and based on the precrisis poverty rate, the number of poor was expected to increase by 36.2% in 2020 [31]. This would correspond to an increase in the poverty rate from 15.2% to 20.7% of the total population. According to a recent report disseminated by the National Institute of Statistics, the poorest households have more difficulties than the better-off, with 62% of those in the lower quintile reporting that they are unable to meet all or part of their expenditures (versus 32% for the upper quintile) [32]. In January 2022, food prices increased by 7.6% year on year [32]. This might classify certain foods or food groups typical of the Mediterranean diet, such as nuts, olive oil, legumes fruits, vegetables, fish and seafoods as luxuries unaffordable for households of the lower and medium tertiles. For example, olive oil was estimated to be four times less consumed by the population living below the poverty line [33]. The price of fruit and legumes has increased by 80% since 2010 [34].

According to our findings, mean adherence to the MD among adults in Tunisia during the pandemic was not adequate and was lower than a healthy score (few high adherents (5.2%), mean MEDAS score $6.6/14 < 9$). Consumption of fish and seafood, legumes and nuts were particularly inadequate for the three MD-adherence classes and has consequently to be primarily considered for an overall improvement in MD adherence. As explained previously, low incomes due to the economic crisis in Tunisia might also be contributing to the low MD adherence, for high adherence requires relatively high expenses [35].

Host nutritional status has been accepted as a key factor in the outcome of a variety of infectious diseases [36]. The literature has recently found a negative association between MD adherence and COVID-19 cases and related deaths [13,37]. According to our results, this inverse association remained robust within the subgroup of unvaccinated obese subjects with no specific diet. Moreover, we observed that the highest MEDAS scores were recorded by healthy adults, while the lowest were among adults presenting a morbid health status with multiple chronic diseases. This result confirms the protective role of this diet against many chronic diseases, such as cardiovascular diseases (arterial hypertension, myocardial infarction, heart attack, etc.), hematological diseases (anemia), endocrine, nutritional, and metabolic diseases (diabetes, hypothyroidism, hyperthyroidism, etc.) and other diseases that are considered common comorbidities in COVID-19 infection [38–40]. Low-grade chronic inflammation underlies COVID-19 comorbidities [40]. It has been proposed that as a plant-based dietary pattern, the MD has anti-inflammatory properties inherent to the high polyphenol content sourced from fruits, vegetables and extra-virgin olive oil [13].

Besides diet and food habits and behaviors, lifestyle changes during COVID-19 lockdown have been the focus of some studies [22,23]. Our study showed a decrease in the number of heavy smokers of 1.4%, i.e., 2.8 times higher than that reported by an Italian survey [22]. This decrease could be explained by a fear of the risk of COVID-19 aggravation and the evolution of the disease towards the severe form observed in heavy smokers [41]. According to our study, tobacco addiction seems to be higher among adults (35–60 years old) and those with low education (primary and secondary level). This could be explained by the social role that smoking plays in these subgroups, which had ended by social isolation due to confinement, as well as attempts to abandon smoking associated with the fear

of a risk of complication in case of coronavirus exposure [42]. Meanwhile, these results may contrast with other studies in which smokers who were most stressed during waves of COVID-19 showed either an increase or decrease in smoking, suggesting that for some smokers, monotony and social isolation may have stimulated smoking, while for others, concern about contracting COVID-19 and becoming seriously ill may have motivated them to quit smoking [42]. Indeed, the subgroup who spent the confinement in solitary mode showed a higher dependence on tobacco compared to those living with their families. This is most likely due to the effect of monotony and boredom associated with the lack of social interaction, as perceived reduced social contact and health fears are linked to poor well-being [41].

With regard to sleep habits, we noticed an overall reduction in sleep hours, especially among women and the elderly. On the other hand, only the unemployed seemed to record more than 9 sleeping hours during the confinement period. The sex difference in the sleep disorder could be explained by higher perception of stress as well as a higher level of anxiety observed more significantly for women than men [43]. An association between stress and poor sleep has been reported, so that the reduction in sleep hours supports the hypothesis of a complex interaction between stress, sleep disturbance, and mental health during COVID-19 confinement [44]. In addition, a preference for late sleep has been observed for youth along with a delayed sleep phase as a result of the use of electronic devices and the decrease in social life [45] as well as the time spent watching TV and surfing on social networks [46], which may impair their perception of time.

Unsurprisingly, a significant decrease in physical activity has been reported during confinement. Nearly 28.3% of our study population gave up sports. As described in another surveys [47–49], outdoor activities such as walking, swimming, soccer, basketball and tennis, were suspended to give way to other home-practiced indoor activities but at a low practice rate.

This study is the first to be conducted in our country and could be considered complementary to another study focusing on the impact of the pandemic on food habits and behaviors [5]. Meanwhile, one of the main limitations of an online survey is that self-reported data can be subject to bias and misreporting. In addition, the use of snowball sampling through social media implies that the sample cannot be considered representative of the general Tunisian population, which leads to selection bias. Indeed, online tools limit access to people who are not used to this technology, such as the elderly and the uneducated. The main strength of cross-sectional studies relies on the fact that they are relatively quick and inexpensive to conduct. We need to consider that the main limitations of this kind of study include inability to make a causal inference and susceptibility to sampling bias, as they often need to select a sample of subjects from a large and heterogeneous study population. Thus, we raise awareness of the need to deeply explore the long-term consequences of COVID-19 confinement on dietary and lifestyle habits on a sample more representative of the Tunisian population.

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Appendix A Questionnaire

Table A1. Sociodemographic and economic data.

Questions	Answers
1. How old are you?	Age in years
2. Sex	Man/woman
3. Nationality	Tunisian/other
4. Place of residence (governorate)	governorate
5. Instruction level	No schooling/primary schooling/secondary schooling/University graduate
6. Occupational status	Unemployed/housewife/student/worker/intermediate executive/upper executive/retiree
7. Number of people in the household	Number
8. What type of housing does the household have?	Housing type
9. Do you have electricity?	Yes/No
10. What is the source of drinking water for the family members?	Source of drinking water
11. Your status with regard to housing?	Owner/in ownership/renter/free accommodation with friends or relatives or company accommodation
12. How many rooms (for sleeping) do you have in your home?	Number of rooms
13. Do you have a refrigerator?	Yes/No
14. Do you have a washing machine?	Yes/No
15. Do you have a dishwasher?	Yes/No
16. Do you have a satellite dish?	Yes/No
17. Do you have internet access?	Yes/No
18. Do you have a television?	Yes/No
19. Do you have a central heating system?	Yes/No
20. Do you have an air conditioner?	Yes/No
21. Do you have a mobile phone?	Yes/No
22. Do you have a car?	Yes/No
23. Do you have a computer?	Yes/No

Table A2. Anthropometrics and medical data.

Questions	Answers
1. Enter your weight (kg) as accurately as possible.	Weight in kg
2. Enter your height (cm) as accurately as possible.	Height in cm
3. Do you suffer from any chronic illness? If yes, quote, otherwise write No.	Yes/No
4. Do you follow a special diet? If yes, specify, otherwise write No.	Yes/No
5. Did you contract COVID-19?	No/Yes, tested positive with mild form of the disease/Yes, tested positive with severe form of the disease/suspected positive/contact case
6. Have you experienced family death due to COVID-19?	Yes/No
7. Have you had a vaccine against COVID-19?	Yes/No

Table A3. MD adherence.

Questions	Answers
1. Is olive oil the main culinary fat used?	Yes/No
2. How many teaspoons of olive oil do you consume per day (including that used for frying, salads, eating out, etc.)?	4 or more/2 or 3/1 or less
3. How many portions of vegetables (cooked and raw potatoes and beans are not included) do you eat per day? (One portion = half a large plate)	3 or more/1 or 2/less than 1
4. How many servings of fresh fruit do you consume per day? (Serving size = one unit of medium-sized fruit, one large cup of sliced fruit, one slice of medium-sized melon/watermelon, or one cup of fresh juice)	3 or more/1 or 2/less than 1
5. How many servings of red meat (veal, beef, mutton)/hamburgers/other meat products (salami, merguez, sausage . . .) do you consume per day? (One serving = 100 to 150 g = one quarter to half of a meal dish)	1 or less/2 to 4/5 to 6/7 or more
6. How many portions of butter, margarine or cream do you consume per day? (One portion = 12 g = one dessert spoon for butter and margarine, 2 tablespoons for cream)	Less than 1/one/more than 1
7. How much sugary drinks (industrial juices)/carbonated drinks/sodas do you consume per day? (One serving equals 330 mL)	Less than 1/1 or more
8. How many glasses/cups of wine do you consume per week?	More than 14 glasses (more than 2 glasses per day)/7 to 14 glasses (1 or 2 glasses per day)/2 to 6 glasses (sometimes but not every day)/one glass or less (occasionally)/none
9. How many portions of legumes (lentils, beans, chickpeas, peas . . .) do you consume per week? (One portion = 150 g = one dish or glass)	3 or more/1 to 2/less than 1
10. How many portions of fish and seafood do you eat per week? (One portion = 100 to 150 g = a quarter to half of a meal dish)	3 or more/1 to 2/less than 1
11. How many times a week do you consume industrial (not homemade) desserts/confectionery/pastries? (Including cakes, biscuits, ice cream, etc.)	Less than 1/1 to 2/3 or more
12. How many servings of nuts (unsalted) do you consume per week? (Including unsalted peanuts, almonds, hazelnuts, walnuts, etc. One serving = 30 g = one handful)	3 or more/1 to 2/less than 1
13. Do you prefer to consume chicken, turkey, or rabbit meat, or a vegetarian protein source, rather than red meat or derived products?	Yes/No
14. How many times a week do you eat dishes cooked with tomatoes or tomato sauce, onion and/or garlic and olive oil?	Twice or more/once or less

Table A4. Eating habits related to Mediterranean diet.

Questions	Answers
1. How many portions of pasta, rice, or other cereals including Oriental and Western pastries do you consume per day?	None/half portion/1 portion/2 portions/more than 2 portions
2. How many servings of whole meal, cracked wheat, cooked wheat, and corn bread do you eat per day? (1 average serving = 80 g or 2 slices)	None/half portion/1 portion/2 portions/more than 2 portions
3. How many servings of milk or yoghurt do you consume per day (1 serving = 150 mL in a cup or 125 g in a pot)?	None/half portion/1 portion/2 portions/more than 2 portions
4. How many servings of cheese or dairy products do you consume per week (1 serving of dairy product = 100 g; 1 serving of ripened cheese = 50 g)?	None/half portion/1 portion/2 portions/more than 2 portions
5. How many eggs do you consume per week?	None/1 egg/2 eggs/4 eggs/more than 4 eggs
6. How much water did you drink per day during the third wave of the pandemic?	Less than 1 L/1 to 2 L/more than 2 L

Table A5. Lifestyle habit changes.

Questions	Answers
1. Did you smoke before COVID-19 (cigarettes, cigars, electronic cigarettes)?	No/Yes, fewer than 5 cigarettes/Yes, between 5 and 10 cigarettes/Yes, more than 10 cigarettes
2. Do you currently smoke?	No/Yes, fewer than 5 cigarettes/Yes, between 5 and 10 cigarettes/Yes, more than 10 cigarettes
3. Your sleeping habits before COVID-19?	Less than 7 h per night/7 h to 9 h per night/More than 9 h per night
4. Your sleeping habits at present?	Less than 7 h per night/7 h to 9 h per night/More than 9 h per night
5. Did you play any sport before COVID-19?	No/gymnastics, yoga, dance, aerobics/walking/swimming/football, basketball, volley, tennis/martial arts
6. Do you currently play sports at home?	No/Yes, training without weights/Yes, weight training at home/yoga/treadmill/postural gymnastics/others
7. How many times did you play sports during COVID-19?	I didn't play sports/1 to 2 times per week/3 to 4 times per week/more than 4 times per week

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Article

Effects of Cardiac Rehabilitation and Diet Counselling on Adherence to the Mediterranean Lifestyle in Patients after Myocardial Infarction

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Abstract: Adherence to the Mediterranean lifestyle—as captured by the Medlife Index Questionnaire (i.e., encompassing a Mediterranean diet as well as other aspects of healthy living, such as food preparation, physical activity, and socializing)—has been associated with reduced cardiovascular events in healthy individuals. In the present study, we sought to determine the adherence to, and the effect of comprehensive cardiac rehabilitation on, Mediterranean lifestyle adherence in patients after myocardial infarction. We included 121 patients (mean age, 55 years; women, 37%) undergoing comprehensive cardiac rehabilitation—i.e., exercise training 3 times per week for 12 weeks plus dedicated workshops promoting the Mediterranean lifestyle. Before and after cardiac rehabilitation, patients completed the Medlife Index Questionnaire. High baseline adherence was associated with favourable glucose (5.39 vs. 6.1 mmol/L; $p < 0.001$), triglycerides (1.1 vs. 1.5 mmol/L; $p = 0.002$), and HDL cholesterol levels (1.32 vs. 1.12 mmol/L; $p = 0.032$). More importantly, the Medlife Score significantly improved following comprehensive cardiac rehabilitation in patients with low baseline adherence (from 13.8 to 16.7 points; $p < 0.001$), but not in patients with high baseline adherence (from 19.4 to 18.8 points; $p = 0.205$). Our findings suggested that Mediterranean lifestyle promotion during cardiac rehabilitation improved adherence to the Mediterranean lifestyle, especially in low-adherence patients.

Keywords: Mediterranean diet; Mediterranean lifestyle; myocardial infarction; coronary artery disease; cardiac rehabilitation; lipid status; physical activity

1. Introduction

Myocardial infarction—a manifestation of coronary atherosclerotic vascular disease—remains a major cause of morbidity and mortality worldwide [1]. The management of myocardial infarction includes immediate revascularisation and the long-term secondary prevention of atherosclerotic vascular disease in the form of prophylactic medication and lifestyle intervention—i.e., smoking cessation, regular exercise, and a Mediterranean-type diet [2]. The Mediterranean diet is entangled with the way of life in Mediterranean countries [3–5] and is associated with favourable glucose and lipid metabolism (i.e., lower fasting plasma glucose levels, lower low-density lipoprotein (LDL) cholesterol and triglycerides levels, and low high-density (HDL) cholesterol levels) [6], improved vascular function [6], and reduced cardiovascular disease morbidity and mortality [7–10]. Pertinent guidelines for cardiovascular disease prevention, therefore, recommend Mediterranean diet for all patients after myocardial infarction [2], as it was shown to be effective for both the primary and secondary prevention of cardiovascular diseases [7,11–13], even as a modifier of genetic risk for cardiovascular disease [14].

Lifestyle in the Mediterranean basin, however, encompasses concepts above and beyond diet, such as food preparation, physical activity and sedentary lifestyle avoidance,

and socializing, which are considered proxy for a healthy way of living [4,15]. These concepts, together with the Mediterranean diet, are captured by the Mediterranean lifestyle (Medlife) Index [16]. The Medlife Index is a recently designed questionnaire that summarises all aspects of the Mediterranean lifestyle and consists of 28 questions in three domains—food consumption, dietary habits, and social habits. So far, it has been applied in various populations in both Mediterranean and non-Mediterranean communities [16–19].

Adherence to the Mediterranean lifestyle, as captured by high scores on the Medlife Index Questionnaire, has been associated with decreased all-cause mortality in healthy individuals [17]. Conversely, data on adherence to the Mediterranean lifestyle in patients after myocardial infarction are scarce. Furthermore, as per guideline recommendations [2], patients after myocardial infarction should undergo comprehensive cardiac rehabilitation, which includes dietary counselling, behavioural support, and exercise; the effects of such programmes on adherence to the Mediterranean lifestyle, however, remain unknown. Therefore, we aimed to (i) assess adherence to the Mediterranean lifestyle in patients after myocardial infarction, (ii) appraise the determinants of adherence to the Mediterranean lifestyle, and (iii) evaluate the effects of a comprehensive cardiac rehabilitation programme on adherence to the Mediterranean lifestyle.

2. Materials and Methods

2.1. Study Population and Design

We included patients after recent myocardial infarction (<4 months prior to the study commencement), who were referred for cardiac rehabilitation programme at Centre for Preventive Cardiology, Department of Vascular Diseases, University Medical Centre Ljubljana, Slovenia. Exclusion criteria were recent acute (<1 month) or uncontrolled non-cardiovascular diseases requiring hospital admission, emergency, or unplanned specialist management; unstable dysrhythmias; pregnancy; and intellectual development disorder. The study was approved by the National Medical Ethics Committee and was performed in accordance with the Declaration of Helsinki and its later amendments. Written consent was obtained from all patients prior to their inclusion.

Before and after the intervention period (i.e., 12 weeks), blood samples were collected after at least 8 h fasting from all the patients and were analysed for blood glucose and lipid levels (direct measurement of total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides levels at the national referral Laboratory for Haemostasis and Atherothrombosis of Department of Vascular Diseases, University Medical Centre Ljubljana). At baseline, demographic characteristics were captured, including educational status (with higher education defined as the completion of >12 years of education) and living place (defined as either urban/suburban or rural as per the national statistics office definition); a thorough medical history and clinical assessment was performed, with the emphasis of cardiovascular appraisal and risk factors. Height and weight measurements were measured using a standard methodology [20]. Data on medication use were based on self-reports and checked in pharmaceutical records as needed (e.g., if a patient could not recall medication use details).

2.2. Medlife Index Questionnaire

The Medlife Index Questionnaire consists of 28 questions divided in three domains: food consumption, dietary habits, and social habits. Adherence to a specific item is scored with 0 or 1. The overall Medlife Index Score, therefore, comprises values between 0 and 28. According to the overall Medlife Index Score, the patient cohort was divided into tertiles [21,22] and was afterward classified into two groups: high-adherence to Medlife (the highest tertile) and low-adherence to Medlife (the lowest and medium tertiles).

All included patients filled out the Medlife questionnaire twice, at baseline and after the completion of the cardiac rehabilitation programme.

2.3. Cardiac Rehabilitation and Dietary Counselling

Cardiac rehabilitation is a comprehensive programme for patients after myocardial infarction. In Slovenia, it is mostly carried out at outpatient clinics and is primarily exercise-based.

This study was carried out at Centre for Preventive Cardiology, Department of Vascular Diseases, University Medical Centre Ljubljana, Slovenia, and lasted 12 weeks for each patient. Besides exercise, patients were included in (i) workshops on general health, risk factors, and cardioprotective therapy (one 90 min mandatory workshop plus as many as needed), (ii) smoking cessation for smokers, (iii) psychological assessment (with counselling on relaxation techniques and stress management as needed), (iv) and dietary counselling (one major 90 min workshop with regular (10–15 min once per week) reassessment and reinforcement during each rehabilitation session). Dietary counselling was provided by an experienced nutrition professional (specialised RN with nutrition counselling expertise). According to guidelines [2], the Mediterranean diet was promoted as the healthiest type, and the following specific suggestions were given: (i) to use olive oil as the main fat for cooking and dressing, (ii) to have at least 2 seasonal, fresh vegetable servings a day (e.g., tomato, broccoli, spinach, pepper, eggplant, cabbage, lettuce, zucchini, etc.), (iii) moderate consumption of fresh fruit (e.g., oranges, tangerines, apples, berries, pears, grapes, peaches, apricots, nectarines, figs, melons), (iv) consumption of fish at least 2 times per week, (v) favouring legumes (e.g., beans, peas, lentils, chickpeas, soybean) to meat (especially red meat), and (vi) having a handful of unsalted nuts every day (e.g., walnuts, hazelnuts, peanuts, almonds, cashews, macadamia nuts). A serving was defined as approximately 1 cup = 250 mL. In addition, advice regarding food preparation was given, e.g., favouring cooking to frying and limiting the use of salt and sugar. Finally, a few examples of healthy meals (including information on ingredients and recipes) were provided.

During the workshops on general health, risk factors, and cardioprotective therapy, patients were advised to have enough sleep during the night and to minimise sedentary lifestyle (e.g., watching television or using the computer) and were encouraged to enjoy outdoor activities (e.g., light gardening).

Despite recommendations on Mediterranean lifestyle regarding exercise being carried out outdoor, due to safety reasons (patients shortly after myocardial infarction), exercise training sessions were held 3 times per week for one hour in the morning hours indoor; exercise training sessions consisted of supervised aerobic exercise and training exercise on exercise bike or treadmill with adjunction of low-weight resistance training.

2.4. Statistical Analysis

The normal distribution of variables was determined using the Shapiro–Wilk test. Normally distributed variables were described with a mean value and standard deviation. Asymmetrically distributed variables were described with medians and interquartile ranges. Categorical variables were described as numbers and/or percentages. Differences between two groups were determined with the independent samples *t*-test and Mann–Whitney U test for normally and asymmetrically distributed variables, respectively. Differences in categorical variables (percentages) were assessed with the chi-squared test. The effects of the intervention were determined with the paired samples *t*-test and Wilcoxon U paired test for normally and asymmetrically distributed variables, respectively. The analysis of covariance (ANCOVA) test was performed to assess the post-rehabilitation Medlife Index Score with respect to baseline pre-rehabilitation values. All data were analysed using IBM SPSS Statistics v. 20 (IBM Corp., Armonk, NY, USA). A *p*-value < 0.05 was considered statistically significant.

3. Results

We included 121 patients with a mean age of 55 years, and 33 (27%) were women. Based on the adherence to the Mediterranean lifestyle, patients were divided into high-

adherence (upper Medlife Index tertile) and low-adherence (first and second Medlife Index tertiles) groups.

There were no significant differences between groups in terms of age, sex, BMI, clinical data, drug prescription, and risk factors (arterial hypertension, dyslipidaemia, cigarette smoking, and diabetes mellitus). In addition, there were no significant differences in terms of living place (Table 1). Conversely, patients in the high-adherence group had significantly more prevalent family history of CVD (55.9% vs. 34.5%; $p = 0.031$) and a larger percentage of higher educated people (47.1% vs. 26.4%; $p = 0.029$) than the low-adherence group.

Table 1. Baseline demographics and clinical data.

	All (n = 121)	Low Adherence (n = 87)	High Adherence (n = 34)	Significance
<i>Demographic</i>				
Age, mean (SD), years	56.0 (9.7)	56.4 (9.1)	54.9 (11.0)	0.501
Female sex, n (%)	33 (27.3%)	23 (26.4%)	10 (29.4)	0.741
Height, mean (SD), cm	173.0 (8.8)	172.7 (8.3)	173.6 (10.0)	0.655
Weight, mean (SD), kg	85.9 (17.0)	86.6 (17.5)	84.2 (15.7)	0.464
BMI, mean (SD), kg/m ²	28.6 (4.5)	28.9 (4.7)	27.8 (4.0)	0.221
Normal weight, n (%)	24 (19.8%)	16 (18.4%)	8 (23.5%)	0.524
Overweight, n (%)	58 (47.9%)	40 (46.0%)	18 (53.0%)	0.491
Obese, n (%)	39 (32.2%)	31 (35.6%)	8 (23.5%)	0.200
<i>Risk factor for CVD</i>				
Arterial hypertension, n (%)	55 (45.5%)	38 (43.7%)	17 (50.0%)	0.530
Dyslipidaemia, n (%)	70 (57.9%)	54 (62.1%)	16 (47.1%)	0.133
Family history of CVD, n (%)	49 (40.5%)	30 (34.5%)	19 (55.9%)	0.031
Diabetes mellitus, n (%)	13 (10.7%)	10 (11.5%)	3 (8.8%)	0.670
Current cigarette smoker, n (%)	16 (13.2%)	13 (15.0%)	3 (8.8%)	0.216
Former cigarette smoker, n (%)	42 (34.7%)	33 (37.9%)	9 (26.5%)	
Never smoked, n (%)	63 (52.1%)	41 (47.1%)	22 (64.7%)	
<i>Clinical data</i>				
STEMI, n (%)	75 (62.0%)	54 (62.1%)	21 (61.7%)	0.975
Ejection fraction, median (Q1–Q3), %	55 (53–56)	55 (54–56)	55 (50–59)	0.998
PCI LAD, n (%)	65 (53.7%)	49 (56.3%)	16 (47.1%)	0.358
<i>Medication</i>				
ACEi/ARB, n (%)	100 (82.6%)	72 (82.8%)	28 (82.3%)	0.958
Beta blockers, n (%)	105 (86.8%)	76 (87.4%)	29 (85.3%)	0.763
Statins, n (%)	121 (100%)	87 (100%)	34 (100%)	1.000
Antidiabetic therapy, n (%)	10 (8.3%)	8 (9.2%)	2 (5.9%)	0.552

Table 1. Cont.

	All (n = 121)	Low Adherence (n = 87)	High Adherence (n = 34)	Significance
<i>Laboratory test</i>				
HbA1c, mean (SD), %	5.81 (0.75)	5.89 (0.82)	5.60 (0.48)	0.027
<i>Educational level</i>				
Lower, n (%)	82 (67.8%)	64 (73.6%)	18 (52.9%)	0.029
Higher, n (%)	39 (32.2%)	23 (26.4%)	16 (47.1%)	
<i>Living place</i>				
Urban and suburban, n (%)	89 (45.4%)	62 (40.2%)	27 (58.8%)	0.361
Rural, n (%)	32 (26.4%)	25 (28.7%)	7 (20.6%)	

SD—standard deviation; BMI—body mass index; CVD—cardiovascular disease; STEMI—ST-elevation myocardial infarction; Q1–Q3—interquartile range; PCI LAD—percutaneous coronary intervention of left anterior descending artery; ACEi/ARB—angiotensin-converting enzyme inhibitor/angiotensin receptor blocker; HbA1c—glycated haemoglobin.

At baseline, patients in the high-adherence group had significantly lower blood glucose (5.39 vs. 6.08 mmol/L; $p = 0.001$), HbA1C (5.60% vs. 5.89%; $p = 0.027$), and triglyceride levels (1.17 vs. 1.54 mmol/L; $p = 0.002$) than patients in the low-adherence group. These differences mostly remained significant at follow-up, with 5.41 vs. 6.12 mmol/L ($p < 0.001$) for blood glucose levels, 1.32 vs. 1.14 mmol/L ($p = 0.007$) for HDL, and 1.05 vs. 1.70 mmol/L ($p < 0.001$) for triglyceride levels, when high- and low-adherence groups were compared, respectively (Table 2).

Table 2. Effects of cardiac rehabilitation and diet counselling on blood glucose and lipid status in both examined groups.

	Low Medlife Adherence (n = 87)			High Medlife Adherence (n = 34)			Comparison between Groups (p)	
	Baseline	Follow-Up	p	Baseline	Follow-Up	p	Baseline	Follow-Up
Glucose	6.08 (1.47)	6.12 (1.43)	0.569	5.39 (0.63)	5.41 (0.71)	0.903	0.001	<0.001
TChol	3.44 (0.87)	3.45 (0.91)	0.928	3.32 (0.83)	3.10 (0.67)	0.236	0.505	0.034
HDL	1.12 (0.33)	1.14 (0.36)	0.630	1.26 (0.35)	1.32 (0.34)	0.045	0.032	0.007
LDL	1.61 (0.70)	1.57 (0.66)	0.431	1.54 (0.74)	1.34 (0.60)	0.272	0.398	0.068
Triglycerides	1.54 (0.71)	1.70 (1.28)	0.313	1.17 (0.63)	1.05 (0.37)	0.338	0.002	<0.001

All measurements are described as mean (SD) and expressed in mmol/L. TChol—total cholesterol; HDL-c—high-density lipoprotein cholesterol; LDL-c—low-density lipoprotein cholesterol.

Cardiac rehabilitation and diet counselling were not associated with a significant post-intervention change in the lipid status in either of the groups (Table 2), except for a significant increase in HDL levels in the high-adherence group (from 1.26 to 1.32 mmol/L; $p = 0.045$).

Figures 1–3 and Table A1 depict adherence to the specific domains in the Medlife questionnaire of both high- and low-adherence groups at baseline and follow-up. Patients in the high-adherence group were more adherent to most Mediterranean food consumption domains than the low-adherence group. Patients in the low-adherence group significantly improved adherence in several domains, while patients in the high-adherence did not. Similarly, the Medlife Index Score significantly improved only in the low-adherence group (from 13.8 to 16.7 points; $p < 0.001$), while in the high-adherence group, it did not (from 19.4 to 18.8 points; $p = 0.205$).

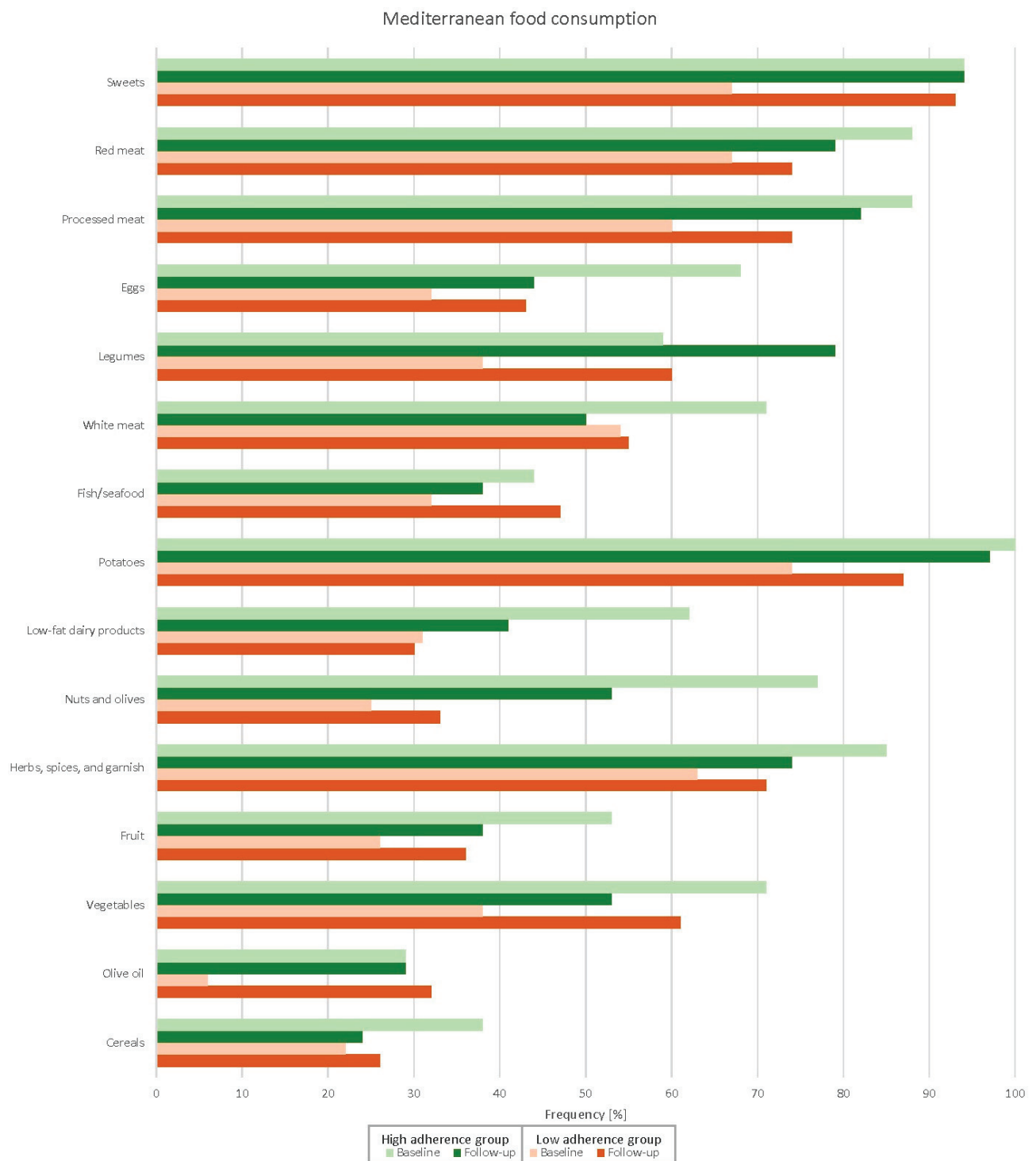


Figure 1. Changes in the Mediterranean food consumption subdomains after the cardiac rehabilitation programme.



Figure 2. Changes in the Mediterranean dietary subdomains after the cardiac rehabilitation programme.

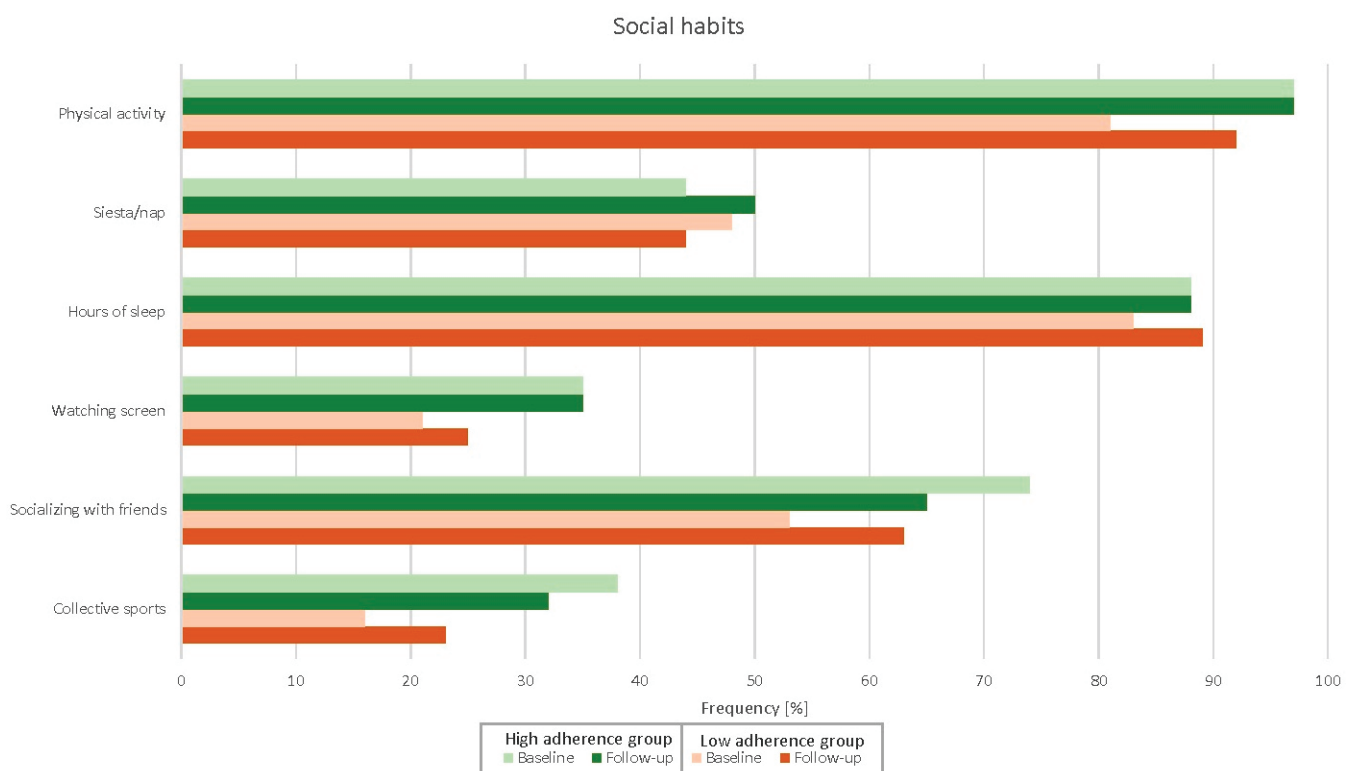


Figure 3. Changes in the social habits subdomains after the cardiac rehabilitation programme.

Table 3 shows the ANCOVA tests using follow-up overall Medlife Index Score (a) and overall Medlife Index Score difference (b) as dependent variables, high vs. low adherence as a fixed factor, and baseline overall Medlife Index Score as a covariate in both models. The covariate, i.e., the baseline overall Medlife Index Score, was significantly related to both dependent variables. There was also a significant effect of the high- vs. low-adherence groups on both dependent variables (Table 3).

Table 3. ANCOVA tests using high vs. low adherence as a fixed factor, baseline overall Medlife Index score as a covariate, and (a) follow-up overall Medlife Index score and (b) overall Medlife Index score difference, as dependent variables.

(a) Dependent Variable: Follow-Up Overall Medlife Index Score						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected model	347.806 *	2	173.903	30.255	0.000	0.339
Intercept	69.921	1	69.921	12.164	0.001	0.093
Baseline overall Medlife Index Score	240.060	1	240.060	41.764	0.000	0.261
High adherence vs. Low adherence	31.369	1	31.369	5.457	0.021	0.044
Error	678.260	118	5.748			
Total	37,299.000	121				
Corrected total	1026.066	120				
(b) Dependent Variable: Overall Medlife Index Score Difference						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected model	355.211 **	2	177.605	30.899	0.000	0.344
Intercept	69.921	1	69.921	12.164	0.001	0.093
Baseline overall Medlife Index Score	47.355	1	47.355	8.239	0.005	0.065
High adherence vs. Low adherence	31.369	1	31.369	5.457	0.021	0.044
Error	678.260	118	5.748			
Total	1486.000	121				
Corrected total	1033.471	120				

Legend: * R squared = 0.339 (adjusted R squared = 0.328), ** R squared = 0.344 (adjusted R squared = 0.333).

4. Discussion

Our study showed that adherence to the Mediterranean lifestyle was associated with more favourable glucometabolic and lipid profiles in patients after myocardial infarction. More importantly, our findings suggest that poor adherence to the Mediterranean lifestyle—as captured by a low score on the Medlife Index Questionnaire—may be improved by a comprehensive cardiac rehabilitation programme, which provides dietary counselling as part of a multifaceted lifestyle intervention through cardiac rehabilitation. To the best of our knowledge, this is the first study in patients after myocardial infarction to assess the adherence to, and improvement of, a Mediterranean lifestyle, which encompasses diverse aspects of living beyond and above food consumption (i.e., food preparing, physical activity, and socializing).

Patients who adhered to the Mediterranean lifestyle after myocardial infarction had better glucose and lipid profiles. In our study, high adherence (i.e., highest-tertile Medlife Index Score) was associated with lower levels of fasting blood glucose, as well as lower triglycerides and higher HDL-C levels, which corroborated previous findings in healthy individuals [6,18,23]. Conversely, in contrast to previous studies in healthy individuals [24], we found no associations with LDL-C levels. This is in line with our present understanding of derangements in lipid metabolism. Unfavourable triglyceride/HDL cholesterol levels are associated with obesity, diabetes, unhealthy dietary habits, and sedentary lifestyle; are predictors of atherosclerotic vascular disease onset and progression (“atherogenic dyslipidaemia”); and—as opposed to LDL cholesterol—are less amendable via lipid-lowering medication [25] but responsive to lifestyle interventions [26–28]. In our study, all patients were on guideline-directed high-intensive statin therapy, which likely overshadowed the effect of lifestyle on LDL cholesterol; conversely, favourable triglyceride/HDL cholesterol levels were strongly associated with adherence to the Mediterranean lifestyle, underscoring the importance of lifestyle in addressing residual cardiovascular risk, which is not amendable via lipid-lowering medication.

More importantly, adherence to the Mediterranean lifestyle significantly improved after a comprehensive cardiac rehabilitation programme—but only in patients with a lower baseline Medlife Index Score. The difference between the low- and high-adherence groups diminished to non-significant levels after cardiac rehabilitation, except for legume, vegetable, nut, olive, olive oil, and whole-grain product consumption; this represents the largest room for improvement in patients with poor baseline adherence. While intuitive in terms of a “regression to the mean” phenomenon, our findings remained significant after ANCOVA adjustment for baseline Medlife Index Scores. Moreover, some aspects of the Mediterranean lifestyle—such as legume and whole-grain product consumption—improved even in patients with high baseline adherence. Conversely, patients with low baseline adherence improved in most Mediterranean diet and dietary pattern domains. Altogether, these findings suggest that patients with low adherence to the Mediterranean lifestyle, as appraised with the Medlife Index Questionnaire, may benefit the most from lifestyle interventions. In addition, there is room for improvement even in patients with high baseline adherence—especially in below-satisfactory domains, such as the consumption of nuts and olives (53%), vegetables (53%), and olive oil (29%).

Two findings in our study merit further addressing. Firstly, family history of cardiovascular disease was associated with higher adherence to the Mediterranean lifestyle. It seems that experiencing a cardiovascular event in the immediate family may impact lifestyle choices—either through alarming (i.e., offspring of parents with cardiovascular disease trying to defer atherosclerotic events) or empowerment (i.e., vertical sharing and spreading the Mediterranean lifestyle from parents’ secondary prevention experience). However intriguing, these speculations require further appraisal with properly designed studies. Secondly, sociodemographic factors were important determinants of Mediterranean lifestyle adherence. Education level was associated with a higher Medlife Index Scores in our study—a finding comparable to some [29,30]—but not all [18]—reports on healthy individuals. Lower household income [31] and socioeconomic status [32] were also previously associated with poor adherence, whereas our study did not show a possible impact of living place (urban/suburban vs. rural). While most reports in healthy individuals suggested a better adherence to the Mediterranean diet in rural communities [33], the uptake of other Mediterranean lifestyle domains, such as regular exercise, may be less pronounced [29,30]. The promotion of the Mediterranean lifestyle, therefore, should be applied even to communities with a perceived Mediterranean way of life, which may be prone to a “westernization” or “nutrition transition” of lifestyle patterns [34,35].

We identified some limitations of our study—firstly, sample size, design, and the single-centre setting. Its quasi-experimental pre–post-test series design limited the data quality and the evidence that could be derived from them. Although the sample size in this report was fairly large as compared with similar prospective cohorts, larger and randomised,

controlled, multicentre studies are warranted to confirm and further explicate our results. Secondly, the extrapolation of our results to populations beyond coronary artery disease patients, especially in terms of lipid status, should be performed with caution, as all our patients after myocardial infarction exhibited lipid profiles compatible with high-intensity statin management. Thirdly, using categorical variables—not continuous scale values—was already identified as a limitation of the original study that introduced the Medlife Index Questionnaire [10]. Fourthly, data were obtained from the questionnaire, so imprecision and the response and social desirability biases could not be ruled-out. Finally, even at follow-up, a large percentage of patients did not adopt the classical recommendations of the Mediterranean lifestyle (e.g., olive oil, vegetables, or nuts) suggesting that the implementation strategies for improvement should be refined.

5. Conclusions

In conclusion, we showed that baseline adherence to the Mediterranean lifestyle was associated with more favourable glucose and lipid profiles in patients after myocardial infarction. More importantly, a comprehensive cardiac rehabilitation programme, which included Mediterranean lifestyle promotion, effectively improved adherence to the Mediterranean lifestyle—especially in low-adherence patients—which was a main strength of our report. The Mediterranean lifestyle in patients after myocardial infarction is a pivotal guideline-derived intervention, which is associated with a favourable cardiometabolic profile and should be especially encouraged in patients with poor baseline adherence.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and was approved by the National Medical Ethics Committee of the Republic of Slovenia (0120-547/2018).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declare no conflict of interest.

Previous Presentations: Parts of these results were presented as an abstract at the European Society of Cardiology-Preventive Cardiology Congress 2021 (abstract 11491).

Appendix A

Table A1. Changes in the specific domains of the Medlife questionnaire after the cardiac rehabilitation pro-programme and comparison between groups.

	High Adherence to Mediterranean Lifestyle (n = 34)			Low Adherence to Mediterranean Lifestyle (n = 87)			Comparison between Groups	
	Baseline	Follow-Up	p	Baseline	Follow-Up	p	Baseline	Follow-up
Medlife index	19.4 (1.8)	18.8 (3.0)	0.205	13.8 (2.2)	16.7 (2.7)	<0.001	<0.001	0.001
Mediterranean food consumption								
Sweets (≤ 2 servings/week)	94%	94%	1.000	67%	93%	<0.001	0.004	1.000
Red meat (< 2 servings/week)	88%	79%	0.257	67%	74%	0.083	0.030	0.663
Processed meat (≤ 1 serving/week)	88%	82%	0.414	60%	74%	0.014	0.005	0.435
Eggs (2–4 servings/week)	68%	44%	0.033	32%	43%	0.061	0.001	1.000
Legumes (≥ 2 servings/week)	59%	79%	0.008	38%	60%	<0.001	0.060	0.068
White meat (2 servings/week)	71%	50%	0.090	54%	55%	0.847	0.145	0.756
Fish/seafood (≥ 2 servings/week)	44%	38%	0.414	32%	47%	0.007	0.307	0.496
Potatoes (≤ 3 servings/week)	100%	97%	0.317	74%	87%	0.003	0.002	0.175
Low-fat dairy products (2 servings/day)	62%	41%	0.071	31%	30%	0.841	0.004	0.331
Nuts and olives (1–2 servings/day)	77%	53%	0.021	25%	33%	0.194	<0.001	0.075
Herbs, spices, and garnish (≥ 1 servings/day)	85%	74%	0.102	63%	71%	0.144	0.032	0.981
Fruit (3–6 servings/day)	53%	38%	0.197	26%	36%	0.170	0.011	0.954
Vegetables (≥ 2 servings/day)	71%	53%	0.058	38%	61%	<0.001	0.002	0.551
Olive oil (≥ 3 servings/day)	29%	29%	1.000	6%	32%	<0.001	0.001	0.938
Cereals (3–6 servings/day)	38%	24%	0.132	22%	26%	0.414	0.108	0.922
Mediterranean dietary habits								
Water or infusions	65%	50%	0.197	53%	56%	0.480	0.329	0.589
Wine	18%	6%	0.102	9%	5%	0.206	0.214	0.673
Limit salt in meals	91%	94%	0.317	79%	95%	<0.001	0.200	0.673
Preference for whole-grain products	79%	91%	0.046	63%	72%	0.059	0.134	0.047
Snacks	100%	100%	1.000	91%	97%	0.096	0.104	0.558
Limit nibbling between meals	91%	94%	0.564	74%	92%	<0.001	0.061	1.000
Limit sugar in beverages	100%	100%	1.000	87%	96%	0.005	0.033	0.558

Table A1. Cont.

	High Adherence to Mediterranean Lifestyle (n = 34)			Low Adherence to Mediterranean Lifestyle (n = 87)			Comparison between Groups	
	Baseline	Follow-Up	p	Baseline	Follow-Up	p	Baseline	Follow-up
Social habits								
Physical activity 150 min/week	97%	97%	1.000	81%	92%	0.012	0.043	0.439
Siesta/nap	44%	50%	0.480	48%	44%	0.394	0.834	0.671
Hours of sleep	88%	88%	1.000	83%	89%	0.166	0.641	1.000
Watching television	35%	35%	1.000	21%	25%	0.248	0.150	0.381
Socializing with friends	74%	65%	0.317	53%	63%	0.050	0.062	1.000
Collective sports	38%	32%	0.480	16%	23%	0.134	0.017	0.437

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Article

Association among Premenstrual Syndrome, Dietary Patterns, and Adherence to Mediterranean Diet

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Abstract: Premenstrual syndrome (PMS) adversely affects the physiological and psychological health and quality of life of women. Mediterranean diet (MD) could be helpful for managing and preventing PMS, but evidence on the association between dietary patterns and PMS in Asian women is limited. This study aimed to investigate the association of dietary patterns and adherence to MD with PMS in Korean women. This cross-sectional study recruited 262 women aged 20–49 years via an online survey. PMS was diagnosed using the American College of Obstetricians and Gynecologists diagnostic criteria. MD adherence was assessed using the Korean version of the Mediterranean Diet Adherence Screener. Mediterranean Diet Score (MDS) was classified into tertiles (T) (T1: 0–3, T2: 4–5, and T3: ≥ 6). Dietary pattern was assessed with the Food Frequency Questionnaire. Multiple logistic regression analyses were conducted to evaluate the association between dietary pattern scores and PMS prevalence. The proportion of PMS was significantly lower in MDS tertile (T) 3 than in T1 (55.4% in T3 vs. 74.4% in T1, $p = 0.045$). After adjusting for confounders, participants in the highest tertile of the bread/snack pattern had a higher risk of PMS (odds ratio [95% CI]: 2.59 [1.32–5.06]), while traditional dietary pattern and meat/alcohol pattern were not associated with PMS. In conclusion, we found that low adherence to MD and higher bread/snack dietary pattern were associated with increased risk of PMS, respectively.

Keywords: premenstrual syndrome; Mediterranean diet; dietary habit

1. Introduction

Premenstrual syndrome (PMS), which occurs during the luteal phase of the menstrual cycle and resolves spontaneously after the onset of menstruation, is a common psychological and somatic disorder among women of reproductive age [1]. This syndrome consists of psychiatric or physical symptoms that significantly impair the normal daily functioning of women at any stage in her reproductive life, including work, relationships, and personal activities, and negatively impact quality of life [1–3]. Although the exact etiology of PMS remains unknown, the symptoms may be related to hormonal variations [4]. Genetic backgrounds, psychosocial factors, and lifestyle factors such as aerobic exercise and dietary pattern are known to be associated with prevalence of PMS [1,5–7]. For example, excessive consumption of sweet-tasting food, fast food, deep-fried meals, coffee, and alcohol are significantly associated with the development of PMS [8,9]. In contrast, adequate intake of vegetables and fruits could alleviate PMS symptoms [8,9].

The Mediterranean diet (MD) is characterized by high consumption of vegetables, fruits, whole grains, legumes, nuts, and olive oil. Importantly, it has consistently been validated for its beneficial influence on health [10,11]. Thus, the MD could have beneficial

effects on management and prevention of PMS [12]. However, there has been a limited amount of research investigating the association between dietary patterns and PMS in Asian women. Therefore, we aimed to investigate this association, particularly between PMS and MD adherence.

2. Materials and Methods

2.1. Study Design and Population

This cross-sectional study was conducted among Korean women of childbearing age who participated in an online survey in September 2021. The survey was conducted using survey panels from dataSpring (<https://ko.d8aspring.com/contact>). A total of 262 women aged 20–49 years voluntarily participated in the online survey. The exclusion criteria were as follows: (1) age <20 years or >49 years; (2) known history of thyroid disease; (3) current medical history of oral contraceptives or hormone replacement therapy; and (4) unwillingness to provide informed consent.

2.2. Data Collection

Data were collected during the online survey using a self-administered questionnaire. The participants were first assigned to a pre-screening procedure. The pre-screening questionnaire included anthropometric variables (height and weight), demographic characteristics, underlying medical conditions (hypertension, diabetes mellitus, and dyslipidemia), and menstrual history. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Menstrual history included age of menarche (years), menstrual length (days), duration of flow (days), menstrual regularity (regular/irregular), amount of flow (little/moderate/heavy), and dysmenorrhea (present/none).

Demographic information included age (years), education status (under-graduate or graduate), marital status (single or married), and personal lifestyle habits (smoking, alcohol drinking, and physical activity). Smoking and alcohol drinking habits were classified as never/former/current smoker and never/current drinker, respectively. Physical activity was assessed using the Godin Leisure-Time Exercise Questionnaire [13]. Participants were asked to recall the number of strenuous, moderate, or mild physical activity exceeding 15 min in duration, considering a 7-day period (a week). The intensity of each physical activity was described as “heart beats rapidly (e.g., running or vigorous swimming)”, not exhausting (e.g., fast walking, easy bicycling, and folk dancing)”, and “minimal effort (e.g., yoga or easy walking)”. Total weekly leisure activity score was calculated by multiplying the number of 15-min episodes by the weights of 9, 5, and 3, and summing those values into an overall score. Based on the total score, participants were categorized into three groups: active (≥ 24 units), moderately active (14–23 units), and insufficiently active/sedentary (< 14 units).

The subjects who qualified in the pre-screening procedure proceeded to the main survey. The main survey questionnaire included: (1) American College of Obstetricians and Gynecologists (ACOG) diagnostic criteria for PMS [14,15]; (2) Korean version of the Mediterranean Diet Adherence Screener (K-MEDAS) [16]; and (3) Food Frequency Questionnaire (FFQ) [17].

2.3. Diagnosis of Premenstrual Syndrome

PMS was confirmed following the ACOG diagnostic criteria proposed in 2000 [14], as follows. PMS was diagnosed if the patient reported at least one affective symptom (i.e., depression, angry outbursts, irritability, anxiety, confusion, or social withdrawal) and at least one somatic symptom (i.e., breast tenderness, abdominal bloating, headache, or swelling of extremities) during the 5 days before menses in each of the three prior menstrual cycles. In addition, these symptoms should have been relieved within 4 days from the onset of menses, without recurrence until at least cycle day 13. The ACOG PMS questionnaires translated into Korean by the National Institute of Food and Drug Safety Evaluation [15]

were used. Based on the ACOG diagnostic criteria, participants with or without PMS were classified into the PMS and non-PMS groups.

2.4. Assessment of Mediterranean Diet Adherence

Adherence to the MD was assessed using a 14-item questionnaire developed by Ji-won Lee et al. which was a modification of the validated Mediterranean Diet Adherence Screener [16]. The K-MEDAS questionnaire is a validated tool for assessing adherence to the Mediterranean diet in the Korean population. One point is given for using perilla oil or olive oil as the principal source of fat for cooking (question; Q1) and for preferring white meat over red meat (Q13). One point is also given in the following cases: for Q2, ≥ 3 teaspoons of perilla oil or olive oil per day; for Q3, ≥ 2 servings of vegetables per day; for Q4, ≥ 1 pieces of fruit per day; for Q5, < 1 serving of red meat or sausages per day; for Q6, < 1 serving of butter, margarine, or cream per day; for Q7, < 1 serving of sugar-sweetened beverages per day; for Q8, ≥ 7 servings of wine per week; for Q9, ≥ 3 servings of beans or tofu per week; for Q10, ≥ 3 servings of fish or seafood per week; for Q11, < 3 times of sweets, bread (except whole wheat bread), cakes, and cookies per week; for Q12, ≥ 3 times of nuts per week; and for Q14, ≥ 3 times of whole grain per week. Failure to meet each parameter is given a 0 score. The resulting modified MD scores ranged from 0 to 14, with higher score indicating higher adherence to the MD.

2.5. Assessment of Nutritional Intake and Dietary Patterns

The nutritional survey was conducted using the semi-quantitative FFQ, which has been validated for 112 food items [17]. Participants were required to report the frequency of usual food items consumed in the preceding year on a daily, weekly, or monthly basis, considering the average amount of intake for each food item. Based on the nutrient composition similarity, food items were categorized into 21 food groups: rice, mixed grain rice, noodles/dumplings, breads/rice cakes, soup/stew, soybeans, eggs, red meat, white meat, fish/seafood, vegetables, fermented foods, kimchi, seaweed, potatoes, milk/dairy products, fruits, beverages, snacks, nuts, and alcoholic beverages. Total energy and nutrient intake values were derived from this FFQ. Detailed information regarding the specific food items is available on the KNHANES website [18].

2.6. Statistical Analysis

Normally distributed continuous variables were presented as the mean $\pm$ standard deviation or median (25th, 75th), while categorical variables were presented as the number (percentage, %). Between-group comparisons were performed using an independent two-sample t-test or Mann–Whitney U test for continuous variables and using chi-square test for categorical variables. For continuous variables, analysis of variance was performed to compare the differences among tertile groups. Dietary patterns were generated by factor analysis using the principal component method. Twenty-one food items were entered into the factor analysis. Eigenvalues > 1.0 were used to determine the number of factors to retain by scree plot. To increase interpretability, factors were rotated by orthogonal transformation (varimax).

Foods with factor-loading > 0.5 were considered to contribute to the dietary patterns. Finally, 15 food items were used in the factor analysis. Three major dietary patterns were extracted, which explained 49.5% of the total variance. The Kaiser–Meyer–Olkin (KMO) value was 0.826. Factor scores for each dietary pattern were categorized into tertile. Multiple logistic regression analyses were conducted to evaluate the association between each dietary pattern score and prevalence of PMS. Model 1 was an unadjusted model. Model 2 was adjusted for age and BMI. Model 3 was adjusted for all factors in model 1 with the addition of smoking status, alcohol drinking status, and physical activity. All statistical analyses were performed using Statistical Package for the Social Sciences version 23.0 (IBM Corp., Armonk, NY, USA). All tests were two sided, and p values < 0.05 were considered statistically significant.

3. Results

3.1. Baseline Participant Characteristics

In total, 171 (65.3%) and 91 (34.7%) participants did and did not have PMS, respectively. The baseline participant characteristics by group are shown in Table 1. There were significant between-group differences in age, BMI, education, marital status, physical activity, and underlying conditions. The participants with PMS were likely to be current smokers and current drinkers ($p = 0.046$ and $p < 0.001$, respectively). Table 2 shows the menstrual characteristics by group. Although there were no differences in age of menarche, menstrual length, duration of flow, menstrual regularity, and amount of flow (little/moderate/heavy), the prevalence of dysmenorrhea was significantly higher in the PMS group (95.3% vs. 80.2%, $p < 0.001$).

Table 1. Baseline participant characteristics by PMS group.

Characteristics	Non-PMS Group	PMS Group	<i>p</i> -Value
N	91 (34.7%)	171 (65.3%)	
Age (years)	33.0 (26.0–37.0)	31.0 (26.0–37.0)	0.213
BMI (kg/m ²)	21.1 (19.6–23.1)	20.5 (19.1–22.9)	0.460
Education, N (%)			
Undergraduate	26 (28.6%)	46 (26.9%)	0.773
Graduate	65 (71.4%)	125 (73.1%)	
Marital status, N (%)			
Single	63 (69.2%)	123 (71.9%)	0.647
Married	28 (30.8%)	48 (28.1%)	
Smoking status, N (%)			
Never smoker	78 (85.7%)	126 (73.7%)	0.046
Former smoker	8 (8.8%)	19 (11.1%)	
Current smoker	5 (5.5%)	26 (15.2%)	
Alcohol drinking status, N (%)			
Never drinker	31 (34.1%)	23 (13.5%)	<0.001
Current drinker	60 (65.9%)	148 (86.5%)	
Physical activity, N (%)			
Active	22 (24.2%)	55 (32.2%)	0.389
Moderately active	28 (30.8%)	45 (26.3%)	
Insufficiently active/sedentary	41 (45.1%)	71 (41.5%)	
Underlying conditions, N (%)			
Hypertension	3 (3.3%)	1 (0.6%)	0.122
Diabetes mellitus	0 (0.0%)	3 (1.8%)	0.554

PMS, premenstrual syndrome.

Table 2. Menstrual characteristics of the study participants by PMS group.

Characteristics	Non-PMS Group	PMS Group	<i>p</i> -Value
Age of Menarche (years)	14.0 (13.0–15.0)	13.0 (12.0–13.0)	0.227
Menstrual Length (days)	28.0 (28.0–30.0)	29.0 (28.0–30.0)	0.275
Duration of flow (days)	5.0 (5.0–7.0)	6.0 (5.0–6.0)	0.771
Menstrual regularity, N (%)			
Regular	61 (67.0%)	124 (72.5%)	0.354
Irregular	30 (33.0%)	47 (27.5%)	
Amount of flow, N (%)			
Mild	10 (11.0%)	18 (10.5%)	0.993
Moderate	72 (79.1%)	136 (79.5%)	
Heavy	9 (9.9%)	17 (9.9%)	
Dysmenorrhea, N (%)			
Present	73 (80.2%)	163 (95.3%)	<0.001
None	18 (19.3%)	8 (4.7%)	

PMS, premenstrual syndrome.

3.2. Nutritional Status and Dietary Patterns

Table 3 presents the nutritional intake status according to the presence of PMS. Compared with the non-PMS group, the PMS group had a significant higher intake of total calorie, carbohydrate, fiber, vitamin A, vitamin K, and Ca. However, after adjusting for the total calorie intake, no significant difference in nutritional status between two groups was observed. Weekly consumption frequency of food groups in accordance with the presence of PMS is shown in Table 4. A comparison of weekly consumption frequency showed a significant difference with respect to the food groups of white meat, snacks, and alcoholic beverages. Weekly consumption frequency of white meat was significantly higher in the PMS group than in the non-PMS group (0.32 ± 0.46 times/wk vs. 0.23 ± 0.17 times/wk, $p = 0.024$). In addition, the PMS group showed significantly higher weekly consumption frequency of snacks (1.52 ± 2.07 times/wk vs. 1.05 ± 1.49 times/wk, $p = 0.033$) and alcoholic beverages (0.44 ± 0.86 times/wk vs. 0.25 ± 0.45 times/wk, $p = 0.022$).

Table 3. Nutritional status by PMS group.

Variables	Non-PMS Group	PMS Group	<i>p</i> -Value
N	91	171	
Total calorie	1667.6 $\pm$ 931.2	1950.6 $\pm$ 1121.5	0.041
Carbohydrate (g/day)	255.7 $\pm$ 128.3	295.7 $\pm$ 168.8	0.049
Protein (g/day)	45.9 $\pm$ 4.8	43.5 $\pm$ 3.3	0.112
Fat (g/day)	45.5 $\pm$ 34.3	55.1 $\pm$ 39.2	0.050
Fiber (g/day)	10.7 $\pm$ 1.1	13.3 $\pm$ 1.0	0.036
Saturated fatty acids (%)	1.93 $\pm$ 0.2	1.90 $\pm$ 2.0	0.584
Polyunsaturated fatty acids (%)	5.36 $\pm$ 2.90	5.35 $\pm$ 1.95	0.961
Monounsaturated fatty acids	5.34 $\pm$ 1.90	5.50 $\pm$ 2.20	0.539
Omega-3 fatty acids	0.30 $\pm$ 0.31	0.29 $\pm$ 0.20	0.692
Omega-6 fatty acids	0.01 $\pm$ 0.02	0.01 $\pm$ 0.01	0.783
Omega-6/Omega-3	0.08 $\pm$ 0.19	0.06 $\pm$ 0.11	0.268
Vitamin A	298.6 $\pm$ 236.4	386.2 $\pm$ 304.2	0.011
Vitamin C	93.4 $\pm$ 83.5	115.2 $\pm$ 121.1	0.088
Vitamin D	3.2 $\pm$ 5.0	4.2 $\pm$ 11.5	0.473
Vitamin E	13.4 $\pm$ 10.7	15.9 $\pm$ 11.5	0.098
Vitamin K	116.1 $\pm$ 121.0	166.2 $\pm$ 177.9	0.008
Riboflavin	1.3 $\pm$ 0.9	1.5 $\pm$ 1.0	0.250
Niacin	9.8 $\pm$ 7.3	11.5 $\pm$ 7.2	0.075
Vitamin B6	1.7 $\pm$ 3.8	1.5 $\pm$ 1.0	0.660
Ca	375.2 $\pm$ 280.0	460.3 $\pm$ 307.9	0.029
Na	2327.4 $\pm$ 2287.3	2783.7 $\pm$ 2068.3	0.103
K	2216.4 $\pm$ 1547.6	2584.9 $\pm$ 1665.6	0.082
Zinc	8.6 $\pm$ 7.7	9.7 $\pm$ 5.8	0.222

PMS, premenstrual syndrome.

Figure 1 presents the proportions corresponding to each tertile. MDS was classified into tertiles (T1: 0–3, T2: 4–5, and T3: ≥ 6). The proportions of PMS was significantly lower in T3 than in T1 (55.4% vs. 74.4%, $p = 0.045$), while the proportion of non-PMS was significantly higher in T3 than in T1 (44.6% vs. 25.6%, $p = 0.045$). Figure 2 shows the proportion of PMS according to adherence to each of the 14 MDS components. The non-PMS group showed a higher adherence rate to MD component 6 (<1 serving of butter, margarine, or cream per day) (81.3% vs. 66.7%, $p = 0.012$), component 7 (<1 serving of sugar-sweetened beverages per day) (59.3% vs. 45.6%, $p = 0.034$), and component 11 (<3 times of sweets, bread (except whole wheat bread), cakes, and cookies per week) (52.7% vs. 39.8%, $p = 0.044$). There were no significant differences in adherence to other components. Adherence to MDS component 8 (≥ 7 servings of wine per week) was zero for all participants.

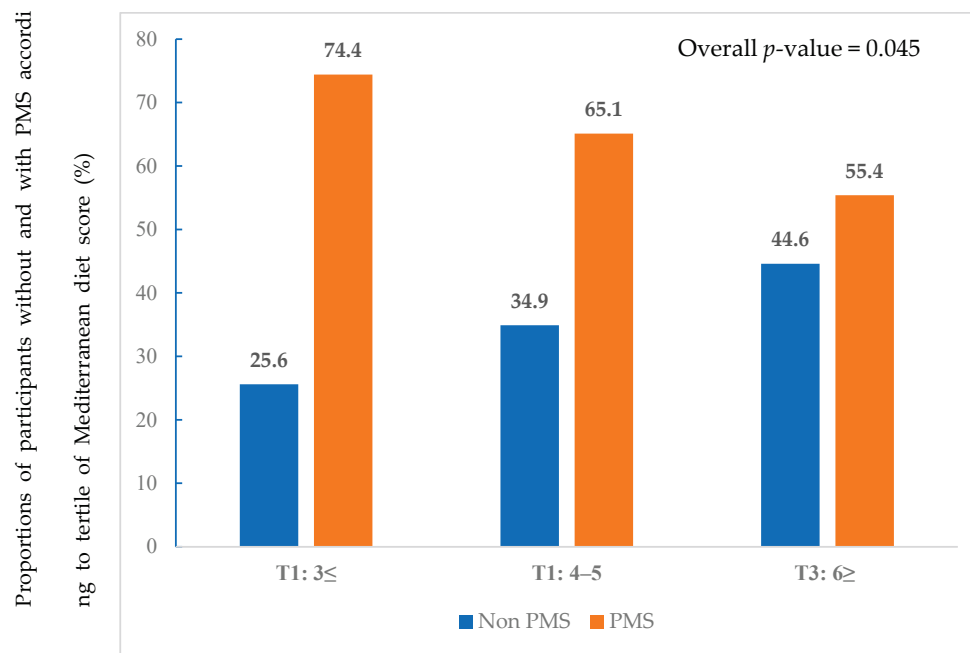


Figure 1. Proportions of participants without and with PMS according to Mediterranean diet score tertiles.

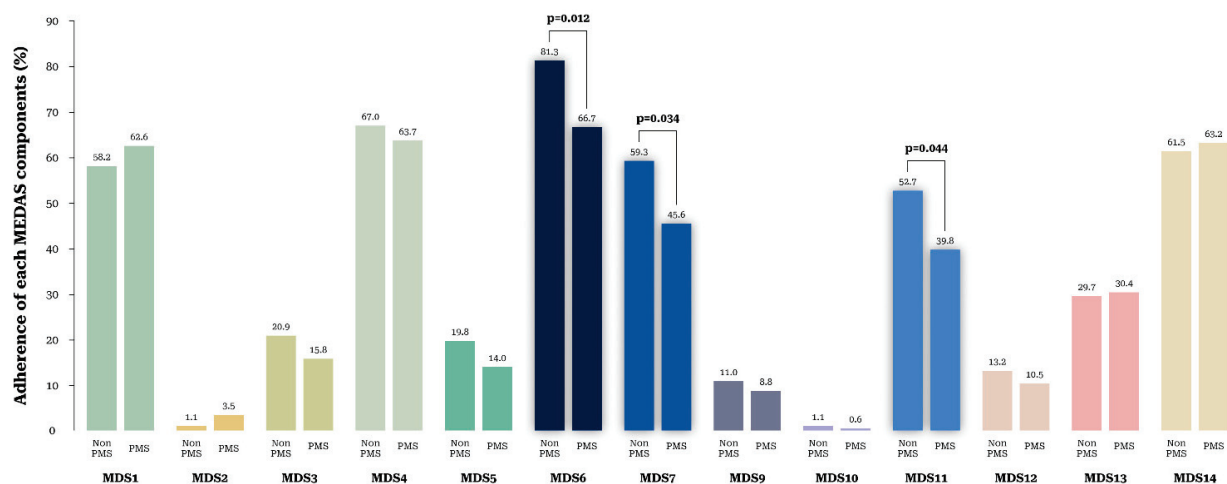


Figure 2. Proportion of participants without and with PMS according to adherence to each of the 14 components of the Mediterranean diet score.

Table 4. Weekly consumption frequency (times/week) of food groups by PMS group.

Variables	Non-PMS Group	PMS Group	p-Value
N	91	171	
Rice	2.31 ± 1.67	2.43 ± 1.73	0.598
Mixed grain rice	5.19 ± 5.88	5.04 ± 5.67	0.84
Noodles/dumplings	0.50 ± 0.49	0.58 ± 0.58	0.27
Breads/rice cakes	0.42 ± 0.48	0.51 ± 0.49	0.166
Soup/stew	0.36 ± 0.56	0.40 ± 0.41	0.492
Soybeans	0.67 ± 0.98	0.75 ± 1.08	0.56
Eggs	1.91 ± 1.89	1.90 ± 1.74	0.953
Red meat	0.48 ± 0.66	0.47 ± 0.44	0.833
White meat	0.23 ± 0.17	0.32 ± 0.46	0.024
Fish/seafood	0.38 ± 0.68	0.37 ± 0.61	0.957

Table 4. Cont.

Variables	Non-PMS Group	PMS Group	<i>p</i> -Value
Vegetables	0.59 ± 0.70	0.64 ± 0.74	0.623
Fermented foods	0.83 ± 1.65	0.96 ± 1.37	0.53
Kimchi	2.64 ± 3.00	2.92 ± 3.22	0.492
Seaweed	0.69 ± 1.37	0.66 ± 1.16	0.876
Potatoes	0.34 ± 0.64	0.35 ± 0.66	0.986
Milk/dairy products	1.37 ± 1.55	1.72 ± 1.97	0.11
Fruits	0.51 ± 0.53	0.57 ± 0.60	0.401
Beverages	1.80 ± 1.65	2.03 ± 1.72	0.289
Snacks	1.05 ± 1.49	1.52 ± 2.07	0.033
Nuts	0.19 ± 0.51	0.34 ± 1.35	0.171
Alcoholic beverages	0.25 ± 0.45	0.44 ± 0.86	0.022

PMS, premenstrual syndrome.

3.3. Association between Dietary Patterns and PMS

We identified three major dietary patterns using factor analysis: (i) traditional diet pattern, which was high in fish/seafood, vegetables, kimchi, seaweed, potatoes and fruits; (ii) meat/alcohol pattern, which was high in rice, noodles/dumplings, red meat, and alcoholic beverages; and (iii) bread/snack pattern, which was high in breads/rice cakes and snacks. The identified dietary patterns explained 49.5% of dietary food intakes in the study population. The factor loadings associated with 15 food groups for the major dietary patterns are presented in Table S1.

Table 5 presented the odds ratio (OR) and 95% confidence intervals (CI) for PMS across tertiles (T) of the three major dietary patterns. The bread/snack pattern was associated with prevalence of PMS in model 1 (T3 vs. T1, OR, 95% CI 2.63 (1.39–5.00), *p*-value = 0.003). After adjusting for age, body mass index, smoking status, alcohol drinking status, and physical activity, this significant association was remained (T3 vs. T1, OR, 95% CI, 2.59 (1.32–5.06), *p*-value = 0.006). Traditional diet pattern was not associated with prevalence of PMS in model 1 (T3 vs. T1, OR, 95% CI 0.91, (0.49–1.68), *p*-value = 0.905). Meat/alcohol pattern was not associated with prevalence of PMS in model 1 (T3 vs. T1, OR, 95% CI, 1.82 (0.97–3.40), *p*-value = 0.060). After adjusting for same confounders, similar trends were shown (traditional diet pattern; T3 vs. T1, OR, 95% CI, 1.13 (0.56–2.30), *p*-value = 0.735 and meat/alcohol pattern; T3 vs. T1, OR, 95% CI 1.60 (0.83–2.09) *p*-value = 0.160).

Table 5. Odds ratio and 95% confidence intervals for PMS according to the major dietary patterns.

	Traditional Diet Pattern		Meat and Alcohol Pattern		Bread and Snack Pattern	
	OR (95% CI)	<i>p</i> -Value	OR (95% CI)	<i>p</i> -Value	OR (95% CI)	<i>p</i> -Value
Model 1						
T1	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
T2	1.07 (0.57–2.00)	0.831	1.75 (0.94–3.26)	0.076	1.91 (1.03–3.53)	0.039
T3	0.91 (0.49–1.68)	0.905	1.82 (0.97–3.40)	0.060	2.63 (1.39–5.00)	0.003
Model 2						
T1	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
T2	1.11 (0.59–2.09)	0.751	1.80 (0.96–3.36)	0.068	1.87 (1.01–3.48)	0.048
T3	1.03 (0.54–1.98)	0.927	1.86 (0.99–3.49)	0.053	2.61 (1.37–4.97)	0.003
Model 3						
T1	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
T2	1.17 (0.61–2.29)	0.635	1.53 (0.79–2.95)	0.205	1.88 (0.98–3.58)	0.056
T3	1.13 (0.56–2.30)	0.735	1.60 (0.83–2.09)	0.160	2.59 (1.32–5.06)	0.006

Ref, reference; PMS, premenstrual syndrome; OR, odds ratio; 95% CI, 95% confidence interval. Model 1: unadjusted model. Model 2: Adjusted for age and body mass index. Model 3: Adjusted for age, body mass index, smoking status, alcohol drinking status, and physical activity.

4. Discussion

Research on the association between dietary patterns and PMS in Asian women is scarce. This cross-sectional study found that participants with PMS have lower adherence to MD. In addition, we identified three dietary patterns among Korean women: bread/snack, traditional diet, and meat/alcohol diet. Importantly, bread/snack dietary pattern was adversely associated with PMS, whereas traditional dietary pattern and meat and alcohol diet pattern were not associated with PMS.

A recent systemic review and meta-analysis conducted in India and Turkey reported that PMS was prevalent in 43–52.2% of women of reproductive age [19,20]. A Spanish cohort study also reported a PMS prevalence of 73.7% [21] and a meta-analysis in Iranian women of reproductive age reported a prevalence rate of 70.8% [95% CI: 63.8–77.7] [22]. This difference in prevalence among the studies might be due to the difference in the study population, and cultural contexts, as well as differences in study methodology such as sample size, design, and methods of PMS measurement. In our study, majority of participants had PMS (65.3% of 262 participants). Although it cannot be excluded that ACOG diagnostic criteria overestimated the prevalence of PMS [23], the rate was consistent with the results of previous studies that reported a high prevalence of PMS in Korea [24,25].

Dietary factors are influential but modifiable parameters that can be included in the management of PMS, and several studies have found a significant association between dietary habit and PMS [5,26]. A study conducted in the United Arab Emirates reported a high prevalence of PMS among university students with high calorie/fat/sugar/salt food consumption [26]. Western dietary pattern, which is characterized by high consumption of fast foods, soft drink, and desserts, is significantly associated with a higher likelihood of PMS, whereas healthy and traditional dietary pattern is associated with a lower risk [5,9]. The MD is rich in high-complex carbohydrate and monounsaturated fatty acids, rather than simple sweets and saturated and trans-fatty acids [10,11]. Many studies have shown that the MD has beneficial effects in preventing chronic diseases, such as metabolic syndrome, type 2 diabetes, inflammatory disease, cardiovascular disease, and cancer [27]. In a recent Spanish study, low adherence to the MD was associated with longer menstrual cycles [28]. Women who ate less than two pieces of fruit per day had a higher risk of menstrual pain (OR: 2.984; 95% CI = 1.390–6.406; $p < 0.05$) [28]. The amount of menstrual bleeding was lower in women who consumed olive oil daily, while it was more severe in those who consumed ham weekly.

However, only few studies have found a significant association between MD habit and PMS, especially in Asians. Our findings are consistent with those of previous studies. We found a significantly higher MD score in the non-PMS group than in the PMS group. The proportions of participants consuming butter, margarine, or cream less than one serving/day; sugar-sweetened beverage less than one serving; and sweets, bread, cakes, and cookies less than three times per week were significantly higher in the non-PMS group than PMS group. Further, highest bread/snack pattern was associated with a 2.59 times higher risk of PMS. Although the exact etiology of PMS still remains unknown, several possible mechanisms could support our findings. First, high consumption of bread and snacks with excess sodium [29] may decrease serum magnesium (Mg) levels [30,31].

Reduced Mg levels have been reported in women with PMS [32], and a randomized clinical trial confirmed that Mg supplementation alleviated PMS symptoms related to mood [32]. The traditional Korean diet pattern is characterized by high consumption of vegetables, legumes, whole grains, fish and fermented foods (kimchi, soy sauce, and soybean paste), and these components are similar to those of MD [33]. However, traditional fermented foods of Korean diet also contain high sodium [34]. Therefore, in the current study, traditional diet pattern might not be associated with prevalence of PMS. Second, individuals with PMS are prone to have depressive mood and stronger cravings for refined carbohydrate and fat foods [35,36]. Third, a western dietary pattern is closely related to chronic low-grade inflammation, and several studies proved significant positive associations between serum levels of inflammatory markers (e.g., high-sensitivity C-reactive

protein, interleukin-12, and interferon- γ) and menstrual symptom severity in women with PMS [37,38]. In addition, the common psychiatric features of PMS have been associated with chronic inflammation [38]. Fourth, a previous study demonstrated that oxidative stress results from an oxidant/antioxidant imbalance is closely associated with various symptoms of PMS [39]. MD, a representative antioxidant diet, has abundant polyphenols and monounsaturated and polyunsaturated fatty acids. Therefore, MD could help to reduce PMS symptoms.

Our study has some limitations. First, the causal relationship between dietary patterns and PMS could not be investigated owing to the nature of cross-sectional study. Second, nutritional status and dietary pattern were assessed using the FFQ, which has disadvantages of lacking accuracy for absolute nutrient values, especially micronutrients and possibility of over and underreporting of consumption of certain foods [40]. In addition, dietary patterns do not represent all possible patterns and people may fit into more than one pattern. Third, we only included the participants who participated in the online survey panel. Therefore, our results have limited generalizability to the overall population of Korean women, other races, and other countries. Fourth, during the survey, we allowed subjects to freely report their diseases in addition to hypertension, dyslipidemia, diabetes, and cardiovascular disease. Participants who have mental illness are likely to understate their situation and report falsely. Therefore, we could not fully evaluate the individuals' psychological status. Finally, the effect of unmeasured factors such as physiological condition (e.g., menopause status) and comorbidities cannot be completely ruled out. Despite these limitations, to our best knowledge, this study is the first to investigate the association between dietary pattern and PMS and examine adherence to MD as it relates to the prevalence of PMS among Korean women. Further investigations into the biological mechanisms by which dietary factors influence the pathogenesis of PMS are required.

5. Conclusions

This is the first study investigating the association between dietary pattern and PMS among Korean women. Our result suggested that bread/ snack pattern is associated with higher prevalence of PMS in Korean women. We also suggest that low adherence to MD is associated with PMS. Our findings suggest that adherence to MD and avoiding bread/snack consumption could be helpful for managing and preventing PMS. Further investigations into the biological mechanisms of dietary factors' action on the pathogenesis of PMS are required.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/nu14122460/s1>, Table S1: Factor loadings of the dietary patterns derived from factor analysis.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and was approved by the Institutional Review Board of Yongin Severance Hospital (protocol code 9-2021-0110).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Article

Association of the APOA-5 Genetic Variant rs662799 with Metabolic Changes after an Intervention for 9 Months with a Low-Calorie Diet with a Mediterranean Profile

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Abstract: In cross-sectional studies, the genetic variant rs662799 of the APOA5 gene is associated with high serum triglyceride concentrations, and in some studies, the effect of short-term dietary interventions has been evaluated. The aim of the present investigation was to evaluate the role of this genetic variant in metabolic changes after the consumption of a low-calorie diet with a Mediterranean pattern for 9 months. A population of 269 Caucasian obese patients was recruited. Adiposity and biochemical parameters were measured at the beginning (basal level) and after 3 and 9 months of the dietary intervention. The rs662799 genotype was assessed with a dominant analysis (TT vs. CT + CC). The APOA5 variant distribution was: 88.1% ($n = 237$) (TT), 11.5% ($n = 31$) (TC) and 0.4% ($n = 1$) (CC). There were significant differences only in triglyceride levels at all times of the study between the genotype groups. After 3 and 9 months of dietary intervention, the following parameters improved in both genotype groups: adiposity parameters, systolic pressure, total cholesterol, LDL cholesterol, leptin, adiponectin and the leptin/adiponectin ratio. The intervention significantly decreased insulin levels, HOMA-IR and triglyceride levels in non-C allele carriers (Delta 9 months TT vs. TC + CC). i.e., insulin levels (delta: -3.8 ± 0.3 UI/L vs. -1.2 ± 0.2 UI/L; $p = 0.02$), HOMA-IR levels (delta: -1.2 ± 0.2 units vs. -0.3 ± 0.1 units; $p = 0.02$), triglyceride levels (delta: -19.3 ± 4.2 mg/dL vs. -4.2 ± 3.0 mg/dL; $p = 0.02$). In conclusion, non-C allele carriers of rs662799 of the APOA5 gene showed a decrease of triglyceride, insulin and HOMA-IR levels after consuming a low-calorie diet with a Mediterranean pattern; we did not observe this effect in C allele carriers, despite a significant weight loss.

Keywords: ApoA5 gene; rs662799; low-calorie diet; triglycerides

1. Introduction:

Dyslipidemia is a metabolic problem commonly detected in obese subjects [1], and obese individuals usually presents elevated triglyceride levels and decreased HDL-cholesterol, secondarily associated with atherosclerosis [2]. The apolipoprotein A5 (ApoA5) gene is located on chromosome 11q23.3 in the APOA1/C3/A4A5 gene cluster. This gene encloses four exons coding for a 366-amino acid protein called APOA5. This apolipoprotein is secreted from the liver, regulates triglyceride metabolism and is part of HDL-cholesterol particles [3,4]. The role of APOA5 in the metabolism of triglycerides involves different pathways; this protein reduces the synthesis of very low density lipoprotein (VLDL) triglycerides and increases the hydrolysis of VLDL triglycerides, secondary to the action of lipoprotein lipase [3,4]. Some studies have reported that the APOA5 gene is clearly associated with the establishment of cardiovascular events [5] and metabolic syndrome [6,7].

Moreover, single-nucleotide variants (SNVs) in the APOA5 gene, such as rs651821 and rs3135506, have been associated with metabolic syndrome and its traits, [8–12]; recently, the involvement of rs662799 ($-1131T > C$) [13] in lipid profile and metabolic syndrome

has been shown too. This last genetic variant of the APOA5 gene has been described to be a functional-tag SNV [11,12]. The C allele of rs662799 deteriorates ribosomal translation efficiency, and this decreases the concentrations of APOA5 and decreases lipoprotein lipase activity [8,9]. In the literature, there are few intervention studies evaluating the role of this SNV, but they have shown some interesting findings. In a cross-sectional design with hypertriglyceridemic patients, C allele carriers reported higher levels of triglycerides than non-C allele carriers, without a relation to total energy intake and total saturated and polyunsaturated fat intake [14]. After a well-designed normocaloric dietary changes during 4 weeks in hypertriglyceridemic subjects, only carriers of the T allele showed an improvement in the lipid profile [15]. Another short-term study (3 months) with a high-carbohydrate/low-fat hypocaloric diet (1500 cal/day) reported that postprandial triglyceride elevation is modulated by the T allele [16]. In other short-term (3 months) dietary intervention with a hypocaloric diet (1500 cal/day) with a Mediterranean profile, C allele carriers showed a lack of response after weight loss of the triglyceride levels [17]. Finally, another cross-sectional design [18] reported a relationship between the C allele and a high fat intake; it reported a relationship between triglyceride levels and a high dietary fat intake in subjects with the minor allele.

At this moment, there are no long-term nutritional interventions that have evaluated changes in the relationship of rs662799 with the metabolic. This is an interesting investigation topic, since it was shown that weight loss following a Mediterranean dietary pattern decreased the lipid levels [14]. The Mediterranean diet is a primarily plant-based eating plan that includes the daily intake of whole grains, olive oil, fruits, vegetables and legumes, nuts, herbs, and spices. Other foods like animal proteins are eaten in small quantities, with the animal protein being preferentially obtained from fish and seafood. These types of foods contain high amounts of dietary unsaturated fats and have potential effects on the lipid profile s they may affect directly the lipids levels [15,16].

The aim of our study was to evaluate the role of SNV rs662799 on metabolic variables in obese subjects after a low-calorie diet with a Mediterranean pattern during a period 9 months.

2. Subjects and Methods

2.1. Subjects and Clinical Investigation

The present study enrolled a total of 269 unrelated obese subjects from our health area during a routine check-up of obesity, with a body mass index $>30 \text{ kg/m}^2$. The selection method was consecutive non-probabilistic. All subjects agreed to enroll in the investigation and provided written informed consent. Inclusion criteria were body mass index $>30 \text{ kg/m}^2$ and age > 18 years. Exclusion criteria were the presence of any associated condition, coronary events, renal or hepatic disorders, alcohol intake more than 20 g/day in women and more than 30 g/day in men or within the 12 months before the study, and receiving medications known to influence the lipid levels (statins, fibrates, hormonal therapy, glucocorticoids and anti-inflammatory drugs).

All measurements were realized in our department of investigation by physicians. Anthropometric parameters were measured for all subjects for body weight, height, body mass index (BMI) and waist circumference at the beginning and at 3 and 9 months of dietary intervention. Total body fat mass was measured by electric impedance determination. For the analysis of biochemical parameters and the genotype of rs662799, 15 mL of venous blood was drawn and aliquoted in ethylenediaminetetraacetic acid (EDTA)-coated probes after 10 h of overnight fast. Lipid profile, C reactive protein (CRP), adipokine levels (leptin, total adiponectin, resistin and the adiponectin/leptin ratio), fasting glucose, insulin were measured, and insulin resistance was calculated with the homeostasis model assessment (HOMA-IR) method.

2.2. Adiposity Parameters and Blood Pressure

Height (cm) was determined using a non-elastic measuring tape (Quimild, Spain). Waist circumference (cm) was measured using a non-elastic measuring tape (Quimild, Spain). Body weight was measured using a digital scale (Quimild, Spain) and recorded to the nearest 10 g. Body mass index (BMI) was determined with the equation (body weight (in kg) divided by height (in m²)). Total fat mass was measured by impedance with an accuracy of 5 g [19] (EFG BIA 101 Anniversary, Akern, It), and this parameter was calculated with the equation $(0.756 \text{ Height}^2 / \text{Resistance}) + (0.110 \text{ Body mass}) + (0.107 \text{ Reactance}) - 5.463$.

Blood pressures (systolic and diastolic) were determined by averaging four measurements (Omrom, Los Angeles, CA, USA), after a rest time of 10 min.

2.3. Analytical Parameters

Glucose concentrations were determined by an automated hexokinase oxidase system (COBAS INTEGRA 400 analyzer (Roche Diagnostic, Montreal, QC, Canada). Insulin concentrations were determined by an electrochemiluminescence assay (COBAS INTEGRA 400 analyzer (Roche Diagnostic, Montreal, QC, Canada). Insulin resistance was calculated with the formula of the homeostasis model assessment for insulin resistance (HOMA-IR) $(\text{glucose} \times \text{insulin} / 22.5)$ [20]. Different components of the lipid profile (triglycerides, total cholesterol and HDL-cholesterol) were measured using the COBAS INTEGRA 400 analyzer (Roche Diagnostic, Montreal, QC, Canada). LDL cholesterol was obtained in an indirect way through the Friedewald formula $(\text{LDL cholesterol} = \text{total cholesterol} - \text{HDL cholesterol} - \text{triglycerides} / 5)$ [21]. C-reactive protein (CRP) was measured by immunoturbimetry (Roche Diagnostics GmbH, Mannheim, Germany).

All adipokines were determined by the Enzyme-Linked Immuno Sorbent Assay (ELISA) as follows: leptin (Diagnostic Systems Laboratories, Inc., Webster, TX, USA) (DSL1023100) [22], adiponectin (R&D systems, Inc., McKinley, MN, USA) (DRP300) [23] and resistin (Biovendor Laboratory, Inc., Brno, Czech Republic) (RD191016100) [24].

2.4. Genotyping of the APOA5 Gene Variant

DNA was isolated from leucocytes, using a commercially available DNA isolation kit (Biorad, Los Angeles, CA, USA). Genotyping was realized by polymerase chain reaction real-time analysis. Primers were designed with the Sequenom Assay Design v4 (SEQUENOM, Inc. San Diego, CA, USA). The polymerase chain reaction (PCR) was realized with 50 ng of genomic DNA and 0.25–0.30 µL of each oligonucleotide primer for rs662799 (primer forward: 5'-GAGCCCCAGGAACTGGAGCGAAAGT-3' and reverse 5'-AGATTTGCCCCATGAGGAAAAGCTG-3', in a 3.0 µL final volume (Lifetechnologies, LA, CA). DNA was denatured at 90 °C for 10 min; subsequently, 45 cycles at 65 °C for 15 s, and annealing at 59 °C for 40 s were performed. PCRs were run in a 2.5 µL volume containing 0.15 µL of iPLEX Termination mix (Bio-Rad®, San Diego, CA, USA) with hot start Taq DNA polymerase. Then, 15% of the samples were randomly re-evaluated to confirm accuracy.

2.5. Dietary Intervention

Obese subjects were assigned to consume a low-calorie diet (around 1000 calories per day) with a pattern of a Mediterranean diet for 9 months. The target distribution of macronutrients in this diet was 53% carbohydrates, 27% fats, and 20% proteins. The target percentage of dietary fats was 65% of monounsaturated fats, 20% of saturated fats, and 15% of polyunsaturated fats. Food tables were utilized, including legumes, vegetables, poultry, whole grains, fish, fresh fruit, olive oil and limiting unhealthy fats such as margarines, fatty meats, snacks, industrial pastries. All the subjects were given written instructions by a registered dietitian on completion of a 3-day (1-weekend and 2-week days) dietary record and basal time and after 3 and 9 months of dietary intervention. These records were analyzed with a software (DietSource®, Geneve, Switzerland) with food references

for our country [25]. All participants attended four sessions (30 min each, with example menu plans) at the start of the trial and during the intervention to explain the diet. The physical exercise activity indicated included sessions of aerobic exercise at least 3 times per week (45–60 min each), and the patient recorded it on a self-reported questionnaire. All subjects were interviewed weekly by phone to evaluate whether they were following the Mediterranean diet and the physical exercise activity plan.

2.6. Statistical Analysis

The sample size ($n = 250$) was obtained to determine differences over 10 mg/dL of triglyceride concentrations with 90% power and 5% significance. Statistical evaluation was performed using SPSS for Windows, version 23.0 software package (SPSS Inc. Chicago, IL, USA). We performed the analysis with a dominant genetic model (TT vs. TC + CC). Descriptive statistics are presented as mean $\pm$ standard deviation for continuous variables and as a percentage for categorical variables. Two-tailed Student's *t*-test was used to analyze continuous variables with normal distribution. Chi-square test, with Yates correction as necessary, was used to analyze categorical variables. In order to reduce Type I errors in the association analysis, the Bonferroni test was applied for multiple testing. The statistical analysis to evaluate the interaction between the gene and the dietary intervention was performed using ANCOVA (covariance analysis) adjusted by age, sex, and BMI modeling the dependent variable with the starting values. Hardy–Weinberg equilibrium was determined with Chi-square test to compare our expected and observed data.

2.7. Ethical Approval

All procedures were in accordance with the ethical standards of the institutional research committee (HVUVA committee 7/2020) and with the 1964 Helsinki declaration and its later amendments. Written informed consent was obtained from all individual participants included in the study.

3. Results

The rs662799 distribution was as follows: 88.1% ($n = 237$) of the participants (TT) were homozygous for the T allele, 11.5% ($n = 31$) (TC) were heterozygous, and 0.4% ($n = 1$) (CC) were homozygous for the C allele. Finally, the T allele frequency was 0.94 and the C allele frequency was 0.06; therefore, throughout the analysis we used a dominant evaluation (TT vs. TC + CC). This variant was in Hardy–Weinberg equilibrium ($p = 0.43$).

The average age of our sample was 51.3 ± 5.2 years (range: 25–66), and the average BMI was 40.7 ± 3.2 kg/m² (range: 33.3–43.4). Gender distribution was 199 females (74.0%) and 70 males (26.0%). The average age of both genotype groups was similar (TT; 51.9 ± 4.9 years vs. TC + CC; 51.0 ± 4.2 years: ns) as well as the gender proportion (TT 25.7% males vs. 74.3% females vs. TC + CC 28.1% males vs. 71.9% females). Patients followed the caloric restriction and macronutrient recommendations as indicated by the dietitian (Table 1). The percentages of macronutrients were those indicated in the initial objectives, and the same happened with the total fat intake and the amount of unsaturated and saturated fats. Basal and post-intervention physical exercise levels were similar in both groups (Table 1).

3.1. Modifications in Anthropometry and Blood Pressure

Adiposity variables and systolic/diastolic pressure were not significantly related to the rs662799 variant, as shown in Table 2. When we compared both genotype groups at baseline, there were no significant differences in any parameter. After the dietary intervention during a period of 9 months and in both genotype groups, all the following parameters improved at 3 and 9 months (Delta 9 months TT vs. TC + CC); BMI (delta: -4.5 ± 0.5 kg/m² vs. -4.4 ± 0.3 kg/m²; $p = 0.51$), body weight (delta: -6.6 ± 2.0 kg vs. -6.4 ± 1.9 kg; $p = 0.43$), fat mass (delta: -5.1 ± 0.8 kg vs. -5.0 ± 1.1 kg; $p = 0.59$), waist circumference (delta: -6.2 ± 1.9 cm

vs. -6.0 ± 1.8 cm; $p = 0.51$) and systolic pressure (delta: -4.8 ± 1.0 mmHg vs. -5.2 ± 2.0 mmHg; $p = 0.49$). These changes were the same in the two genotype groups.

Table 1. Average daily intakes and physical activity determined at three times (basal and 3 and 9 months after the intervention) (mean $\pm$ SD).

	TT ($n = 237$)			TC + CC ($n = 32$)		
	0 Time	3 Months	9 Months	0 Time	3 Months	9 Months
Calorie intake (kcal/day)	1728.9 $\pm$ 210.8	1012.1 $\pm$ 21.1 *	1008.1 $\pm$ 13.1 *	1823.9 $\pm$ 129.1	1010.1 $\pm$ 18.1 *	1001.9 $\pm$ 12.1 *
Carbohydrate intake (g/day) (PTC%)	207.8 $\pm$ 51.9 (48.9%)	137.5 $\pm$ 20.1 \$ (54.3%)	130.1 $\pm$ 18.1 \$ (53.9%)	218.1 $\pm$ 41.1 (47.8%)	133.1 $\pm$ 19.1 \$ (53.7%)	130.9 $\pm$ 12.1 \$ (52.6%)
Fat intake (g/day) (PTC%)	58.9 $\pm$ 10.3 (30.6%)	31.1 $\pm$ 8.1 # (26.7%)	32.2 $\pm$ 5.1 # (26.9%)	62.4 $\pm$ 8.3 (30.8%)	30.2 $\pm$ 4.3 # (26.9%)	31.1 $\pm$ 5.1 # (27.4%)
Protein intake (g/day) (PTC%)	88.6 $\pm$ 14.1 (20.5%)	50.6 $\pm$ 7.3 & (20.0%)	51.9 $\pm$ 7.1 & (20.2%)	92.9 $\pm$ 10.1 (22.4%)	51.2 $\pm$ 6.9 & (20.5%)	52.3 $\pm$ 6.0 & (21.0%)
Saturated Fat intake (g/day) (PFC%)	36.2 $\pm$ 6.0 (61.4%)	6.1 $\pm$ 4.9 ** (19.6%)	6.4 $\pm$ 4.0 ** (19.0%)	37.8 $\pm$ 5.1 (60.5%)	5.9 $\pm$ 2.2 (19.5%)	5.3 $\pm$ 1.1 (19.1%)
Monounsaturated Fat intake (g/day) (PFC%)	15.2 $\pm$ 3.1 (25.8%)	21.3 $\pm$ 2.9 \$\$ (63.2%)	22.1 $\pm$ 2.0 \$\$ (64.1%)	15.8 $\pm$ 5.2 (25.3%)	19.9 $\pm$ 4.0 (65.9%)	20.1 $\pm$ 4.1 (66.1%)
Polyunsaturated Fat intake (g/day) (PFC%)	7.5 $\pm$ 6.0 (13.8%)	5.3 $\pm$ 4.9 ## (17.2%)	5.4 $\pm$ 3.2 ## (16.9%)	8.8 $\pm$ 4.2 (14.2%)	4.4 $\pm$ 2.2 (15.6%)	4.7 $\pm$ 0.9 (15.8%)
Physical activity (min/week)	121.2 $\pm$ 12.1	127.8 $\pm$ 12.5	130.1 $\pm$ 9.9	125.9 $\pm$ 4.2	132.1 $\pm$ 11.2	133.2 $\pm$ 10.2

PTC: Percentage of total calories; PFC: Percentage of fat calories. Statistical differences $p < 0.05$, in each genotype group (* Daily Calorie intake, \$ Daily Carbohydrate intake, # Daily fat intake, & Daily protein intake, ** Saturated fat intake, \$\$ monounsaturated fat intake, ## Polyunsaturated fat intake).

Table 2. Adiposity parameters and pressure at three times (basal and 3 and 9 months after the intervention) (mean $\pm$ SD).

	TT ($n = 237$)			TC + CC ($n = 32$)		
	0 Time	3 Months	9 Months	0 Time	3 Months	9 Months
BMI	40.8 $\pm$ 2.1	38.3 $\pm$ 3.2 *	36.1 $\pm$ 1.1 *	40.6 $\pm$ 2.3	38.4 $\pm$ 2.1 *	36.2 $\pm$ 1.0 *
Weight (kg)	91.4 $\pm$ 3.2	86.9 $\pm$ 2.0 \$	84.8 $\pm$ 2.0 \$	90.9 $\pm$ 2.1	86.8 $\pm$ 2.0 \$	84.5 $\pm$ 2.1 \$
Fat mass (kg)	36.3 $\pm$ 1.1	33.1 $\pm$ 1.0 #	31.2 $\pm$ 1.1 #	35.9 $\pm$ 1.2	33.0 $\pm$ 1.8 #	30.9 $\pm$ 1.8 #
WC (cm)	111.7 $\pm$ 4.3	107.1 $\pm$ 3.1 &	105.5 $\pm$ 3.0 &	110.8 $\pm$ 6.0	107.6 $\pm$ 4.1 &	104.9 $\pm$ 3.1 &
SBP (mmHg)	127.0 $\pm$ 3.0	123.1 $\pm$ 3.1 **	122.8 $\pm$ 4.0 **	126.9 $\pm$ 4.1	122.1 $\pm$ 3.1 **	121.7 $\pm$ 2.9 **
DBP (mmHg)	81.3 $\pm$ 3.1	78.8 $\pm$ 4.1	79.1 $\pm$ 4.0	81.4 $\pm$ 3.0	78.1 $\pm$ 3.0	78.9 $\pm$ 4.1

BMI: body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; WC, waist circumference; Statistical differences $p < 0.05$, in each genotype group with basal value (* BMI, \$ Weight, # fat mass, & WC, ** SBP). No statistical differences between genotype groups.

3.2. Changes in Classical Biochemical Parameters

When we compared the biochemical parameters in the two groups at baseline, only the triglyceride (TG) levels showed significant differences. These TG concentrations were higher in patients with the C allele (TT vs. TC + CC in basal levels: -19.9 ± 3.1 mg/dL; $p = 0.02$), (TT vs. TC + CC in post treatment 3 months levels: -20.1 ± 3.2 mg/dL; $p = 0.03$) and (TT vs. TC + CC in post treatment 9 months levels: -34.7 ± 5.8 mg/dL; $p = 0.03$), see Table 3.

Table 3. Biochemical parameters at three times (basal and 3 and 9 months after the intervention) (mean $\pm$ SD).

	TT (<i>n</i> = 237)			TC + CC (<i>n</i> = 32)		
	0 Time	3 Months	9 Months	0 Time	3 Months	9 Months
Glucose (mg/dL)	103.8 $\pm$ 4.0	100.8 $\pm$ 3.1	99.8 $\pm$ 4.2	101.3 $\pm$ 6.0	99.9 $\pm$ 4.2	98.7 $\pm$ 4.1
Total ch. (mg/dL)	208.8 $\pm$ 4.6	195.2 $\pm$ 4.1 \$	193.1 $\pm$ 3.1 \$	208.7 $\pm$ 4.1	194.1 $\pm$ 3.1 \$	192.1 $\pm$ 3.0 \$
LDL-ch. (mg/dL)	137.2 $\pm$ 8.1	120.8 $\pm$ 2.2 #	118.1 $\pm$ 4.0 #	140.5 $\pm$ 3.9	122.5 $\pm$ 4.2 #	118.9 $\pm$ 3.1 #
HDL-ch. (mg/dL)	55.8 $\pm$ 1.6	54.7 $\pm$ 1.9	55.1 $\pm$ 1.2	55.9 $\pm$ 2.1	55.5 $\pm$ 1.3	56.1 $\pm$ 1.2
TG (mg/dL)	123.2 $\pm$ 11.0	110.9 $\pm$ 9.2 *	104.1 $\pm$ 6.2 *	143.1 $\pm$ 10.2 x	140.8 $\pm$ 4.1 x	139.1 $\pm$ 7.8 x
Insulin (mUI/L)	10.8 $\pm$ 2.1	9.2 $\pm$ 1.3 &	7.0 $\pm$ 1.0 &	12.3 $\pm$ 2.1	10.8 $\pm$ 2.2	11.0 $\pm$ 2.3
HOMA-IR	3.7 $\pm$ 1.0	2.1 $\pm$ 0.9 **	1.5 $\pm$ 0.7 **	3.9 $\pm$ 0.8	3.5 $\pm$ 1.1	3.6 $\pm$ 1.2
CRP (mg/dL)	4.3 $\pm$ 1.0	4.3 $\pm$ 1.3	4.2 $\pm$ 1.1	4.4 $\pm$ 2.1	4.5 $\pm$ 1.3	4.3 $\pm$ 1.1

HOMA-IR (homeostasis model assessment). CRP (C-reactive protein). Statistical differences $p < 0.05$, in each genotype group with basal values (total cholesterol \$, LDL cholesterol #, triglycerides *, insulin &, HOMA IR **). Statistical differences $p < 0.05$, between genotype groups (triglycerides x).

Considering glucose metabolism after the dietary intervention (Delta 9 months TT vs. TC + CC), insulin concentrations (Delta: -3.8 ± 0.3 UI/L vs. -1.2 ± 0.2 UI/L; $p = 0.02$) and HOMA-IR (Delta: -1.2 ± 0.2 units vs. -0.3 ± 0.1 units; $p = 0.02$) decreased significantly in non-C allele carriers. After the dietary intervention (Delta 9 months TT vs. TC + CC), TG concentrations (Delta: -19.3 ± 4.2 mg/dL vs. -4.2 ± 3.0 mg/dL; $p = 0.02$) improved in non-C allele carriers, too. Total cholesterol and LDL cholesterol concentrations decreased in both genotype groups.

3.3. Adipokine Levels

Table 4 reports changes of serum adipokines and the adiponectin/leptin ratio. The serum levels of these adipokines were similar in TT and TC + CC genotype groups, in baseline values and in levels throughout the intervention. After the dietary intervention and in both genotypes (delta 9 months TT vs. TC + CC), the serum adiponectin levels increased (delta: 24.2 ± 3.1 ng/dL vs. 21.9 ± 3.9 ng/dL; $p = 0.42$), and the leptin levels decreased (delta: -19.2 ± 5.1 ng/dL vs. -20.1 ± 4.1 ng/dL; $p = 0.39$). Finally, the calculated adiponectin/leptin ratio increased in both genotypes (delta 9 months TT vs. TC + CC: 0.55 ± 0.2 vs. 0.61 ± 0.1 ng/dL; $p = 0.49$). The resistin levels remained unchanged.

Table 4. Circulating adipocytokines at three times (basal and 3 and 9 months after the intervention) (mean $\pm$ SD).

	TT (<i>n</i> = 237)			TC + CC (<i>n</i> = 32)		
	0 Time	3 Months	9 Months	0 Time	3 Months	9 Months
Adiponectin (ng/mL)	4.8 $\pm$ 1.0	4.7 $\pm$ 2.0	4.9 $\pm$ 1.8	4.5 $\pm$ 2.0	4.4 $\pm$ 1.3	4.7 $\pm$ 1.2
Resistin (ng/mL)	39.1 $\pm$ 5.0	68.1 $\pm$ 4.2 \$	63.1 $\pm$ 4.1 \$	34.9 $\pm$ 4.1	67.2 $\pm$ 4.2 \$	66.1 $\pm$ 3.1 \$
Leptin (ng/mL)	88.1 $\pm$ 8.1	71.2 $\pm$ 4.1 *	68.9 $\pm$ 4.1 *	89.1 $\pm$ 8.1	70.1 $\pm$ 3.1 *	69.9 $\pm$ 6.1 *
Ratio Adiponectin/leptin	0.36 $\pm$ 0.2	0.92 $\pm$ 0.1 #	0.91 $\pm$ 0.1 #	0.18 $\pm$ 0.1	0.92 $\pm$ 0.3 #	0.93 $\pm$ 0.2 #

Statistical differences $p < 0.05$, in each genotype group (* leptin \$ adiponectin, # ratio adiponectin/leptin). No statistical differences between genotype groups.

4. Discussion

The results of this interventional study in Caucasian obese subjects showed that the single-nucleotide variant (SNV) rs662799 of the APOA5 gene modulated the metabolic response of weight change secondary to a long-term low-calorie diet with a Mediterranean pattern. In C allele carriers, this dietary intervention generated significantly lower improvements in insulin, HOMA-IR and triglyceride levels than in non-C allele carriers.

Investigations in the literature evaluating the role of this SNV on metabolic modifications are scarce and with a very short duration. For example, in the literature there is

only one study that evaluates the role of rs662799 in the response to statins [26]. This study reported that dyslipidemic subjects with the C allele benefited less from statin treatment compared with non-C allele carriers. Despite these few studies, this SNV has been associated with high levels of triglycerides (TG) [27] and a high occurrence of cardiovascular events [28,29]. These scientific findings can be explained by the fact that the C allele reduces ribosomal translation activity, thereby decreasing the concentrations of APOA5 [30], and this fact negatively affects lipoprotein lipase activity and raises serum TG levels [31].

The relationship of different diets with this SNV have been evaluated, although in short-term interventions. In a previous study with borderline to moderate hypertriglyceridemic Chinese patients [15], Jang et al. showed that a dietary intervention during 3 months decreased the triglyceride levels in TT subjects, with a lack of action in C allele carriers. The dietary changes consisted in increasing the vegetable amount to at least 180–420 g/day and substituting by 1/3 the intake of refined rice with legumes three times per day, as a carbohydrate source. The decrease in TG levels in this study was 20% secondary to changes in serum APOA5 concentration. In our study the improvement was 16%, very similar to that of the study previously discussed [15]. In this previous work, insulin resistance was not evaluated, and also there were differences in the design with respect to our work; in fact, a low-calorie diet was not used, the nutritional intervention was shorter, while the evaluated subjects were not normolipemic obese as in our case. In another short-term intervention trial of 6 days [16], healthy Korean volunteers followed a high-carbohydrate/low-fat diet, in which the percentages of carbohydrates, proteins and fats were 70%, 15% and 15%, respectively. The energy intake of the patients was dependent on their satiety. Subjects with the C allele had higher TG levels after this short intervention trial than TT allele subjects. An intervention trial of 3 months with a hypocaloric Mediterranean diet in a Caucasian population [17] reported a decrease in HOMA-IR, insulin levels and triglycerides after a significant weight loss in non-C allele carriers, with a lack of metabolic effect in C allele carriers. In this study, there was a caloric restriction of 500 calories per day, and it was carried out in obese subjects. The dietary distribution was 50% of the calorie value from carbohydrates, 30% from lipids and 20% from proteins. The proportion of fats was 55% from monounsaturated fats, 30% from saturated fats and 15% from polyunsaturated fats. The caloric restriction in our present study was higher than that in this previous study [17], while the distribution of macronutrients was similar, with a Mediterranean pattern, though with a higher percentage of monounsaturated fats. Despite these differences in the study design, the metabolic results of both studies are similar, with significant improvements in triglyceride, HOMA-IR and insulin levels in the non-carriers of the C allele. The postprandial state can play a relevant role in the above-mentioned findings, as Kim et al. [18] reported using a meal tolerance test. In non-obese healthy Korean subjects, C allele carriers showed a deferred peak time of TG and higher postprandial chylomicron levels and free fatty acids (FFA) and insulin levels after a high-fat meal compared to a low-fat meal [18]. This decreased clearance of TG in carriers of the C allele may produce a decrease in APOA5 function. In the postprandial situation, the delivery of FFA into the circulation is modulated by the intracellular hormone-sensitive lipase (HSL) and the way that lipoprotein lipase-derived fatty acids are not captured by adipose tissue and muscle [32]. Therefore, this metabolic change detected in the postprandial state after a high-fat meal could be secondary to a decrease of the postprandial suppression of HSL by insulin in C allele carriers, due to an insulin resistance state. This fact could be related to the lack of response to insulin and HOMA-IR in patients carrying the C allele in our study after the dietary intervention. In agreement of this hypothesis, recent investigations in Caucasian healthy subjects [33] reported that this SNV modulated postprandial lipidemia after a breakfast containing 75 g of fat, though the addition of glucose (25 g) to the tested breakfast suppressed this change. Perhaps, the secretion of insulin after a glucose overload could explain these findings, because insulin action was shown to decrease APOA5 expression and even to downregulate ApoA-5 levels in plasma [34].

In our present study in a Caucasian population, we used a diet with a Mediterranean profile based on its beneficial findings reported in other observational studies. Sanchez- Moreno et al. [35] detected a genotype–dietary fat interaction involving this genotype for obesity phenotypes in a Mediterranean population in a cross-sectional study. Hubacek et al. [36] reported that TG levels were higher in C allele carriers with the higher energy intake than in non-C allele carriers. Lai et al. [37] demonstrated that the n-6 (but not n-3) polyunsaturated fatty acids intake increased the fasting triglyceride concentrations in C allele carriers; therefore, the quality of the fat in the Mediterranean diet can also have an influence. Beyond the caloric restriction of the diet, some foods of this diet, such as lean meats, fish and olive oil with large amounts of polyphenols, monounsaturated fats, polyunsaturated, minerals and vitamins, could also explain the benefits observed in the metabolic parameters in non-C allele carriers [14]. Moreover, other nutrients could play an important role, for example, carriers of the minor allele with a low calcium intake (<500 mg/day) showed elevated triglyceride concentrations, in a cross-sectional study [38].

There are some limitations in our investigation. Firstly, the dietary intake was based on self-reports from the patients; these self-reported data could overestimate or underestimate the actual dietary intake. Second, considering the small allelic frequency of the C allele, the data of the genetic analyses should be evaluated with caution. Thirdly, the study was designed for Caucasian obese subjects aged 20–60 years without dyslipidemia and with a high BMI, so the data are not generalizable to the healthy population. Fourthly, our sample consisted of Caucasian obese patients, and our results could be generalized only to this subpopulation. Fifthly, the absence of a healthy control group decreases the power of the results obtained with our design. Sixthly, we used the same caloric restriction in males and females, and perhaps this is a potential bias, too. Finally, the circulating levels of ApoA5 were not been determined, and the determination of this apolipoprotein would have helped us to interpret our results also from a physiological perspective.

5. Conclusions

In conclusion, this long-term study demonstrated that the minor C allele of the APOA5 gene (rs662799) is negatively related to the effect on triglycerides, insulin levels and HOMA-IR after consumption of a low-calorie diet with a Mediterranean pattern. Further studies are necessary to evaluate the importance of this SNV in the lipid profile and metabolic syndrome in different populations [39]. In the future, clinical guidelines will incorporate this knowledge to improve the design of nutritional interventions for obese patients, using panels of SNVs and other omic technologies [40].

Author Contributions: D.d.L.R. and R.A. designed the study and realized the statistical analysis; O.I. performed the anthropometric evaluation and the control of the dietary intake; D.P. realized the biochemical and genotype evaluation. All authors have read and agreed to the published version of the manuscript.

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Article

Differential Glycemic Effects of Low- versus High-Glycemic Index Mediterranean-Style Eating Patterns in Adults at Risk for Type 2 Diabetes: The MEDGI-Carb Randomized Controlled Trial

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Abstract: A Mediterranean-style healthy eating pattern (MED-HEP) supports metabolic health, but the utility of including low-glycemic index (GI) foods to minimize postprandial glucose excursions remain unclear. Therefore, we investigated the relative contribution of GI towards improvements in postprandial glycemia and glycemic variability after adopting a MED-HEP. We conducted a randomized, controlled dietary intervention, comparing high- versus low-GI diets in a multi-national (Italy, Sweden, and the United States) sample of adults at risk for type 2 diabetes. For 12 weeks, participants consumed either a low-GI or high-GI MED-HEP. We assessed postprandial plasma glucose and insulin responses to high- or low-GI meals, and daily glycemic variability via continuous glucose monitoring at baseline and post-intervention. One hundred sixty adults (86 females, 74 males; aged 55 ± 11 y, BMI 31 ± 3 kg/m², mean $\pm$ SD) with $\geq$ two metabolic syndrome traits completed the intervention. Postprandial insulin concentrations were greater after the high-GI versus the low-GI test meals at baseline ($p = 0.004$), but not post-intervention ($p = 0.17$). Postprandial glucose after the high-GI test meal increased post-intervention, being significantly higher than that after the low-GI test meal (35%, $p < 0.001$). Average daily glucose concentrations decreased in both groups post-intervention. Indices of 24-h glycemic variability were reduced in the low-GI group as compared to baseline and the high-GI intervention group. These findings suggest that low-GI foods may be an important feature within a MED-HEP.

Keywords: Mediterranean diet; metabolic syndrome; metabolic health; impaired glycemic control; metabolic risk factors; insulinemia; glycemic variability; continuous glucose monitoring; oral glucose tolerance test; meal glucose tolerance test

1. Introduction

Type 2 diabetes is a dire metabolic condition that has a profound impact on the estimated ~400 million individuals afflicted worldwide [1]. Without a rapid and robust response, type 2 diabetes is projected to continue along this course of precipitous increases in cases during the upcoming decades [1,2]. The deleterious effects of type 2 diabetes are further magnified when its contributions towards cardiovascular disease, the leading cause of mortality in Western nations, are considered [3,4]. Given the societal burden, research

investigating the potential for lifestyle and dietary interventions for those who are at risk of developing type 2 diabetes, must be fully leveraged if there is to be hope in slowing the steep rise in cases [5].

Postprandial glycemia may contribute as much or more than fasting blood glucose to the pathogenesis of impaired insulin sensitivity and insulin secretion seen in the progression towards type 2 diabetes [6,7]. Indeed, the loss of postprandial glucose control often manifests before derangements in fasting indices, along the path towards type 2 diabetes in at-risk individuals [8]. Therefore, strategies to attenuate postprandial glucose excursions may be of particular importance in the effort to reduce the global burden of disease. Proposed 40 years ago, the glycemic index (GI) of carbohydrate-containing foods is posited to play a substantial role in postprandial glucose excursions [9]. However, there is still not a consensus on the relevance and utility of GI in non-diabetic people [10], particularly in the context of a healthy eating pattern (HEP) where other health-promoting dietary factors may take precedence [11]. It is unclear whether differences in postprandial glucose and insulin responses that are induced acutely by various carbohydrate foods may disappear after a few weeks due to compensatory mechanisms operative in non-diabetic people [12].

Consuming a Mediterranean-style (MED) HEP is supported by considerable evidence suggestive of a reduced risk of type 2 diabetes development [13,14]. The preponderance of the evidence is observational in nature, with relatively little experimental evidence, particularly outside of the geographical Mediterranean region, supporting this HEP. Fat quality, dietary fiber, and different bioactive compounds have been emphasized as the core elements behind the observed health effects of a MED-HEP [15]. However, to the best of our knowledge, there have been no studies assessing the relative contributions of high-versus low-GI foods to improvements in indices of glucose control and cardiometabolic health in the context of a MED-HEP.

Therefore, we conducted a 12-week, multi-center, randomized, controlled intervention assessing the potential differential effects of iso-caloric, weight-maintaining, high- versus low-GI MED-HEPs on indices of glucose control among participants at risk for developing type 2 diabetes. We hypothesized that the low-GI group would present with lower postprandial glucose and insulin responses relative to the high-GI group at baseline and that these differential responses would be maintained at the end of the dietary intervention. Further, we hypothesized that the low-GI group would present with improvement in indices of glycemic variability from baseline to post-intervention, relative to the high-GI group.

2. Materials and Methods

The MEDGI-Carb trial is an international multi-center randomized, controlled, parallel-group, 15-week trial including a 3-week baseline period followed by 12 weeks of controlled dietary intervention. This research study was initiated in January 2018 and the trial continued through December 2019. This study was conducted at three centers: (1) Federico II University, Naples, Italy (2) Chalmers University of Technology, Gothenburg, Sweden, and (3) Purdue University, West Lafayette, IN, USA. The study protocol was approved by the institutional review boards at Purdue University and Federico II University and by the Swedish Ethical Review Authority. This study was registered in the public trial registry Clinicaltrials.gov (accessed on 7 December 2021) as NCT03410719. Detailed descriptions of study protocols for all centers are published [16].

2.1. Experimental Design

During the 12-week intervention period, subjects consumed a controlled, iso-energetic, weight-maintenance diet and were instructed to consume intervention-specific foods to achieve a low-glycemic or high-glycemic MED-HEP (low-GI or high-GI, respectively). Outcome measurements were obtained on standardized testing days to determine markers of glucose homeostasis by completion of an eight-hour meal glucose tolerance test (MGTT), including both breakfast and lunch resembling food choices of the assigned diet, an oral glucose tolerance test (OGTT), and 24-h continuous glucose monitoring on separate days

at baseline and post-testing. Primary outcomes are postprandial plasma glucose and insulin responses during the MGTT; secondary outcomes include fasting and OGTT plasma glucose and insulin; blood HbA_{1c}; and indices of 24-h glycemic variability.

2.2. Eligibility Criteria

The eligibility criteria were designed to select middle-aged and older adults at risk for developing type 2 diabetes. Therefore, adults with a waist circumference >102 cm (males) or >88 cm (females) and one additional trait of the metabolic syndrome, according to the National Cholesterol Education Program's Adult Treatment Panel III [17], were recruited. The additional traits could include blood pressure >130/85 mmHg or taking medication to control high blood pressure; fasting plasma glucose 5.6–7.0 mmol/L; fasting triglycerides 1.7–4.5 mmol/L; HDL < 1.0 mmol/L (males) or <1.3 mmol/L (females). A member of the research team at each of the three testing sites, who was not involved in data collection or analysis, generated the random allocation sequence and assigned subjects to the interventions. Each subject was randomly assigned to one of two dietary groups using either a stratified block pattern (Italy & US; $n = 8$, 10 blocks; 4 randomized to each group per block of 8, using an online randomization plan generator (<http://randomization.com/> (accessed on 31 January 2022) or a mixed size of the block pattern (Sweden; 4, 6, and 8 subjects per block in random block order, using Rstudio software version 2.4.0 (RStudio, Boston MA, USA) with package 'blockrand' version 1.3)). The randomization code remained unrevealed until all participant testing and analyses of samples for a priori primary outcomes were completed. Full inclusion criteria details, recruitment, and consent procedures can be found elsewhere [16].

2.3. Dietary Control

During the 3-week baseline period, all subjects consumed their habitual, self-chosen, unrestricted diets. Throughout the 12-week intervention period, each subject was counseled to follow their assigned iso-caloric MED-HEP using a combination of prescribed menus (breakfast, lunch, and snacks) and an item-specific version of the 'Dinner Recipe Builder' for dinner. The Dinner Recipe Builder is a mechanism to self-efficacy by which participants were given the flexibility to mix and match ingredients, while still following a MED-HEP. The two group-specific diet plans contained primarily the same foods and beverages in their MED-HEPs, except for substitutions of major sources of starch in the meals. All participants were advised to consume the same quantity of metabolizable carbohydrate (270 g/d) and fiber (35 g/d). Higher or lower energy content was achieved through the modulation of dietary fat and protein. One-half of daily carbohydrate (135 g) was the same for both the low- and high-GI groups, including the carbohydrates in fruits, vegetables, and other foods that all subjects consumed. The other one-half of the daily carbohydrate intake (135 g) was specific to the low-GI and high-GI groups. Specifically, 135 g of carbohydrate in the low-GI group came from foods with GI values < 55, while 135 g of carbohydrate in the high-GI group came from foods with GI values > 70. These GI cut-points correspond with those indicative of low-GI foods (<55) and high-GI foods (>70) [18]. The intervention-specific carbohydrates were distributed as 35 g at breakfast, 40 g at lunch, and 60 g at dinner. Complete descriptions of dietary controls can be found elsewhere [16]. Briefly, all participants were provided with group-specific instructions on the quantities of specific foods to consume. They were also given selected food items to use for their meals (high-GI jasmine rice, potato, mashed potatoes, couscous, wholegrain bread, and rusks; low-GI pasta, brown rice, flatbread, all bran, and wheat, plus rye bread and seeds). Dietary counseling was given bi-weekly and included group dinner meal preparation sessions.

2.4. Postprandial Assessments

Visits for clinical assessments at baseline and post-intervention included an 8-h MGTT and a 2-h OGTT (Supplemental Figure S1). Prior to all testing days, participants were instructed not to eat or drink anything (except a small amount of water) from 22:00 h the

evening before the visit. Participants were counseled to refrain from vigorous physical activity for 48 h prior to testing days, avoid alcohol 24 h prior to testing days, and avoid caffeinated beverages the morning of testing days. After arriving at the testing facilities, participants would be seated in a chair/bed to rest. A catheter was placed in an antecubital vein and remained in place for the remainder of the testing day. Blood pressures were taken in duplicate after 15 min of rest.

For the OGTT and MGTT, double baseline fasting blood samples were collected at the minus 15-min time-point and the minus 5-min time-point following 15 min of rest (pooled ((mean value)) and denoted as time-point -10). The 75 g glucose drink (OGTT) or test meal (MGTT) were consumed at time-point 0. Full test meal contents are provided in Supplemental Table S1. Each participant consumed a test meal consistent with the composition of the assigned diet, at baseline, and at the end of the intervention. During the OGTT, blood samples were collected at 60 min and 120 min after consumption of the test glucose beverage. Subjects were not permitted additional fluid consumption during the test. During the MGTT, blood samples were collected immediately following the test breakfast meal (time-point $+15$) and then at intervals progressing from 15 min to one hour from time-points $+30$, 45, 60, 90, 120, 180, and 240. The second test meal was provided following the time-point $+240$ blood draw. The blood draw pattern of timing was repeated after the second meal was consumed.

2.5. Continuous Glucose Monitoring

Medtronic iPro2 Professional continuous glucose monitoring devices (Northridge, CA, USA) were used to obtain 24-h interstitial glucose concentrations in 5-min intervals during the baseline and post-testing weeks. Data were entered into the EasyGV platform (University of Oxford, Oxford, England) for calculation of the relevant indices of glycemic variability in non-diabetic individuals (mean amplitude of glucose excursions; continuous overall net glycemic action; mean absolute glucose; and lability index).

2.6. Blood Collection and Analysis

During the 12-week intervention period, subjects consumed a controlled, iso-energetic, weight-maintenance diet and were instructed to consume intervention-specific foods to achieve a low-glycemic or high-glycemic MED-HEP (low-GI or high-GI, respectively). Complete descriptions of blood sample collection analyses can be found elsewhere [16]. Briefly, blood samples were obtained from an antecubital vein and placed in tubes containing a clot activator to obtain serum or sodium/lithium heparin to obtain plasma. Serum tubes were held at room temperature for at least 15 min and then centrifuged at $4000 \times g$ at 4°C for 15 min ($3000 \times g$ at 4°C for 10 min in Sweden). EDTA plasma, serum, and heparinized plasma samples were immediately refrigerated/kept on ice, processed, and aliquoted into microtubes. Plasma and serum aliquots were frozen at -20°C within 2 h of sample collection, stored at this temperature for a maximum of one week, and then stored at -80°C until thawed for analysis. EDTA plasma samples were used to assess insulin and glucose concentrations. All samples were analyzed at the end of the study to minimize batch effects.

2.7. Statistical Analyses

The primary analyses followed the intention-to-treat plan. A repeated measures linear mixed model was used to model the main effects of group, time, and group $\times$ time interactions as well as the effect of the study center. Least square means for outcomes of interest were calculated via the *LSMESTIMATE* statement using the *PROC MIXED* procedure in SAS statistical software version 9.4 (SAS Institute, Cary, NC, USA). Power calculations indicated that 180 subjects total (90 low-GI, 90 high-GI; 60 from each testing center) would provide greater than 80% power to detect a 30% differential response between the dietary interventions for the primary endpoint (postprandial insulin) with similar variation reported in the study by Giacco et al. [19]. Postprandial glucose and insulin

averages were assessed by setting the minimum-reported value of the participant as the baseline, whereby all subsequent values were firstly subtracted from the minimum values, and then were averaged. The same standard was used in the calculation of postprandial glucose and insulin areas under the curve (AUC, using the trapezoidal rule). This method minimizes the conceptual errors associated with large glucose excursions washing out and not being represented in postprandial assessments, i.e., high glycemic variability where large peaks (hyperglycemia) are followed by large depressions below fasting concentrations (hypoglycemia). Calculation with the AUC_{min} method consistently resulted in the lowest intra- and inter-individual coefficients of variation in previous assessments of postprandial glycemia [20].

When confronted with missing data from postprandial assessments, multiple imputation using chained equation procedures were followed in accordance with Rubin's rules [21] to combine the statistical results from each individual imputed complete data. Co-variables accounted for in all statistical models included BMI, waist circumference, age, sex, smoking status (smoker/non-smoker), and testing center (Italy, Sweden, or USA). When no significant group $\times$ time interaction was observed, data from both groups were pooled to assess the overall effect of the dietary intervention. Significance was set at $p < 0.05$.

3. Results

The CONSORT participant flow diagram is presented in Figure 1. During the clinical testing phase (February 2018 to December 2019), 584 participants were screened for eligibility. Of the initially enrolled 213 participants, 27 (low-GI; $n = 12$, high-GI; $n = 15$) dropped out prior to commencing the dietary intervention, and 26 (low-GI; $n = 8$, high-GI; $n = 18$) dropped out during the dietary intervention, resulting in 160 (low-GI; $n = 86$, high-GI; $n = 74$) participants completing the dietary intervention.

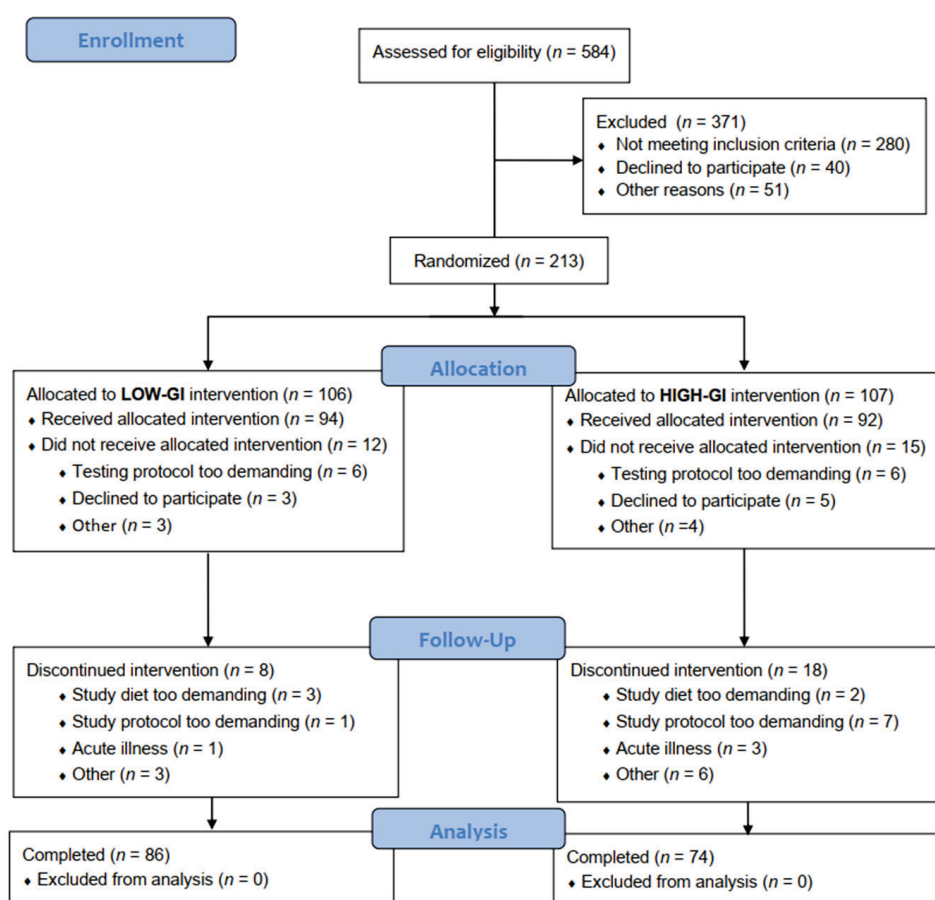


Figure 1. CONSORT participant flow diagram.

3.1. Baseline Characteristics

There were 119 female participants and 94 male participants (Table 1). In our sample of adults at risk for type 2 diabetes, all participants presented with elevated waist circumference (by design). Forty-four percent of participants had two traits of the metabolic syndrome, 37% had three traits (and thus are classified as having the metabolic syndrome), 16% presented with four traits, and 3% had all five traits. Elevated blood pressure was the most common secondary trait (60%), followed by elevated fasting glucose (46%), low HDL (38%), and elevated triglycerides (33%).

Table 1. Fasting clinical characteristics of participants at screening.

Demographic Characteristics	Low-GI (<i>n</i> = 102)	High-GI (<i>n</i> = 111)
Age at randomization (years)	55 ± 10	55 ± 11
Female <i>n</i> (%)	55 (53.9%)	64 (57.7%)
Weight (kg)	92 ± 14	88 ± 14
BMI (kg/m ²)	31.1 ± 3.1	30.3 ± 3.0
Waist Circumference (cm)	106 ± 8	105 ± 9
Metabolic characteristics		
Glucose (mmol/L)	5.4 ± 0.6	5.4 ± 0.7
Total cholesterol (mmol/L)	4.9 ± 1.0	5.0 ± 0.9
Triglycerides (mmol/L)	1.3 ± 0.6	1.3 ± 0.6
HDL (mmol/L)	1.3 ± 0.4	1.3 ± 0.4
LDL (mmol/L)	3.2 ± 0.8	3.3 ± 0.7
Systolic blood pressure (mm Hg)	126 ± 14	126 ± 14
Diastolic blood pressure (mm Hg)	82 ± 9	83 ± 8

Data are means ± SD. There were no statistically significant differences at baseline.

3.2. Dietary Composition

At baseline, the two groups did not present with differences in any dietary features (Supplemental Table S2). Post-intervention, both groups reduced their intakes of total saturated and polyunsaturated fat, while their intakes of fiber, monounsaturated fat, and carbohydrates increased. Post-intervention there was no difference in energy or nutrient composition between the low- and high-GI groups. Targeted differences in the glycemic index were achieved with average glycemic index values of 46.8 ± 3.1 vs. 66.2 ± 4.7 reported in the low- and high-GI groups, respectively.

3.3. Postprandial MGTG Glucose and Insulin Responses

Postprandial insulin and glucose responses to the 8-h MGTG are presented in Figure 2. Average postprandial insulin was greater after the high-GI test meals compared to the Low-GI test meals at baseline ($p = 0.004$), but this difference was no longer present post-intervention ($p = 0.17$). From baseline to post-intervention, postprandial insulin decreased in the high-GI group, but not in the low-GI group (group $\times$ time; $\Delta -30.6 \pm 15.3$ pmol/L; $p = 0.046$). Postprandial glucose was greater (~17%) after the high-GI vs. the Low-GI test meals at baseline ($p = 0.02$), and more so post-intervention (35%; $p < 0.001$). This greater difference between low- and high-GI groups post-intervention was attributable to increases in average postprandial glucose over the 12 weeks in the high-GI group ($\Delta 0.2 \pm 0.1$ mmol/L; $p = 0.03$). Similar to postprandial insulin responses, glucose responses in the low-GI group were unchanged after the intervention. Overall, there were more robust differential effects of GI between the groups at the lunch meals, while glucose and insulin responses were less different after the breakfast meals (Supplemental Figure S2). Postpran-

dial glucose and insulin AUC results were comparable to those seen for postprandial averages (Supplemental Figure S3).

Our study was not designed to determine differences in glycemic responses based on sex or race/ethnicity per se. Most participants were Caucasian non-Hispanic ($n = 158$, 99%), therefore, no meaningful post hoc analyses can be conducted. Sex effects were detected during modeling postprandial insulin, but not postprandial glucose responses.

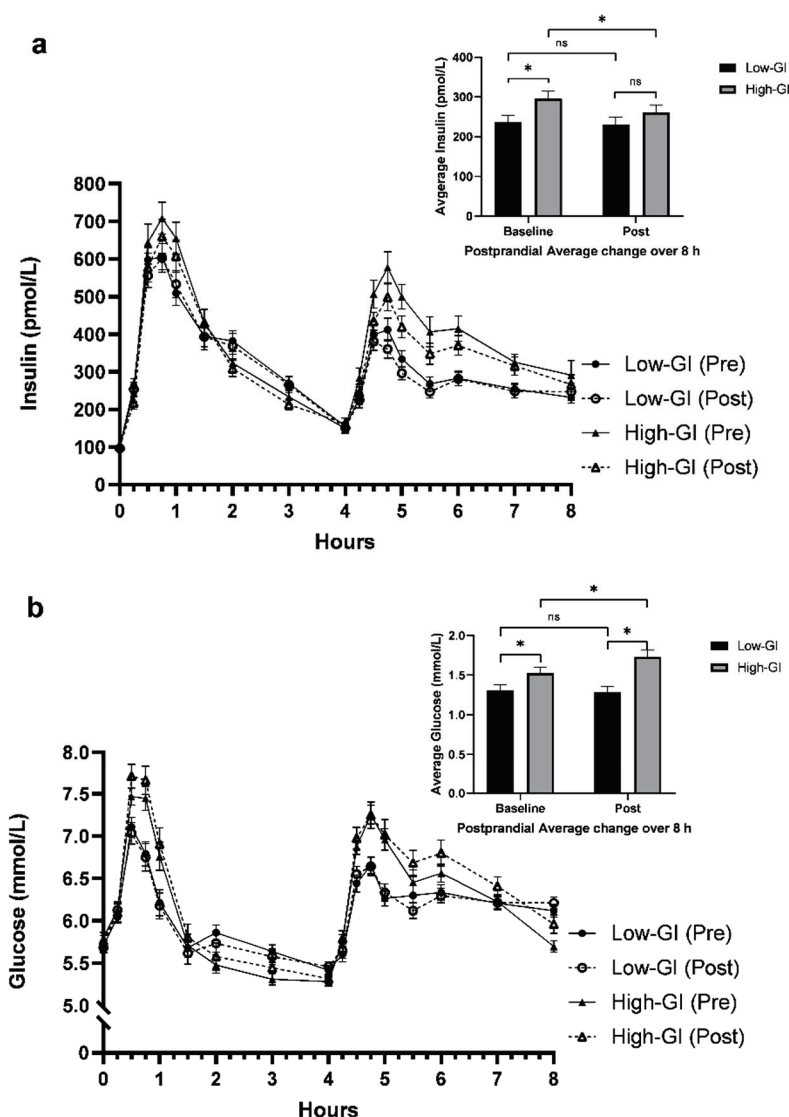


Figure 2. Insulin (a) and glucose (b) responses to a low-GI and high-GI 8-h meal glucose tolerance test at baseline and after a 12-week dietary intervention. Inset bar graphs display average postprandial insulin and glucose elevations above fasting concentrations over the 8-h period. Data are means $\pm$ SEM. * Statistically significant, $p < 0.05$. ns, no significance.

3.4. 24-h Glycemic Variability

At baseline, all 24-h continuous glucose monitoring variables were not different between the groups (Figure 3). Average 24-h glucose concentrations decreased in both groups, with indices of glycemic variability improving in the low-GI group, but not in the high-GI group. The low-GI group presented with reductions in 24-h standard deviation, mean amplitude of glucose excursions, and mean absolute glucose. Lability index ($p = 0.04$), mean amplitude of glucose excursions ($p < 0.01$), and mean absolute glucose ($p = 0.02$) were lower in the low-GI group compared to the high-GI group post-intervention.

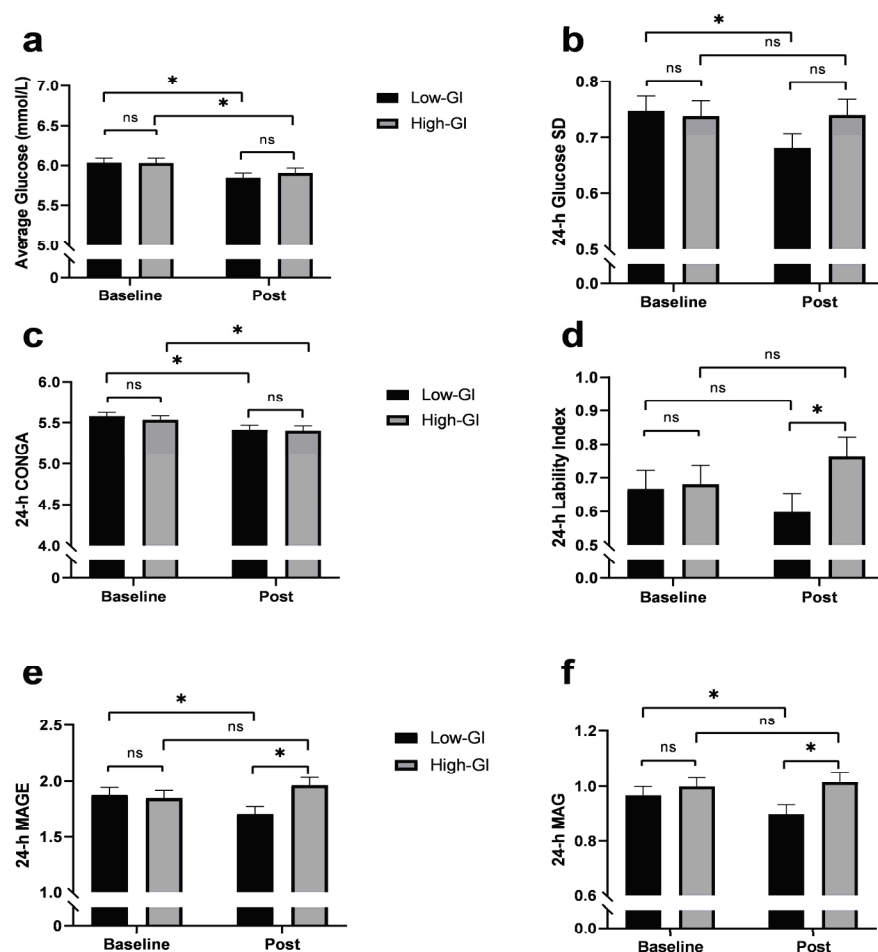


Figure 3. 24-h continuous glucose monitor-derived measures of glycemic variability at baseline and after a 12-week dietary intervention. (a) Average 24-h glucose concentration, (b) standard deviation (SD), (c) continuous overall net glycemic action (CONGA), (d) lability index, (e) mean amplitude of glucose excursions (MAGE), and (f) mean absolute glucose (MAG). Presented data are means $\pm$ SEM. * Statistically significant, $p < 0.05$. ns, no significance.

3.5. Fasting and OGTT Glucose and Insulin Responses

There were no differences in 1-h or 2-h glucose (Supplemental Figure S4a) and insulin (Supplemental Figure S4b) between the low- and high-GI groups at either baseline or post-intervention. Irrespective of GI, glucose responses, but not insulin, decreased over the course of the intervention (pooled analysis; $p = 0.02$). The GI of the MED-HEP did not influence changes over time in the fasting risk factors for type 2 diabetes, including fasting glucose, insulin, HOMA-IR, and HbA_{1c} (Supplemental Table S3).

4. Discussion

This study demonstrated that consuming a Mediterranean-style eating pattern with low-GI foods reduces daily glycemia and indices of glycemic variability in adults at risk for type 2 diabetes. As hypothesized, postprandial glycemia was lower after the low-GI test meals relative to the high-GI test meals at baseline, and the magnitude of this difference increased during the dietary intervention. This widened difference was primarily attributable to increases in the high-GI group's postprandial glucose, while the low-GI group's glucose responses were not different between baseline and post-intervention. This suggests that a diet based on high-GI foods, including 50% of carbohydrates from foods with GI values > 70 , significantly increases the postprandial glucose response, not only in an acute setting but also in the long term despite adherence to an overall healthy eating pattern.

Contrary to our hypothesis, while postprandial insulin was lower in the low-GI group compared to the high-GI group at baseline, this difference was no longer present post-intervention; in fact, postprandial insulin decreased at post-intervention more in the high-GI group relative to the low-GI group. However, this decline in the postprandial insulin occurred only at breakfast, while plasma insulin concentrations after lunch remained significantly higher during the intervention in the high-GI group. Indeed, the difference in postprandial insulin at lunch was more than 50% higher in the high-GI group. Postprandial plasma insulin concentrations are generally considered a marker of increased cardiovascular risk and therefore the increased postprandial insulin concentrations after lunch might be considered as a potential untoward effect of the high GI diet [22].

In contrast to the clear benefits of a low-GI diet over a high-GI diet regarding its effects on postprandial glucose metabolism, we detected no improvement in fasting glucose, OGTT, or HbA_{1c}. This was unsurprising, as fasting plasma glucose concentrations are typically unaffected by weight-maintaining dietary interventions, particularly when they are not clearly elevated [23]. In addition, HbA_{1c} is an imprecise marker of glucose control for values below 5.5% (37 mmol/mol), which is the range in which most of our participants were included [24]. Regarding OGTT responses, improvements in this marker are only reported in long-term weight-loss interventions in individuals with diagnosed impaired glucose tolerance [25].

Our study results are concordant with the outcomes of one previous study [26] where individuals with prediabetes displayed improved postprandial glucose control in response to a low-GI diet, but they are not completely concordant with the OmniCarb trial, a similar intervention involving manipulation of GI within a dietary intervention in which plasma insulin concentrations were not influenced by GI [11]. While low-GI diets have clinical utility in individuals with type 2 diabetes [27], our findings support the hypothesis that replacing high-GI foods for low-GI foods might also be useful for improving indices of glycemic control in people with traits of metabolic syndrome, thereby potentially reducing the risk of progression to type 2 diabetes and/or cardiovascular disease [28].

The results from the 24-h continuous glucose monitoring add important context to our postprandial findings. We report that while the mean 24-h glucose concentrations decreased comparatively in both groups following MED-HEPs, changes in the indices of glycemic variability favored the low-GI group. While there is currently a limited consensus on the relative importance of different indices of glycemic variability [29], the consistent improvement observed in the low-GI group across classical and contemporary indices strongly suggests a more stable blood glucose profile after the low-GI diet. The classical indices include the mean amplitude of glucose excursions, mean absolute glucose, and standard deviation, while the contemporary indices include lability index and continuous overall net glycemic action. Mechanistic support for the relevance of glycemic variability can be found in research demonstrating that acute glucose excursions induce oxidative stress to a greater degree than sustained hyperglycemia [6]. However, the clinical relevance of reducing blood glucose excursions remains unresolved, with some [30], but not all [31] evidence supporting glycemic variability as an independent risk factor for overall mortality and cardiovascular diseases. Assessment of glucose variability in individuals at risk for type 2 diabetes, such as our participants, may be particularly important as perturbations in glucose homeostasis may be an early manifestation of glucose dysregulation [32].

We chose to assess the effects of GI in the context of a MED-HEP because the MED-HEP has been linked with a reduced progression to type 2 diabetes in at-risk populations [33]. Indeed, the MED-HEP appears to be particularly effective in improving glycemic control relative to other dietary patterns such as DASH which may be comparatively more effective in reducing blood pressure [34]. Components of the MED-HEP, such as abundant monounsaturated fatty acids in olive oil, may also particularly improve postprandial glycemia via an improved postprandial insulin sensitivity [35].

Strengths of the MEDGI-Carb study include strong clinical design features such as randomization, blinding of the analytical procedures, and an appropriate sample size with

respect to effect size and power based on previous research. Further, the robustness of the primary manipulated variable (GI of the carbohydrate foods) was ensured through independent analyses to ensure that the GI of the carbohydrate foods were sufficiently different between the low- and high-GI groups, which had been intended but was difficult to achieve in previous studies. A potential limitation stems from our study sample consisting almost entirely of participants of Caucasian ethnicity. Certain populations, such as Asian individuals, display unique metabolic characteristics that may predispose them to benefit more from the interventions posited to influence the postprandial carbohydrate metabolism [36]. Therefore, care should be taken that these findings are not overgeneralized to people from other racial groups. In addition, our study was tailored to assess the metabolic effects of the dietary intervention but it was not powered for a duration and sample size that would allow for the evaluation of the impact of the diets on clinical events. Lastly, we cannot discount that the health-promoting effects of GI reduction may be greater in the context of a higher-carbohydrate pattern, such as a typical 'dietary approaches to stop hypertension' HEP [37], compared with a lower-carbohydrate diet, such as a MED-HEP [38]. Therefore, future studies should investigate both the potential modulation of postprandial glycemia by the monounsaturated fats commonly found in MED-HEPs and assess the potential GI-induced differences from MED-HEP varieties featuring a higher percentage of daily energy from carbohydrates.

5. Conclusions

In conclusion, the acute superiority in the indices of postprandial glucose control of participants who emphasized low-GI foods relative to those who emphasized high-GI foods is sustained and was further amplified over time in the context of a background MED-HEP. However, this amplified difference over time is primarily due to a worsening of postprandial glucose control among participants whose diets emphasized high-GI foods. Unexpectedly, the higher insulin responses to consuming high-GI versus low-GI meals at baseline were not apparent after adopting the MED-HEP. Consuming a MED-HEP was sufficient to reduce daily glycemia, but only the low-GI diet resulted in improvements in the indices of daily glycemic variability. Collectively, these findings demonstrate the relevancy of GI within a mixed diet with healthy features resembling those of a traditional Mediterranean-style diet among non-diabetic individuals. Since low-GI foods are an inherent element of a traditional Mediterranean diet [39], our findings suggest that low-GI foods may contribute to the health benefits seen from the MED-HEP.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/nu14030706/s1>, Figure S1: Meal- and oral glucose tolerance testing day schematic, Figure S2: Insulin and glucose elevations above fasting concentrations in response to low-GI and high-GI Breakfast and Lunch Meals During 8-h Meal Glucose Tolerance Tests at Baseline and After a 12-week Dietary Intervention, Figure S3: Insulin and Glucose Postprandial Areas Under the Curve in Response to low-GI and high-GI Meals During 8-h Meal Glucose Tolerance Tests at Baseline and After a 12-week Dietary Intervention, Figure S4: 1-h and 2-h glucose and insulin responses at a 2-h oral glucose tolerance test (75 g glucose bolus), Table S1: Food Composition of Standardized Meal Glucose Tolerance Test Meals, Table S2: Dietary Composition Before And After A low- And high-GI Mediterranean Dietary Intervention, Table S3: Effect of low- versus high-GI Healthy Eating Patterns on Risk Factors for Type 2 Diabetes.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: G.R. is a member of the Scientific Advisory Board of the Nutrition Foundation of Italy, the Barilla Foundation for Food and Nutrition, and the Istituto Nutrizionale Carapelli Foundation; he is a member of the Health and Wellbeing Advisory Board of the Barilla G. e R. Fratelli Company. R.B. is currently employed by ADM. Research presented in this paper was conducted in a former role and has no connection with ADM. I.B. is currently employed by AstraZeneca. Research presented in this paper was conducted in a former role and has no connection with AstraZeneca.

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Review

Can Mediterranean Diet Have a Positive Impact on Kidney Health? A Pending Answer to a Long-Time Question

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Abstract: Dietary studies conducted in chronic kidney disease (CKD) patients were focused on the quantities of single nutrients, however it is possible that the excessive attention put on the restriction of proteins, sodium, potassium and phosphorus may compromise the overall quality of the diet in terms of micronutrients and palatability. Instead, concentrating on the nutritional quality healthy dietary patterns, may provide a better approach to improve nutritional prescriptions in CKD patients. All these dietary regimens share common features as reduced content of red meat, salt and saturated fatty acids, and higher fiber content, but may differ in terms of single nutrients consumption. In particular, Mediterranean Diet (Med Diet) has been associated with reduced incidence of diabetes, cardiovascular diseases and obesity, all conditions that are also strictly related to CKD. Given its low content of animal proteins and high contents of fiber it is possible that Med Diet may exert also positive effects on CKD as well as on its metabolic complications. In this review we summarize the role of Med Diet in primary prevention of CKD and on its progression.

Keywords: med diet; chronic kidney disease; nutrition; prevention

1. Introduction

Chronic kidney disease (CKD) is a worldwide health problem that affects about 10% of the general adult population [1–3]. This disease has global implications, ranking as the 17th leading cause of life-expectancy loss in 2015 [4]. In Italy the prevalence of this disease assesses around 8%, with little inter-sex variation. CKD is characterized by a decline in kidney function that is exacerbated by type 2 diabetes (T2DM), obesity, hypertension but also by inflammation and oxidative stress [5–7]. There is a strong association between CKD and cardiovascular disease (CVD) considering that CKD seems to be an independent risk factor for cardiovascular events and for all-cause of cardiovascular (CV) death, as it is associated with increased levels of inflammatory factors, abnormal apolipoprotein levels, elevated plasma homocysteine, enhanced coagulability, anemia, left ventricular hypertrophy, increased arterial calcification, endothelial dysfunction, and arterial stiffness [1,2,8]. On the other hand, also patients affected by primary heart diseases are more prone to develop CKD [PMID: 20037146]. Moreover, in diabetic patients renal damage is part of macro and microvascular complications that typically characterize disease progression, and contributes to increase the overall mortality rate [1,9]. For these reasons, there is a growing need to define preventive strategies that may reduce CKD incidence and slow down its progression. Lifestyle and dietary habits can influence kidney function, playing a crucial role in the prevention and development of CKD. A healthy diet decreasing CKD incidence and progression would in turn significantly decrease the burden on healthcare, with the adjunctive benefit of avoiding or reducing pharmacological intervention. Generally, dietary

studies in CKD concentrated on single nutrients and foods [10–12]. The study of healthy dietary patterns instead, may provide a more powerful tool to evaluate the synergistic and cumulative effects of certain nutrients on CKD [13,14].

The Med Diet (Med Diet) was first examined in medical literature in 1975 as a model of healthy eating. There is not actually a single model of Med Diet, but the main composition is modeled after the historic diet of Crete's island [15]. This type of diet focused its nutrients intake especially on plant-based foods, including: fruits, vegetables, legumes and non-saturated fats derived from olive oil, nuts, seeds and fish. At the same time poultry, red meat and eggs were limited, but not fully excluded. Fruits were consumed especially at the end of the meal. Wine was consumed with moderation along with dairy products. Whole grain-based foods were preferred over more processed one [12,13]. Therefore, this whole food diet can lead to many physiological changes that may contribute to preserve renal health. Specifically, it can lead to improvements in blood pressure control, plasma lipid profile, systemic inflammation, body weight maintenance [10,14–16]. A detailed and up to date composition of Med Diet is presented in Figure 1, along with guidelines for specific nutrients intake.

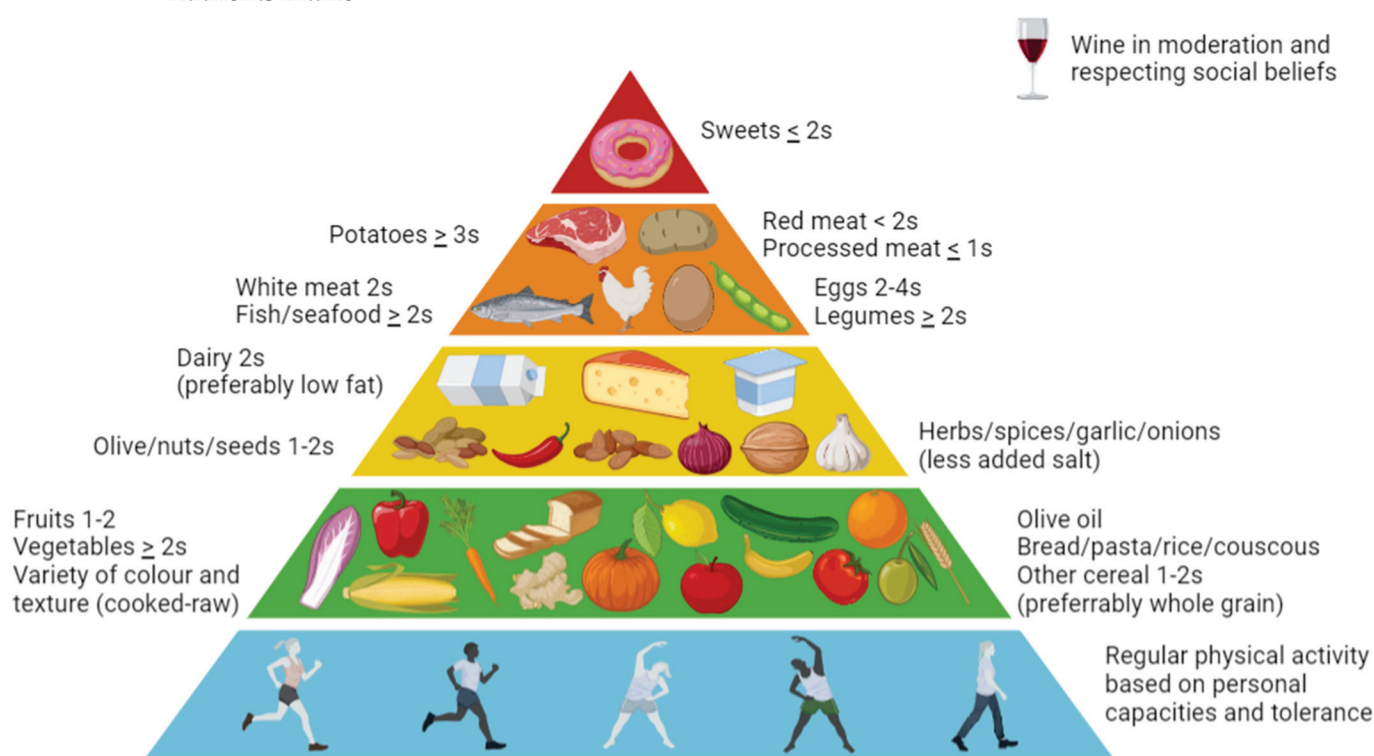


Figure 1. Med Diet pyramid: updated indications and guidelines for adult population. **Note:** s: servings; Adapted from “Med Diet as the diet of choice for patients with chronic kidney disease” [17].

The aim of the present review is to evaluate and discuss recent evidence concerning the potential benefits of Med Diet on kidney health, focusing on primary and secondary prevention of CKD.

2. Med Diet in Primary Prevention of CKD

Different clinical studies, both observational and interventional, have been conducted to assess the efficacy of Med Diet in preserving kidney function [18].

Most of cross-sectional and prospective studies investigated the associations between this dietary pattern and renal outcomes using scores for adherence to Med Diet [17,19–21]. Dietary adherence has been assessed through a diet score of up to nine most-characteristic foods groups from the pyramid that are health-promoting and/or health-harming. A semi-quantification/frequency intake of these items results in a total score (from 0 to 9)

used to quantify the adherence to Med Diet pattern [22]. Therefore, we should consider that in this way, the Med Diet score is population specific. In this context, two cross-sectional studies in healthy Greek populations showed that a better observance to the Med Diet was associated with a lower albuminuria and higher creatinine clearance rate, according respectively to the Med Diet Quality Index for children and adolescents [20] and the MDS (Med Diet Score) [17,19]. Furthermore, in a cohort of 1110 Swedish elderly men, after a follow-up of 10-year it was demonstrated, according to the MDS [13,17,19], that following Mediterranean Diet was associated with a better kidney function. eGFR calculated as an estimate of serum concentrations of cystatin C (mg/L) [23]. Patients with eGFR < 60 mL/min/1.73 m² were considered to have manifest CKD according to KDOQI definition [24]. Individuals with high (6–8 Mediterranean Diet Score) and medium (3–5 Mediterranean Diet Score) MDS were 42% and 23% less likely to have CKD, respectively (adjusted odds ratio [95% confidence interval] = 0.77 [0.57 to 1.05] and 0.58 [0.38 to 0.87], respectively, *P* for trend = 0.04).

During the ten-year follow-up, 168 (33%) CKD individuals died. Kaplan–Meier curves were used to assess survival rates in low- (≤ 2 points), medium- (3–5 points), and high-adherent (≥ 6 points) individuals. CKD Individuals with high and medium MDS adherence have a 23% and 25% lower risk of all-cause mortality, respectively [13].

More recently in an Iranian study, Asghari and colleagues analyzed the relationship between adherence to Med Diet and the 6-year incidence of CKD in 1212 adults aged 30 to 71 years. They demonstrated a significant inverse association between Med Diet and the risk of incident CKD. In particular, patients more adherent to the Med Diet pattern had a 50% lower risk of developing CKD, regardless of the presence of diabetes, hypertension, or high BMI [25]. After further adjustment for baseline eGFR, the inverse association between high MDS and incidence of CKD remained significant (OR = 0.53; 95% CI, 0.31–0.91). Furthermore, every 1-point increase in the MDS was associated with an 11% reduced risk of incident CKD at follow-up ((OR = 0.89; 95% CI, 0.80–1.00), *p* = 0.05). In the Atherosclerosis Risk in Communities (ARIC) study, involving 12,555 participants aged 45–64 years, emerged that greater adherence to healthy dietary patterns, including the Med Diet, was associated with a lower risk of developing CKD. The primary outcome was incident CKD, outlined using a composite definition: an eGFR <60 mL/min/1.73 m² accompanied by $\geq 25\%$ eGFR decline at a subsequent visit after baseline, and hospitalization or death related to CKD stage ≥ 3 , or end-stage renal disease or eGFR <60 mL/min/1.73 m² at a subsequent study visit accompanied by $\geq 30\%$ eGFR decline relative to baseline. During the 24-year follow-up, there were 3980 cases of incident CKD. Of these cases, 56% (2237) showed a decline in eGFR. A modified adherence score for the U.S. population (aMed) assessed the adherence to the Med Diet pattern. After adjustment for BMI, diabetes, systolic blood pressure, antihypertensive medication, and HDL, patients in the highest quintile of aMED score had a 13% lower risk of incident CKD compared with those in quintile 1 [26]. Even in a German cohort [27] following Med Diet was associated with a lower prevalence of diabetes mellitus and a higher glomerular filtration rate, in contrast to what was observed in people who adhered to the diet proposed by the German Food Pyramid Index (GFPI). From single nutrients standpoint, higher eGFR was observed in people who consumed more nuts, whole grains, fish, and legumes, while higher whole-fat dairy consumption was associated with lower eGFR [25,28].

There are few dietary intervention clinical studies assessing the impact of a Med Diet on parameters associated to kidney function in a CKD prevention perspective. An earlier report from a primary prevention study, the PREDIMED (Prevención con Dieta Mediterránea) study, evaluated, in a 1-year follow-up, the impact on kidney function in elderly people without coronary heart disease (CHD) but at high risk of CVD of three different dietary interventions: two Med Diets supplemented, respectively, with EVOO and nuts and a control low-fat diet [29]. Participants randomly assigned to MedDiet groups were given free virgin olive oil (15 L every 3 months) or mixed nuts (30 g/d; 15 g/d of walnuts, 7.5 g/d of almonds, and 7.5 g/d of hazelnuts). Participants assigned to the control diet

received recommendations regarding the intake reduction of all types of fats, both animal and vegetable, as recommended by the American Heart Association guidelines [30]. The authors found that eGFR improved in the 3 treatment groups compared to baseline, with no differences between dietary interventions even when the presence/absence of T2DM was considered separately. Supplemental analyses performed on PREDIMED trial cohort confirmed the positive impact of Med Diet on kidney function seen previously [31]. For this study were enrolled 5675 patients aged 55–75 with overweight or obesity ($\text{BMI} \geq 27 \text{ kg/m}^2$ and $<40 \text{ kg/m}^2$) and free from cardiovascular disease who satisfied at least 3 criteria for the metabolic syndrome (MetS) definition. Patients at extremes of energy intake (women < 500 and $>3500 \text{ kcal/die}$, and men < 800 and $>4000 \text{ kcal/die}$) were excluded. Dietary intake was evaluated using food frequency questionnaires (FFQ). Some were prescribed an erMed Diet (energy-reduced Med Diet), checked with a modified energy-reduced Med Diet Score (erMDS) [32]. The other two diet regimes evaluated were DASH (Dietary Approach to Stop Hypertension) [33,34] and classic MDS [17,19]. They assessed the associations between categories of changes in dietary patterns and changes in eGFR (mL/min/1.73 m^2). Besides, they assessed the association between categories of changes in dietary patterns and eGFR decline ($\geq 10\%$). In both analyses, the decrease or maintenance of changes category was used as reference. Participants were categorized in four groups according to changes in dietary pattern scores after 1 year of follow-up: decrease or maintenance of changes and tertiles of increasing changes.

Participants in the highest tertile of increase in 17-item erMedDiet Score showed higher changes in eGFR (β : $1.87 \text{ mL/min/1.73 m}^2$; 95% CI: $1.00\text{--}2.73$) and had lower odds of $\geq 10\%$ eGFR decline (OR: 0.62 ; 95% CI: $0.47\text{--}0.82$) compared to subjects in the decrease/maintenance category, while MedDiet and DASH Scores were not associated with any outcomes regarding CKD. Not significant results were observed with the Trichopoulou-MedDiet and DASH Score. As stated by the authors, these inconsistencies could be in part explained by their differences in the score calculation, which in contrast to the 17-item erMedDiet depends on cut-off points based on the study population distribution of each item. The greatest contributors to the association between changes in erMDS [32] and eGFR changes were the consumption of ≥ 2 units/day of vegetables, ≥ 3 servings/week of legumes and moderate consumption of wine. Conversely, in the CORDIOPREV trial [1], the efficacy of Med Diet on the prevention of kidney dysfunction was demonstrated. In this study, 1002 coronary artery disease patients were divided into two groups. One group followed Med Diet, while the other group a low-fat diet. Renal function was assessed at baseline and after five years of dietary intervention. The results of the study showed that although eGFR decreased after both dietary interventions compared with baseline, Med Diet produced a smaller decrease in eGFR than the low-fat diet even when correcting for diabetes. So, a long-term consumption of Med Diet, rich in extra virgin olive oil and other non-saturated fats, compared with a low-fat diet, may contribute to preserve renal function.

Instead, a study that focused on Hispanic ethnicity obtained contrasting results. The HCHS/SOL (The Hispanic Community Health Study/Study of Latinos) enrolled 8711 patients, age 18–74, without baseline CKD. Diet habit was evaluated with FFQ and patients were subjected to three different diet regimes: Alternative Healthy Eating Index-2010 (AHEI-2010) [33], DASH [34] and Med Diet evaluated with MDS [13,19]. On average, eGFR declined by $0.65 \text{ mL/min/1.73 m}^2$ per year. Lower AHEI-2010 [35] diet score was associated with greater decline in eGFR dose-response ($p \text{ trend} = 0.02$). In contrast, DASH [34] and MDS [19] were not statistically different in regard to annualized change in eGFR. None of the three dietary prescriptions seemed to have an impact on the development of CKD [35].

3. Role of Med Diet in CKD Secondary Prevention

Another important point of interest is whether adherence to Med Diet could impact the progression of CKD.

Picard et al. evaluated 50 patients, aged 18–80, with diabetes mellitus and CKD stage 1–4. There were no significant differences in age, CKD stage, diabetes type and duration

among patients selected [36]. The FU was of 5 years. A 3-day food record was used to assess dietary habits. Adherence to Med Diet was evaluated with MDS. In this study a lower stage of CKD was associated with higher MDS score. However, Med Diet seemed not to be significantly associated with eGFR variations during the follow-up. Higher MDS was associated with higher potassium intake, but, most notably, not with higher serum potassium concentrations. Conversely, patients with higher serum potassium levels had worse renal function and generally consumed less potassium-rich nutrients.

Another study, conducted by Moradi et al. in 270 patients with Diabetic Nephropathy (DN), produced contrasting results. The study included DN patients with serum creatinine (sCr) of 1–2.5 mg/dL, and blood urea nitrogen (BUN) of 20–40 mg/dL. Patients on a specific diet or with a daily energy intake <800 Kcal or >4200 kcal were excluded. Diet was checked by using FFQ. A trained dietitian evaluated all participants. MDS was assessed based on the eight characteristics of a traditional Med diet [17]. A maximum MDS of 9 could be obtained by summation, and participants were grouped into tertiles based on Med diet score. In this research, eGFR, sCr and BUN seemed to be not associated with Med Diet, either in crude or multivariable-adjusted models [37].

On the other hand, a very recent work, conducted within the frames of the ongoing CORDIOPREV study (a prospective RCT) found very interesting results [1]. This study enrolled 1002 patients with established coronary heart disease, but without events in the past 6 months and without any other serious illnesses. Patients were then randomized to two different diets: Med Diet and a low-fat diet (in accordance with the recommendations of the National Cholesterol Education Program). No interventions to increase physical activity or aimed to obtain weight loss were made. The evaluation of the patients lasted 5 years. Estimated GFR declined in both groups after dietary intervention but the rate of eGFR loss was significantly lower in patients who were randomized to Med Diet ($p = 0.033$). When considering baseline eGFR, this effect remained significant only among patients with eGFR between 60–90 mL/min. In particular, eGFR decline rate was 2.49 mL/min/1.73 m² lower in Med Diet group compared to those that were prescribed a low-fat diet ($p = 0.040$). No changes in albuminuria were observed after any of the two dietary interventions. Moreover, Med Diet produced a significantly lower decay of renal function also in diabetic patients (eGFR decline rate was 2.07 mL/min/1.73 m² lower compared to the low-fat diet). However, the most important result was the independent association of Med Diet with eGFR changes even after correction for baseline eGFR, diabetes and age ($B = 1.474$, $p = 0.041$).

Heindel et al. in a cohort of 2813 patients [27], obtained similar results. This prospective observational cohort study enrolled patients with CKD stage 1–3. Dietary habits were collected using FFQ derived from the European Prospective Investigation into Cancer and Nutrition (EPIC) study [38]. In this cohort 3 different dietary patterns were evaluated: DASH diet, [34] Med Diet [19] and GFPI [39] (German Food Pyramid Recommendation). In particular, to evaluate Med Diet, a mMDS (modified MDS) for non-Mediterranean population was used [17]. In this study every SD (standard deviation) increase of mMDS was directly associated with higher eGFR ($\beta = 0.932$, $p = 0.007$), even after adjustment for significant risk factors as: gender, age, BMI, caloric intake, smoking status, alcohol consumption, school education, HDL-cholesterol, LDL-cholesterol, hypertension and diabetes. In particular, high intakes of cereals ($\beta = 1.733$, $p = 0.020$), fish ($\beta = 1.630$, $p = 0.017$), and unsaturated fats ($\beta = 1.755$, $p = 0.011$) were associated with higher eGFR. Only higher intake of dairy products (low and whole-fat dairy) was associated with lower eGFR ($\beta = 21.633$, $p = 0.018$).

Med Diet seems to play a favorable effect on renal outcomes even in patients who underwent renal transplantation. Gomes et al. evaluated 632 patients with renal transplantation [40]. The primary end point was graft failure, defined as return to dialysis or re-transplantation. Secondary end points were the composite end points defined as: doubling of serum creatinine or graft failure, and graft loss defined as graft failure or death with a functioning graft. No specific dietary counselling prior the beginning of the

trial was given, except for lowering sodium intake and to lose weight if a patient had high BMI. No peri-transplant complications were present in patients of this cohort. Diet intake was assessed through the validated FFQ and adherence to Med Diet was evaluated through the MDS, median follow-up was 5.4 years. MDS was inversely associated with graft failure (HR: 0.76–95% CI: 0.58 to 0.98—5-year absolute risk reduction (ARR), 3%), kidney function decline (HR: 0.76–95% CI: 0.62 to 0.93—5-year ARR, 4%), and graft loss (HR: 0.78–95% CI: 0.66 to 0.92—5-year ARR, 5%). These associations were still significant after adjustment for age, sex, BSA, eGFR, urinary protein excretion, time since transplantation, primary kidney disease, HLA mismatches, living versus deceased donor kidney, and preemptive transplantation (graft failure: HR: 0.68–95% CI: 0.50 to 0.91—kidney function decline: HR: 0.68–95% CI: 0.55 to 0.85—and graft loss: HR: 0.74–95% CI: 0.63 to 0.88). These associations remained valid even after further correction for immunosuppressive treatment, cardiovascular risk factors, and lifestyle factors. Higher intake of legumes and nuts was inversely associated with graft failure (HR: 0.57–95% CI: 0.36 to 0.91) and higher than median intake of cereals (HR: 0.66–95% CI: 0.49 to 0.89) and moderate alcohol intake (HR: 0.63–95% CI: 0.44 to 0.89) were both inversely associated with graft loss. Finally higher MDS was associated with lower risk of graft failure especially in patients with higher proteinuria and shorter transplant time.

4. Med Diet and Metabolic Complications in Patients with CKD

In patients with CKD, Med Diet has several metabolic effects that deserve to be analyzed. Patients with CKD, especially those in more advanced stages, are at high risk of hyperkalemia [41], which is associated with increased mortality [42]. Med Diet, like other healthy diets, contains a significant amount of foods with an high potassium content [43] such as whole grains, fruits and vegetables [44]. In addition, it is worth noting, that also dairy products, animal proteins and beverages contribute significantly to dietary potassium intake [45] but their consumption is limited in Med Diet. Picard et al. [36] in their post-hoc analysis of a RCT assessed the relationship between Med Diet and potassium intake in CKD patients (study where diets were sorted for potassium intake above 2000 mg/day vs. below 2000 mg/day). Of the 50 participants, 9 had hyperkalemia. Five participants had hyperkalemia once, four had hyperkalemia more than once, for a total of 15 events. No association between dietary potassium intake and serum potassium levels was found. Interestingly, they found that those with better diet quality consumed more potassium than those with poor diet quality but did not have higher rates of hyperkalemia or higher serum potassium levels. In another study Heindel et al. [27] evaluated three dietary patterns rich in potassium: Med Diet, DASH and a diet proposed by the German Food Pyramid Index. Their data suggest that these three dietary patterns could be safe since they didn't observe any hyperkalemia in their patients. Lack of correlation between potassium from whole foods and serum levels can be explained with the lower bioavailability of potassium in unprocessed and minimally processed foods. In fact, when plant cell walls remain intact, this could blunt the effect on serum potassium levels [46]. Additionally, it has been proposed that plant foods, that are base-inducing foods, tend to shift potassium intra-cellularly minimizing the impact on serum levels [47]. Thereof, we may summarize that although Med Diet is generally characterized by a high content of potassium there are not evidences suggesting that in CKD patients it may be associated with higher incidence of hyperkalemia. However, it is noteworthy that none of these studies was performed in subjects with advanced CKD where a severe reduction of eGFR may interfere with urinary potassium excretion and may induce potassium accumulation and hyperkalaemia.

Med Diet is rich in monounsaturated fats (MUFA) from olive oil (mainly VOO or EVOO) and poor in saturated fats (SFA) from meat and dairy products. For this reason, Med Diet is widely recognized for its benefits in reducing cardiovascular risk factors by improving lipid profile [1]. Moradi et al. in their cross-sectional study measured serum lipids, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triacylglycerols (TG) in 270 patients with diabetic nephropathy stage 1–2. A trained dietitian evaluated

all participants. Med Diet score was assessed on basis of the eight characteristics of a traditional Med Diet that were outlined by Trichopoulos et al. [21]. Participants were given one point for: daily portion of fruit, fish, vegetables, whole grains, legumes or nuts, if the ratio of MUFA to SFA was equivalent to or greater than the median intake of the study population, and if the daily portion of meat (red meat, poultry and processed meats) and dairy products were less than the median intake of the study population. They found that lipid profile was not significantly associated with Med Diet observance in either crude or multivariate-adjusted models. Also Picard et al. and in the CORDIOPREV study did not find any association between diet quality and TG, LDL or HDL [1,36].

Thanks to the presence of high-quality carbohydrates provided by whole grains, nuts, fruits, and vegetables [22] Med Diet is characterized by a low glycemic index, with positive effects on glucose control, hyperinsulinemia, insulin response and decreased appetite. Furthermore, Med Diet provides 30 to 50 g of fibers per day [22,48] that can contribute to improve postprandial glycaemia and to improve insulin sensitivity. Heindel et al. [27] also in a cohort of 2813 German CKD patients, aged over 60 years, confirmed these evidences. High Med Diet score was associated with lower incidence of diabetes and lower HbA1c in contrast to what was observed in people who adhered to the diet proposed by the German food pyramid (GFPI) [27]. This is consistent with the results found in another large German prospective cohort study in which better adherence to Med Diet was associated with lower prevalence of diabetes mellitus in the general population [49]. In a small observational study [50] conducted in 99 Australian CKD patients (stages 3 to 5), adherence to Med Diet was associated with an improved glycemia as well as with a lower incidence of type 2 diabetes.

As for other potential benefits of Med Diet on hyperphosphatemia and metabolic acidosis in CKD patients, we did not find any study specifically addressing these aspects. See Table 1 for reviewed studies.

Table 1. Summary of principal studies and paper evaluating Med diet impact on kidney function.

Study	Type	Methods	Results
Primary prevention studies			
Adherence Med Diet and albuminuria levels in Greek adolescents: data from the Leontio Lyceum ALbuminuria (3L study). Mazaraki et al. [20]	Longitudinal prospective cohort study	365 healthy adolescents aged 12–17. Adherence to Med Diet was assessed using the KIDMED score [25].	Lower adherence to Med Diet was significantly and independently associated with higher ACR.
Adherence Med Diet is Associated With Renal Function Among Healthy Adults: The ATTICA Study. Chrysoshoou et al. [19]	Longitudinal epidemiological cohort study	3042 healthy patients, age 18–88. 1 year FU. Diet was evaluated using EPIC score [36]. Adherence to Med diet was evaluated with MDS [9]	Creatinine clearance was directly and independently associated with higher MDS. In unadjusted analyses urea and creatinine were inversely associated with MDS.
Med Diet, Kidney Function, and Mortality in Men with CKD. Huang et al. [13]	Longitudinal prospective cohort study (based on ULSAM)	1110 male patients, average age 70 years, with and without baseline CKD. 10 years FU. Dietary habits evaluated with SNFA [40]. Adherence to Med Diet was evaluated with adapted MDS [9].	Higher MDS was significantly and independently associated with lower OR of developing CKD. Moreover, higher MDS was associated to significantly better survival in patients with CKD.

Table 1. Cont.

Study	Type	Methods	Results
Adherence to Med Diet is associated with reduced risk of incident chronic kidney diseases among Tehranian adults. Ashgari et al. [25]	Longitudinal prospective cohort study (based on TLGS)	1212 patients (age 30–70). Patients with CKD, previous MI, stroke, cancer or <800 Kcal/die/>4200 Kcal/die were excluded. Diet was assessed using FFQ, adherence to Med Diet was assessed with MDS [9].	Higher MDS was significantly and independently associated with a lower risk of CKD development, even after adjustment for eGFR.
Dietary patterns and risk of incident chronic kidney disease: the Atherosclerosis Risk in Communities study. Hu et al. [26]	Longitudinal prospective cohort study (based on ARIC)	12155 patients (age 45–64). Patients with CKD at baseline, history of MI, cancer or females with <500 or >3500 kcal/die or males with <700 or >4500 kcal/die were excluded. Diet was assessed with FFQ. Diet adherence was assessed with HEI-2015 [17], AHEI-2010 [28] and aMed [23] (modified from standard MDS). Median FU 24 yrs.	Adherence to Med Diet was significantly and independently associated with lower risk of incident CKD. In particular nuts, whole grain and moderate alcohol consumption were associated with a lower risk of CKD development, while red/processed meat was associated with higher risk of CKD.
Effects of Med Diets on Kidney Function: A Report From the PREDIMED Trial. Diaz-Lopez et al. [29]	Longitudinal prospective RCT	665 Patients aged 55–80. Patients with history of CVD, severe chronic illness, substance addiction were excluded. 1 year FU. 3 types of diet: Med Diet + olive oil, Med Diet + nuts, control low fat diet.	eGFR increased significantly in all 3 groups. No significant between group difference in eGFR and albuminuria progression was noted.
Association of Diet Quality Indices with Longitudinal Changes in Kidney Function in U.S. Hispanics/Latinos: Findings from the Hispanic Community Health Study/Study of Latinos (HCHS/SOL) Missikpode et al. [35]	Prospective observational cohort study	8771 patients from the HCHS/SOL study [22,23]. Median FU 6 years. Healthy diet regime adherence evaluated with AHEI-2010 [29], DASH [28], MDS [9]. Patients with CKD at baseline were excluded.	Lowest AHEI-2010 quartiles were associated with greater decline in eGFR. In particular, higher consumption of whole fruit was associated with a slower annual decrease in eGFR. DASH and Med Diet quartiles were not significantly correlated with annualized eGFR variation. None of 3 dietetic patterns were associated with incident CKD
Prospective associations between a priori dietary patterns adherence and kidney function in an elderly Mediterranean population at high cardiovascular risk Valle-Hita et al. [31]	Prospective observational cohort study	5675 patients age 55–75, overweight or obese, from PREDIMED-Plus study [27]. Patients excluded if extreme low/high energy intake (<500 Kcal/die w./<800 Kcal/die m. - >3500 Kcal/die w./>4000 Kcal/die m.). Diet evaluated using FFQ. Energy reduced Med Diet was evaluated using erMDS [26] classic MDS [9]. Diet regimes also evaluated with DASH [29]. 1 year FU.	Adherence to erMed Diet was significantly and independently associated with lower decrease in eGFR at 1 year, in a dose dependent fashion. The same was true when considering the OR of 10% eGFR decline. Nutrients mostly associated with eGFR changes were vegetables, legumes, and moderate wine consumption. No significant association were found for classic Med Diet [19] or DASH [17].

Table 1. Cont.

Study	Type	Methods	Results
Recent secondary prevention studies			
Med Diet and Kidney Function Loss in Kidney Transplant Recipients. Gomes et al. [40]	Longitudinal cohort study	632 Rtx-p with a functioning graft for ≥ 1 -year. Standard IS and overall therapy, no prior specific dietary counseling. Diet evaluated using FFQ, Med Diet evaluated using Med Diet Score (MDS) [22] using a 9-point scale	MDS was inversely associated with graft failure (HR 0.76; 5 yrs ARR 3%), kidney function decline (HR 0.76; 5 yrs ARR 4%) and graft loss (HR 0.78; 5 yrs ARR 5%) independently from confounders. MDS also associated with longer time to graft failure, especially if high Prot-U was present.
Low Med Diet scores are associated with reduced kidney function and health related quality of life but not other markers of cardiovascular risk in adults with diabetes and chronic kidney disease. Picard et al. [36]	Post-Hoc analysis of a RCT longitudinal study	50 patients with diabetes and CKD stage 1–4, age from 18–80. 5-year FU. 3-day food record was used to assess nutrients intake. MDS calculated with a 9-point scale [22].	Stage 1–2 vs. Stage 3–5 CKD was associated with higher MDS, but no association of MDS in and eGFR decline rate. Higher K ⁺ intake was associated to higher MDS but not to higher serum K ⁺ . Hyperkalemic patients had lower K ⁺ intake and worse eGFR.
Association between adherence to the Med Diet and renal function biomarkers and cardiovascular risk factors among diabetic patients with nephropathy. Moradi et al. [37]	Cross-sectional study	270 patients with DN, Prot-U 300–1000 mg/dL, sCr 1–2.5 mg/dL. Diet evaluated using FFQ. MDS was calculated with a 9-point scale [22]. Patients with daily intake <800 Kcal or >4200 Kcal were excluded.	sCr, BUN, eGFR, CRP not significantly associate to Med diet adherence. No relevant association also with CVD markers.
Long-term consumption of a Med Diet or a low-fat diet on kidney function in coronary heart disease patients: The CORDIOPREV randomized controlled trial. Podadera-Herreros et al. [1]	RCT	1002 CHD patients, last CV event >6 mths before enrolling, age 20–75. Randomization based on age, sex and previous MI between Med diet and low-fat high complex carbohydrate diet according to (National Cholesterol Education Program). 5 yrs FU.	eGFR declined significantly after both dietary interventions, Med Diet was independently (vs baseline eGFR, sex, DM) and significantly associated with a lower eGFR decline both in DM and non-DM patients, especially in patients with baseline eGFR between 60–90 mL/min
Association Between Dietary Patterns and Kidney Function in Patients With Chronic Kidney Disease: A Cross-Sectional Analysis of the German Chronic Kidney Disease Study Heindel et al. [27]	Prospective observational cohort study	2813 all-cause CKD patients, stage 1–3, or stage 1–2 with overt Prot-U. 2 years FU. Diet evaluated using FFQ adapted from EPIC study. 3 different diets: DASH [28], Med diet and GFPI. Med Diet evaluated through modified MDS [8].	Med Diet was independently associated with higher eGFR. In particular, higher intake of cereals, fish, unsaturated fats were significantly associated with higher eGFR, while dairy products were associated with lower eGFR.

Note: ACR: albumin to creatinine ratio; EPIC: European Prospective Investigation into Cancer and Nutrition; ULSAM: Uppsala Longitudinal Study of Adult Men; SNFA: Swedish National Food Administration; TGLS: Teheran Glucose and Lipid Study; ARIC: Arteriosclerosis Risk In Communities; HEI: Healthy Eating Index; RTx-p: renal transplant patients; IS: immunosuppressive therapy; FFQ: Food frequency questionnaire; MDS: Med Diet score; HR: hazard ratio; ARR: absolute risk ratio; Prot-U: urinary protein excretion in 24 h; RCT: randomized controlled trial; CKD: chronic kidney disease; FU: follow-up; eGFR: estimated glomerular filtration rate; DN: diabetic nephropathy; BUN: blood urea nitrogen; CRP: c-reactive protein; sCr: serum creatinine; CVD: cardiovascular disease; CV: cardiovascular; MI: myocardial infarction; DM: diabetes mellitus; DASH: dietary approach to stop hypertension; GFPI: german food pyramid index; AHEI: alternate healthy eating index; erMDS: reduced energy Med Diet score.

5. Conclusions

From the data presented in this review the potential beneficial effect of Med Diet on CKD seems evident, both in terms of prevention of the development of established disease as well as in terms of slowing disease progression. In particular, positive renal outcomes seem to be more dependent on the consumption of specific nutrients, as legumes, nuts and vegetables. Conversely, red/processed meat and high alcohol intake had in general a negative impact on renal health. Furthermore, Med Diet seems to have a positive effect also on metabolic control of diabetic patients, could help to reduce the incidence of renal and cardiovascular complications in this high-risk population.

However, further studies are needed, especially to clarify the effectiveness of Med Diet in slowing CKD progression, and the impact of ethnicity and previous dietetic regimes on Med Diet efficacy. In fact, a relevant limitation that emerged from our review is that very few studies have evaluated the benefits of the Med Diet in slowing the progression of disease especially in advanced stages of CKD. Moreover, it would be interesting to evaluate the influence of Med Diet on the evolution of CKD based on different primary renal diseases and/or in the presence of different comorbidities.

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Review

Ketogenic and Modified Mediterranean Diet as a Tool to Counteract Neuroinflammation in Multiple Sclerosis: Nutritional Suggestions

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Abstract: Ketogenic Diet is a nutritional pattern often used as dietotherapy in inflammatory diseases, including neurological disorders. Applied on epileptic children since 1920, in recent years it has been taken into account again as a tool to both reduce inflammatory burdens and ameliorate the nutritional status of patients affected by different pathologies. Multiple sclerosis (MS) is considered an immune-mediated neuro-inflammatory disease and diet is a possible factor in its pathogenesis. The aim of this work is to investigate the main potential targets of MS-related impairments, in particular the cognitive deficits, focusing on the alteration of biomarkers such as the Brain Derived-Neurotrophic Factor and the Tryptophan/Kynurenine ratio that could play a role on neuroprotection and thus on MS progression. Furthermore, we here propose nutritional suggestions which are useful in the development of a ketogenic diet protocol that takes advantage of the anti-inflammatory properties of low-carbohydrate foods from the Mediterranean diet to be applied to subjects with MS. In conclusion, this approach will allow one to develop the ketogenic diet combined with a modified Mediterranean diet as a possible tool to improve neuroinflammation in multiple sclerosis.

Keywords: Mediterranean; ketogenic; multiple sclerosis; diet; neuroinflammation; Tryptophan/Kynurenine ratio; brain derived neurotrophic factor

1. Introduction

Multiple sclerosis (MS) is a neurological disease characterized by an autoimmune response, inflammation, demyelination, gliosis, and neuronal loss [1]. It has a multifactorial nature and various environmental factors or metabolic conditions may have a role in its etiology [1]. Nutrition is now recognized as one of the possible risk factors for the development of MS and has potential applications in the management and treatment of the disease: eating habits and lifestyles can exacerbate or improve the symptoms of the disease, modulating the inflammation, interacting with human cells and the commensal gut microbiota [2].

In this context, the ability of dietary factors to interact with enzymes, transcription factors, and nuclear receptors of human cells is of striking importance [3,4]. Food can have medium and long-term effects on the production of circulating hormones which influence

many physiological mechanisms responsible for the development of an inflammatory state and for an alteration of the redox cellular state [4,5]. Many MS patients are malnourished, clinically manifesting weight loss, overweight or obesity, as well as vitamin deficiency [6]. In patients with MS, malnutrition has been associated with impairment of the immune system, with negative impacts on cognitive function, inducing a chronic inflammatory state that increases the frequency of relapse and worsens the progression of the disease [7]. In addition, nutritional status negatively influences the effectiveness of drug therapy [8]. Several dietary strategies have been proposed over the years for the treatment of MS, complementary to drug therapy. Some of the models proposed appear particularly restrictive and difficult to implement over time, potentially leading to deficiencies in some nutrients. Despite the differences between the various diets, there are also some aspects in common, such as avoiding processed foods, foods with a high glycaemic index and foods rich in saturated fat; reducing the consumption of fatty red meat; and increasing the consumption of fruit and vegetables. However, clear evidence is lacking to support the benefit of any specific dietary regimen and well-designed, randomized controlled trials are needed [9]. The subject affected by MS often asks for a specific dietary regimen, but without guidelines, it could lead to malnutrition [6].

The Mediterranean diet (MeDi) in MS modulates the gut microbiota and low-grade inflammatory state. It has also recently been shown to reduce the risk of mental disorders, including depression and cognitive decline [10]. The influence of MeDi, rich in polyphenols, is reflected in Brain Derived Neurotrophic Factor (BDNF) levels, improving cognitive function, as shown in clinical trials [11]. This link becomes important if we consider the promising role of BDNF on neuroprotection demonstrated in experimental models of MS and clinical studies.

MeDi foods, especially vegetables, are rich in nutraceuticals. The use of nutraceuticals has been proposed for the enhancement of cognitive performances and reduction of neurodegenerative impairments, considering inflammation and oxidative stress as substantial factors that could induce neurological alterations [12–14]. Upon this view, nutraceuticals can find applications in neurodegenerative diseases such as MS.

Similarly, the application of the Ketogenic Diet (KD) protocol on mice with autoimmune encephalomyelitis (EAE) showed beneficial effects on disease progression, disability, cognitive status, and inflammatory markers by reducing the expression of enzymes involved in the biosynthesis of pro-inflammatory molecules [6]. A recent study has observed that the eight-week intervention with the very-low-calorie KD produced significant weight loss in obese patients, decreasing pro-inflammatory cytokine production, increasing adiponectin serum levels, and improving metabolic profile [15].

Hence, this evidence supports the interest in the development of a KD protocol modified and strengthened by the use of typical foods of the MeDi with lower concentrations of carbohydrates, in order to appreciate the beneficial properties of these two integrated dietary regimens on MS.

The aim of this review is to provide a comprehensive overview of the key points related to MS in order to pave the way to the application of a new dietary protocol that would be beneficial for the management of this neurological disorder. To this purpose, we have firstly investigate the main potential targets of MS-related impairments, in particular the cognitive deficits, focusing on the alteration of biomarkers such as BDNF and the Tryptophan/Kynurenine ratio whose neuroprotective metabolites can be modulated by nutritional approaches. Indeed, these biomarkers, that could play a role in neuroprotection and thus on MS progression, could be influenced by ketogenic and Mediterranean dietary protocols. Then, we focus on the consequences of malnutrition and its comorbidities on MS severity and progression. Finally, the potential anti-inflammatory properties of MeDi and KD on MS will be investigated, highlighting the effect of both protocols on BDNF production, and on inflammatory markers. In the light of all this evidence, nutritional suggestions based on the modified MeDi, and the KD will be proposed, comprising macro- and micro-nutrients that would be beneficial for the enhancement of MS patient compliance.

2. Multiple Sclerosis: Cognitive and Nutritional Factors

MS is a central nervous system (CNS) disease comprising macro and microscopic alterations: a focal inflammation resulting in macroscopic plaques and injury to the blood-brain barrier; and neurodegeneration with the microscopic impairment of axons, neurons, and synapses [1]. Macroscopically, myelin loss, edema, and axonal injury are encountered in MS plaques; whilst microscopical MS lesions strongly activate auto-immunity response recruiting macrophages, T and B cells, and initiating cytotoxic activities of microglia [16]. The MS disease course has been classified into seven categories, as in Lublin et al., 2014. The main four are the following: (1) relapsing-remitting (RR) is a very common disease course in which neurological symptoms develop over days to weeks and last 24 to 48 h; (2) primary progressive regards patients whose symptoms gradually deteriorate from the onset, without relapses; (3) secondary progressive shows an initial RR course followed by a slower neurologic deterioration; (4) progressive-relapsing typically shows a gradual deterioration with superimposed relapses. Furthermore, MS patients could present (5) a clinically isolated syndrome with a single episode of inflammatory CNS demyelination; (6) a fulminant syndrome with severe MS symptoms, multiple relapses and rapid progression; lastly (7) a benign clinical course with an overall mild disability.

2.1. The Role of BDNF in MS and the Influence of Dietary Factors on Its Regulation

Once it was established that the impaired myelination process plays a crucial role in MS-induced neurodegeneration, the importance of neurotrophic growth factors in the myelin repair were given attention [17]. Among them, BDNF represents a gold standard in this context. Indeed, it is critical for neurodevelopment, for neuronal function and survival in the adult brain [18,19], but also for synaptic plasticity, especially in discrete brain regions such as the hippocampus, which is particularly important for behavioral and physiological processes [20,21]. It has been shown that BDNF contributes to neuroprotection via several effects, among which we encounter the impact on myelin integrity [22,23]. Several authors revealed that it can influence the distribution pattern of myelin structural proteins implicated in the integrity of the intact myelin sheath [24–26]. This myelin-protective effect helps guarantee mechanisms of myelin repair and eventually the degree of remyelination by downstream BDNF-induced endogenous pathways [27]. What has been unveiled is that it induces oligodendrocytes precursor proliferation, migration, and differentiation in the myelin damage foci [22,28], but also constitutes a survival factor for neurons by promoting remyelination of damaged axons [29].

Not surprisingly, recent research has explored its promising impact in the neuroprotection of MS in experimental models and clinical studies [24,25]. Indeed, some authors supported BDNF contribution to the remyelination of MS-induced lesions [26,30]. In particular, its levels resulted in decreased MS patients during RR phases, thus limiting the opportunity of individuals to repair myelin damage before a subsequent MS attack [31]. Whereas, BDNF serum levels are higher during the MS attack, though not sufficient to promote complete remyelination [26,32]. Collectively, these data could demonstrate its protective up-regulated activity to promote neuronal recovery in MS compared with healthy subjects and this could represent a reliable biomarker especially for MS diagnosis [33]. Even though some authors blame the putative discrepancy between serum and brain levels of BDNF [34], others indicate that plasma concentration reflects the brain altered levels in neurological disorders [35,36]. However, it is undeniable that the results obtained so far prompted novel pharmacological strategies in order to attempt the amelioration of MS disease by increasing endogenous or exogenous BDNF levels [37,38]. Intriguingly, its levels also seem to be implicated in cognitive deficits typical of neurodegenerative disorders such as AD, psychiatric disorders and MS [36]. Indeed, Hori et al., 2017 revealed that BDNF levels are strictly linked to the cognitive domains of memory and verbal learning, verbal fluency, and executive function. Moreover, its polymorphisms in MS have been correlated with cognitive performance and measures of brain atrophy [39,40].

In this context, the evaluation of BDNF biomarker in MS patients could be powerfully influenced by specific dietary protocols. Indeed, BDNF is implicated in glucidic homeostasis and energy balance [41], since an inverse correlation between blood glucose levels and BDNF release was found. Particularly, in rodent models, BDNF expression was correlated with glucose actions in the ventromedial hypothalamic nucleus. More intriguingly, KD was specifically implicated in the modification of plasma BDNF levels [42]. Indeed, beta-hydroxybutyrate (BHB) produced by this dietary protocol is able to cross the blood brain barrier (BBB) inducing the increase in the mitochondrial respiration and in turn NF- κ B, that ultimately activates histone acetyltransferase p300/EP300 and consequently BDNF synthesis [42]. In addition to KD effects on BDNF levels, polyphenols present in the MeDi have been correlated to BDNF levels. It was outlined that the assumption of polyphenol-rich foods in MeDi activates nuclear factor CREB and thus BDNF levels, with positive outcomes on spatial memory performance in murine models. In addition, clinical trials pointed out that the consumption of high levels of polyphenols boosts cognitive function influencing neurogenesis via the specific activation of ERK/CREB/BDNF axis [11].

In the light of this evidence, KD and MeDi effects on BDNF levels could constitute a crucial turning point for supporting the cognitive relapse of MS through the control of specific dietary protocols.

2.2. Tryptophan-Kynurenine Metabolism in MS and the Influence of KD

Tryptophan (Trp) is an essential amino acid whose metabolites play key roles in several physiological processes; due to its very low reserves in the body, its deficiency rapidly manifests under various catabolic conditions. In a recent review its roles as a source of serotonin and melatonin, as a regulator of neurotransmission and its capability to influence circadian rhythm and cognitive functions have been highlighted and discussed [43]. It also influences the regulation of skeletal muscle mass and primarily influences immune responses. Trp has a stimulatory effect on proteosynthesis and its supplementation increases muscle mass and reduces adipose tissue. Trp blood levels decrease with age and inflammation: such decrease of Trp and the accumulation of its catabolite Kynurenine (Kyn) contribute to the development of sarcopenia. Kyn functions via numerous metabolic intermediates modulating inflammatory responses, oxidative stress and nicotinic and glutamatergic receptors [44]. In addition, Kyn serves important signaling functions in inter-organ communication and modulates endogenous inflammation. Several metabolites target the *N*-methyl-D-aspartate receptor as antagonists such as kynurenic acid (K+*A*) or as agonists such as quinolinic acid (QA) and 3-hydroxykynurenine (3-HK), thus putatively exerting neuroprotective and neurotoxic effects on neuronal excitability [44,45].

It is possible to assume that in MS disease the indoleamine 2,3 dioxygenase (IDO) could influence TRP effects on immune functions since IDO can be activated during inflammation (stimulated by tumor necrosis factor- α (TNF- α), interferon (INF- α), etc.) to form Kyn, thus diminishing the amount of Trp available for the synthesis of serotonin, melatonin (increase in the Kyn/Trp ratio) and other important azoles. This leads to Trp depletion and consequently attenuation of proteosynthesis; such an effect could result in the rapid progression of muscle atrophy, sarcopenia, and polyneuromyopathy. Noteworthy, IDO was evidenced as a regulator of T cells' response in MS' clinical course via modulation of Th1/Th2 ratio. For instance, in RRMS patients' IDO expression, that is augmented in relapsing phases, can be decreased by glucocorticoids administration together with its catalytic activity [46].

The effect of the Kyn/Trp ratio was evaluated on inflammatory states and neuronal excitability, highlighting that reductions in the Kyn/Trp ratio in favor of Trp through the consumption of tryptophan-rich foods improve skeletal muscle mass and ameliorate endogenous inflammation in MS patients [43]. The modulation of Trp-Kyn metabolism through lifestyle (diets, Branched-Chain Amino Acid (BCAA), aerobic exercise) could modify the balance in favor of Trp and its neuroprotective metabolites, ultimately supporting the treatment of MS disease with low grade chronic inflammation. Indeed, the KD protocol

was found to downregulate the Kyn pathway in the hippocampus and at plasma level in rat models, revealing beneficial effects on neurodegenerative processes [47]. Lastly, BHB produced in the KD protocol determines a reduction of Kyn levels, an increase in KA and also in the KA/KyN ratio which sustains the neuroprotective of KD by inhibiting the kynurenine 3-monooxygenase enzyme that ultimately synthesizes KA [48].

2.3. Malnutrition in MS

Malnutrition is defined as an acute, subacute, or chronic state of overnutrition or undernutrition with or without inflammatory activity that leads to a change in body composition and functional variation. In patients with MS, malnutrition has been associated with impairment of the immune system. It affects mental function, respiratory muscle strength, and it contributes to exacerbate already existing symptoms, such as muscle wasting and weakness, fatigue and muscle spasm [49,50].

Many MS patients suffer from various forms of malnutrition, including weight loss, obesity or vitamin deficiency [6]. Malnutrition in MS is independent of the disease course and duration, number of attacks, disability status, and functional system involvement [49]. However, its incidence has not been well determined and there is a paucity of information regarding its functional consequences to MS patients. Epidemiological data show that the prevalence of chronic malnutrition was 11.8% in MS patients and only 2% in patients with other chronic disorders [49]. Our recent study of patients with RR-MS found that 70% of patients had a nutritional risk, of these 20% were undernourished and 50% were overnourished (unpublished data). Weight loss and cachexia are often present in patients with MS [51]. Evidence shows that there is a correlation between significant weight loss, cachexia, and a demyelinating lesion in the lateral hypothalamus [52]. Accordingly, a state of undernutrition can worsen the disease. When considering the condition of undernutrition in MS patients it is important to evaluate the impact of certain vitamin deficiencies: vitamin D deficiency has been widely reported, whereas folic acid and vitamin B12 deficiency has not. A recent review showed that vitamin D deficiency may be relevant to the development of the disease and to its severity [8,53]. In addition, the same authors also observed that low vitamin D levels also affect therapeutic response to medications. The above highlights the importance of vitamin D supplementation in MS patients in order to achieve plasma levels appropriate to the demands of the disease. Recently, it has been shown that vitamin D levels below 40 ng/mL are too low to keep the clinical condition under control and not to observe new lesions on MRI [8]. According to the literature [8], extensive controlled clinical studies would be necessary to establish standard levels of vitamin D supplements which are useful for patients with MS.

Overnutrition (defined as BMI > 24.9) includes overweight or obesity, that in infancy and adolescence can predispose to the onset of MS [54]. The Nurses' Health Study I and II found that women with a BMI ≥ 30 kg/m² at age 18 had a 2.25-fold increased risk of developing MS compared to those with a BMI in the normal range after adjusting for age, latitude, race/ethnicity, and smoking [55]. An epidemiological study on MS has estimated that eliminating childhood obesity could prevent approximately 15% of MS cases [56]. The condition of obesity and overweight in patients with MS increases the risk of comorbidities such as diabetes and cardiovascular diseases (CVDs) [57–59]. Both induce a chronic inflammatory state that worsens the disease, increasing the frequency of relapse with negative impact on cognitive function [7]. Obesity, overweight, and insulin resistance are interrelated components of the Metabolic Syndrome (MetS). Indeed, in a recent study on MS the onset of MetS was observed in 22% of patients and insulin resistance in 46% with a higher incidence in comparison with healthy controls [60,61]. Although the association between MetS or insulin resistance and degree of disability has not been unequivocally demonstrated, it has been observed that insulin resistance can exacerbate MS-related conditions as well as neurocognitive dysfunction and inflammatory and immune responses [62].

2.4. Role of Adipokines in the Pathogenesis of MS Associated with Obesity

Adipokines released from adipose tissue directly and indirectly control appetite, energy balance, immunity, angiogenesis, insulin sensitivity and lipid metabolism [63]. The balance between proinflammatory (such as leptin) and anti-inflammatory (such as adiponectin-APN) mediators seems to play an important role in the pathogenesis of MS. Some studies have reported increased levels of leptin, resistin, and visfatin as well as decreased levels of APN in patients with RR-MS in comparison with healthy controls. Leptin and APN show an opposite role in the immune response. Leptin is a potent proinflammatory molecule. Its serum concentration increases in the active phase of RR-MS in untreated patients as compared to controls [64]. In the past, this adipokine has been considered a marker of disease activity and response to therapy but this was disproved in a 2018 randomized control trial [65,66]. The calorie restriction (CR) reduces leptin serum levels, by repairing the production of pro-inflammatory cytokines, demyelination, and axonal injury [59].

Otherwise, APN exhibits anti-inflammatory activity in immune system cells [67]; in fact, it inhibits the activation and proliferation of T and B lymphocytes and the phagocytic activity of macrophages, as well as the synthesis of both pro- and anti-inflammatory cytokines [68,69]. Reduced APN concentration was observed in patients with MS compared with healthy controls; interestingly, APN levels were higher in female patients than in male patients [70,71].

3. Mediterranean Diet

MeDi is associated with numerous health effects; it is one of the most varied and balanced diets and has been proven to be effective in terms of health protection, reducing the risk of CVD, diabetes mellitus and some types of cancer [72]. A recent cohort of Southern Italian patients have shown that the MeDi can have beneficial effects on MS long-term disability outcomes by positively modulating gut-microbiota and the low-grade chronic systemic inflammation, including CVD [10]. HELENA study has investigated the effects of MeDi on inflammatory state highlighting a counteracting effect of stress on inflammatory biomarkers with high MeDi adherence [73]. Finally, recent research has shown that a higher adherence to the MeDi is associated with a lower risk of mental disorders, including cognitive decline and depression [74]. Thus, the MeDi has a strong rationale for use in progressive MS [75].

3.1. Anti-Inflammatory Effect of MeDi

The Mediterranean dietary pattern is composed of fruit, vegetables, whole grains, nuts, seeds, legumes, and extra virgin olive oil (EVOO). Moreover, the traditional one is low in calorie intake as well as in animal proteins, especially in red and cured meat, with low amounts of saturated fatty acids and sugars and a high content of fibers [76]. It is a nutritional pattern and includes a lifestyle leading to successful aging and to a reduction in the onset of diseases and disabilities typical of the aging process [77,78]. The explanation could be found in the great variety of phytochemical compounds of its foods with proven nutraceutical properties [78]. These molecules showed antioxidant and anti-inflammatory effects, highlighted by reduction in serum levels of inflammatory mediators such as C-reactive protein, Interleukin-(IL)-6, as well as many inflammatory biomarkers found in many chronic diseases, including MS [10,79,80]. Diet may influence the gut microbial composition and its metabolites that induce changes in progression and severity of MS disease. Additionally, many studies have demonstrated that MS patients exhibit intestinal dysbiosis with decreased abundance of *Clostridium*, *Bacteroidetes* and *Adlercreutzia* microbes [81]. Studies have shown that low-calorie diets comprising of high levels of fruits, vegetables and fish, which are typical in MeDi, promote beneficial gut microbiota and reduce inflammation in the body [82]. Gut commensal bacteria exert both pro- and anti-inflammatory responses by regulating T cell differentiation and immune responses in the gut [83].

The MeDi is rich in nutraceuticals, such as phenolic acids, flavonoids, stilbenes and lignans, terpenoids such as carotenoids and tocopherols, and unsaturated fatty acids. All foods containing these compounds can be considered as “functional foods”. Although a universal definition does not exist, the Functional Food Center defined them as “Natural or processed foods that contain known or unknown biologically active compounds; which, in defined, effective non-toxic amounts provide a clinically proven and documented health benefit for the prevention, management, or treatment of chronic diseases” [84]. Many studies have investigated how health status is directly affected by nutraceuticals, providing evidence that the increased intake of some nutraceuticals, above the habitual and recommended dose levels, can decrease both inflammatory status and reactive oxidative species [79]. In addition, it has been proposed that the use of nutraceuticals can improve cognitive performance and reduce neurological impairments considering inflammation and oxidative stress as substantial factors that could induce neurological alterations [85]. Upon this view, nutraceuticals can find applications in neurodegenerative diseases such as MS.

3.2. MeDi Foods Suggested for the Protocol to Be Developed

Foods of the MeDi that we will take into consideration for our proposed suggestions will be the ones with lower-carbohydrate concentrations that are richer in lipids, in order to enhance the anti-inflammatory properties of the MeDi with a modified approach. In particular, EVOO, the connecting food between Mediterranean countries, is the main source of fat in that diet and is a particularly rich source of phytochemical compounds, especially polyphenols. The main lipid constituents of olive oil are triglycerides. There are three main fatty acids in the triglyceride fraction: a monounsaturated fatty acid (MUFA), oleic acid (73.6%); a saturated fatty acid, palmitic acid (13.7%); and a polyunsaturated fatty acid (PUFA), linoleic acid (7.85%). The percentage ratio of fatty acids is 16.2%, 74.4% and 9.4% for SFA, MUFA and PUFA, respectively [86].

The remaining fraction contains about 230 bioactive molecules. These include lipophilic phenols (whose levels fall as olives grow up), sterols, color pigments (mainly chlorophylls and carotenoids), alcohols, waxes, aldehydes, esters, ketones, and phenolic compounds such as hydrophilic phenols [87,88]. EVOO might exert beneficial effects reducing the levels of markers of inflammation and conferring neuroprotection [88,89]. Hydroxytyrosol, oleuropein, and oleocanthal, polyphenols widely studied in vivo, in vitro and directly in human, inhibit Nuclear Factor- κ B (NF- κ B) pathway, showing ibuprofen-like activity and inhibiting cyclooxygenases 1 and 2, that are responsible for prostaglandin production [78,90,91]. Moreover, the phenolic, oleuropein aglycone inhibits TNF- α -induced matrix metalloproteinase 9 in a monocyte cell line with an interesting role in the development of inflammatory diseases [92]. In particular, a claim on the scavenging effect of EVOO compounds in radical species exists. A daily intake of 20 g of olive oil, which contains at least 5 mg of hydroxytyrosol and its derivatives (e.g., oleuropein and tyrosol) provides the expected beneficial effects. It relates to the impact of olive phenolic compounds on the protection of blood lipids from oxidative stress [93]. Nonetheless, it is to be considered that EVOO comes from olives. Although few studies exist on this food, it seems to exert anti-inflammatory and antioxidant effects, decreasing IL-6 and the levels of malondialdehyde, the main product of the PUFA peroxidation, and important index of oxidative stress [94]. Fish is another traditional MeDi food, although only in seaside countries and not in the countryside. The main components with anti-inflammatory properties are the omega-3 fatty acids, in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), especially in blue fish. Its meat seems less prone to induce pro-inflammatory cytokine production during digestion compared to red meat, due to the difference in lipid composition. Moreover, fish seems to modulate inflammatory cytokine production as well, with a possible positive effect on autoimmune disorders. In addition, stearidonic acid found in fish is a better precursor of α -linoleic acid for the synthesis of EPA whose effects on human health have been recognised for several years [95].

Of note, the benefits of fish consumption exceed the potential risks of heavy metal exposure [96,97]. Nuts and seeds are recognized as healthy food in MeDi, as well. They are rich in MUFA, PUFA, fibers, and omega-3. Almonds, pumpkin seeds, pistachio, and walnuts are frequently consumed by people of the Mediterranean basin, as a snack or in many recipes [98,99]. Herbs and spices, for example, parsley, oregano, rosemary, thyme, cinnamon, chili, and sage, are used daily in the Mediterranean area, both in the seaside and countryside villages. Their abundance in phytochemicals such as flavonoids, anthocyanins, isoflavones, terpenes, and isothiocyanates, confer the antioxidant and anti-inflammatory properties to these foods [100]. It is also noteworthy the caper. It is a plant that grows wild in the Mediterranean basin that offers two edible options: the bud, the caper, and the fruit, the so-called “cucuncio”. In addition, in this case, anti-inflammatory and antioxidant properties were investigated, especially due to polyphenols [101]. In mice, caper fruit inhibits cytokine gene expression, including (IFN- γ , IL-17 and IL-4, probably due to saponins, flavonoids and alkaloids [102].

4. Ketogenic Diet

KD is a high-fat, low-carbohydrate diet that results in ketosis, elevations of fatty acids, serum levels, modulation of glycemia, and relative CR. In clinical practice, KD is an established treatment for drug-resistant epilepsy and the treatment of choice for Febrile Infection-Related Epilepsy Syndrome, a presumed inflammatory condition. KD may work by targeting “out of control” immune activation. There is a growing list of potential inflammatory pathway targets of KD including adenosine, ketone bodies, mechanistic target of rapamycin pathways, peroxisome proliferator-activated receptor-gamma (PPAR- γ), NLR Family Pyrin Domain Containing 3 (NLRP3) inflammasome, and gut microbiota [103]. KD is based on a drastic reduction in carbohydrates, associated with an increase in the proportion of proteins and fats. This condition pushes the body into ketosis, that is, into a metabolic state characterised by increased concentration of ketone bodies in the blood. Studies on mice with experimental EAE showed the beneficial effects of KDs on disease progression, disability, cognition and inflammatory markers, KDs could reduce the expression of enzymes involved in the biosynthesis of pro-inflammatory molecules [104,105].

4.1. Anti-Inflammatory Factors in the KD

Some features typical of KD could be responsible for its anti-inflammatory effects.

CR is a dietary restriction that drastically reduces energy intake without malnutrition [106]. It can modulate some inflammatory regulators such as NF- κ B inhibitor alpha (Nfkbia), tissue inhibitor of metalloproteinases-3 TNF- α , IL-6, COX-2, iNOS, VCAM-1, and ICAM-1 [107,108]. Thus, CR can regulate inflammation reducing the level of many pro-inflammatory mediators and pathways.

Omega-3 (n-3) and the omega-6 (n-6) are the two main groups of PUFAs. In nutrition, we recognize three types of omega-3 fatty acids in foods, which are alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). ALA consists of 18 carbon atoms while EPA and DHA are considered long carbon chains. These are essential fatty acids that we can only introduce through diet. ALA is a precursor of EPA and DHA but is capable of producing quantities of less than 15%. To increase the share of EPA and DHA, we can introduce them through diet. ALA is present in vegetable oils while EPA and DHA are represented in fish coming from the microalgae on which fish feed. DHA plays an important role as a component of cell membranes and is present in high concentrations in the retina and brain. EPA, on the other hand, plays an important role in the synthesis of eicosanoids and competes with arachidonic acid to produce prostaglandins (PG), thromboxanes (TX), and leukotrienes (LT). Higher concentrations of EPA than arachidonic acid drive the synthesis of eicosanoids with less inflammatory activity. Moreover, n-3 PUFA indirectly regulates transcription factors involved in the expression of inflammatory genes. In addition, n-3 PUFA might influence the composition of gut microbiota, enhancing the production of anti-inflammatory compounds [109]. n-6 PUFAs are found in a variety of animal

products and in vegetable oils, such as canola and corn oil. They produce pro-inflammatory effects and due to their sources, the Western diet provides an excess of n-6 PUFAs, compared to n-3. Thus, it can be considered pro-inflammatory. Not enough data are available to establish the recommended dose of omega 3, the IOM (National Academy of Medicine) has been able, however, to establish the adequate intake in a healthy population based on age (Institute of Medicine, Food and Nutrition Board). Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids (macronutrients). Washington, DC: National Academy Press; 2005). According to the European Food and Drug Authority, the total daily intake of DHA and EPA should not exceed 5 g per day [110]. Dietary reference intakes for energy, carbohydrate, fibre, fat, fatty acids, cholesterol, protein, and amino acids (macronutrients). Washington, DC: National Academy Press; 2005). The adequate values are 1.6 g of ALA per day for males (age >14 years) and 1.1 g of ALA per day for females (age >14 years)[110]. The KD increases the levels of specific PUFAs that can bind and activate the considered potential anticonvulsant drug targets [111].

The KD's anti-inflammatory properties could also be due to the variation of adenosine levels, as this molecule has anti-inflammatory activity [112]. It was demonstrated that adenosine can reduce systemic inflammation by modulating LPS-induced transmigration of polymorphonuclear cells and lowering pro-inflammatory mediators, such as TNF- α , IL-6, and CXCL2/3 [113]. Astrocytic adenosine kinase (ADK) can phosphorylate adenosine and lead it to clearance from the extracellular space. KD is able to lower the expression of ADK, consequently raising extracellular levels of adenosine and triggering the activation of inhibitory adenosine A1 receptor (A1AR). Most important, the effect of the KD on adenosine was associated with a decrease in electrographic seizure activity [114]. Both A1ARs and the adenosine A2 receptor are involved in inflammation, thus providing an opportunity for pharmacological intervention [115].

Ketone bodies, BHB and Acetoacetic Acid (AcAc), exert their neuroprotective role through the reduction of oxidative stress and ROS production by enhancing NADH oxidation and by inhibiting mitochondrial permeability transition. Ketone bodies seem to target inflammatory signaling cascade both by direct action on the inflammasome and ROS reduction [116]. Recently, the relevant role of microbiome in KD bioactive effects was also demonstrated. It can act in the antiseizure effect of the KD, using both an acute seizure model and an epilepsy mouse model. The gut microbiota transplantation after KD confers seizure protection in mice fed with a standard diet, with an increase of the GABA in the hippocampus [117].

4.2. KD and Neuroinflammation: The Role of NLRP3 Inflammasome

BHB, represents another anti-inflammatory mediator produced following the KD. It reduces nucleotide-binding oligomerization domain leucine-rich repetition and NLRP3 inflammasome-mediated activation of IL-1 β [118]. This could be one of the reasons to explain the anti-inflammatory effects of the KD.

It exerts antidepressant-like effects, possibly by inhibiting NLRP3-induced neuroinflammation in the hippocampus [119].

NLRP3 is a pattern recognition receptor of the innate immunity, belonging to the NOD-like receptor (NLR) subfamily. Together with the adaptor ASC protein PYCARD, it forms a protein complex able to activate caspase-1 and, consequently IL-1 β , initially translated as pro-IL-1 β . The inflammasome dysregulation has been implicated in different autoimmune diseases [120]. Recent studies show that the NLRP3 acts as a bridge between the innate and adaptive immune responses in the initial stages of MS and EAE by promoting the migration of macrophages, dendritic cells and myelin-specific autoreactive CD4⁺ T cells to the CNS [121,122]. Therefore, it can be considered a critical factor in the development of neuro-inflammation and an interesting therapeutic target in immune-related disorders [123].

5. Nutritional Suggestions to Develop a Dietary Protocol for MS Patients

Studies on mice with EAE showed beneficial effects of KD on disease progression, disability, cognition and inflammatory markers. The ketone bodies produced by KD are an alternative energy source for the brain and they are useful to reduce neuroinflammation possibly by inhibiting NLRP3. In addition, it seems ketone bodies are able to stimulate mitochondrial biogenesis and to reduce NLRP3 permeability by improving redox balance.

This section provides nutritional suggestions for developing a diet plan based on the KD that includes many foods from the MeDi, which are rich in polyphenolic compounds, to be applied to patients with MS type RR during the remission phases.

The nutritional plan, that could be developed, could have anti-inflammatory, immunomodulatory, and neuroprotective properties that could bring improvements on neuroinflammation and the redox state of the MS patient with benefits on the progression and course of the disease. Assuming that dietary intervention can (i) modulate the inflammatory state; (ii) protect against neurodegeneration; or (iii) promote nervous system repair [124], the choice of nutrients and their proportions is critical. This section pays particular attention to the importance for the MS patient to introduce macronutrients in the diet in the appropriate ratios. The effects of diet on MS may be the consequence of the direct action of metabolites produced by food or the effect of metabolites synthesized by the gut microflora or even diet-mediated changes in gut microbial composition.

The nutrition plan should include a classical KD protocol which is modified in some aspects. It could involve two steps: (1) an adaption phase and (2) a maintenance phase. The adaptation phase is divided into two periods: the first will last four weeks during which patients will be instructed to limit the intake of carbohydrate to 20 g/day, in order to establish the ketosis condition. In the second period of the maintenance phase, lasting one month, the patient will be asked to increase carbohydrate intake by 5 g each week until the maximum level of 40 g per day is reached. The carbohydrates used will be with glycemic index and glycemic load below 50 and six respectively to maintain an adequate state of ketosis and constant levels of blood sugar and insulin.

The maintenance phase lasts one month and is characterized by a constant state of nutritional ketosis (Figure 1). The ketone body levels will be maintained between 0.5 and 3 mM.

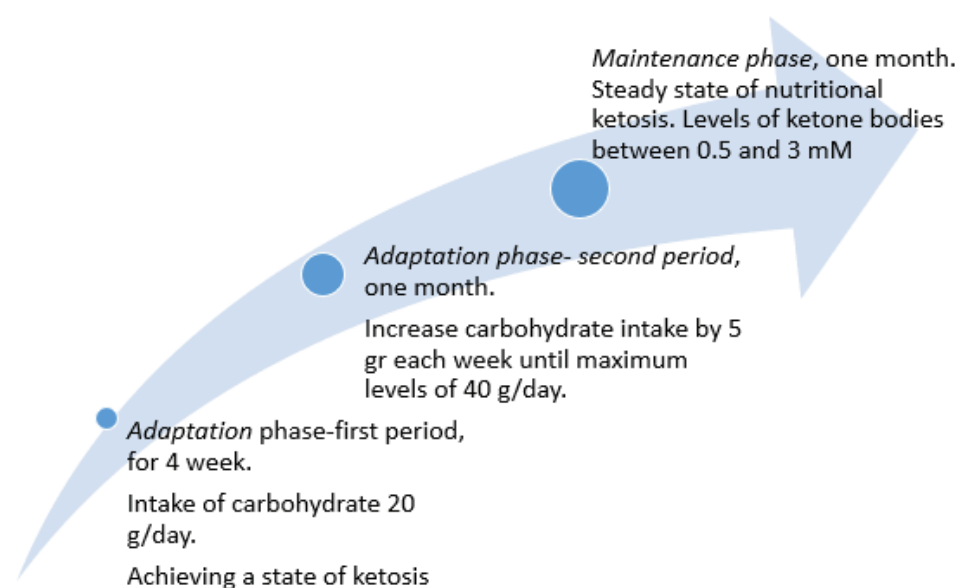


Figure 1. Trend of daily carbohydrate intake in the protocol to be developed to achieve proper levels of ketosis.

A similar nutritional approach was applied in a clinical trial and achieved good compliance in patients with MS. In this study the authors concluded that the KD diet is a

safe and inexpensive complementary treatment option for MS. The implementation of the protocol is based on the international guidelines of ketogenic protocols [125], providing a caloric deficit that can vary from 300 to 500 Kcal depending on the BMI. It is also expected to have a daily intake of water equal to 0.4 mL per kg of body weight.

5.1. Macronutrients

Macronutrients will be divided into approximately 5–10% carbohydrates; 15–20% proteins; 70–80% fats (compared to a traditional KD characterized by 90% fat, 6% protein, and 4% carbohydrates).

Considering the effects of KD on the state of ketosis, on the lipid profile, and on the glycemic profile, taking into account the protective effect of BHB on neuroinflammation previously mentioned, it becomes important to pay attention to the fatty acid composition.

The percentage of SFA and PUFA in the KD influences ketosis status, lipid and glycemic profile. Short-term administration of a KD richer in PUFAs (15% SFA, 25% MUFA, 60% PUFA) induces a greater level of ketosis and improves insulin sensitivity without negatively affecting total or LDL cholesterol levels compared with a traditional KD high in saturated fats (60% SFA, 25% MUFA, 15% PUFA) [125]. A prospective study in pediatric MS has suggested that the increase in energy intake from saturated fat tripled the risk of relapse rate [126]. On the contrary, animal studies have found that PUFAs, especially alpha linolenic acid (ALA) and EPA and DHA have a beneficial effects on EAE by reducing the production of inflammatory cytokines and inducing PPAR in CNS infiltrating T cells [125,127]. In addition to immunomodulatory effects, PUFA's prevent demyelination and promote neuroprotection and remyelination [124]. For the reasons outlined above, the protocol suggested will provide high concentrations of PUFAs with a qualitative distribution of fatty acids as follows: 15% SFA, 25% MUFA, 60% PUFA, the ratio between omega-3 and omega-6 will be 1:4 [128].

In a diet, the intake of proteins must be strictly controlled and animal proteins must be limited because of their proven pro-inflammatory effects, precisely for this reason Hernández et al. proposed a ratio between vegetable and animal proteins of 70 to 30. Data in the literature show that increased consumption of plant protein may be associated with a reduced risk of CVDs [129], type 2 diabetes, and inflammation [130,131]. Recently in the eighth edition of the Dietary Guidelines for American it was suggested to shift the ratio towards proteins of vegetable origin [132]. Regarding the amount of protein to be administered, it was demonstrated that a slightly higher-protein diet was able to both positively influence body weight regulation and reduce insulin resistance. This aspect becomes important if we consider that insulin resistance as well as other parameters of the MetS, which affects 30% of MS patients, have negative effects on the progression of the disease [130].

In addition, a recent meta-analysis of 32 randomized controlled trials showed a long-term positive effect of higher-protein diets on body weight management, which in turn could lead to lower glycated hemoglobin (HbA1c) [133]. Another recent study showed that a protein score with a higher protein energy percentage (E%) within the acceptable macronutrient distribution range for protein in combination with a higher plant to animal protein ratio, would be associated with a lower HbA1c level [134].

The daily protein intake provided should be between 0.9 and 1.2 g/Kg of body weight and will be characterized by a high ratio of plant to animal protein.

In brief, Figure 2 shows the qualitative and quantitative nutritional distribution suggested for the protocol to be developed.

Carbohydrates 5–10%	Protein 15–20%	Lipids 70–80%
•Glycemic Index, below 50 •Glycemic load, below 6	•0,9–1,2 g/Kg body weight •High ratio of plant to animal protein	•15% SFA, 25% MUFA, 60% PUFA •$\Omega 3/\Omega 6=1/4$

Figure 2. Qualitative and quantitative daily distribution of macronutrients into the future protocol proposed to be applied to patients with MS.

When selecting foods for inclusion into the future protocol, particular attention will be paid to those rich in tryptophan in order to increase the Trp/Kyn ratio.

Therefore, taking into consideration that the recommended daily intake for an adult is estimated to be around 250–425 mg, corresponding to 3.5–6.0 mg/Kg/day, foods included in the modified MeDi and that we suggest to include in the protocol are: eggs which have an average content of (197 mg/100 g) and in particular the yolk which has a content of 237 mg/100 g; fish, with particular reference to sea bass (249 mg/100 g), tuna (237 mg/100 g), sea bream (259 mg/100 g), sardine (250 mg/100 g), sole (220 mg/100 g). Among meats, chicken (240 mg/100 g) and turkey rump (333 mg/100 g). Other recommended foods include almonds (394 mg/100 g), pine nuts (300 mg/100 g), cashews (240 mg/100 g), flaxseed (298 mg/100 g), sesame seeds, pumpkin seeds (Tabella di composizione degli alimenti, aggiornamento 2000, CEd EDRA). In our modified MeDi food suggestions, dark chocolate can also be added, despite not being part of the typical MeDi protocol, because it is particularly rich in Trp. The aforementioned foods will supplement those in Table 1 so that you could have a synergistic effect with the ketogenic diet on the antioxidant and anti-inflammatory properties.

Table 1. Foods to be included in the nutritional suggestions and their nutraceutical compounds with anti-inflammatory and antioxidant properties.

Food	Main Anti-Inflammatory/Antioxidant Components	Reference
Pistachio nut	Proanthocyanidins	[99]
EVOO/olive	Polyphenols (e.g., oleocanthal, hydroxytyrosol, tyrosol, oleuropein).	[88]
Almonds	Vitamin E, MUFA, PUFA	[88,98]
Capers	Phenolic compounds (e.g., rutin), tocopherols (e.g., gamma-tocopherol), carotenoids (e.g., lutein and beta-carotene), vitamin C.	[101]
Cinnamon	E-cinnamaldehyde, o-methoxycinnamaldehyde	[135]
Turmeric	Curcumin	[136,137]
Green tea	Epigallocatechin 3-gallate (EGCG)	[138]

Legend: EVOO: extra virgin olive oil. MUFA: monounsaturated fatty acid. PUFA: polyunsaturated fatty acid.

5.2. Bowel Dysfunction in MS and Fiber Nutritional Suggestions

The protocol will need to pay special attention to the contribution of fiber. A patient with MS has a high prevalence of intestinal dysfunction whose origin is multifactorial, in fact it may depend on neurological alterations, polypharmacy, behavioral problems or motor skills. Constipation and fecal incontinence can coexist and alternate, impacting the patient's quality of life and social interactions [139,140]. The percentage of patients with neurological bowel dysfunction varies from 39 to 73% and the bowel symptoms seem to be correlated to the Expanded Disability Status Scale (EDSS) and disease duration, but not with the type of MS [141]. However, it is not said that patients with a mild degree of malignancy do not present intestinal disorders; on the contrary, it seems that constipation may be a symptom which MS manifests itself with [142]. The regulation of stool consistency

is important and can be modulated by the intake of both water and fiber. The management of the fiber amount (and laxative as well) is essential because on the one hand they need the formation of soft stools, preventing incontinence and constipation. On the other hand, the excess of both can cause bloating, in the presence of pan-gut dysmotility. Scant evidence exists on the use of laxatives, mostly from studies of neurological conditions or idiopathic bowel symptoms.

KDs are typically low in fiber which is needed not only for healthful intestinal function but also for the microbial production of beneficial colonic short-chain fatty acids [143], which enhance nutrient absorption, stimulate the release of satiety hormones, improve immune function, and have anti-inflammatory and anti-carcinogenic effects [144]. It has been suggested that the supplementation of KD with fiber and non-digestible carbohydrates might be advisable although data to confirm that supplementation could counteract the effects of very-low-carbohydrate diets on the gut microbiota are lacking [145].

Given the difficulty of achieving the appropriate amount of fiber through a KD protocol and the intestinal dysfunction that can be seen in the MS patient, it is planned to supplement our suggestions with natural substances with a laxative function to help intestinal function.

Laxative agents that can be considered include: (i) psyllium that is a bulking agent which might work for slow-transit constipation as shown in patients with Parkinson's disease (PD) [146]; (ii) polyethylene glycol equally acts as an osmotic agent but does not rely on bacterial fermentation for its activation. The advantage is that it can be quite effective, and the dose can be adjusted within a wide range according to the patient's need. In patients with PD, it has been proven to relieve constipation [147]; (iii) a stimulant laxative such as bisacodyl has been shown to be very effective in patients with chronic idiopathic constipation [148].

According to recent work, these laxatives may be effective in MS patients [139].

5.3. Micronutrients

A critical aspect of KD that must be kept under control regards micronutrients, vitamins and minerals which can easily become defective. Extreme carbohydrate restriction can profoundly affect diet quality, typically curtailing or eliminating fruits, vegetables, whole grains, and legumes and increasing the consumption of animal products. Very-low-carbohydrate diets may lack vitamins, minerals, fiber, and phytochemicals found in fruits, vegetables, and whole grains [149]. Low-carbohydrate diets are often low in thiamin, folate, vitamin A, vitamin E, vitamin B6, calcium, magnesium, iron, and potassium [150]. Physiologically, it has been shown that the deficiency of certain vitamins and minerals affects energy production and results in physical and mental fatigue and impaired cognitive functions, consequences that aggravate the clinical symptoms of MS [151].

Other suggestions include strict control of the intake of vitamins and minerals playing a key role in controlling inflammation and whose deficiency is related to a worse prognosis of the disease [151]. Given the deficiency of some micronutrients associated with KDs, in Table 2 are listed the micronutrients that will be added in the form of supplements in the recommended daily amounts.

Table 2. Suggestions of micronutrients to include in the protocol, amounts that have shown effects on fatigue and cognitive function and recommended intake and tolerance levels for a healthy population.

Micronutrients	Effect of Supplementation with Vitamins and Minerals on Mental Fatigue and Cognitive Functions	RDA, AI, or UL in Healthy Subjects According to LARN
Vitamin D	Dose > 100 µg/die Effect = toxicity [152,153] Serum concentrations >150 ng/mL Effect = clinical condition of vitamin D toxicity characterized by hypercalcemia and hypercalciuria) [154]	RDA = 15 µg/die (adult 18–74 yo) RDA = 20 µg/die (>74 yo) UL = 100 µg/die [155]

Table 2. Cont.

Micronutrients	Effect of Supplementation with Vitamins and Minerals on Mental Fatigue and Cognitive Functions	RDA, AI, or UL in Healthy Subjects According to LARN
Thiamine (B1)	Dose = 50 mg/die for 2 months (120 young women) Effect = improvement of attention threshold and mood [156]	RDA = 1.2 mg/die (men > 18 yo) RDA = 1.1 mg/die (women > 18 years) UL not defined [155]
Niacin (B3)	Dose = 250 mg/die Effect = modulation of NIACR1 expression on peripheral immune cells by improving sleep spectrum disorders in Parkinson's disease [157]	RDA = 18 mg/die UL (Nicotinamide) = 10 mg/die UL (Nicotinic acid) = 900 mg/die [155]
Vitamin C	Dose > 2000 mg/die Effect = diarrhea or kidney damage [152,153]	RDA = 105 mg/die (men > 18 yo) RDA = 85 mg/die (women > 18 yo) UL not defined [155]
Pyridoxine (B6)	Dose > 1000 mg/die Effect = might mimic MS symptoms Dose < 50 mg/die Effect = nervous symptoms [152,153]	RDA = 1.3 mg/die (18–29 years) RDA = 1.7 mg/die (men) RDA = 1.5 mg/die (women, 60–74 yo) UL = 25 mg/die [155]
Vitamin E	Dose > 1500 IU/die Effect = possible toxicity	AI = 13 mg α -TE (men > 18 yo) AI = 12 mg α -TE (women > 18 yo) UL = 300 mg α -TE [155]
Folic acid (B9)	Dose = 1000IU of of alpha-tocopherol twice daily for three years Effect = No significant effect cognitive function, speed of processing, clinical global impression, functional performance, adverse events, or mortality [158] Dose = 800 μ g/day for 3 years Effect = improvements of global cognitive functions, information-processing speed and memory storage [159] Dose = 400 μ g/day for 2 years (+100 μ g/day of B12) Effect = improvement of cognitive functions particularly long- and short-term memory [160]	RDA = 400 μ g/die UL = 1000 μ g/die [155]
Vitamin B12	Dose = 400 μ g/day for 2 years (180 subjects with mild cognitive impairment) Effect = improved cognitive performance (full scale and verbal intelligence, memory) [160]	RDA = 2,4 μ g/die (men and women) UL not defined [155]
Calcium		RDA = 1000 mg/die (18–59 yo) RDA = 1200 mg/die ($\geq$ 60 yo) UL = 2500 mg/die [155]
Zinc	Dose = 15.30 mg/die (387 healthy adults 55–87 yo) Effects = better on spatial working memory [161]	RDA = 12 mg/die (men) RDA = 9 mg/die (women) UL = 25 mg/die [155]
Iron	Dose = 60 mg/die for 4 months (149 iron-deficient American women) Effect = 5-fold improvement in cognitive performance [162]	RDA = 10 mg/die (men $\geq$ 18 yo) RDA = 18 mg/die (women 18–49 yo) RDA = 10 mg/die (women > 50 yo) [155]
Magnesium	Dose = 20 mg/day Effect = reduced risk of depression [163]	AI = 2.7 mg/die (men > 18 yo) AI = 2.3 mg/die (adult women > 18 yo) UL not defined [155]

Legend. RDA, Recommended Daily Allowance; UL, Tolerable Upper intake Level; AI, Adequate Intake; LARN, Nutrients and Energy for Italian population; yo, years old; NIACR1, Niacin receptor 1; α -TE, Tocopherol Equivalent.

6. Conclusions

The present review explored putative biomarkers implicated in MS-related alterations such as the role of BDNF and the Tryptophan/Kynurein ratio on cognitive deficits and

eventual neuroprotection. Furthermore, we described the effects on the immune system, neuroinflammation and redox balance of both the modified MeDi and the KD regimen in order to develop a potential dietary protocol that could be applied to MS patients. We think that this nutritional approach may exert an enhanced effect compared to the individual dietary regimens on which it is based. The encouraging preclinical data on KD in MS disease and the result of the recent clinical trial on KD conducted in MS patients confirm and support our idea.

The application of the protocol and the possible confirmation of its effectiveness could be useful to formulate guidelines on proper nutrition for MS patients.

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