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RESEARCH PAPER

The OMNIVEG STUDY: Health outcomes of shifting from a traditional to a vegan Mediterranean diet in healthy men. A controlled crossover trial

Miguel López-Moreno [a,b], Ujué Fresán [c], Juan Del Coso [d], Millán Aguilar-Navarro [e], María Teresa Iglesias López [f], Javier Pena-Fernández [e], Alejandro Muñoz [e,*], Jorge Gutiérrez-Hellín [e]

[a] Diet, Planetary Health and Performance, Faculty of Health Sciences, Universidad Francisco de Vitoria, 28223, Pozuelo, Spain
[b] School of Physiotherapy, Faculty of Health Sciences, Universidad Francisco de Vitoria, 28223, Pozuelo, Spain
[c] ISGlobal, 08024, Barcelona, Spain
[d] Sport Sciences Research Centre, Rey Juan Carlos University, 28943, Madrid, Spain
[e] Exercise and Sport Science, Faculty of Health Sciences, Universidad Francisco de Vitoria, 28223, Pozuelo, Spain
[f] Wellness and Health, Universidad Francisco de Vitoria, 28223, Pozuelo, Spain

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Abstract *Background and aim:* The Mediterranean diet is a plant-based dietary pattern with well-established health benefits such as the reduced risk of cardiovascular disease. Additionally, incorporating more plant-based foods into a Mediterranean diet may provide further health benefits. The study aimed to assess the effect of shifting from a traditional Mediterranean diet to a vegan Mediterranean diet on cardiorespiratory fitness and lipid profile in physically active and healthy men.

Methods and Results: Participants underwent a baseline period with adhesion to the general patterns of the Mediterranean diet for three weeks and then they changed to an isocaloric vegan version of the Mediterranean diet for four weeks, with a 7-day washout period between diets. The shift from the traditional Mediterranean diet to the vegan Mediterranean diet required substituting animal-based foods with plant-based foods that contain comparable amounts of protein and fat. Fourteen participants with a mean age of 24.6 ± 7.0 years (range: 18–37 years), completed the study protocol. The change from the traditional to the vegan Mediterranean diet reduced blood concentration of total cholesterol (−22.6 mg/dl, $p < 0.01$, Effect size [ES] = 1.07) and low-density lipoprotein cholesterol (−12.8 mg/dl, $p < 0.01$, ES = 0.72). An inverse correlation was observed between the intake of dietary fibre and LDL-C (partial rho = −0.43, p = 0.040).

Conclusions: The adoption of a vegan Mediterranean diet with plant-based proteins and fats instead of the traditional Mediterranean diet improved several cardiometabolic health outcomes in physically active and healthy men.

Clinical Trial Registry: NCT06008886.

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* Corresponding author.
E-mail address: Alejandro.munoz@ufv.es (A. Muñoz).

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1. Introduction

Plant-based diets emphasize the consumption of foods from plant sources (fruits, grains, legumes, roots and seeds, among others) instead of animal sources (meat and poultry, fish and dairy products). Among the most popular plant-based diets worldwide is the Mediterranean diet, a variety of culinary traditions common in countries of the Mediterranean Sea. The term "Mediterranean diet" was first introduced in research by Ancel Keys, based on the dietary patterns observed in southern Italy during the 1950s. This definition was formalized in 1993 at the International Conference on the Diets of the Mediterranean, describing a diet abundant in plant-based foods, using olive oil as the main fat source, and including low to moderate amounts of meat, dairy products, eggs, and wine [1]. Epidemiologic studies have confirmed that those who adhere to the Mediterranean diet obtain several health benefits such as reduced risks of cardiovascular disease [2], type 2 diabetes [3], and certain types of cancer [4]. The Mediterranean diet has been identified as one of the recommended dietary patterns for the promotion of cardiovascular health in the main dietary guidelines [5,6].

The PREDIMED (Prevención con Dieta Mediterránea) study reported that among participants who followed the Mediterranean diet, those who consumed more plant-based foods had a lower risk of all-cause mortality [7]. These data suggest that, within the general pattern of the traditional Mediterranean diet, the adoption of a provegetarian food pattern may result in even greater health benefits. In this regard, it has been found that a low-fat vegan diet may exceed the benefits of the Mediterranean diet on body weight, lipid concentrations, and insulin sensitivity [8]. A review of observational and interventional studies using plant-based diets revealed that a high proportionof whole plant-based foods is linked to an improvement in different cardiometabolic risk factors, such as blood pressure, lipid and inflammatory profile [9]. Similarly, isocaloric substitution studies have shown that replacing the consumption of red meat protein or processed meat protein with plant sources of protein (e.g., legumes and nuts) reduces the risk of cardiovascular disease [10,11].

Collectively, this evidence suggests that a provegan Mediterranean diet, that maintains the general patterns of the traditional Mediterranean diet but substitutes the animal sources of protein and fat contained in fish, poultry, red meat and dairy products with plant-based protein and fat may increase the cardiovascular protection of this diet pattern. However, although the evidence favouring the health benefits of vegetarian diets is wide, it has not been established if adopting a vegan Mediterranean diet by the substitution of the animal sources of protein and fat of the traditional Mediterranean diet by plant-based foods can improve cardiometabolic and inflammatory parameters in healthy an active individual. For this reason, the primary objective of this study was to assess the effect of shifting from a traditional Mediterranean diet to a vegan Mediterranean diet on cardiorespiratory fitness and lipid profile in physically active and healthy men. Similarly, the secondary objectives were to evaluate the impact on blood pressure and immune status.

2. Methods

2.1. The OMNIVEG study

The OMNIVEG study was a controlled crossover trial conducted between June and August 2023 in Madrid, Spain. Participants underwent a baseline period with adhesion to the general patterns of the Mediterranean diet for three weeks. After this period, they changed to an isocaloric vegan version of the Mediterranean diet for four weeks, with a 7-day washout period between diets. Both, the traditional and vegan Mediterranean diets were tailored and designed according to the food preferences of each participant and to maintain the same macronutrient distribution. The modification from the traditional to the vegan Mediterranean consisted simply of the substitution of animal-based foods containing protein and fat for plant-based foods with comparable amounts of protein and fat. Just after the baseline period with the traditional Mediterranean diet and after the end of the vegan version of the Mediterranean diet, maximal oxygen uptake (VO_2max), body mass and composition, blood pressure and blood lipid and immune profiles were measured (Fig. 2). Participants were instructed to maintain their physical exercise routines throughout the study period which was confirmed by training diaries. To ensure standardization of the measurements, all tests were completed at the same laboratory, using the same testing devices, and handled by the same researchers.

2.2. Participants

The sample size was estimated with the G *Power® software (3.1.9.7; Heinrich Heine University of Düsserdorf, Germany), assuming a medium effect size for the primary variables, statistical power of 80% and an α error probability of 0.05, resulting in a sample size of 14 participants. To achieve the sample size required, taking into account potential dropouts, a total of 17 participants were initially recruited, although only 14 participants completed the whole experiment. Participants were recruited through flyers located on the university campus (Universidad Francisco de Vitoria, Madrid) and through social media advertising. Because the effects of dietary intervention on cardiometabolic health determinants appear to differ by sex, limiting the study to a male population helped to reduce the variability due to these gender differences, allowing a clearer and more specific interpretation of the effects of dietary intervention on cardiometabolic health in men [12]. Inclusion criteria were: men, aged between 18 and 40 years, physically active (moderate-intensity exercise 3–5 days per week) according to the recommendations of the World Health Organization (WHO), with a body mass index (BMI) between 18.5 and 24.9 kg/m^2, no tobacco use, no or low alcohol consumption and no

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orthopaedic limitations. Exclusion criteria were: any type of chronic cardiovascular, metabolic, gastrointestinal, respiratory and musculoskeletal disease or serious musculoskeletal injuries within the prior six months (Fig. 1). Information to screen participants who fulfilled these criteria was obtained by an *ad hoc* pre-participation questionnaire and screening, which included a collection of medical and physical exercise histories. Before enrolment in the study, potential participants received comprehensive information about the associated risks and potential discomforts of the research protocol. Subsequently, they provided written informed consent to participate in the study voluntarily. The study protocol and design were approved by the Ethics Committee of the University Francisco de Vitoria (20/2023), and it fully complied with the 1964 Helsinki Declaration and its subsequent modifications (last update 2013). The trial was registered on ClinicalTrials.gov (ID: NCT06008886).

2.3. Diet modification to change from a traditional to a vegan Mediterranean diet

Before the onset of the study, participants went to the laboratory for an initial dietary evaluation conducted by a

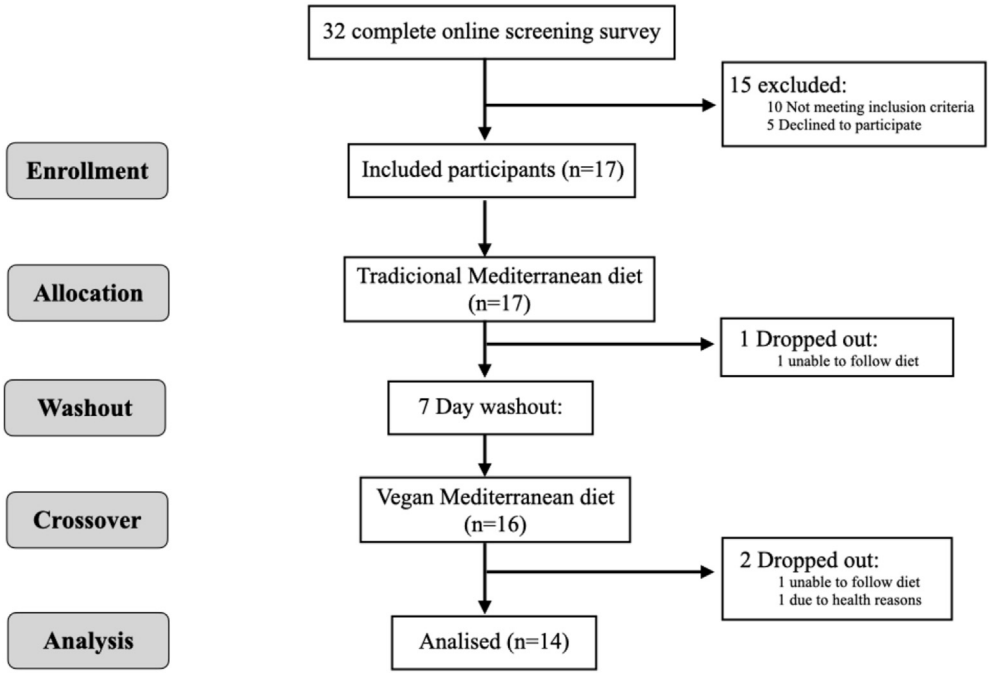


Figure 1 Flow diagram of participants through the study.

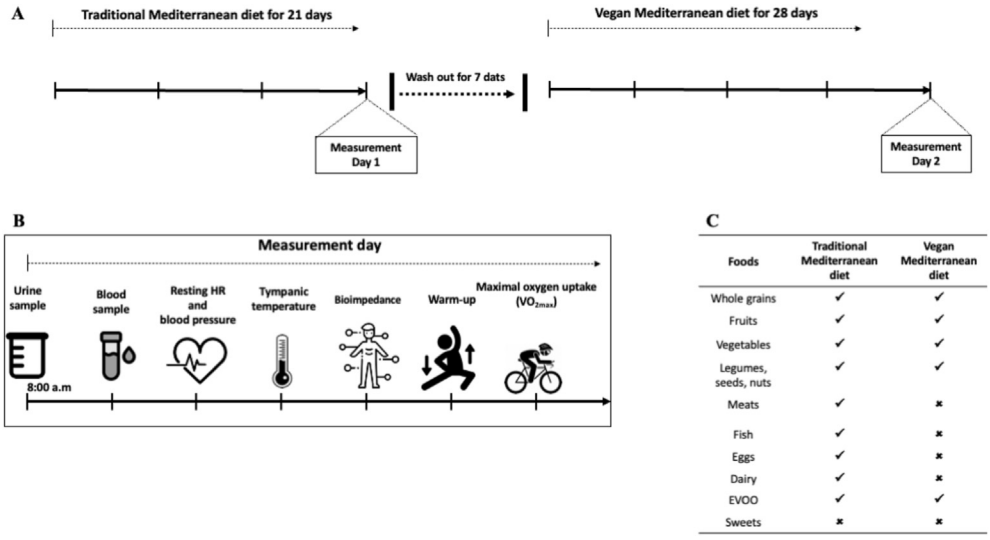


Figure 2 Experimental design of the study (A), assessment obtained on each measurement day (B) and foods included in the traditional and vegan versions of the Mediterranean diets of the study.

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registered dietitian to assess body characteristics and main dietary patterns. Participant's total daily energy expenditure was estimated using the Harris-Benedict equation to determine the basal metabolic rate multiplied by 1.55 to compensate for the moderate physical activity. With this data, a normocaloric traditional Mediterranean diet was designed to contain an abundant intake of whole plant-based foods with moderate to low consumption of fish, poultry, low-fat dairy products and eggs, very low consumption of red and processed meats and no sweets (Supplemental material Table S1). Olive oil was the main added fat and animal protein accounted for 60% of total protein intake [13]. Participants implemented the Mediterranean diet for 3 weeks and deviations in the foods recommended by the dietitian were noted and analysed. For this analysis, the dietary intake throughout the intervention was monitored using three 24-h dietary recalls weekly (2 non-consecutive weekdays and one weekend day) and participants registered in these dietary recalls the foods consumed, weights and/or household measurements, cooking method and timing of intake. Food consumption data were analysed through a specific software (Dietopro, Valencia, Spain) [14]. In the case that the deviation in the diet affected the main pattern of the traditional Mediterranean diet for more than one day, the participant was discarded from the analysis (n = 1).

After a 7-day washout period, participants adopted a vegan version of the same Mediterranean diet for four weeks. This vegan diet was isocaloric concerning the previous diet and maintained the macronutrient distribution. Overall, the vegan diet was mainly composed of whole plant-based foods as the previous diet, but the animal sources of protein and fat were substituted by plant foods rich in protein and fat. During the vegan diet, the participants were supplemented with 1000 μg of cyanocobalamin (vitamin B12) twice a week (Harrison Sports Nutrition, Granada, Spain) to compensate for the deficiency in the intake of this vitamin induced by the vegan diet [15]. The same methods described to note and analyse deviations in the foods recommended by the dietitian described above were applied to the vegan diet. In the case that the deviation in the diet affected the main pattern of the vegan Mediterranean diet for more than one day, the participant was discarded from the analysis (n = 1). For any of the diet intervention protocols, participants had continuous feedback from the researchers to resolve any questions and to ensure proper adherence.

2.4. Experimental procedures in the measurement days

One day after the baseline period of the traditional Mediterranean diet and one day after the vegan version of the Mediterranean diet, participants underwent a battery of measurements to assess their cardiorespiratory fitness, cardiometabolic health and immune status. For these measurements, participants were told that they had to abstain from vigorous exercise and maintain the diet and fluid intake regimen as recommended by the dietitians for 24 h before testing. Participants were also instructed to avoid the consumption of *p*-synephrine, orange juice, citrus-fruit derivatives, alcohol, caffeine, and other stimulants within the 24 h preceding each test. Compliance with these standardized procedures was confirmed using food and training diaries. On each measurement day after completing each dietary intervention, participants arrived at the laboratory at 08:00 a.m., having fasted for a minimum of 8 h. Upon participants' arrival, they were instructed to urinate, and their euhydration status was confirmed by assessing urine specific gravity, with a threshold of <1.020 [16]. After ensuring all the necessary standardizations were met, participants rested supine for 10 min a capillary blood sample was withdrawn from a fingertip for the measurement of several blood parameters as explained in the next section. Then, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured with a digital sphygmomanometer (Omron, Japan) in triplicate. The mean blood pressure (MAP) of each participant was calculated according to the equation: $MAP = DBP + 1/3(SBP - DBP)$. The resting heart rate was recorded for 2 min using the Wearlink + V800 device (Polar, Finland). Subsequently, the participant's tympanic temperature was measured at rest, also in triplicate, following the removal of any earwax from the left auditory canal. An infrared tympanic thermometer (Thermoscan 7 IRT6520, Braun, Germany) was utilized for this measurement. Then, participants underwent a full anthropometric analysis by bioimpedance. After the resting measurements, participants were dressed in a T-shirt and shorts and a heart rate belt (H10, Polar, Finland) was attached to their chest to record heart rate during exercise. Participants sat on a cycle ergometer (Ergoselect 4, Ergoline, Germany) and performed an 8-min warm-up at an intensity that elicited 45% of their age-predicted maximum heart rate, followed by joint mobility work. Then, participants underwent a ramp exercise test until reaching voluntary fatigue to assess their maximal oxygen consumption (VO2 max). Throughout the test, the workload was incrementally increased by 25 W/min, while oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were continuously monitored using a breath-by-breath analyser (Ergostik, Geratherm Respiratory, Germany) calibrated with certified gases and a syringe before each test. The VO2 max was defined as the highest recorded value of VO_2 attained during the test. The ramp exercise test was considered maximal and valid when the following criteria for VO_{2max} were met after the test: stable VO_2 despite increasing power output, a respiratory exchange ratio exceeding 1.10, a rating of perceived exertion (measured using the 6-to-20-point Borg scale) surpassing 19 points, and a peak heart rate surpassing 80% of the age-adjusted estimate of maximum heart rate [17]. Additionally, the peak cycling power obtained during the test was recorded (Wmax).

2.4.1. Analytical procedure

A portion of the capillary blood samples was initially used to measure blood glucose concentration with a glucometer (Accu Check Advantage®, Roche). Afterwards, the concentrations of total cholesterol (TC), LDL-C, HDL-C,

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triglycerides (TG) and haemoglobin (Hb) were measured with a POC multi-parameter lux meter (Biochemical Systems International, Italy). The atherogenic index was calculated as the logarithm of TG to HDL-cholesterol concentration [18]. 25-Hydroxyvitamin D (25-(OH)D) concentration was measured by immunochromatography with a portable analyser (Microcaya, Spain). Monocyte, lymphocyte, eosinophils and neutrophil concentrations were measured with the HemoCue® WBC DIFF system (HemoCue AB, Ängelholm, Sweden). The neutrophile-lymphocyte ratio (NLR) was calculated as a measure of immune status [19]. The monocyte-to-high-density lipoprotein-cholesterol ratio (MHR) was calculated as a measurement of the inflammatory status of the participant as an inflammatory marker [20].

2.4.2. Anthropometric measurements

The anthropometric measurement with bioimpedance was taken following the standards described previously [21]. Body mass, fat mass, body fat percentage, visceral fat index, lean mass and fat-free mass (FFM) were measured by bioelectrical impedance analysis (Inbody Co., Ltd., Seoul, Korea) using manufacturer predictive equations. Body height was measured with the participant standing fully erect, feet together, head in the Frankfort plane and arms hanging freely to the nearest millimetre (0.1 cm). BMI was calculated using the formula weight (kg)/[height $(m)^2$]. Anthropometric data were the result of the mean of a minimum of three repeated measurements.

2.5. Statistical analysis

Continuous variables were expressed as the mean and the standard deviation (SD) for the whole group of participants. Normal distribution of the dataset for each variable was confirmed using a Shapiro-Wilk test and parametric statistics were employed as all variables presented a normal distribution. Student's t-test for paired samples was used to compare dietary intake data, anthropometric variables, cardiovascular fitness variables, cardiometabolic health, immune status after the traditional and the vegan versions Mediterranean diet. Partial correlation coefficients were calculated for dietetics factors and biomarker cardiometabolic values adjusted for age, BMI, lean mass, FFM and energy intake per day. The effect size (ES) was assessed with Cohen's *d* and the following criteria were established: trivial (0−0.19), small (0.20−0.49), medium (0.50−0.79) and large (≥0.80) [22]. The significance level was set at $p \leq 0.050$. The statistical analysis was conducted using the IBM Statistical Package for Social Sciences (SPSS) version 22.0 (IBM, Chicago, IL, USA).

3. Results

3.1. Participants

Fig. 1 depicts the flow diagram of the participants. Thirty-two participants were initially assessed for eligibility. Ten did not meet the inclusion criteria and, five declined to participate in the study after the initial interview. Seventeen participants started the intervention protocols. One participant withdrew during the traditional Mediterranean diet intervention due to difficulties in adhering to the dietary guidelines. During the vegan version of the Mediterranean diet, two participants dropped out of the study (one due to lack of time to cook the prescribed meals and one due to mild health issues unrelated to the research protocols). Therefore, a final sample of 14 participants with a mean age of 24.6 ± 7.0 years (range 18−37 years) completed satisfactorily all research protocols.

3.2. Dietary assessments

Both interventions complied with the targeted dietary recommendations regarding total energy intake and macronutrient distribution (Table 1). In the vegan phase, participants consumed more legumes, grains, nuts, seeds, and plant-based meat alternatives$p < 0.05$). Conversely, higher intakes of animal protein foods, including dairy products, eggs, fish, white meat, red meat, and processed meat, were reported in the traditional Mediterranean diet ($p < 0.05$) (Supplemental material Table S2). No differences in carbohydrate, protein and dietary fat intakes were observed between both groups. As expected, dietary fibre intake was higher in the vegan version compared to the traditional version of the Mediterranean diet ($p < 0.01$).

Table 1 Dietary characteristics of the traditional and vegan versions of the Mediterranean diet.

	Traditional Mediterranean diet	Vegan Mediterranean diet	*p*-value	ES
Energy, kcal/day	2599.6 (180.8)	2634.9 (148.3)	0.14	0.25
CHO, g/day	311.3 (29.7)	320.8 (52.4)	0.39	0.22
CHO, % of energy	47.9 (3.3)	48.9 (5.1)	0.17	0.23
Dietary fibre, g/day	30.7 (2.8)	41.1 (4.9)	<**0.01**	**2.61**
Protein, g/kg/day	1.60 (0.1)	1.56 (0.1)	0.239	0.40
Protein, g/day	120.2 (5.1)	115.3 (4.8)	<**0.01**	**0.99**
Protein, % of energy	18.5 (1.5)	17.5 (0.9)	<**0.01**	**0.81**
Animal protein, % of protein	62.9 (8.2)	0 (0.0)	<**0.01**	**10.8**
Fat, g/day	97.0 (17.8)	99.0 (13.2)	0.62	0.13
Fat, % of energy	33.6 (5.2)	33.8 (2.1)	0.90	0.05

Data are presented as mean (standard deviation). CHO = Carbohydrates, ES = Effect size.

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Table 2 Anthropometric characteristics of the participants after the baseline period with the traditional Mediterranean diet and after the period with the vegan version of the Mediterranean diet.

	Traditional Mediterranean diet	Vegan Mediterranean diet	*p*-value	ES
Weight, kg	74.9 (8.9)	74.0 (8.4)	0.54	0.11
BMI, kg/m^2	23.5 (1.6)	23.1 (1.7)	0.54	0.26
Fat body, %	15.8 (3.8)	15.1 (4.1)	0.16	0.20
Mass fat, kg	11.9 (3.8)	11.1 (3.5)	0.13	0.22
Visceral fat,	3.2 (1.9)	2.9 (1.9)	0.10	0.15
Lean mass, kg	59.9 (6.1)	59.6 (6.1)	0.07	0.09
Fat-free mass, kg	63.0 (6.4)	62.9 (6.5)	0.09	0.06

Data are presented as mean (standard deviation). ES = Effect size.

Table 3 Physiological variables during a maximal graded cycling test after the baseline period with the traditional Mediterranean diet and after the period with the vegan version of the Mediterranean diet.

	Traditional Mediterranean diet	Vegan Mediterranean diet	*p*-value	ES
VO_2max, ml/kg/min	43.7 (10.1)	44.4 (6.5)	0.68	0.07
VO_2max, l/min	3.4 (0.7)	3.3 (0.6)	0.54	0.15
Wmax	296.4 (54.5)	294.6 (55.6)	0.72	0.03
HRmax, bmp	188.2 (8.0)	187.6 (9.6)	0.62	0.07
Maximal RER	1.2 (0.1)	1.3 (0.1)	0.10	0.47

Data are presented as mean (standard deviation). Wmax = Peak cycling power, HRmax = Maximum heart rate, RER = Respiratory Exchange Ratio, ES = Effect size.

The percentage of animal protein was lower in the vegan version compared to the traditional version of the Mediterranean diet ($p < 0.01$). Although the protein intake per day and kg of body mass were maintained during the vegan phase compared to the traditional Mediterranean diet, the percentage of energy provided in the form of the protein was slightly lower in the vegan phase than in the Mediterranean diet group ($p < 0.01$).

3.3. Anthropometrics measurements

Table 2 presents data on anthropometric variables after the dietary interventions. No difference in any anthropometric variable was observed in the comparison after the baseline period with the traditional Mediterranean diet and after the period with the vegan version of the Mediterranean diet.

3.4. Cardiorespiratory fitness

The values of the different physiological variables measured during the maximal graded cycling test are shown in Table 3. No differences in absolute and relative VO_2max, Wmax, HRmax and maximal respiratory exchange ratio were observed in the comparison after the baseline period with the traditional Mediterranean diet and after the period with the vegan Mediterranean diet.

3.5. Cardiometabolic health

Table 4 shows cardiometabolic health variables after the baseline period with the traditional Mediterranean diet and after the period with the vegan version of the Mediterranean diet. The vegan version of the diet significantly reduced TC (−22.6 mg/dl, p < 0.01, Effect size [ES] = 0.72) and LDL-C concentrations (−12.8 mg/dl, p < 0.01, ES = 0.72), systolic blood pressure (−7.3 mm Hg; $p = 0.02$; ES = 0.88), diastolic blood pressure (−4.9 mm Hg; $p = 0.01$; ES = 0.93) and mean arterial pressure (−5.7 mm Hg; p < 0.01; ES = 1.03). In addition, an inverse correlation was found between dietary fibre intake with LDL-C (partial rho = −0.43, $p = 0.040$) and diastolic blood pressure (partial rho = −0.42, $p = 0.045$).

3.6. Immune status

Table 5 shows immune status variables after the baseline period with the traditional Mediterranean diet and after the period with the vegan version of the Mediterranean

Table 4 Cardiometabolic health variables after the baseline period with the traditional Mediterranean diet and after the period with the vegan version of the Mediterranean diet.

	Traditional Mediterranean diet	Vegan Mediterranean diet	*p*-value	ES
Glucose, mg/dl	86.5 (5.8)	89.1 (7.2)	0.17	0.40
Triglycerides, mg/dl	109.9 (50.1)	105.4 (48.2)	0.81	0.09
Total Cholesterol, mg/dl	145.4 (21.1)	122.9 (20.9)	<**0.01**	**1.07**
LDL-Cholesterol, mg/dl	76.4 (17.3)	63.6 (17.2)	<**0.01**	**0.72**
HDL-Cholesterol, mg/dl	46.7 (14.3)	43.2 (14.9)	0.05	0.24
Haemoglobin, g/dL	14.3 (1.3)	14.1 (1.5)	0.68	0.14
Atherogenic Index	2.5 (1.1)	2.6 (1.1)	0.86	0.09
Vitamin D, ng/mL	21.7 (8.6)	26.8 (8.8)	0.06	0.59
Resting heart rate, bpm	64.3 (9.8)	65.5 (7.4)	0.54	0.17
Systolic blood pressure, mm Hg	132.4 (6.4)	125.1 (9.8)	**0.02**	**0.88**
Diastolic blood pressure, mm Hg	76.3 (5.5)	71.4 (5.0)	**0.01**	**0.93**
Mean arterial pressure, mm Hg	94.8 (5.2)	85.8 (6.0)	<**0.01**	**1.03**

Data are presented as mean (standard deviation). ES = Effect size.

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Table 5 Immune status variables after the baseline period with the traditional Mediterranean diet and after the period with the vegan version of the Mediterranean diet.

	Traditional Mediterranean diet	Vegan Mediterranean diet	*p*-value	ES
Monocytes, 10^9/L	0.3 (0.1)	0.3 (0.1)	0.82	0.06
Lymphocyte, 10^9/L	2.7 (1.0)	2.7 (1.0)	0.98	0.01
Eosinophils, 10^9/L	0.2 (0.1)	0.2 (0.1)	1.00	0.00
Neutrophils, 10^9/L	3.9 (3.8)	3.3 (1.4)	**0.04**	**0.48**
NLR	2.4 (1.6)	1.5 (0.5)	**0.03**	**0.77**
MHR	6.7 (2.8)	7.9 (4.5)	0.14	0.32

Data are presented as mean (standard deviation). NLR = Neutrophil to lymphocyte ratio, MHR = Monocyte to HDL ratio, ES = Effect size.

diet. Although the distribution of white blood cells was similar after both dietary interventions, neutrophils concentration significantly decreased after the vegan version of the diet (−0.66 10^9/L; $p = 0.04$; ES = 0.48; Table 5). As a result, the NLR was reduced after the vegan Mediterranean diet when compared to the traditional Mediterranean diet (−0.9; $p = 0.03$; ES = 0.77).

4. Discussion

In the present study, we evaluated the effect of shifting from a traditional Mediterranean diet to a vegan Mediterranean diet on cardiorespiratory fitness, metabolic health and immune status in physically active and healthy men. After a 3-week with a traditional version of the Mediterranean diet that included moderate amounts of dairy products, eggs, fish and meat, participants shifted to an isocaloric vegan Mediterranean diet with only plant-based protein and fat for 4 weeks. Despite the diets having a similar macronutrient distribution, the diet shifting towards a vegan Mediterranean diet improved several cardiometabolic health parameters including a reduced TC and LDL-C concentrations and lower blood pressure while enhancing the inflammatory profile of the participants. Interestingly, these benefits were obtained while participants maintained their VO2 max and without measurable changes in body mass and body fat percentage. Greater physiological changes might have been observed if the participants had been allowed to eat to satiety rather than maintaining a specified energy intake. However, in this study, the aim was to isolate the effects of the foods in each intervention while keeping energy intake constant. These findings suggest that the adoption of a vegan Mediterranean diet instead of the traditional Mediterranean diet improved cardiometabolic health and the inflammatory profile without affecting cardiorespiratory fitness in healthy and active men over the short term. These health benefits were obtained only after 4 weeks of diet intervention, confirming the fast body response to a diet with plant sources of protein and fat.

Previous studies have reported the beneficial effect of both Mediterranean and vegan diets on different cardiometabolic risk factors in participants with hyperlipidaemia, coronary artery disease, type 2 diabetes mellitus or obesity [8,13,23–27]. However, in these investigations, a hypocaloric diet [26] or an *ad libitum* diet was prescribed when following the prescription dietary pattern, which ultimately led to a reduction in energy intake [8,24,25,27]. It is known that a modest weight loss is sufficient to induce a decrease in TC, LDL-C and TG levels [28] and therefore, the cardiometabolic benefits of adopting a Mediterranean or vegan diet may be more associated with body mass loss than a pure effect of using new food elements [29]. Barnard et al. [8] took a step forward as they compared the effect of a low-fat vegan diet with a Mediterranean diet in overweight individuals to ascertain which of these two dietary patterns would be more beneficial. The data of Barnard et al.'s study showed a greater reduction of plasma lipids with the vegan diet compared to the Mediterranean diet which suggests a superiority of using vegetables as the unique source for energy intake (*i.e.,* vegan diet) over the allowance of moderate amounts of proteins in fats of the Mediterranean diet (*i.e.,* omnivorous diet) [8]. The current investigation is innovative because it studies for the first time the benefits of the traditional Mediterranean diet with a combined dietary pattern of vegan and Mediterranean to determine if there is an additive effect when combining these two beneficial dietary patterns. This combination of diets was feasible as this study was performed in Spain and participants already had dietary patterns compatible with the Mediterranean style even before recruitment. In this case, the shifting to a vegan version of the Mediterranean diet produced rapid benefits on the blood cholesterol profile and blood pressure regulation over the traditional Mediterranean diet, even in participants that were healthy and active and in the absence of body mass changes. These outcomes suggest that combining the patterns of the Mediterranean diet that includes vegetables, fruits, whole grains, legumes and olive oil as the primary source of fat, and those of a vegan diet that uses these plant. based products as the unique source of protein and fat may lead to a more favourable impact on the cardiometabolic health of healthy participants, at least when compared with the traditional Mediterranean diet that includes small amounts of dairy products, eggs, fish and meat.

The present work reported a reduction in TC (−22.6 mg/dl) and LDL-C (−12.8 mg/dl) just 4 weeks after shifting to a vegan version of the Mediterranean diet. These results are clinically relevant when considering that a reduction of 18 mg/dl in LDL-C decreases the relative risk of atherosclerotic cardiovascular disease by 10% in the first year and 20% after 3 years [30]. This occurs because cardiovascular risk depends on the time of exposure to LDL-C, emphasizing the need to maintain optimal LDL-C levels from an early age [31]. Dietary interventions to reduce TC and LDL-C levels include restricting the intake of dietary saturated fat and dietary cholesterol and increasing the intake of fibre and bioactive compounds such as phytosterols. These dietary modifications to reduce TC and LDL-C can be achieved when adopting a 100% plant-based diet [6] as happened in the current study. Specifically, we observed an inverse relationship between LDL-C levels and dietary

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fibre intake. Although both diets reported a similar intake of energy and macronutrients, the greater inclusion of whole vegetables in the Mediterranean vegan diet could have provided a higher intake of some dietary components such as dietary fibre. This finding could explain the reduction in LDL-C observed with the vegan diet. Multiple physiological mechanisms could be linked to the lipid-lowering effects of dietary fibre, such as inhibiting intestinal cholesterol uptake, improving the elasticity of blood vessel walls or encouraging the production of short-chain fatty acids (SCFAs) by the microbiota [32,33]. In addition to dietary fibre, plant foods contain other compounds with anticholesterolemic properties such as glycosylates, phytates or bioactive compounds (e.g. polyphenols and saponins) [34–36]. Overall, adopting a vegan version of the Mediterranean diet may produce further benefits on the cholesterol profile of healthy individuals already following a traditional Mediterranean diet.

The antihypertensive effects reported with the vegan version of the Mediterranean diet in the present study could be linked to some of the components previously described. In particular, nitrates present in vegetables such as beetroot, rocket lettuce, spinach or radishes have demonstrated antihypertensive effects. Dietary nitrate intake from plant sources promotes the increase of nitric oxide (NO) with vasodilator properties, which improves vascular function and regulates blood pressure [37,38]. Previous studies have suggested that a nitrate-rich plant-based meal could reduce SBP in healthy subjects [39]. Unfortunately, in our study, we did not evaluate dietary nitrate intake and it remains as a speculation the link between reduced blood pressure and the increase in the dietary intake of nitrates. Although the current investigation does not reveal the mechanism behind the modification in blood pressure regulation with the adoption of a vegan version of the Mediterranean diet, the reduction in blood pressure reported only after 4 weeks of a vegan Mediterranean diet points towards the potential benefits of this dietary pattern to maintain a healthier blood pressure regulation.

In the current study, participants were healthy and active men with at least 60 min/day of exercise training at least 3–5 days/week and they were encouraged to maintain their exercise training routines during the whole experiment. Exercise training diaries indicated that participants fulfilled this guideline as the parameters of exercise training volume, frequency, intensity and type of exercise discipline practised were similar in the 3 weeks with the traditional Mediterranean diet and in the 4 weeks with the vegan version of the Mediterranean diet. Interestingly, the shifting from the traditional to the vegan version of the Mediterranean diet did not affect VO_2max measured during the incremental exercise test performed after each diet, suggesting that the aerobic performance was unaffected by the adoption of the vegan diet. In a previous study in patients with type 2 diabetes, a whole food plant-based diet led to a significant increase in peak VO_2 and maximum power output [40]. These improvements in cardiorespiratory fitness could be linked to the concomitant reduction in body weight loss that habitually accompanies the adoption of a vegan diet [41]. In addition, it has been suggested that a vegan diet could be better than an omnivorous diet for optimizing glycogen stores due to the presence of carbohydrate-rich foods, which could subsequently improve performance by delaying fatigue and increasing workload [42]. In our study, no changes in body mass and no differences in carbohydrate intake were found between diets which may explain the lack of changes in VO_2max. Taken all these outcomes together, it seems that the adoption of a vegan diet *per se* does not affect cardiorespiratory fitness in the short term unless it produces a significant reduction in body mass or increases the proportion of carbohydrates in the diet.

Another important finding of the present study was the improvement in the NLR after the adoption of the vegan Mediterranean diet. No study to date has evaluated the effect of a plant-based diet on this marker of inflammation in healthy subjects. In the study conducted by Sha et al. [23] no difference in NLR was observed after a vegan diet for 8 weeks in patients with coronary artery disease. This could be derived from the plant foods included in the dietary intervention. The intake of healthy plant foods (fruit, vegetables, whole grains and nuts) has been associated with a reduction of different inflammatory biomarkers such as NLR in healthy young women. Specifically, polyphenols contained in foods such as fruits, coffee or olive oil, have been suggested as dietary factors involved in the reduction of NLR [43]. On the other hand, a lower beta diversity of the gut microbiota has been correlated with higher NLR values [44]. Dietary fibre could also be important in modulating the gut microbiota and, therefore, the release of anti-inflammatory factors [45]. The outcomes of this study should be further confirmed with the assessment of other inflammatory markers in both healthy and clinical populations but the use of vegan versions of the Mediterranean diet to reduce inflammation constitutes an interesting starting point for those seeking anti-inflammatory measures through diet.

The study exhibits several strengths, including its employment of a crossover design, the similarity between diets in terms of total energy and macronutrient contents and weekly follow-up to ensure participants' adherence during the intervention. Moreover, the diets were designed according to the preferences and requirements of the participants, which would facilitate their long-term maintenance. Nevertheless, certain limitations warrant consideration. First, the study was conducted in healthy men, so these results cannot be extrapolated to women and/or individuals with pathologies. To have a homogenous sample, women were not included in this study due to gender differences in health determinants and physiological processes (e.g. menstrual cycle) [46,47]. Although similar findings would be expected in a female sample, this should be corroborated in further analysis. Second, the relatively short duration of the intervention and relatively small number of participants who completed the study could limit the generalizability of the results. However, the present study could be considered as a pilot study, which set the basis for being scaled up to a larger population and

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followed up in a larger period. Additionally, further analyses should determine the biological mechanisms involved in our findings. In the current experiment, the order of the diets was not randomized and a progressive effect of being monitored through the experiment may explain the changes from the traditional to the vegan version of the Mediterranean diet. If this happened, it was not accompanied by a reduction in body mass or body fat which limits the bias of not randomizing the order of the diets. Last, we only tested the effect of the adoption of the vegan Mediterranean diet on aerobic exercise through a maximal incremental exercise test on a cycle ergometer. Further experiments should test if the adoption of a vegan version of the Mediterranean diet influences other forms of exercise.

In conclusion, the adoption of a vegan version of the Mediterranean with only plant-based sources of protein and fat for 4 weeks resulted in improvements in the lipid profile, blood pressure regulation and inflammatory status in physically active healthy men already following a traditional Mediterranean diet. These benefits were obtained without affecting cardiorespiratory fitness which constitutes interesting proof that a vegan diet may be a suitable dietary pattern for physically active individuals.

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Author Disclosures

The authors declare there is no conflict of interest about the content of the present study.

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ML-M, JG-H and JDC designed research; ML-M, JG-H, AM, MMA-N, MTIL and JP conducted research; ML-M, UF and JG-H analysed data; and ML-M, UF, JG-H and JG-H wrote the paper. ML-M, JG-H and JDC had primary responsibility for the final content. All authors read and approved the final manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2024.08.008.

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