

Special Issue Reprint

Nutritional Epidemiology of Non- Communicable Diseases

Feature Papers

Edited by
Hassan Vatanparast

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Nutritional Epidemiology of Non-Communicable Diseases— Feature Papers

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Guest Editor

Hassan Vatanparast



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About the Editor

Hassan Vatanparast

Hassan Vatanparast is a Professor at the University of Saskatchewan, jointly appointed in the College of Pharmacy and Nutrition and the School of Public Health. His research interests center on nutritional epidemiology, public health nutrition, and health equity, with a particular focus on vulnerable populations including immigrants, Indigenous communities, and children. He has led and collaborated on numerous national and international projects examining dietary patterns, nutrient intake, and chronic disease prevention. Dr. Vatanparast has contributed extensively to the development of nutrition policies and programs in Canada and abroad, and his work has been recognized for its impact on improving population health through evidence-based interventions.

Preface

Non-communicable diseases (NCDs) remain the leading cause of morbidity and mortality worldwide, placing a substantial burden on individuals, communities, and health systems. Even in the context of emerging infectious diseases, such as the COVID-19 pandemic, individuals living with non-communicable diseases (NCDs) have been shown to be at significantly higher risk of severe outcomes, including increased morbidity and mortality. Among the modifiable determinants of NCDs, nutrition plays a central and increasingly recognized role, influencing the development, progression, and prevention of conditions such as cardiovascular diseases, cancer, metabolic disorders, and age-related health outcomes. This Special Issue, *“Nutritional Epidemiology of Non-Communicable Diseases”*, brings together a diverse collection of research that advances our understanding of these complex relationships through rigorous epidemiological approaches.

The studies included in this volume span a broad range of topics, including dietary patterns, micronutrient intake, and their associations with chronic disease outcomes across different populations and settings. Collectively, they highlight the importance of population-level dietary assessment and the use of large-scale epidemiological data to identify nutritional gaps, risk factors, and opportunities for intervention related to NCD prevention and control.

A key strength of this collection is its attention to both biological and social determinants of health. Some of the papers in this Reprint emphasize the role of dietary quality within broader contexts, including socioeconomic disparities, cultural influences, and evolving food environments. These perspectives are essential for informing equitable and context-sensitive strategies aimed at reducing the burden of NCDs globally.

Importantly, the evidence presented in this volume extends beyond the academic environment. It has direct implications for public health practice and policy, including the development of dietary guidelines, nutrition interventions, and health promotion strategies. By bridging research and its implication toward policy and practice, the studies contribute to ongoing efforts to strengthen prevention and control of NCDs through evidence-based, multisectoral approaches.

This Reprint is intended for researchers, clinicians, public health professionals, and policymakers who are engaged in advancing population health through nutrition. It is our hope that the insights presented here will foster interdisciplinary collaboration, support informed decision-making, and inspire further research in the evolving field of nutritional epidemiology.

Hassan Vatanparast
Guest Editor

Article

Comparative Analysis of Dietary and Supplemental Intake of Calcium and Vitamin D among Canadian Older Adults with Heart Disease and/or Osteoporosis in 2004 and 2015

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Abstract: Despite the role of calcium and vitamin D in osteoporosis and heart disease, little research has examined changes in the intake of calcium and vitamin D among individuals with these conditions over time. Using data from the 2004 and 2015 Canadian Community Health Surveys, we investigated changes in dietary and supplemental intake of calcium and vitamin D among Canadian older adults aged ≥ 50 years, both with and without heart disease and/or osteoporosis, between 2004 and 2015. Notable declines in dietary calcium intake occurred, particularly among non-supplement users. Surprisingly, individuals with osteoporosis and heart disease, who are at higher nutritional risk, were less likely to use calcium supplements in 2015 compared to 2004. Among calcium supplement users, those with osteoporosis or both conditions experienced significant reductions in their usual calcium intake in 2015, with an increased proportion failing to meet recommended intake levels. Conversely, vitamin D supplement users experienced a substantial rise in vitamin D intake in 2015. In 2015, only a small proportion of supplement users did not meet the recommended vitamin D intake levels. These findings underscore the importance of public health initiatives to facilitate safe increases in calcium and vitamin D intake for older adults, particularly those with heart disease and osteoporosis.

Keywords: calcium; vitamin D; dietary intake; supplement; heart disease; osteoporosis; older adults; Canadian population

1. Introduction

According to the World Health Organization, chronic diseases are the leading cause of mortality globally, responsible for approximately 63% of all annual deaths [1]. These chronic diseases encompass non-communicable diseases (NCDs), long-term mental health disorders such as schizophrenia and major depression, and certain communicable diseases like HIV/AIDS. The escalating prevalence of chronic diseases poses a significant challenge to healthcare systems worldwide, both in terms of mortality rates and healthcare expenditures [2]. In Canada, chronic diseases have become a growing concern, accounting for approximately 88% of total deaths and constituting approximately one-third of direct healthcare expenses [3,4].

Among these chronic conditions, heart disease and osteoporosis are particularly notable. Heart disease, encompassing a range of cardiovascular conditions, is a leading cause of death globally [5]. It is characterized by conditions like coronary artery disease, heart failure, and arrhythmias, each contributing a substantial burden on healthcare systems [5]. Osteoporosis, on the other hand, is a bone disease that leads to increased fracture risk due

to decreased bone mass and the deterioration of bone tissue [6]. It is especially prevalent in the aging population and contributes significantly to morbidity and healthcare costs [6].

The frequent co-occurrence of heart disease and osteoporosis in older adults, along with their shared underlying mechanisms such as inflammation and hormonal changes, highlights the importance of exploring their interrelations [7,8]. The substantial socioeconomic burden imposed by these chronic diseases underscores the complexities involved in managing them within the Canadian healthcare system [9,10].

Accumulating evidence underscores the significance of diet, a modifiable risk factor, in both the development and management of heart disease and osteoporosis [11,12]. In the context of bone health, extensive research has primarily focused on specific dietary components, notably calcium and vitamin D [13,14]. In November 2010, the Institute of Medicine (IOM), in collaboration with the American and Canadian governments, established the most recent dietary reference intakes (DRIs) for calcium and vitamin D. These recommendations serve as vital guidelines for maintaining bone health [15,16]. Our previous research, utilizing data from the Canadian Health Measures Survey (CHMS), has highlighted that approximately one-quarter of Canadians fail to meet the recommended dietary allowance (600 to 800 IU for those aged ≥ 1 year) for vitamin D. Notably, the use of supplements has contributed positively to achieving better plasma vitamin D levels [17]. Furthermore, studies have shown that, even with supplement usage, the average daily calcium intake among Canadian adults, particularly older adults, remains below the recommended levels essential for optimal bone health. For instance, women and men aged 25 years or older have reported mean daily calcium intakes of 1038 ± 614 mg and 904 ± 583 mg, respectively, which fall short of the recommended levels [18].

Emerging evidence indicates that micronutrients such as calcium and vitamin D are not only crucial for bone health but also play a role in reducing the risk of developing heart disease [19,20]. However, the literature offers varying reports concerning the relationships between dietary calcium/vitamin D intake, calcium/vitamin D supplementation, and the risk of heart disease. In a stratified analysis, Larsson and colleagues observed an inverse association between dietary calcium intake and the risk of stroke in populations with a low to moderate average calcium intake. Conversely, they noted a positive association in populations with a high calcium intake [21]. Furthermore, while certain studies have reported a connection between high intake of supplemental calcium and an increased risk of heart disease [22,23], others have not provided substantial support for this hypothesis [24,25].

To the best of our knowledge, there is limited research examining changes in the dietary and supplemental intake of calcium and vitamin D among individuals with heart disease and/or osteoporosis over time. Therefore, using data from the 2004 and 2015 Canadian Community Health Surveys, our primary objective in this study was to investigate alterations in the dietary and supplemental intake of calcium and vitamin D among older Canadian adults, both with and without heart disease and/or osteoporosis, between 2004 and 2015. Given the growing body of evidence linking calcium supplement usage to an increased risk of cardiovascular events, we hypothesize that the total calcium intake has declined among older adults with heart disease and/or osteoporosis between 2004 and 2015. Conversely, in light of the evidence supporting the cardioprotective effects of vitamin D, we anticipate that the total vitamin D intake has increased among older adults with heart disease and/or osteoporosis over the same period.

2. Materials and Methods

2.1. Study Population

This study utilizes data from the Canadian Community Health Survey (CCHS) Nutrition data for the years 2004 and 2015. The CCHS is a cross-sectional health survey conducted in Canada, designed to gather comprehensive health information from Canadian residents. Data on food consumption were collected using a 24 h dietary recall technique, with both 2004 and 2015 surveys capturing information on two separate days (day 1 and day 2). The 24 h dietary recall portion of the CCHS employs a sophisticated computer-assisted

interviewing tool known as the Automated Multiple-Pass Method (AMPM). This method is designed to assist respondents in more accurately recalling and reporting the foods they consumed within the 24 h period preceding the interview, spanning from midnight to midnight [26,27]. For the 2015 dataset, a total of 20,487 individuals provided detailed information regarding their food consumption and socio-demographic variables on day 1, while 7623 individuals reported similar information on day 2. In the case of the 2004 dataset, the sample size for day 1 was 35,107 individuals, with a weighted frequency of 31,030,722, and for day 2, it was 10,786 [26,27]. As this study involved analyzing de-identified secondary data from the CCHS obtained via the Research Data Centre (RDC) of Statistics Canada, it was exempt from ethics approval per policies governing secondary data analysis.

2.2. Analytical Sample

This study represents a nationally representative sample of Canadian individuals aged 50 years and older. The data used in this analysis were drawn from a total of 8,887,312 (weighted frequency) and 12,399,600 (weighted frequency) individuals from the CCHS surveys conducted in 2004 and 2015, respectively. To ensure the robustness and comparability of the dataset, we applied specific exclusion criteria. Individuals under the age of 50, pregnant or lactating women, those with exceptionally high nutrient intakes, individuals who did not report any food intake during the 24 h recall, and those reporting a daily caloric intake outside the range of 200–8000 kcal were excluded. These exclusion criteria were consistently applied to both the 2004 and 2015 survey data.

2.3. Dietary and Supplemental Calcium and Vitamin D

The survey participants were queried about various aspects of their dietary and supplement intake, including the type of food consumed, the quantity of food, meal timing, location of consumption, and the use of dietary supplements. In the 2015 survey, information on supplement consumption, including frequency and quantity, was collected specifically for the preceding 24 h. In contrast, the 2004 survey collected supplement intake data differently. The participants were asked about their supplement usage over the 30 days leading up to the day of the interview. This approach allowed us to derive detailed information regarding supplement frequency, duration, and the daily amount consumed over this 30-day period.

2.4. Calcium and Vitamin D Requirements

The dietary reference intakes of calcium and vitamin D used in this study were defined by the IOM Food and Nutrition Board [16]. For individuals aged 50 and over, the estimated average requirements (EAR) and upper tolerable levels (UL) for calcium are as follows: male, 51–70 years: EAR 800 mg, UL 2000 mg; female, 51–70 years: EAR 1000 mg, UL 2000 mg; male, 71 years or older: EAR 1000 mg, UL 2000 mg; and female, 71 years or older: EAR 1000 mg, UL 2000 mg. For vitamin D, the EAR is 10 µg, and the UL is 100 µg for individuals aged 50 years and above.

2.5. Chronic Diseases

In both the CCHS surveys conducted in 2004 and 2015, the participants aged 19 years and older were inquired about their current status regarding specific chronic health conditions, all of which were diagnosed by healthcare professionals. These conditions included high blood pressure, diabetes, heart disease, cancer, and osteoporosis. Notably, the query about osteoporosis was directed exclusively to respondents aged 50 years and older.

2.6. Definitions and Covariates

The categorical variables used in this study include osteoporosis—self-reported (yes, no), heart disease—self-reported (yes, no), age (50–70 years, ≥71 years), sex (male, female), ethnicity (non-indigenous, indigenous), immigrant (yes, no), calcium supplement user (yes, no), vitamin D supplement user (yes, no), weight status (normal weight, overweight,

obese), smoker (yes, no), education (<secondary graduate, secondary graduate, some post-secondary graduate, university graduate), region of residence (Atlantic, Quebec, Ontario, Prairies, British Columbia), and household income (decile 1–2, 3–4, 5–6, 7–8, 9–10) unless otherwise specified. We also calculated the percentages of individuals who met the recommended food guide serving per day of four food groups (vegetables and fruit, grain products, milk and alternatives, meat and alternatives) based on Canada's 2007 Food Guide recommendations [28].

2.7. Statistical Analyses

All statistical analyses were completed using SAS, version 9.4. To produce population-level estimates, Statistics Canada used weighting and bootstrapping weights as per the recommendations. To calculate the usual calcium and vitamin D intakes and the percentages of the population below the EAR and above the UL from food only and from both food and dietary supplements for ages 50–70 years and >70 years, the National Cancer Institute (NCI) method was used in CCHS 2004 and 2015 with the following covariates:

- (i) The sequence of the 24 h recall, categorized as day 1 or day 2,
- (ii) The day of the week when the 24 h recall data were obtained and categorized as weekday or weekend,
- (iii) Energy consumption from food during the 24 h recall period.

For the NCI estimation, SAS macros developed by the National Cancer Institute (Usual Dietary Intakes: SAS Macros for the NCI Method, 2018) were used with a slight modification to account for bootstrapping weights. More details about the NCI method and the SAS macros can be found on the NCI website [29]. To compare the 2004 and 2015 results, the absence of overlapping 95% confidence intervals concept was applied [30]. Alpha was set at 0.05. The values are represented as percentages (SE) and means (SE) where required. The association between both chronic conditions (i.e., osteoporosis and heart disease) and sociodemographic variables was evaluated separately for each disease using multiple logistic regression models. Descriptive analyses were also carried out. Permission to access and conduct an analysis of the CCHS 2004 and 2015 data was obtained from the Saskatchewan Research Data Center Program of Statistics Canada.

3. Results

The results are presented in the following subsections, including descriptive data and the results from the statistical modelling.

3.1. Sociodemographic Characteristics

Table 1 presents the sociodemographic characteristics of the Canadians aged 50 and over who reported having osteoporosis or heart disease in 2004 and 2015. In 2004, the prevalence of osteoporosis was 11.3%, which decreased to 10.6% in 2015. Similarly, the prevalence of heart disease was 10.9% in 2004 and 9.3% in 2015. In 2015, a significant, 4.6% reduction in the proportion of female individuals with osteoporosis was reported compared to 2004. While there were no significant differences in vitamin D supplement use among individuals with osteoporosis between 2004 and 2015, there was a significant decrease in calcium supplement use in 2015. In 2004, 56.5% of individuals with osteoporosis used calcium supplements, compared to only 45.2% in 2015. The prevalence of osteoporosis significantly increased among smokers in 2015 compared to 2004, with rates of 12.5% in 2004 and 20.2% in 2015. Regarding education levels, the prevalence of osteoporosis significantly decreased among individuals with less than secondary education and university graduates. Conversely, it increased significantly among secondary graduates and post-secondary graduates in 2015 compared to 2004. A significant reduction was observed in the percentage of osteoporosis patients among those who met the recommended serving sizes for “grains” (5% decrease in 2015) and “meat and alternatives” (6% decrease in 2015) according to the Canada Food Guide 2007. Significant changes were also noted across income deciles. The lowest income group (decile 1–2) experienced a 27% increase in the prevalence of

osteoporosis in 2015; the highest increase among all income categories. Additionally, the second-lowest income group (decile 3–4) saw an approximately 10% increase. However, the remaining middle- and high-income groups all experienced significant decreases in 2015.

Table 1. Sociodemographic descriptions of individuals with osteoporosis and heart disease in 2004 and 2015 among Canadians 50 years and older ¹.

Sociodemographic Variables	Osteoporosis				Heart Disease			
	2004 (n = 1,061,034) (11.3%)		2015 (n = 1,375,730) (10.6%)		2004 (n = 1,028,909) (10.9%)		2015 (n = 1,211,896) (9.3%)	
	% ± SE	95% CI	% ± SE	95% CI	% ± SE	95% CI	% ± SE	95% CI
Age								
50–70 years	54.1 ± 2.0	(50.2, 58.1)	55.4 ± 2.4	(50.6, 60.1)	48.0 ± 2.2	(43.4, 52.6)	48.6 ± 2.9	(42.9, 54.4)
≥71 years	45.9 ± 2.0	(42.0, 49.8)	44.6 ± 2.4	(39.9, 49.4)	52.0 ± 2.2	(46.8, 56.0)	51.4 ± 2.9	(45.6, 57.1)
Sex								
Male	11.0 ± 1.4	(8.2, 13.8)	15.6 ± 1.8 [¥]	(12.1, 19.2)	57.4 ± 2.1	(53.4, 61.5)	55.6 ± 2.9	(49.9, 61.3)
Female	89.0 ± 1.4	(86.2, 91.8)	84.4 ± 1.8 [¥]	(80.8, 88.0)	42.6 ± 2.1	(38.5, 46.6)	44.4 ± 2.9	(38.7, 50.1)
Ethnicity								
Non-indigenous	98.6 ± 0.4	(97.7, 99.4)	97.3 ± 0.8	(95.7, 99.0)	97.9 ± 0.5	(97.0, 98.9)	97.7 ± 0.8	(96.2, 99.3)
Indigenous	1.5 ± 0.4	(0.6, 2.3)	2.7 ± 0.8	(1.0, 4.3)	2.1 ± 0.5	(1.1, 3.0)	2.3 ± 0.8	(0.7, 3.8)
Immigrant								
Yes	27.3 ± 2.3	(22.5, 31.5)	24.0 ± 4.4	(18.8, 29.3)	20.6 ± 1.9	(16.9, 24.3)	24.0 ± 2.8	(18.5, 29.4)
No	73.3 ± 2.3	(68.5, 77.5)	76.0 ± 3.3	(70.8, 81.2)	79.5 ± 1.9	(75.8, 83.2)	76.0 ± 2.8	(70.6, 81.5)
Calcium Supplement user								
Yes	56.5 ± 1.2	(52.1, 60.8)	45.2 ± 4.4 [¥]	(39.2, 51.2)	33.9 ± 1.9	(29.5, 38.2)	24.5 ± 2.6 [¥]	(19.5, 29.6)
No	43.5 ± 0.2	(39.2, 47.9)	54.8 ± 3.3 [¥]	(48.8, 60.8)	66.2 ± 0.2	(61.8, 70.5)	75.5 ± 2.6 [¥]	(70.4, 80.5)
Vitamin D Supplement user								
Yes	55.1 ± 1.3	(50.7, 59.5)	56.5 ± 4.4	(50.5, 62.5)	32.9 ± 1.2	(28.7, 37.2)	38.0 ± 2.9	(32.2, 43.7)
No	44.9 ± 0.3	(40.5, 49.4)	43.5 ± 3.3	(37.5, 49.5)	67.1 ± 0.2	(62.8, 71.3)	62.0 ± 2.9	(56.3, 67.8)
Weight Status								
Normal weight	39.7 ± 4.3	(34.4, 45.0)	37.5 ± 4.7	(30.3, 44.8)	26.7 ± 4.2	(21.3, 32.1)	28.0 ± 3.5	(21.1, 34.8)
Overweight	38.5 ± 5.3	(33.3, 43.7)	38.9 ± 5.1	(31.0, 46.9)	43.3 ± 5.4	(37.2, 49.5)	42.7 ± 4.3	(34.3, 51.0)
Obese	21.8 ± 6.2	(16.8, 26.8)	23.5 ± 6.8	(18.1, 29.0)	30.0 ± 6.3	(24.1, 35.9)	29.4 ± 3.9	(21.8, 37.0)
Smoking status								
Yes	12.5 ± 1.4	(9.9, 15.2)	20.2 ± 3.0 [¥]	(14.3, 26.1)	18.6 ± 2.0	(14.6, 22.6)	15.6 ± 2.4	(10.9, 20.3)
No	87.5 ± 1.4	(84.8, 90.1)	79.8 ± 3.0 [¥]	(73.9, 85.8)	81.4 ± 2.0	(77.5, 85.4)	84.4 ± 2.4	(79.8, 89.1)
Education								
<secondary graduate	41.7 ± 2.4	(37.1, 46.4)	26.3 ± 2.5 [¥]	(21.5, 31.2)	44.7 ± 2.3	(40.2, 49.1)	29.6 ± 2.7 [¥]	(24.4, 34.8)
secondary graduate	16.1 ± 1.8	(12.6, 19.5)	32.6 ± 3.0 [¥]	(26.8, 38.4)	13.4 ± 1.4	(10.7, 16.2)	24.0 ± 2.5 [¥]	(19.1, 28.9)
some post-secondary graduate	5.1 ± 0.9	(3.4, 6.8)	29.1 ± 2.7 [¥]	(23.7, 34.5)	5.4 ± 0.9	(3.6, 7.2)	30.5 ± 3.1 [¥]	(24.4, 36.6)
university graduate	37.1 ± 2.2	(32.8, 41.4)	12.0 ± 1.6 [¥]	(8.8, 15.2)	36.5 ± 2.1	(32.3, 40.7)	15.9 ± 2.3 [¥]	(11.3, 20.5)
Region of residence								
Atlantic	7.5 ± 0.8	(5.9, 7.7)	8.2 ± 0.8	(6.6, 8.8)	10.0 ± 0.9	(8.2, 10.9)	10.9 ± 1.0	(9.0, 12.9)
Quebec	24.3 ± 2.2	(20.0, 24.2)	24.7 ± 2.6	(19.6, 24.7)	24.4 ± 2.2	(20.1, 24.6)	29.7 ± 3.0	(23.7, 35.7)
Ontario	38.6 ± 2.0	(34.7, 38.4)	41.1 ± 2.8	(35.5, 41.6)	36.6 ± 1.9	(33.0, 36.3)	35.5 ± 3.0	(29.5, 41.4)
Prairies	16.6 ± 1.3	(14.1, 16.1)	15.1 ± 1.6	(12.0, 15.3)	13.6 ± 1.2	(11.3, 13.0)	14.3 ± 1.6	(11.1, 17.4)
British Columbia	13.1 ± 1.4	(10.3, 13.1)	10.9 ± 1.5	(8.0, 10.9)	15.3 ± 1.7	(12.0, 15.6)	9.7 ± 1.5 [¥]	(6.7, 12.6)
Met daily grain product requirement								
Yes	23.2 ± 2.2	(18.2, 27.5)	18.0 ± 2.4 [¥]	(13.4, 22.6)	23.6 ± 2.2	(19.2, 28.0)	19.5 ± 2.6	(14.4, 24.6)
No	76.8 ± 2.2	(72.8, 81.1)	82.0 ± 2.4 [¥]	(77.4, 86.6)	76.4 ± 2.2	(72.0, 80.8)	80.5 ± 2.6	(75.5, 85.6)
Met daily meat and alternatives requirement								
Yes	30.7 ± 2.2	(26.4, 35.0)	24.5 ± 4.6 [¥]	(19.4, 29.6)	23.1 ± 1.9	(19.4, 26.8)	20.6 ± 2.7	(15.2, 25.9)
No	69.3 ± 2.2	(65.0, 73.6)	75.5 ± 3.6 [¥]	(70.4, 80.6)	76.9 ± 1.9	(73.2, 80.6)	79.4 ± 2.7	(74.1, 84.8)

Table 1. Cont.

Sociodemographic Variables	Osteoporosis				Heart Disease			
	2004 (n = 1,061,034) (11.3%)		2015 (n = 1,375,730) (10.6%)		2004 (n = 1,028,909) (10.9%)		2015 (n = 1,211,896) (9.3%)	
	% ± SE	95% CI	% ± SE	95% CI	% ± SE	95% CI	% ± SE	95% CI
Met daily milk and alternatives requirement								
Yes	9.3 ± 1.0	(7.3, 11.3)	11.0 ± 1.7	(7.7, 14.4)	14.9 ± 1.7	(11.5, 18.3)	9.6 ± 1.7 [‡]	(6.3, 13.0)
No	90.7 ± 1.0	(88.7, 92.7)	89.0 ± 1.7	(85.6, 92.3)	85.1 ± 1.7	(81.7, 88.5)	90.4 ± 1.7 [‡]	(87.1, 93.7)
Met daily vegetable and fruit requirement								
Yes	20.9 ± 1.8	(17.4, 24.3)	16.9 ± 2.6	(11.9, 22.0)	21.8 ± 1.9	(18.1, 25.4)	14.8 ± 2.2 [‡]	(10.4, 19.1)
No	79.1 ± 1.8	(75.7, 82.6)	83.1 ± 2.6	(78.0, 88.1)	78.2 ± 1.9	(74.6, 81.9)	85.2 ± 2.2 [‡]	(80.9, 89.6)
Income decile								
Decile 1–2	6.2 ± 1.8	(2.7, 6.7)	33.3 ± 3.1 [‡]	(27.2, 39.5)	3.3 ± 0.7	(1.8, 4.7)	28.0 ± 2.5 [‡]	(23.1, 32.9)
Decile 3–4	11.4 ± 1.4	(8.6, 11.3)	21.1 ± 2.0 [‡]	(17.1, 25.0)	13.5 ± 1.6	(10.4, 16.6)	29.3 ± 3.0 [‡]	(23.5, 35.1)
Decile 5–6	30.5 ± 2.2	(26.2, 30.7)	23.2 ± 2.5 [‡]	(18.2, 28.2)	36.3 ± 2.3	(31.7, 40.8)	22.2 ± 2.7 [‡]	(16.9, 27.4)
Decile 7–8	34.7 ± 2.5	(29.9, 34.6)	14.0 ± 2.4 [‡]	(9.4, 18.7)	28.8 ± 2.2	(24.6, 33.1)	10.8 ± 1.7 [‡]	(7.5, 14.1)
Decile 9–10	17.2 ± 2.1	(13.1, 17.2)	8.4 ± 1.6 [‡]	(5.3, 11.5)	18.2 ± 2.0	(14.4, 22.0)	9.7 ± 2.3 [‡]	(5.1, 14.3)

[‡] Values are mean ± SE, and the analyses were conducted separately for the 2004 and 2015 data sets. [‡] Significant differences (*p*-value < 0.05) between CCHS 2004 and 2015 using the confidence interval overlapping technique.

A similar pattern was observed among heart disease patients and sociodemographic variables. In 2015, there was a significant decrease of approximately 8.5% in the percentage of heart disease patients taking calcium supplements compared to 2004. Regarding education levels, significant decreases were found in the proportion of heart disease patients among individuals with less than secondary education and university graduates, with reductions of 15.1% and 20.6%, respectively. In contrast, significant increases were observed among secondary graduates and some post-secondary graduates, with increases of 10.6% and 25.1%, respectively. A reduction in the percentage of heart disease patients was also noted among individuals who consumed the recommended servings of “vegetables and fruit” and “milk and alternatives” in 2015, with decreases of 7% and 5.3%, respectively. Similar to the pattern observed for osteoporosis, there were significant increases in the prevalence of heart disease among those in the lowest income groups, specifically decile 1–2 (25%) and 3–4 (16%). Conversely, the middle- and high-income groups experienced significant decreases in the proportion of heart disease patients, with reductions ranging from 8% to 18%.

3.2. Factors Associated with Osteoporosis and Heart Disease among Canadians Aged 50 and Older in 2004 and 2015

Table 2 presents the results of a multivariate logistic regression analysis examining the association between osteoporosis/heart disease and sociodemographic variables among the Canadian population aged 50 years and older. This includes individuals with and without these conditions, reflecting a comprehensive overview of the prevalence and associated factors across this demographic segment for the years 2004 and 2015. In 2004, individuals aged ≥71 years had 2.3 times higher odds of having osteoporosis, while females had 8.1 times higher odds compared to males. Additionally, individuals who consumed vitamin D supplements exhibited 1.9 times higher odds of having osteoporosis. Regarding income, those in the lowest income group (decile 1–2) were 2.9 times more likely to have osteoporosis than those in the highest income decile (9–10). Similarly, individuals in the middle-income groups (deciles 3–4, 5–6, and 7–8) were 1.7 times more likely to have osteoporosis. No significant effects were observed for ethnicity, immigration status, calcium supplement use, smoking status, region of residence, education, weight status, or food group consumption.

Table 2. Factors associated with osteoporosis and heart disease among Canadians (≥ 50 years) in 2004 and 2015 ¹.

	Osteoporosis		Heart Disease	
	2004 (n = 1,061,034) (11.3%)	2015 (n = 1,375,730) (10.6%)	2004 (n = 1,028,909) (10.9%)	2015 (n = 1,211,896) (9.3%)
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Age				
50–70 years	1	1	1	1
≥ 71 years	2.3 * (1.8, 2.9)	2.3 * (1.7, 3.1)	3.1 * (2.4, 4)	3.4 * (2.4, 4.8)
Sex				
Male ²	1	1	1.7 * (1.3, 2.2)	1.6 * (1.2, 2.2)
Female ³	8.1 * (5.5, 12)	4.6 * (3.1, 6.6)	1	1
Ethnicity				
Indigenous ⁴	1	1	1	1
Non-indigenous	0.6 (0.2, 1.6)	0.6 (0.3, 1.3)	0.8 (0.4, 1.8)	0.995 (0.4, 2.6)
Immigrant				
No ⁴	1	1	1	1
Yes	1.1 (0.8, 1.5)	0.8 (0.5, 1.3)	0.8 (0.6, 1)	0.9 (0.6, 1.3)
Calcium supplement User				
No ⁴	1	1	1	1
Yes	1.1 (0.8, 1.6)	1.2 (0.8, 1.7)	1 (0.6, 1.6)	0.8 (0.5, 1.3)
Vitamin D supplement User				
No ⁴	1	1	1	1
Yes	1.9 * (1.3, 2.8)	1.5 (1.0, 2.1)	0.9 (0.6, 1.5)	1.1 (0.7, 1.8)
Weight Status				
Normal weight ⁴	1	1	1	1
Overweight	0.9 (0.7, 1.2)	1.1 (0.8, 1.6)	1.2 (0.9, 1.6)	1.1 (0.7, 1.6)
Obese	0.7 (0.5, 0.9)	0.9 (0.6, 1.3)	1.4 (1, 1.9)	1.5 (1, 2.2)
Smoking status				
No ⁴	1	1	1	1
Yes	0.8 (0.6, 1.1)	1.6 (1, 2.5)	1.04 (0.7, 1.5)	1.1 (0.7, 1.8)
Education				
University graduate ⁴	1	1	1	1
<Secondary graduate	1.3 (1, 1.8)	1.6 (1, 2.6)	1.2 (0.9, 1.7)	1.1 (0.7, 1.7)
Secondary graduate	0.9 (0.6, 1.3)	1.2 (0.8, 1.8)	1 (0.7, 1.4)	0.8 (0.5, 1.1)
Some post-secondary graduate	1.1 (0.7, 1.8)	1.7 (1, 2.9)	0.9 (0.6, 1.5)	0.9 (0.6, 1.5)
Region of residence				
Ontario ⁴	1	1	1	1
Atlantic	1 (0.7, 1.4)	1 (0.7, 1.5)	0.9 (0.7, 1.3)	1.6 * (1.1, 2.5)
Quebec	1 (0.7, 1.5)	0.9 (0.6, 1.4)	0.8 (0.6, 1)	1.4 (0.9, 2.3)
Prairies	1.2 (0.9, 1.6)	0.9 (0.6, 1.3)	0.8 (0.6, 1)	1.2 (0.8, 1.8)
British Columbia	0.7 (0.5, 1.1)	0.8 (0.5, 1.2)	1.2 (0.8, 1.8)	0.9 (0.6, 1.5)
Income				
Decile 9–10 ⁴	1	1	1	1
Decile 1–2	2.9 * (1.2, 6.9)	2.4 * (1.3, 4.5)	1.7 (0.9, 3.4)	1.8 (0.9, 3.6)
Decile 3–4	1.7 * (1, 2.7)	1.6 (0.9, 2.8)	2.7 * (1.7, 4.2)	1.6 (0.8, 3.2)
Decile 5–6	1.7 * (1.2, 2.5)	2.5 * (1.3, 4.5)	2 * (1.4, 2.8)	1.5 (0.7, 3)
Decile 7–8	1.7 * (1.2, 2.4)	1.9 (1, 3.5)	1.4 (1, 1.9)	0.9 (0.5, 1.9)
Met daily grain product requirement				
No ⁴	1	1	1	1
Yes	1.1 (0.8, 1.6)	1.1 (0.7, 1.7)	1.3 (0.9, 1.8)	0.9 (0.6, 1.4)
Met daily meat and alternatives requirement				
No ⁴	1	1	1	1
Yes	1 (0.7, 1.3)	0.8 (0.6, 1.2)	0.8 (0.6, 1.1)	0.8 (0.5, 1.1)
Meet daily milk and alternatives requirement				
No ⁴	1	1	1	1
Yes	0.7 (0.5, 1.1)	1.2 (0.7, 2.1)	1.3 (0.9, 2)	1 (0.6, 1.7)
Meet daily vegetable and fruit requirement				
No ⁴	1	1	1	1
Yes	0.8 (0.6, 1.1)	1.2 (0.8, 1.9)	1 (0.7, 1.3)	1 (0.7, 1.6)

¹ Values are OR (95% CI), and the analyses were conducted separately for the 2004 and 2015 data sets. ² Reference group for osteoporosis. ³ Reference group for heart disease. ⁴ Reference category of the odds ratio. * Significant differences (p -value < 0.05) within categories.

In 2015, the odds of having osteoporosis were 2.3 times higher among older adults (≥ 71 years) compared to adults aged 50–70 years, and they were 4.6 times higher among

females compared to males. Individuals in the lowest income group (decile 1–2) and middle-income group (decile 5–6) were 2.4 times and 2.5 times more likely to have osteoporosis, respectively, compared to the highest income group (decile 9–10). No significant effects were observed for ethnicity, immigration status, vitamin D and calcium supplement use, smoking status, region of residence, education, weight status, or food group consumption.

In 2004, the individuals in the oldest age group (≥ 71 years) were 3.1 times more likely to report having heart disease compared to individuals aged 50–70 years, and males were 1.7 times more likely to have heart disease compared to females. Individuals in the middle-income deciles of 3–4 and 5–6 were 2.7 times and 2 times more likely to have heart disease compared to individuals in the highest income decile 9–10. The likelihood of having heart disease was not significantly affected by ethnicity, immigration status, calcium supplement use, vitamin D supplement use, smoking status, region of residence, education, weight status, or food group consumption in 2004.

In 2015, the odds of having heart disease were 3.4 times higher among individuals aged ≥ 71 years compared to the younger group, and they were 1.6 times higher among males compared to females. Residents of the Atlantic region were 1.6 times more likely to have heart disease compared to individuals who lived in other regions. Moreover, ethnicity, immigration status, vitamin D and calcium supplement use, smoking status, income, education, weight status, and food group consumption did not have any significant effect on heart disease reporting in 2015.

3.3. Dietary and Supplemental Intake of Calcium among Individuals with Heart Disease, Osteoporosis, or Both Conditions in 2004 and 2015, Comparing Supplement Users and Non-Users

Table 3 presents the mean usual calcium intake among Canadians who reported having heart disease, osteoporosis, or both conditions using the NCI method in both 2004 and 2015. The percentage of individuals reporting both chronic conditions was 1.8% in 2004 and 1.7% in 2015. Among the individuals who did not take calcium supplements, there was a significant decrease in usual calcium intake from food, with a reduction of approximately 78 mg for those with heart disease and a similar decrease for those without heart disease between 2004 and 2015. Similar trends were observed among non-supplement users with and without osteoporosis, where usual calcium intake from food significantly decreased by 68 to 75 mg in 2015 compared to 2004. Among the calcium supplement users, we observed significant decreases in the usual calcium intake among individuals with osteoporosis (302 mg) and those with both osteoporosis and heart disease (205 mg).

Table 3. Dietary and supplemental intake of calcium among Canadians (≥ 50 years) who had heart disease, osteoporosis, and both diseases combined among supplement users and non-users in 2004 and 2015 ¹.

Chronic Diseases	Calcium Supplement Non-Users		Calcium Supplement Users			
	2004 (61.5%)	2015 (67.9%)	2004 (38.5%)		2015 (32.1%)	
	Calcium (mg) from Food	Calcium (mg) from Food	Calcium (mg) from Food	Calcium (mg) from Food and Supplements	Calcium (mg) from Food	Calcium (mg) from Food and Supplements
No heart disease	732.9 $\pm$ 30.0	654.8 $\pm$ 26.0 [¥]	733.5 $\pm$ 33.5	1244.7 $\pm$ 37.6	738.4 $\pm$ 38.8	1193.9 $\pm$ 57.0
Have heart disease	720.4 $\pm$ 32.7	641.7 $\pm$ 34.6 [¥]	657.8 $\pm$ 34.3	1169.4 $\pm$ 56.6	671.0 $\pm$ 41.5	1126.3 $\pm$ 57.7
No Osteoporosis	737.9 $\pm$ 30.1	662.7 $\pm$ 25.8 [¥]	736.8 $\pm$ 34.0	1204.5 $\pm$ 38.1	744.1 $\pm$ 39.4	1203.3 $\pm$ 57.2
Have Osteoporosis	648.3 $\pm$ 31.9	580.6 $\pm$ 27.8 [¥]	668.1 $\pm$ 34.0	1419.4 $\pm$ 46.2	649.6 $\pm$ 36.4	1117.6 $\pm$ 56.1 [¥]
Have both diseases	594.8 $\pm$ 32.0	592.9 $\pm$ 47.8	637.9 $\pm$ 35.0	1337.9 $\pm$ 67.9	602.8 $\pm$ 47.0	1082.9 $\pm$ 61.1 [¥]

¹ Values are mean $\pm$ SE, and the analyses were conducted separately for the 2004 and 2015 data sets. [¥] Significant differences (p -value < 0.05) between CCHS 2004 and 2015 using the confidence interval overlapping technique.

3.4. Prevalence of Calcium Inadequacy among Individuals with Heart Disease, Osteoporosis, or Both Conditions in 2004 and 2015, Comparing Supplement Users and Non-Users

Figure 1 illustrates the prevalence of calcium inadequacy among Canadians who reported having heart disease, osteoporosis, or both conditions in 2004 and 2015. Among the non-supplement users, the percentage of individuals who did not meet the estimated average requirement for calcium significantly increased by 5.6% for those with heart disease, 6.8% for those without heart disease, and 5.8% for those without osteoporosis in 2015 compared to 2004. For the supplement users, there was a noteworthy increase in the percentage of individuals who did not meet the estimated average requirement for calcium, with a 12% increase among those with osteoporosis and a 15% increase among those with both diseases between 2004 and 2015.

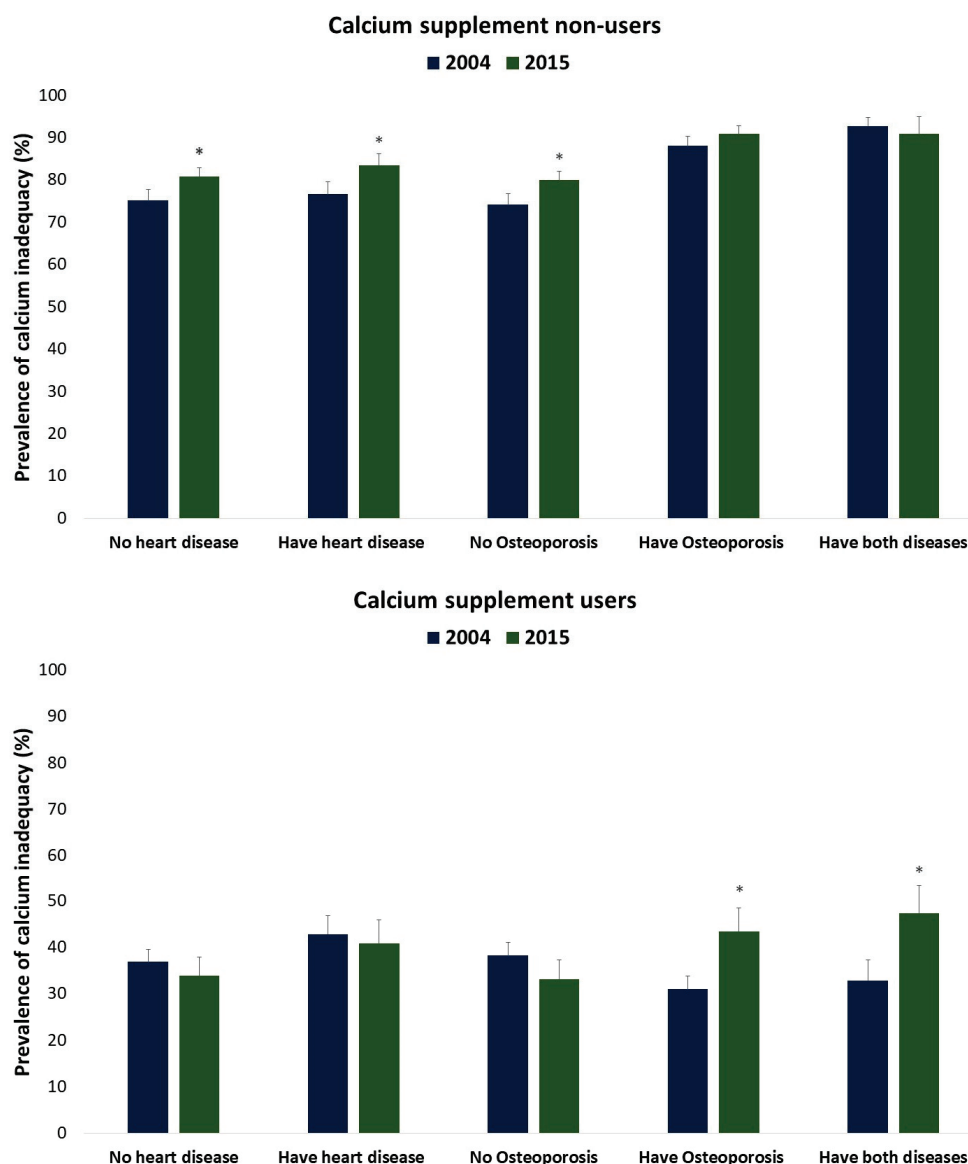


Figure 1. Prevalence of calcium inadequacy among individuals who had heart disease, osteoporosis, or both in 2004 and 2015, comparing supplement users and non-users. Calcium inadequacy was defined as an intake level below the EAR of 800 mg for males aged 51–70 years, 1000 mg for females aged 51–70 years, and 1000 mg for all individuals over 70 years, as specified by the IOM. The analysis was conducted separately for the 2004 and 2015 data sets. * Significant difference ($p < 0.05$) in supplement users between 2004 and 2015 based on age/sex groups using the confidence interval overlap technique.

3.5. Dietary and Supplemental Intake of Vitamin D among Individuals with Heart Disease, Osteoporosis, or Both in 2004 and 2015, Comparing Supplement Users and Non-Users

Vitamin D intake from food remained relatively stable over the 11-year period (from 2004 to 2015) for all the groups, as shown in Table 4. However, across all categories of supplement users, there was a substantial increase in usual vitamin D intake, from both food and supplements, reaching approximately 39 µg in 2015, compared to the range of 16 to 19 µg in 2004.

Table 4. Dietary and supplemental intake of vitamin D among Canadians (≥ 50 years) who had heart disease, osteoporosis, or both diseases combined among supplement users and non-users in 2004 and 2015 ¹.

Chronic Diseases	Vitamin D Supplement Non-Users		Vitamin D Supplement Users			
	2004 (63.7%)		2004 (36.3%)		2015 (42.0%)	
	Vitamin D (µg) from Food	Vitamin D (µg) from Food	Vitamin D (µg) from Food	Vitamin D (µg) from Food and Supplements	Vitamin D (µg) from Food	Vitamin D (µg) from Food and Supplements
No heart disease	5.4 ± 0.3	4.7 ± 0.4	5.3 ± 0.5	16.6 ± 0.6	4.8 ± 0.5	39.6 ± 3.3 [¥]
Heart Disease	5.3 ± 0.4	4.8 ± 0.4	4.8 ± 0.4	16.5 ± 1.2	4.3 ± 0.5	38.6 ± 3.3 [¥]
No Osteoporosis	5.4 ± 0.3	4.8 ± 0.4	5.4 ± 0.5	16.1 ± 0.5	4.8 ± 0.5	39.9 ± 3.3 [¥]
Have Osteoporosis	4.8 ± 0.3	4.2 ± 0.4	4.8 ± 0.4	19.3 ± 1.1	4.4 ± 0.4	38.9 ± 3.3 [¥]
Have both diseases	4.6 ± 0.3	4.5 ± 0.5	4.4 ± 0.4	18.6 ± 3.3	4.0 ± 0.4	39.0 ± 3.2 [¥]

¹ Values are mean ± SE, and the analyses were conducted separately for the 2004 and 2015 data sets. [¥] Significant differences (p -value < 0.05) between CCHS 2004 and 2015 using the confidence interval overlapping technique.

3.6. Prevalence of Vitamin D Inadequacy among Individuals with Heart Disease, Osteoporosis, or Both in 2004 and 2015, Comparing Supplement Users and Non-Users

Figure 2 provides an overview of the prevalence of vitamin D inadequacy among supplement users across all the disease groups in 2004 and 2015. Notably, there was a significant decrease in the proportion of supplement users who did not meet the estimated average requirement for vitamin D, which dropped from 16–19% in 2004 to 6–7% in 2015 among all the disease groups.

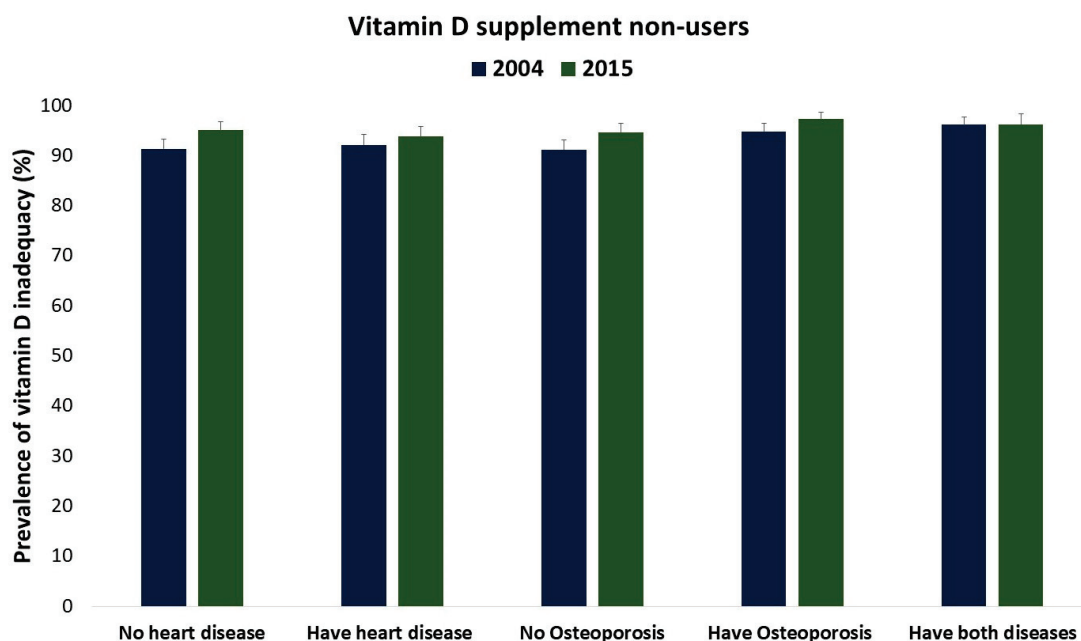


Figure 2. Cont.

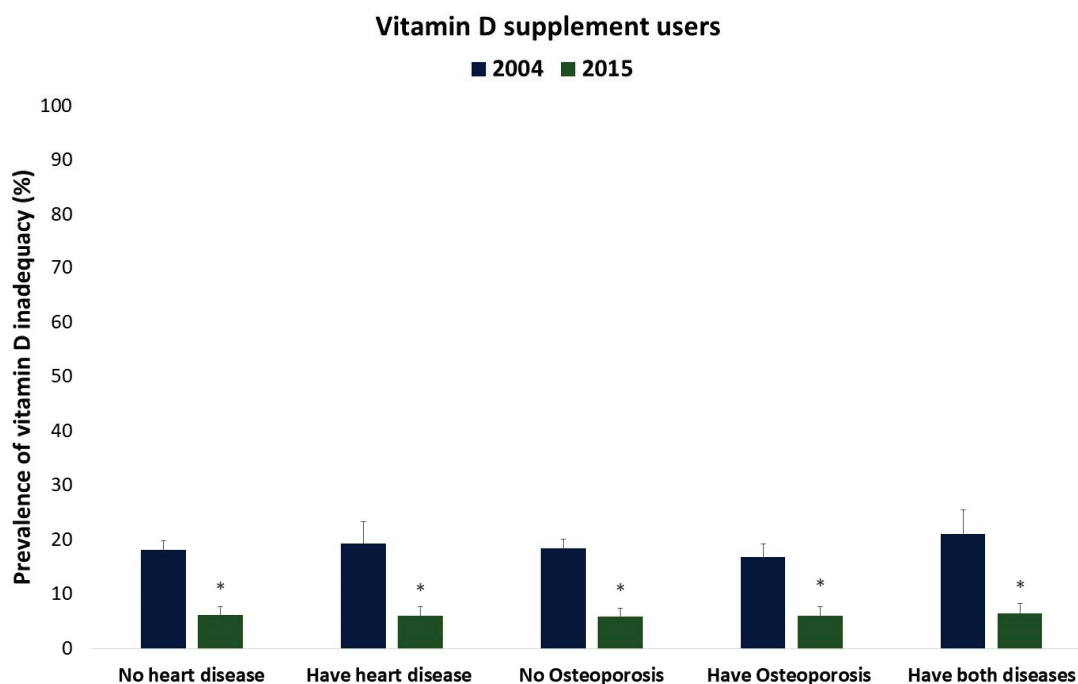


Figure 2. Prevalence of vitamin D inadequacy among individuals who had heart disease, osteoporosis, or both in 2004 and 2015, comparing supplement users and non-users. Vitamin D inadequacy was identified when intake levels were below the EAR of 10 µg (400 IU) for individuals aged 50 years and above, following the IOM recommendations. The analysis was conducted separately for the 2004 and 2015 data sets. * Significant difference ($p < 0.05$) in supplement users between 2004 and 2015 based on age/sex groups using the confidence interval overlap technique.

4. Discussion

Between 2004 and 2015, there was a notable increase in osteoporosis and heart disease prevalence, particularly among the lowest income groups. Among those not taking calcium supplements, calcium intake from food decreased significantly in all the groups except for those with both diseases. Additionally, the proportion of non-supplement users not meeting their calcium requirements increased for those with and without heart disease and osteoporosis. Regarding calcium supplementation, both osteoporosis and heart disease patients were less likely to take calcium supplements in 2015 compared to 2004. Among the supplement users, those with osteoporosis or both diseases had lower calcium intake in 2015, and more individuals did not meet their calcium requirements. While vitamin D intake from food remained unchanged from 2004 to 2015 across all the groups, a significant portion of non-supplement users did not meet their vitamin D requirements. However, the supplement users had increased vitamin D intake in 2015, and fewer failed to meet the requirements during this period.

The observed decrease in dietary calcium consumption between 2004 and 2015 may suggest a decline in overall diet quality among adults aged 50 years and older or a deliberate avoidance of calcium-rich foods in response to confusing media reports about studies indicating cardiovascular health risks associated with high calcium intake, such as the study by Bolland et al. (2008) [31]. This aligns with our previous work, which indicated a decrease in the usual intake of calcium from food sources in calcium supplement nonusers across the general Canadian population aged ≥ 1 year during the same period [32]. Similarly, the findings regarding reduced use of calcium supplements by individuals with osteoporosis and heart disease, as well as the higher likelihood of supplement users with osteoporosis or both diseases not meeting the estimated average requirement for calcium in 2015 compared to 2004, may indicate that older adults with health conditions potentially benefiting from

adequate calcium intake were dissuaded from consuming sufficient supplemental calcium to meet recommended intakes due to the influence of studies like Bolland et al. (2008) [31].

The Bolland et al. (2008) study was the first to report a potential negative effect of calcium supplementation on the risk of cardiovascular disease [31]. In this study, the participants had an average calcium intake of 1900 mg/day, with some individuals consuming amounts exceeding the upper limit, which raised concerns about the risk of hypercalcemia and vascular calcification of preexisting lesions. Excessive calcium intake above the upper limit can lead to hypercalcemia and vascular calcification, increasing the risk of cardiovascular issues [33]. Critics have pointed out several limitations in the Bolland et al. (2008) study [31], including that it was not primarily designed to investigate the relationship between calcium and cardiovascular disease, its small sample size, potential data misinterpretation, the use of a composite cardiovascular endpoint that obscured the actual impact of calcium supplementation on myocardial infarction, the failure to control for potential confounding factors, and limited generalizability due to the characteristics of the study sample [34]. Upon reanalysis of the Bolland et al. (2008) data using only cardiovascular event data from hospital records, no explicit adverse cardiovascular risk associated with calcium supplementation was found [34]. It has been estimated that a study would require 20,000 participants over 5 years to detect a 20% difference in cardiovascular risk related to calcium supplementation [35].

Following their 2008 publication, Bolland and colleagues conducted a meta-analysis that reaffirmed their initial findings [36]. However, it is important to note that this meta-analysis excluded trials of combined calcium and vitamin D supplementation, which significantly restricted the applicability of their conclusions. This limitation is significant, because the recommended approach to managing osteoporosis involves ensuring adequate intake of both calcium and vitamin D [37]. Subsequently, several other studies expressing concerns about the relationship between calcium intake and cardiovascular health have been published [22,38,39]. However, it is worth acknowledging that these studies faced criticisms, including issues related to selective data reporting and inconsistent interpretation, as highlighted in various letters to the editors.

Several recent meta-analyses have provided evidence suggesting that calcium intake within recommended levels does not pose an increased risk of cardiovascular events. One meta-analysis, which focused on prospective observational studies examining dietary calcium intake and cardiovascular disease risk, found that individuals with the highest dietary calcium intake had a pooled relative risk of 0.92 for any coronary artery disease (CAD) and 0.86 for any stroke when compared to those with the lowest intake [40]. Another meta-analysis, which included randomized controlled trials of calcium supplementation with and without vitamin D (including the Bolland et al. 2008 study), reported a pooled relative risk of 1.14 for cardiovascular events among individuals taking calcium supplements compared to placebos. However, the same meta-analysis found that those taking both calcium and vitamin D supplements had a pooled relative risk of 0.99 for cardiovascular events [41]. A meta-analysis conducted by Lewis et al. in 2015 also yielded similar results. They reported a pooled relative risk of 1.02 for coronary heart disease among individuals taking calcium supplementation with or without vitamin D when compared to those not taking calcium supplements. However, the same analysis found a pooled relative risk of 0.96 for all-cause mortality among individuals taking calcium supplementation with or without vitamin D compared to those not taking calcium supplements [42]. These findings, supported by other reviews, collectively suggest that maintaining dietary and supplemental calcium intake within recommended levels does not appear to increase cardiovascular risk and may not provide significant cardiovascular benefits for generally healthy adults [43,44].

A substantial body of literature has highlighted the beneficial effects of dietary and/or supplemental calcium on cardiovascular health, which includes improvements in cholesterol profiles [45], reductions in blood pressure [46], enhanced insulin sensitivity [47], and improved vasorelaxation [45]. Furthermore, recent evidence-based dietary reference intakes continue to recommend adequate calcium and vitamin D intake from food and, when

necessary, supplements, particularly for older adults who may be at risk for osteoporosis and fractures [16]. For instance, a study conducted in postmenopausal women with osteoporosis in France revealed that the average daily dietary vitamin D intake was only 144.8 IU, with 30% of the participants taking a vitamin D supplement. The average daily dietary calcium intake was 966.4 mg, with 38% of the participants using a calcium supplement [48]. In another study among Spanish females aged 50 years and older with osteoporosis, the mean dietary calcium intake was 1239 mg/day, whereas those aged 75 years and older had a significantly lower mean dietary calcium intake of 988 mg/day. Dietary vitamin D intake was reported at 167 IU/day, with even lower intake among participants aged 75 years and older (120 IU/day) [49].

The decline in calcium consumption among older Canadians during a period when the media was highlighting potential cardiovascular risks linked to calcium intake, as indicated in various scientific publications, underscores the importance of responsible research dissemination. Drawing broad conclusions from studies can create unwarranted alarm and may lead the public to discontinue healthy dietary habits, potentially putting them at greater nutritional risk. We have a moral obligation not only to conduct ethical research but also to ethically communicate our findings, with the ultimate goal of safeguarding public health.

Our findings indicate a significant increase in both the prevalence of vitamin D supplement use and the doses of supplemental vitamin D among Canadian adults aged 50 years and older from 2004 to 2015. This aligns with our previous work on vitamin D intake in the general Canadian population aged ≥ 1 year, which showed that, while vitamin D intake from food remained largely unchanged during this period, the prevalence of vitamin D supplement use and the percentage contribution of vitamin D from supplemental sources increased significantly [50]. This upward trend in vitamin D supplement usage and doses could be attributed to the growing body of literature suggesting that vitamin D supplementation may have a protective effect against cardiovascular disease (CVD) [51–53]. For instance, a meta-analysis of eight prospective cohort studies from the United States and Europe by Schöttker et al. found that individuals in the lowest quintile of serum 25(OH)D concentration had an increased risk of cardiovascular and all-cause mortality [54]. However, it is worth noting that while epidemiological evidence [55] and observational studies [56–59] have shown an association between vitamin D deficiency and CVD risk, this has not been confirmed by randomized controlled trials [60]. Another meta-analysis of 21 randomized controlled trials found that vitamin D supplementation did not reduce major adverse cardiovascular events, specific CVD endpoints, or all-cause mortality [61]. Furthermore, concerning bone health and osteoporosis, a recent randomized controlled trial led by Michos et al. discovered that achieving a 25(OH)D concentration of ≥ 40 ng/mL after vitamin D supplementation in adults aged ≥ 70 was not associated with a reduction in falls; in fact, it was associated with an increased risk of consequential falls [62]. It is important to recognize that low dietary vitamin D does not invariably signal a deficiency due to the body's capacity to synthesize it through skin exposure to ultraviolet B (UVB) rays, a process that is subject to seasonal variations in regions like Canada. This consideration is essential when interpreting the non-linear relationship between serum 25(OH)D concentration and health outcomes, which can be influenced by various sources, including diet, UVB exposure (particularly during spring and summer), and supplementation. This multifaceted interplay may explain why randomized controlled trials often fail to confirm findings from observational studies [63]. Additionally, it is worth mentioning that the upper limit recommended by the National Institutes of Health (NIH) and Institute of Medicine (IOM) is 4000 IU daily for individuals aged 9 years and older, underscoring the safe threshold for supplementation [16,64].

Strengths and limitations: In terms of strengths, this study draws on data from two extensive, nationally representative surveys, encompassing both dietary and supplemental intake information. This comprehensive approach enhances the study's credibility and generalizability to the Canadian population. However, there are notable limitations to

consider. Firstly, the study primarily examines changes in calcium and vitamin D consumption among older age groups, and it would be valuable to explore sex-disaggregated data to unveil potential gender-specific trends. Additionally, variations in survey execution between survey cycles, including adjustments to the food booklet used for estimating portion sizes and changes in nutrient databases, may impact the comparability of dietary intake data. Specifically, the shift from line drawings to actual-size photographs in 2015 could introduce a downward bias, particularly affecting estimations of energy, tea, and coffee consumption [65]. Furthermore, the information regarding calcium and vitamin D supplement use was collected differently in 2015, covering the past 24 h, compared to 2004, which included data from the previous 30 days. These reporting period differences should be considered when interpreting the findings. Lastly, there might be some overlap between vitamin D and calcium supplement users, since many supplements contain both nutrients, potentially influencing the study's results. These limitations should be taken into account when interpreting the study's outcomes and planning future research in this area.

5. Conclusions

In conclusion, this study reveals a concerning decline in the usual calcium intake from food among older Canadians between 2004 and 2015. Moreover, a substantial proportion of non-supplement users did not meet the estimated average requirement for calcium, highlighting a nutritional gap. Additionally, among the calcium supplement users, those with osteoporosis or both osteoporosis and heart disease were more likely to fall below the estimated average requirement for calcium in 2015 compared to 2004, indicating potential risk reduction behaviors. Vitamin D inadequacy was also prevalent, with a majority of non-supplement users failing to meet the estimated average requirement in both 2004 and 2015. However, the supplement users demonstrated improved vitamin D intake. The implications of these findings are particularly relevant in light of the recent changes to the Canadian food guidelines, which no longer emphasize dairy as a distinct food group for mandatory consumption. This change may impact calcium intake among those who may reduce or eliminate dairy consumption without adequately replacing it with other calcium-rich foods or supplements. Therefore, it is imperative for public health initiatives to focus on older adults, promoting a balanced intake through both diet and supplementation where needed to bolster heart and bone health. Further research with a longitudinal design is needed to corroborate these results and strengthen this study's conclusions.

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Article

Vitamin D and the Risk of Developing Hypertension in the SUN Project: A Prospective Cohort Study

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Abstract: Vitamin D deficiency has been associated with a higher risk of multiple diseases, including cardiovascular disorders. The purpose of this study was to examine the potential association between predicted levels of serum 25(OH)D and the risk of new-onset hypertension in a large Mediterranean cohort. A validated 136-item food frequency questionnaire was used as the dietary assessment tool. 25(OH)D serum levels were predicted using a previously validated equation. We performed Cox regression models to analyze the association between predicted serum 25(OH)D and the risk of hypertension, according to quartiles of forecasted vitamin D at baseline, after adjusting for multiple potential confounders. Over a median follow-up of 12.3 years, 2338 new cases of hypertension were identified. The analyses revealed a significant inverse association between predicted serum levels of 25(OH)D at baseline and the risk of hypertension. Individuals in the highest quartile showed a 30% relatively lower risk of hypertension compared to the lowest quartile (hazard ratio (HR): 0.70; 95% confidence interval (CI): 0.60–0.80, *p*-trend < 0.001). The outcomes remained significant after performing sensitivity analyses. The findings suggested that higher levels of forecasted vitamin D are inversely and independently associated with the risk of incident hypertension, implying that vitamin D may offer protective benefits against the disease.

Keywords: predicted vitamin D; vitamin D deficiency; hypertension; blood pressure; prospective cohort

1. Introduction

Hypertension, also known as high blood pressure, is the most prevalent cardiovascular disorder in the world [1]. The World Health Organization (WHO) has pointed out that nearly 1.28 billion adults worldwide between the ages of 30 and 79 have been diagnosed with hypertension, two-thirds residing in low-income and middle-income regions [1]. In individuals below 50 years old, hypertension is more commonly found in men. However, the sharper rise in systolic blood pressure (SBP) in women from their third decade of life, particularly after menopause, results in a higher prevalence of high blood pressure among women in groups over 65 years [2,3]. The relevance of hypertension lies in its close association with a higher risk of heart failure, stroke, and the development and progression of chronic kidney disease and coronary artery disease (CAD). Vitamin D deficiency has emerged as a global health concern [4–6]. Vitamin D is a fat-soluble vitamin, which is

synthesized endogenously via sun exposure to the skin. Its metabolites, cholecalciferol and ergocalciferol, are naturally found in various dietary sources (mainly fatty fish—such as salmon, rainbow trout, or herring—and egg yolks, among others) [4]. Beyond its well-established role for calcium in bone metabolism, 1,25(OH)₂D (the active form of 25(OH)D) has been implicated in diverse physiological processes, including metabolic disorders [7,8], cardiovascular diseases (CVD) [9], or the modulation of the immune response [10].

Various observational studies conducted with data from the third National Health and Nutrition Examination Survey (NHANES III) concluded that vitamin D deficiency was inversely associated with essential hypertension [11,12]. Although it remains unclear which exact mechanisms underlie this association, several authors have suggested different hypotheses [13,14]. Nevertheless, other randomized clinical trials (RCTs) showed weak evidence supporting the positive impact of vitamin D supplementation on blood pressure [14–17]. Moreover, results from several published meta-analyses have not been able to reach any common consensus [16,18–23]. In fact, some of them proved a beneficial effect of vitamin D supplementation in specific groups, such as older subjects or obese participants [19,20,23]. Given the potential presence of residual confounding, the task of deducing causality or consequence in the association and establishing a consensus based on these findings becomes challenging [22].

As sunlight constitutes a major source of vitamin D and its availability varies widely based on different factors such as latitude, geography, and lifestyle, the applicability of previous research findings to the Spanish population may be limited. Therefore, this study seeks to shed light on the potential link between forecasted serum 25(OH)D and the likelihood of developing hypertension within a sizable healthy Mediterranean cohort followed over an extended period.

2. Materials and Methods

2.1. Study Population

The SUN project (Seguimiento Universidad de Navarra, Pamplona, Spain) is a dynamic, prospective, and multipurpose cohort, which includes Spanish university graduates. The recruitment of participants started in December 1999 [24]. Data were gathered from participants through validated questionnaires distributed at baseline and every two years. These surveys encompassed information about dietary patterns, lifestyle factors, or the prevalence and incidence of various diseases at both the initial assessment and during subsequent follow-ups, among others. By 31 May 2022, a total of 23,133 individuals completed their initial questionnaire. For this analysis, we excluded 2525 individuals who had previously been diagnosed with hypertension at the time of enrollment. We considered only participants who completed the baseline questionnaire before 31 August 2019 ($n = 20,384$) to warrant at least 2 years and 9 months of follow-up. Additionally, we excluded 1900 individuals with reported daily energy intakes outside the established limits set by Willett (women: <500 kcal/day or >3500 kcal/day; men: <800 kcal/day or >4000 kcal/day) [25]; 757 subjects with prevalent chronic diseases at baseline (diabetes, CVD and cancer) and 1290 participants who were lost to follow-up. Ultimately, our analysis included 16,437 participants and 2338 new cases of hypertension (Figure 1). The overall long-term retention rate in the cohort was 92.7%.

This research followed the principles outlined in the Declaration of Helsinki, and all the procedures were approved by the Research Ethics Committee of the University of Navarra.

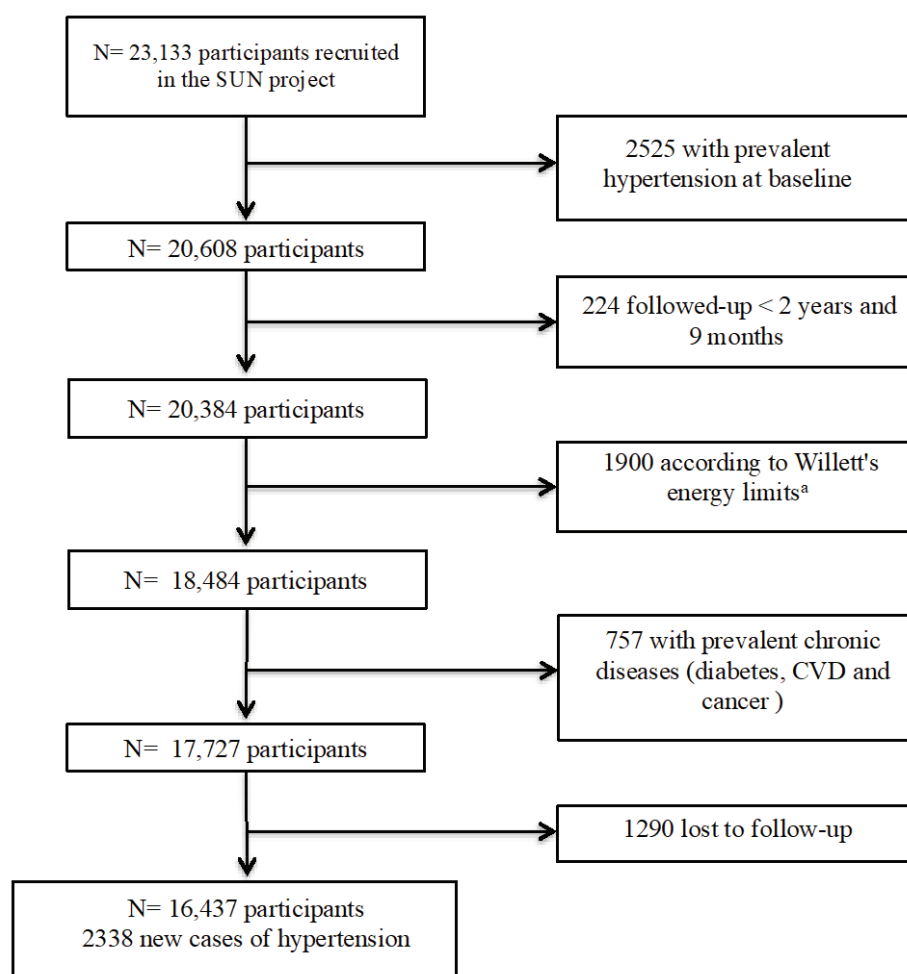


Figure 1. Flowchart of participants included in the analyses. ^a Energy limits predefined by Willett (women: <500 kcal/d or >3500 kcal/d; men: <800 kcal/d or >4000 kcal/d).

2.2. Exposure Assessment

A validated predictive model, established through multiple linear regression analyses [26], was used to estimate serum 25(OH)D levels for each participant. This predictive model incorporated several variables: age, sex, vitamin D intake from diet and supplementation, body mass index (BMI) derived from self-reported and validated weight and height (kg/m^2) [27]; skin reaction to sun exposure (mild or severe reaction); daily time spent walking (minutes per day); and sunlight exposure during summer (hours per day). All these variables were included in the following equation:

$$24.4 + (0.04 \times \text{dietary vitamin D}) + (-0.01 \times \text{age}) + (1.37 \times \text{sex}) + (-0.31 \times \text{BMI}) + (-3.71 \times \text{skin phototype}) \\ + (0.03 \times \text{walking time}) + (0.77 \times \text{summer sun exposure}) + (0.03 \times \text{physical activity})$$

Dietary data were acquired through a validated semiquantitative food frequency questionnaire (FFQ) covering 136 items [28–30]. Vitamin D intake from both dietary sources and supplements was consolidated into a single variable, adjusted for total energy intake (kcal) using the residual method. Summer sun exposure was assessed after calculating a weighted average of the exposure to sunlight each week, during summer months. Physical activity was evaluated through a questionnaire, which were previously validated, in which each physical activity was assigned a weight based on its proportional metabolic equivalents (METs) [31]. Additionally, the duration of each activity was considered to calculate a comprehensive value of MET hours per week for every participant. The methodology of the mentioned predictive model has been thoroughly detailed in previous descriptions [26].

2.3. Outcome Assessment

The main outcome of the current analyses was the incidence of self-reported hypertension. Baseline questionnaires gathered information on any prior diagnosis of hypertension, including the age at diagnosis. Incident cases were defined as those individuals who had no history of hypertension at baseline but declared a new diagnosis during the follow-up period. All this clinical information was requested from participants in their follow-up questionnaires. Hypertension was defined following the 8th Report of the Joint National Committee criteria, which is based on repeated office measurements showing systolic blood pressure (SBP) values of ≥ 140 mmHg and/or diastolic blood pressure (DBP) values of ≥ 90 mmHg [32]. A validation study was previously conducted on a subset of this cohort to verify hypertensive status. This study confirmed the validity of self-reported diagnoses: 82.3% (95% CI: 72.8–92.8) of those reporting hypertension were confirmed through conventional blood pressure measurement, and 85.4% (95% CI: 72.4–89.1) of those who did not report hypertension were confirmed as non-hypertensive [33].

2.4. Covariates Assessment

The baseline questionnaire collected socio-demographic information (age, sex, marital status, educational level), validated anthropometric measures (height and weight) [27], health-related behavioral factors (smoking status, alcohol intake, physical activity [31], total energy intake (kcal/day), specific diets, adherence to the Mediterranean diet, snacking habits, sugar-sweetened beverage consumption, and mineral intake (calcium, sodium, potassium and magnesium), as well as time spent watching television. Additional information about family and personal medical history (prevalence of hypercholesterolemia and hypertriglyceridemia at baseline) and the use of analgesic drugs was also registered. The Mediterranean dietary pattern (MedDiet) was assessed through the Mediterranean diet score based on 9 items, proposed by Trichopoulou et al. [34]. Prior studies conducted within the SUN cohort have proved the reliability of self-reported data [33,35].

2.5. Statistical Analysis

Descriptive statistics were used to summarize baseline characteristics of the participants. Continuous variables were presented as medians and interquartile ranges (non-normal distribution) or as means and standard deviations (normal distribution), while categorical variables were expressed as proportions. Normality of distribution was verified with the Shapiro–Wilk test. The follow-up period was defined as the duration from the initial baseline questionnaire to either the date in which participants were first diagnosed with hypertension or the date of the last follow-up questionnaire, if they did not develop the outcome. To examine the association between predicted 25(OH)D status and hypertension risk, we estimated the incidence rates of hypertension across quartiles of baseline predicted serum 25(OH)D levels. We then employed Cox regression models to calculate hazard ratios and 95% CIs using the lowest quartile as the reference category.

Additionally, we investigated the continuous relationship between incident hypertension and each 1 ng/mL increase in forecasted serum 25(OH)D levels. Initially, HRs were estimated with no adjustments; afterwards, we performed different models adjusted for multiple confounders. The following model included marital status (currently married/others), cumulative tobacco exposure (smoking pack years), smoking status (never, current, or former smoker), years of university studies, daily time spent watching TV (hours), physical activity level (MET-h/week), changes in weight (defined as no change, weight gain prior baseline or prior weight loss), adherence to the Mediterranean diet (low (0–3), moderate (4–6), high (7–9)), total daily calorie intake (kcal/day), daily alcohol consumption (grams/day), adherence to a specific diet (yes/no), daily sugar-sweetened beverage intake (servings/day), intake of vitamin D supplementation (yes/no), snacking habits between meals (yes/no), and daily dietary consumption of sodium, potassium, calcium, and magnesium (mg/day). We also accounted for family history of hypertension (yes/no), prevalent hypercholesterolemia, prevalent hypertriglyceridemia, and analgesic

drug use (yes/no). The third model was additionally adjusted for obesity (≥ 30 kg/m² yes/no). Missing data were handled by simple imputation, using multivariable linear regression for continuous variables and logistic or multinomial regression for categorical variables. We imputed the following variables: daily time spent watching TV (17.8%), time spent sleeping (16.4%), following a specific diet (2.3%), sugar-sweetened beverage consumption (2.6%), weight change (2.9%), and skin reaction after sun exposure (1.6%).

We additionally assessed the potential non-linear relationship between predicted serum 25(OH)D levels and hypertension risk using restricted cubic splines. Furthermore, Nelson–Aalen curves, adjusted for potential confounding variables via the inverse probability weighting method, were performed to illustrate hypertension rates throughout the follow-up period, stratified by sufficient (>20 ng/mL) and insufficient (≤ 20 ng/mL) levels of predicted serum 25(OH)D. We further explored potential modifying effects by age (under or over 50 years old), sex (women/men), and overweight status (overweight/obese or normal weight). Using the likelihood ratio test, we compared the fully adjusted Cox regression model and the same model with the interaction across product terms with quartiles of estimated vitamin D (3 degrees of freedom) to assess interaction significance (p value for interaction). Various sensitivity analyses were conducted to estimate the fully adjusted hazard ratios after comparing the highest and the lowest quartiles of forecasted vitamin D and their association with the onset of hypertension. These analyses were conducted under various assumptions to ascertain robustness and account for potential confounders. We performed new analyses excluding extreme values of total energy intake (<1 st percentile and >99 th percentile), censoring participants with a follow-up period of 14 years or more, excluding those who were diagnosed with hypertension within the first 2 years of follow-up, removing outliers' values of predicted serum 25(OH)D (± 1.5 interquartile range). Further analyses excluded participants with prevalent hypertriglyceridemia and hypercholesterolemia to mitigate potential reverse causality. All p -values were two-tailed, and statistical significance was set at $p < 0.05$. STATA 16.0 (StataCorp, College Station, TX, USA) was used for all analyses.

3. Results

This study included 16,437 participants (63.8% female) with a mean age of 36.4 years (SD ± 11.1 years). The total follow-up period amounted to 193,490 person years, with a median follow-up of 12.3 years. During this time, 2338 participants developed incident hypertension. Baseline characteristics of the participants categorized by quartiles of predicted serum 25(OH)D levels are shown in Table 1. Those in the highest quartile were generally younger, predominantly unmarried, and exhibited a lower prevalence of cardiovascular risk factors such as hypercholesterolemia and hypertriglyceridemia, as well as a lower prevalence of hypertension in their family history. They were also more physically active (3.7 times more active compared to those in the first quartile), had a lower BMI, experienced more summer sun exposure (4 times greater than the lowest quartile), and used fewer analgesic drugs. Regarding dietary habits, they had a higher daily intake of vitamin D; moreover, they were less likely to follow a specific diet and snacked between meals less often than those in the first quartile. Other factors, including time watching TV or sleeping and the intake of various nutrients, minerals, and alcohol, showed no significant differences across the groups.

Most of the cases of hypertension ($n = 842$) were identified in the first quartile, with an incidence rate of 18.2×10^{-3} versus 8.4×10^{-3} years⁻¹ in the highest quartile. Considering the lowest quartile as the reference, we observed a significant, monotonic inverse association between predicted serum 25(OH)D levels and the risk of hypertension throughout the whole period. This association was evident in both the unadjusted model and after adjusting for multiple variables (Table 2).

Table 1. Baseline features according to quartiles of predicted serum 25(OH)D.

	Quartiles of Predicted Serum 25(OH)D			
	Q1	Q2	Q3	Q4
N	4110	4109	4109	4109
Serum 25(OH)D predicted status (range, ng/mL)	7.2; 18.7	18.8; 19.8	19.9; 21.3	21.4; 32.7
Age (years) (p25; p75)	38.8 (31.0; 47.5)	36.0 (29.0; 45.0)	32.3 (26.0; 42.0)	30.0 (24.8; 39.8)
Women (%)	66.1	64.1	63.9	61.0
Smoking status (%)				
Never	45.7	47.8	52.6	56.1
Current	21.9	23.3	23.1	22.0
Former	32.4	28.9	24.3	21.9
Marital status, married (%)	58.1	52.8	42.5	35.6
Years of university (years) (p25; p75)	5.0 (4.0; 5.0)	5.0 (4.0; 5.0)	5.0 (4.0; 5.0)	5.0 (4.0; 5.0)
Body Mass Index (kg/m ²) (p25; p75)	25.2 (22.9; 27.8)	23.1 (21.1; 25.0)	21.9 (20.2; 23.9)	21.5 (19.8; 23.5)
Weight change (%) ¹				
No weight change	21.8	26.3	30.6	30.5
Weight gain	58.6	51.1	43.1	37.9
Weight loss	19.6	22.6	26.3	31.6
Physical activity (METs-h/wk) (p25; p75)	6.9 (1.6; 15.6)	12.4 (4.5; 21.9)	19.2 (9.1; 31.2)	33.4 (18.2; 54.1)
TV (hours/day) (p25; p75) *	1.5 (0.8; 2.0)	1.4 (0.8; 2.0)	1.5 (0.8; 2.0)	1.5 (0.8; 2.0)
Siesta (hours/day) (SD)	0.6 (0.5)	0.5 (0.5)	0.5 (0.5)	0.5 (0.5)
Sleeping hours (hours/day) (p25; p75) *	7.3 (7.0; 8.0)	7.3 (7.0; 8.0)	7.3 (7.0; 8.0)	7.3 (7.0; 8.0)
Walking time (min/day) (p25; p75)	15.0 (15.0; 25.0)	25.0 (15.0; 25.0)	25.0 (15.0; 45.0)	45.0 (25.0; 90.0)
Summer sun exposure (h/day) (p25; p75)	0.4 (0.1; 0.9)	0.8 (0.3; 1.2)	1.0 (0.5; 1.6)	1.6 (0.8; 3.0)
Skin reaction after sun exposure (%) *				
Mild reaction	74.6	98.3	99.1	99.4
Severe reaction	25.4	1.7	0.9	0.6
Energy intake (kcal/d) (p25; p75)	2263.2 (1851.0; 2715.1)	2268.0 (1869.8; 2712.1)	2346.1 (1941.1; 2786.1)	2436.0 (2005.4; 2901.7)
Carbohydrate intake (% of energy) (p25; p75)	43.0 (38.4; 47.8)	43.6 (38.7; 48.1)	43.8 (39.2; 48.2)	43.8 (39.2; 48.4)
Protein intake (% of energy) (p25; p75)	18.1 (16.2; 20.4)	17.9 (16.0; 19.9)	17.8 (16.0; 20.0)	18.0 (16.2; 20.1)
Fat intake (% of energy) (p25; p75)	36.8 (32.4; 40.6)	36.3 (32.6; 40.7)	36.3 (32.2; 40.5)	36.2 (32.0; 40.2)
Monounsaturated fatty acids intake (% of energy) (p25; p75)	15.6 (13.7; 18.1)	15.6 (13.5; 17.9)	15.5 (13.4; 17.8)	15.3 (13.3; 17.6)
Saturated fatty acids intake (% of energy) (p25; p75)	12.4 (10.5; 14.5)	12.5 (10.7; 14.5)	12.5 (10.6; 14.5)	12.4 (10.3; 14.4)
Polyunsaturated fatty acids intake (% of energy) (p25; p75)	5.0 (4.1; 6.0)	5.0 (4.2; 6.0)	5.0 (4.2; 6.1)	5.0 (4.1; 6.1)
Meat consumption (g/d) (p25; p75)	169.2 (122.4; 221.7)	167.4 (121.6; 219.4)	170.7 (124.3; 221.0)	171.6 (123.6; 227.2)
Trichopoulou's Mediterranean diet score (p25; p75)	4.0 (3.0; 5.0)	4.0 (3.0; 5.0)	4.0 (3.0; 5.0)	4.0 (3.0; 6.0)
Sodium intake (mg/d) (p25; p75)	2756 (2064; 3733)	2799 (2077; 3785)	2862 (2148; 3861)	2963 (2191; 3945)
Potassium intake (mg/d) (p25; p75)	4482 (3622; 5468)	4434 (3616; 5393)	4584 (3743; 5601)	4818 (3883; 5937)
Calcium intake (mg/d) (p25; p75)	1145 (886; 1444)	1132 (881; 1417)	1173 (921; 1482)	1231 (953; 1552)
Magnesium intake (mg/d) (p25; p75)	393 (321; 479)	389 (322; 472)	404 (335; 486)	423 (347; 512)

Table 1. Cont.

	Quartiles of Predicted Serum 25(OH)D			
	Q1	Q2	Q3	Q4
Following specific diet (%) *	10.4	7.2	6.3	7.5
Between-meal snacking (%)	38.1	31.9	32.8	34.6
Alcohol intake (g/d) (p25; p75)	2.3 (0.6; 8.1)	2.8 (1.0; 8.6)	3.2 (1.0; 8.5)	3.2 (1.0; 8.5)
Sugar-sweetened beverage (servings/d) (p25; p75) ^{2*}	0.1 (0.0; 0.1)	0.1 (0.0; 0.1)	0.1 (0.0; 0.4)	0.1 (0.0; 0.4)
Total vitamin D intake (mcg/d) ³ (p25; p75)	4.8 (3.5; 6.9)	4.9 (3.6; 7.6)	5.2 (3.8; 10.5)	5.6 (3.9; 11.0)
Vitamin D supplementation (%)	6.4	7.8	9.3	10.9
Vitamin D supplementation (mcg/d) ⁴ (p25; p75)	2.1 (0.3; 5.0)	2.1 (0.7; 5.0)	3.9 (0.7; 5.0)	5.0 (0.7; 5.0)
Total dietary fiber intake (g/d) (p25; p75)	20.3 (15.3; 26.4)	20.0 (15.3; 26.1)	20.8 (16.1; 26.8)	21.9 (16.7; 28.6)
Caffeine intake (mg/d) (p25; p75)	5.0 (2.0; 6.0)	5.0 (2.0; 6.0)	5.0 (1.0; 6.0)	5.0 (1.0; 6.0)
Use of analgesic drugs (%)	12.5	11.2	9.2	9.3
Family History of Hypertension (%)	45.1	41.9	38.0	36.4
Prevalent Hypercholesterolemia (%)	17.9	14.9	12.3	11.6
Prevalent Hypertriglyceridemia (%)	7.4	5.2	3.8	3.6

Categorical variables are described as percentages and continuous variables as medians (interquartile range), except for siesta, which shows a normal distribution and therefore is described as mean (standard deviation).

¹ Weight change prior baseline. ² One serving of sugar-sweetened beverages = 200 mL. ³ Total intake of vitamin D and supplementation, energy-adjusted by residual method (mcg/d). ⁴ Median intake of vitamin D supplementation (mcg/day) was calculated among consumers of vitamin supplements. * Imputed values: watching TV (17.8%), time spent sleeping (16.4%), following a specific diet (2.3%), sugar-sweetened beverages consumption (2.6%), weight change (2.9%) and skin reaction after sun exposure (1.6%).

Table 2. Cox proportional HRs and 95% CI for incident HT according to quartiles of predicted 25(OH)D status at baseline.

	Q1	Q2	Q3	Q4	p Trend	Continuous per + 1 ng/mL ^d
Predicted serum 25(OH)D ^a	17.75 (16.63; 18.33)	19.35 (19.05; 19.63)	20.52 (20.20; 20.87)	22.48 (21.78; 23.59)		
Cases of Incident HT	842	637	447	412		
Person years	46,144	48,251	50,020	49,075		
Incident rate 10 ⁻³ years ⁻¹	18.2	13.2	8.9	8.4		
Age and sex adjusted model	1.00 (reference)	0.79 (0.71–0.87)	0.59 (0.52–0.66)	0.58 (0.52–0.66)	<0.001	0.90 (0.88–0.92)
Multiple-adjusted model1 ^b	1.00 (reference)	0.86 (0.77–0.95)	0.66 (0.58–0.75)	0.67 (0.58–0.77)	<0.001	0.92 (0.90–0.94)
Multiple-adjusted model2 ^c	1.00 (reference)	0.88 (0.79–0.98)	0.68 (0.60–0.77)	0.70 (0.60–0.80)	<0.001	0.93 (0.91–0.95)

^a Predicted serum 25(OH)D status (ng/mL) expressed by p50 (p25; p75). ^b Model adjusted for age, sex, marital status, lifetime smoking (pack years), smoking status (never, current or former), years at university, daily TV consumption, physical activity (MET-h/week), weight change, adherence to Mediterranean diet score, energy intake (kcal/day), alcohol consumption (g/day), following a specific diet, sugar-sweetened beverage intake (servings/day), vitamin D supplementation (%), snacking (%), dietary consumption of sodium, potassium, calcium, and magnesium (mg/day), family history of hypertension, prevalent hypercholesterolemia, prevalent hypertriglyceridemia, and use of analgesic drugs. ^c Model 1 additionally adjusted for obesity. (≥ 30 kg/m² yes, no). ^d HR and 95% CI for incident hypertension for each 1ng/mL increase in predicted serum 25(OH)D ($p < 0.005$).

The results remained significant even after additional adjustment for obesity (BMI ≥ 30 kg/m²) as a potential confounder (HR_{Q4vsQ1} 0.70; 95% CI: 0.60–0.80, p for trend < 0.001). Furthermore, continuous analyses indicated that each 1 ng/mL increase in predicted serum 25(OH)D was linked to a 7% reduction in the risk of incident hypertension (HR 0.93; 95% CI 0.91–0.95). We conducted stratified analyses to examine whether sex, age, and obesity modified the association between predicted serum 25(OH)D and hypertension in the fully adjusted model (Table 3). No significant interactions were evinced

across quartiles of predicted serum 25(OH)D for age (p for interaction = 0.060), sex (p for interaction = 0.280), or overweight status (p for interaction = 0.692).

Table 3. Analysis of effect modification: Adjusted HRs * and 95% CI for the risk of hypertension according to quartiles of predicted levels of serum 25(OH)D stratified by potential confounders.

	N	Incident HT	Q1	Q2	Q3	Q4	p for Interaction
Age							0.060
Age $\geq$ 50 years	2103	703	1.00 (reference)	0.87 (0.71–1.06)	0.85 (0.67–1.06)	0.79 (0.61–1.05)	
Age < 50 years	14,334	1635	1.00 (reference)	0.88 (0.77–0.99)	0.61 (0.53–0.71)	0.64 (0.54–0.76)	
Sex							0.280
Women	10,483	992	1.00 (reference)	0.83 (0.70–0.97)	0.66 (0.54–0.80)	0.75 (0.61–0.93)	
Men	5954	1346	1.00 (reference)	0.94 (0.81–1.09)	0.72 (0.61–0.85)	0.68 (0.56–0.82)	
Overweight							0.692
Overweight/Obese	4267	1155	1.00 (reference)	1.01 (0.87–1.17)	0.84 (0.69–1.03)	0.80 (0.62–1.04)	
Normal weight	12,170	1183	1.00 (reference)	0.93 (0.78–1.10)	0.78 (0.65–0.93)	0.86 (0.70–1.05)	

* Adjusted for age, sex, marital status, lifetime smoking (pack years), smoking status, years at university, daily TV consumption, physical activity, weight change, adherence to Mediterranean diet score, energy intake, alcohol consumption, following a specific diet, sugar-sweetened beverage intake, vitamin D supplementation, snacking, dietary consumption of sodium, potassium, calcium, and magnesium, family history of hypertension, prevalent hypercholesterolemia, prevalent hypertriglyceridemia, use of analgesic drugs, and obesity.

We performed various sensitivity analyses to ensure the reliability of our findings. After excluding participants under different assumptions, we did not observe any significant changes in comparison to the main analysis results (Figure 2).



Figure 2. Sensitivity analyses: HR and 95% CI (Q4 vs. Q1) for incident hypertension according to forecasted serum 25(OH)D status (comparison of upper versus lowest quartile). 1. Fully adjusted

model for age, sex, marital status, lifetime smoking (pack years), smoking status, years at university, daily TV consumption, physical activity, weight change, adherence to Mediterranean diet score, energy intake, alcohol consumption, following a specific diet, sugar-sweetened beverage intake, vitamin D supplementation, snacking, dietary consumption of sodium, potassium, calcium, and magnesium, family history of hypertension, prevalent hypercholesterolemia, prevalent hypertriglyceridemia, use of analgesic drugs, and obesity. 2. Without prevalent hypertriglyceridemia adjustment. 3. Without prevalent hypercholesterolemia adjustment. 4. Without extreme daily energy intake ($<p1$ or $>p99$). 5. Without outliers located within ± 1.5 interquartile range of the average of predicted vitamin D. 6. Without participants who were diagnosed of hypertension during the first 2 years of follow-up. 7. Without participants with a follow-up period of 14 years or more.

To address potential non-linear associations, we performed restricted cubic spline analyses adjusted for the same potential confounders, revealing a statistically significant relationship between predicted serum 25(OH)D and hypertension ($p < 0.001$). This dose-response association is graphically illustrated in Figure 3. Figure 4 exhibits the cumulative incidence of hypertension during follow-up for sufficient (>20 ng/mL) and insufficient (≤ 20 ng/mL) levels of predicted vitamin D, indicating a progressively higher cumulative rate of hypertension as vitamin D levels decrease.

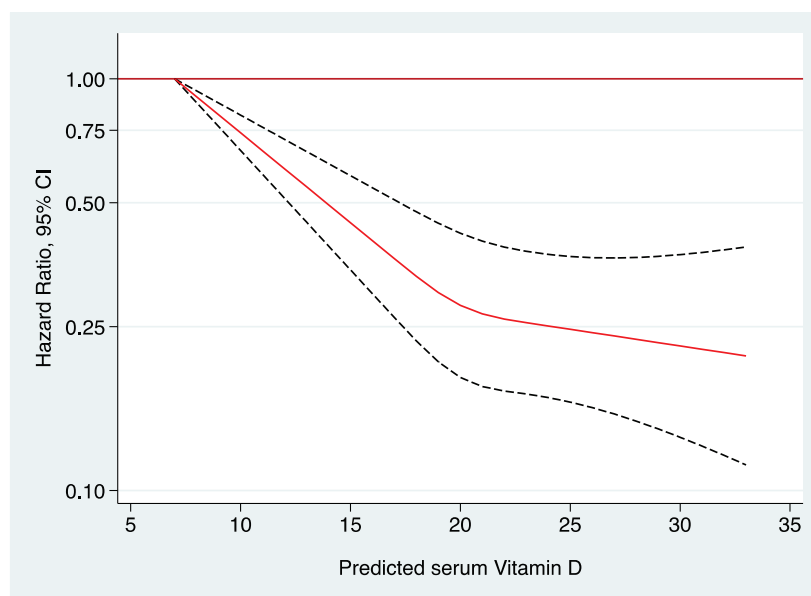


Figure 3. Restricted cubic splines dose-response association: Adjusted HR * and 95% CI for the risk of developing hypertension according to predicted levels of serum 25(OH)D. * Adjusted for age, sex, marital status, lifetime smoking (pack years), smoking status, years at university, daily TV consumption, physical activity, weight change, adherence to Mediterranean diet score, energy intake, alcohol consumption, following a specific diet, sugar-sweetened beverage intake, vitamin D supplementation, snacking, dietary consumption of sodium, potassium, calcium, and magnesium, family history of hypertension, prevalent hypercholesterolemia, prevalent hypertriglyceridemia, use of analgesic drugs, and obesity. Red line represents HR, and dotted lines represent 95% confidence intervals.

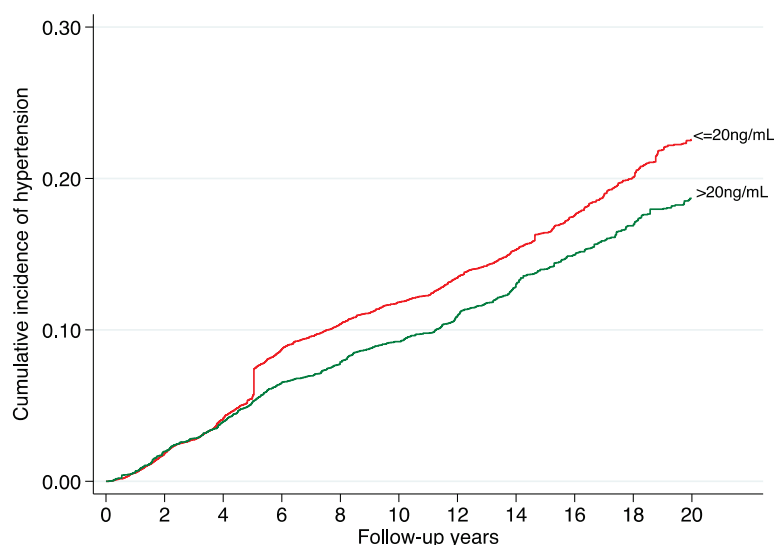


Figure 4. Nelson–Aalen graph: cumulative incidence of hypertension during follow-up according to predicted vitamin D status (cut-off point of 20 ng/mL). Adjusted for age, sex, marital status, lifetime smoking (pack years), smoking status, years at university, daily TV consumption, physical activity, weight change, adherence to Mediterranean diet score, energy intake, alcohol consumption, following a specific diet, sugar-sweetened beverage intake, vitamin D supplementation, snacking, dietary consumption of sodium, potassium, calcium, and magnesium, family history of hypertension, prevalent hypercholesterolemia, prevalent hypertriglyceridemia, use of analgesic drugs, and obesity.

4. Discussion

The current study showed that baseline levels of estimated serum 25(OH)D were inversely related to the development of hypertension over a prolonged follow-up period. The association remained statistically significant after adjusting for multiple potential confounders. The risk of hypertension was also independently associated with predicted 25(OH)D levels after adjusting for obesity, considering the cut-off point of 30 kg/m². BMI was not adjusted as a continuous variable since it was considered a potential intermediate factor in the causal pathway. Results indicated a 30% lower risk of hypertension in the highest quartile compared to the lowest quartile of predicted vitamin D levels (HR 0.70; 95% CI: 0.60–0.80, *p* trend < 0.001). Additionally, a 7% decrease in hypertension incidence was observed for each 1 ng/mL increase in predicted vitamin D levels (HR 0.93; 95% CI: 0.91–0.95). These findings continued to be significant after performing several sensitivity analyses.

Our findings agree with previously published evidence. A comprehensive meta-analysis identified a significant inverse association between incident arterial hypertension and baseline serum levels of 25(OH)D, revealing a relative risk for high blood pressure of 0.70 (95% CI: 0.57–0.86) [18]. Another updated meta-analysis of cohort studies and randomized trials suggested that the risk of new hypertension events decreased by 7% (RR 0.93; 95% CI: 0.89–0.98), after each increase of 10 ng/mL [22]. In addition, a clinical trial conducted among the Black population showed a significant reduction of 0.2 mmHg in systolic pressure per 1 ng/mL increase in serum 25(OH)D (*p* = 0.02), although there was no impact on diastolic pressure [36]. Furthermore, another trial found that vitamin D deficient individuals (<15 ng/mL) had a relative risk of developing hypertension of 2.67 (95% CI: 1.05–6.79) in women and 6.13 (95% CI: 1.00–37.8) in men after four years of follow-up [37]. Analyses of Mendelian randomization supported the causal positive effect of increased serum 25(OH)D in lowering the risk of hypertension [38,39]. One of the analyses documented a reduction in SBP by 0.37 mmHg (95% CI: −0.73 to 0.003; *p* = 0.052) and 0.29 mmHg (95% CI: −0.52 to −0.07; *p* = 0.01) in DBP after each increase of 10% in genetically determined vitamin D levels [38]. Previous evidence has suggested the existence of baseline thresholds for serum vitamin D levels, whereas some researchers

evinced a considerably higher risk of hypertension in those participants with 25(OH)D below 30 ng/mL that remained statistically significant over the range of 30–52 ng/mL [22], while others considered a threshold of 23 ng/mL [40].

Meanwhile, most interventional studies have yielded limited evidence supporting the beneficial impact of vitamin D supplementation on hypertension [14,40–44]. A meta-analysis of 46 clinical trials reported that among participants with a mean baseline vitamin D below 20 ng/mL supplementation did not lead to significant changes in blood pressure [21]. Based on this finding, they concluded that vitamin D supplementation might not be an effective strategy for lowering blood pressure [21]. Similarly, He S et al. [20] conducted another meta-analysis and found no effect on blood pressure among individuals with deficient levels of vitamin D. Nonetheless, their subgroup analyses revealed a significant improvement in blood pressure in both obese and participants over 50 years old after vitamin D3 supplementation [20]. Similarly, Golzarand et al. [19] found positive effects in groups that received daily doses over 800 IU during less than 6 months, as well as in older participants. These discrepancies could be explained by the high heterogeneity of the recruited populations. Therefore, in this study, we limited our recruitment to those apparently healthy participants, leaving aside targeted diseases (such as diabetes, cardiovascular events, or cancer), which could have interfered in the interaction between vitamin D and hypertension. Moreover, differences in dosing regimens can indeed influence the outcomes shown in different studies. On the one hand, studies with more frequent dosing schedules (daily or weekly doses) might better mimic the physiological vitamin D synthesis and provide more consistent benefits for blood pressure regulation [40]. On the other hand, monthly high-dose vitamin D3 supplementation results in large spikes in 25(OH)D status, followed by a gradual decline over the course of the month, which could lead to periods of suboptimal vitamin D levels in-between doses and might potentially negate any consistent effects on hypertension [42].

There are several mainstream mechanisms that can explain how vitamin deficiency may induce hypertension. The first mechanism involves the activation of the renin-angiotensin-aldosterone system (RAAS). As the plasma renin concentration increases, sympathetic activity might become intensified and consequently enhance intra-glomerular pressure, leading to higher blood pressure, a lower glomerular filtration rate (GFR), and cardiovascular damage [13,20,23,45]. Secondly, vitamin D is involved in calcium homeostasis as it stimulates calcium transporters, increasing renal calcium reabsorption and its release from bones. As a consequence, deficient levels of serum vitamin D might result in a lower plasma concentration of calcium, leading to a higher secretion of the parathyroid hormone (PTH), which has proven to be inversely correlated with hypertension [13]. Although the mechanism is still unclear, it is hypothesized that PTH increases the levels of calcium in blood stream, leading to endothelial dysfunction [13]. Moreover, vitamin D may be directly implicated in left ventricular hypertrophy and vascular stiffness [9,46]. Insufficient levels of serum 25(OH)D might lead to a decreased NO (nitric oxide) in blood vessels and a reduction in the calcium influx, affecting vasodilation and causing a subsequent increase in blood pressure [20].

Although evidence does not support vitamin D's antihypertensive therapeutic effect alone, studies examining vitamin D's role as an adjuvant agent to the classical antihypertensive treatment in patients diagnosed with hypertension and vitamin D deficiency seemed to have promising results [20,23,47,48]. If this beneficial effect is confirmed in further research, preventing vitamin D deficiency through food supplementation may be considered as a cost-effective measure to prevent and control hypertension, rather than a widespread screening of vitamin D deficiency as its high prevalence has been clearly demonstrated worldwide [49,50].

Limitations and Strengths

Our study has some limitations that must be noted. The sample primarily consists of young adults with a high level of education, making it unrepresentative of the general

population. Consequently, generalizing our findings requires a deeper understanding of the specific conditions and shared biological mechanisms, rather than relying solely on statistical representativeness [51]. However, this characteristic of our study also enhances its internal validity due to the participants' high educational level and homogeneity, which minimize the influence of confounding factors related to education and their socioeconomic status. Additionally, the use of self-reported data (a validated FFQ to evaluate dietary pattern and lifestyle habits) introduces subjectivity and potential information bias. This is particularly relevant when reporting daily consumption of vitamin D in both diet and supplements, given that collected data consistently indicate consumption below recommended levels. Nevertheless, the prior validation of these self-reported parameters helps ensure the reliability of our results [33,35]. We did not have specific information about issues such as the type of sunscreen used or the clothing, which could be considered in further research. Moreover, our vitamin D prediction model is primarily a research tool more suitable for epidemiological contexts rather than daily clinical practice. It may not be appropriate for specific groups, such as children, pregnant women, or individuals with severe kidney disease, who require tailored prediction methods for accurate outcomes.

To our knowledge, this is one of the few studies to assess the association between forecasted serum 25(OH)D and incident hypertension in a Spanish population and the first one based on a validated predictive model. The study design offers several strengths, including its dynamic recruitment strategy, prospective nature, large sample size with substantial long-term follow-up, and a high retention rate. These factors are essential for establishing a clear cause-and-effect relationship between vitamin D and hypertension. We also accounted for multiple potential confounders and conducted several sensitivity analyses to strengthen the reliability of our findings, minimizing the likelihood of residual bias.

5. Conclusions

Baseline levels of predicted serum 25(OH)D, based on a validated model, appeared to show an independent and inverse association with an increased risk of developing hypertension in a Mediterranean cohort. The association remained strongly significant even after adjusting for multiple confounders, implying that higher levels of forecasted vitamin D at the outset may offer protective benefits against incident hypertension. Nonetheless, it remains uncertain what specific baseline threshold of 25(OH)D might be considered preventive for hypertension, and further investigations should be performed.

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Data Availability Statement: The data from the SUN Project that support our findings are available upon request from the Department of Preventive Medicine and Public Health, School of Medicine, University of Navarra (Spain) at sun@unav.es.

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Article

Adherence to a Cholesterol-Lowering Diet and the Risk of Pancreatic Cancer: A Case–Control Study

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Abstract: Background: Pancreatic cancer risk has been associated with increased serum cholesterol level, which is in turn partially influenced by diet. This study aimed at evaluating the association between pancreatic cancer risk and the adherence to a plant-based cholesterol-lowering diet. Methods: Data were derived from an Italian case–control study including 258 pancreatic cancer patients and 551 controls. The cholesterol-lowering diet score was based on seven components: high intakes of (i) non-cellulosic polysaccharides (a proxy of viscous fibers), (ii) monounsaturated fatty acids, (iii) legumes, and (iv) seeds/corn oils (a proxy of phytosterols); and low intakes of (v) saturated fatty acids, (vi) dietary cholesterol, and (vii) food with a high glycemic index. The score was calculated adding one point for each fulfilled component, thus ranging from zero (no adherence) to seven (complete adherence). The odds ratios (ORs) and 95% confidence intervals (CIs) were estimated through the logistic regression model. Results: Scores 5–7 were associated with reduced cancer risk (OR = 0.30; 95% CI: 0.18–0.52) compared to scores 0–2. Conclusions: Adherence to a plant-based cholesterol-lowering diet was associated with a reduced risk of pancreatic cancer.

Keywords: cholesterol; pancreatic cancer; plant-based diet

1. Introduction

Pancreatic cancer is a fatal cancer affecting approximately 140,000 new patients in Europe, causing approximately 132,000 deaths each year [1]. In both sexes, pancreatic cancer ranked 14th for incidence (age-standardized incidence rate: 7.8/100,000), but 5th for mortality, with rates close to incidence (age-standardized mortality: 7.2/100,000) [1]. Since 2020, the incidence of pancreatic cancer has increased by about 27%, and a further increase is predicted in the near future [1].

Tobacco smoking is the major modifiable risk factor for pancreatic cancer [2]. However, the reduction in smoking prevalence had only a limited impact on pancreatic cancer trends,

suggesting that other etiological factors are sustaining pancreatic cancer incidence. Among other modifiable factors, excessive body weight and dietary factors have been associated with increased risk of pancreatic cancer [3]. Studies on diet quality—measured through common a priori indices such as the Healthy Eating Index (HEI-2005 and HEI-2010), the Alternative Healthy Eating Index-2010 (AHEI-2010), or the Dietary Approaches to Stop Hypertension (DASH)—reported reduced risk of pancreatic cancer among people adhering to a healthy dietary pattern and increased risk in those eating according to a Western dietary pattern [4]. In particular, the favorable patterns are characterized by elevated consumption of fruits and vegetables and by low intake of animal fats. This was confirmed by a dietary modification trial of the Women’s Health Initiative [5], which found a 29% reduction in pancreatic cancer risk in overweight women following a low-fat diet.

However, total fat intake includes different types of fats, which can have different effects on cancer risk. Indeed, a high intake of saturated fatty acids (SFAs) [6–9] and trans-fatty acids [9,10] has generally been associated with an increased risk of pancreatic cancer, whereas monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) have been associated with a reduced risk [7,8,11] or no association [6,9]. Evidence from pre-clinical studies supported the heterogeneity effect on pancreatic cancer risk according to type of fat [12]. Among dietary factors, special attention has been paid to cholesterol. Observational studies found a significant excess in the risk of pancreatic cancer associated with high intakes of dietary cholesterol [13], though this association was mainly found in case–control studies. Studies evaluating serum cholesterol reported similar results [14,15], but the comparison of results on dietary and serum cholesterol is challenging. Indeed, dietary cholesterol is only one contributor to serum cholesterol levels, which may explain the low correlations observed in some studies [16]. Nonetheless, circulating serum cholesterol levels are influenced by dietary habits, which are characterized by different sources of fats, cholesterol-lowering foods, and nutrients.

On this ground, a plant-based cholesterol-lowering Portfolio Diet Score has been recently developed for cardiovascular disease prevention [17]; this was adapted to the Italian diet to investigate the impact of cholesterol-lowering diets in cancer etiology [18] and validated on clinical trial data [19]. We hypothesized that adherence to a cholesterol-lowering diet is inversely associated with the risk of pancreatic cancer, consistent with previous findings for prostate cancer [18]. Specifically, the aims of this study were (a) to investigate the association between the cholesterol-lowering diet score and the risk of pancreatic cancer in an observational study conducted in Italy; and (b) to evaluate the potential modifying effects of common determinants of cancer risk (e.g., sex, age, tobacco smoking, alcohol drinking, overweight/obesity, physical activity).

2. Materials and Methods

2.1. Participants and Study Design

Data were derived from a multicentric case–control study conducted between 1991 and 2008 [7]. Cases were 326 patients (median age 63 years) with incident confirmed pancreatic cancer, admitted to major teaching and general hospitals in the province of Pordenone and in the greater Milan area, Northern Italy. For each case, two controls were frequency-matched by study center, sex, and age, for a total of 652 patients (median age 62 years). They were admitted to the same hospitals as cases for a wide spectrum of acute conditions other than neoplastic or digestive tract diseases, namely trauma (31%), other orthopedic disorders (31%), acute surgical conditions (28%), and other miscellaneous illnesses (10%). Refusal rate was below 5% for both cases and controls. In each of the participating centers, the local institutional authority reviewed the protocol and cleared the conduct of this study, in accordance with the Italian legislation for observational studies in force at the time this study was conceived. Patients signed an informed consent prior to enrollment. To avoid bias due to lipid-lowering medications, 68 cases and 101 controls treated for hypercholesterolemia were excluded from the present analysis, thus leaving 258 cases and 551 controls.

2.2. Diet Assessment and Cholesterol-Lowering Diet Score Computation

Trained interviewers administered a structured questionnaire to both the cases at the time of cancer diagnosis and the controls at hospital admission. The questionnaire assessed socio-demographic characteristics, anthropometric variables, smoking, drinking, physical activity, medical history, and history of cancer in relatives. Current smokers were patients who smoked at least 1 cigarette/day for a minimum continuous period of 1 year. Similarly, current alcohol users were patients who drank at least 1 drink/day for a continuous period of at least 1 year. Former smokers and alcohol users were individuals who had abstained from the habit for at least 1 year before diagnosis (for cases) or interviews (for controls). Overall physical activity, defined by the combination of occupational and recreational physical activities, was categorized as low, medium, and high [20].

The habitual diet during the two years prior to cancer diagnosis (for cases) or hospital admission (for controls) was assessed by a 78-item food-frequency questionnaire (FFQ), which had satisfactory validity (the median value of partial correlation coefficients between nutrient intakes assessed by the FFQ and a 7-day dietary record was 0.46) [21] and reproducibility (the median value of partial correlation coefficients between nutrient intakes assessed by two consecutive FFQs was 0.67) [22]. Participants were asked to indicate the average weekly frequency of consumption of each food item, reporting variation in seasonal consumption of fruit and vegetables. The serving size was defined in “natural” units (e.g., one egg, one apple) or as an average serving in the Italian diet (e.g., 80 g of dry pasta, 150 g of tomatoes). Intakes lower than once a week but at least once a month were coded as 0.5 per week. Total energy and nutrient intakes—including dietary fiber, MUFAs from animal and plant sources, and SFAs—were computed using an Italian food composition database [23]. Dietary fiber was derived using the Englyst method, assessing cellulose separately from soluble and insoluble non-cellulosic polysaccharides. Total MUFA and SFA intakes were then expressed as a proportion of total energy intake, multiplying the intake in grams by 9 kcal and dividing by total energy intake (in kcal). The glycemic index (GI) was expressed as a percentage of the glycemic response elicited by white bread as a standard food, using international tables [24]. The average daily GI of a subject’s diet was computed as the sum of the weighted GI values for each individual food weighted by weekly consumption [25].

The adherence to a cholesterol-lowering diet was assessed through an ad hoc score [18], validated on clinical data [19]. Briefly, the score considered seven a priori dietary components derived from the FFQ: (1) high intake of non-cellulosic polysaccharide soluble fibers (NCPSTs) as a proxy of high viscous fibers; (2) high MUFA intake; (3) high legume intake; (4) low SFA intake; (5) high intake of oils from seeds or corn as a proxy of phytosterols [26]; (6) low dietary cholesterol intake; and (7) low daily GI [27]. One point score was assigned for each of the seven dietary components, and zero elsewhere. The final score was calculated as the sum of all the point scores, and it ranged from 0 (no adherence) to 7 (complete adherence). Low and high intake defined by the current literature [28,29] were scarcely discriminating in the present study population; therefore, for each dietary component, a sex-specific cut-off was identified for women and men separately through a receiving operating characteristic (ROC) analysis predicting “case” status [18]; the point score was assigned accordingly.

2.3. Statistical Analysis

The original sample size of 326 cases and 652 controls (case-to-control ratio: 1:2) was calculated at the time this study was conceived to estimate an odds ratio of pancreatic cancer ≥ 1.5 for several dichotomous exposures (e.g., ever vs never tobacco smoking, diabetes), fixing the a priori probabilities $\alpha = 0.05$ and $\beta = 0.20$ (power = 80%).

Odds ratios (ORs) and the corresponding 95% confidence intervals (CIs) for categories of cholesterol-lowering diet score (i.e., 0 to 2; 3 to 4; and 5 to 7) were computed through logistic regression models conditioned on sex. A model with minimum adjustments including terms for study center, age (<60, 60–64, 65–69, ≥ 70 years), education (<7, 7–11,

≥ 12 years), year of interview (1991–1999, 2000–2008), and daily total energy intake (< 2029 , 2029–2459; 2460–2984; ≥ 2985 kcal/day) was firstly estimated. Subsequently, to account for potential confounding, a fully adjusted model was estimated, including additional terms for smoking habits (never, former, current < 20 cigarettes/day, current ≥ 20 cigarettes/day), drinking habits (never, former, current), diabetes mellitus, family history of pancreatic cancer in first-degree relatives, and physical activity (low/medium, high). Differences in the distribution of cholesterol-lowering diet score across strata of socio-demographic characteristics as well as lifestyle factors among controls were evaluated by means of the chi-square test of independence.

In addition, the ORs and 95% CIs for a 1-point increment in the cholesterol-lowering diet score were further estimated in the overall study sample and across strata of potential effect modifiers, such as socio-demographic characteristics (i.e., sex, age, education, period of enrollment) and lifestyle factors (i.e., tobacco smoking, alcohol drinking, physical activity) that might have an impact on diet or lipid profile. Diabetes mellitus was not considered given the small number of subjects reporting this condition. Heterogeneity across strata was assessed by means of the likelihood-ratio test comparing models with and without the interaction term. All tests were two-sided, and the significance level was set at 0.05. All analyses were conducted using R version 4.0.5.

3. Results

The distribution of socio-demographic characteristics and potential confounders is reported in Table 1. The cases reported high education, diabetes mellitus, and current smoking more frequently, whereas no differences emerged for other characteristics.

For each dietary component, the gender-specific optimal cut-off discriminating cases and controls is reported in Table 2. A reduced pancreatic cancer risk was found in people with high intakes of dietary fiber (NCPSPFs) (i.e., ≥ 6.9 g/day for women and ≥ 7.3 g/day for men; OR = 0.43; 95% CI: 0.29–0.65) and in those with low glycemic index (i.e., < 71.0 for women and < 70.9 for men; OR = 0.40; 95% CI: 0.27–0.59). A non-significant association emerged for the remaining dietary components. Combining the seven dietary components, we evaluated the overall adherence to the cholesterol-lowering diet, as follows: high adherence, defined as a total score of 5 to 7, was reported in 17.1% of the cases and in 27.2% of the controls (Table 2), leading to an OR of 0.30 (95% CI: 0.18–0.52) compared to patients with 0–2 scores. Results were consistent when the patients with treated hypercholesterolemia were included in the analysis (Table S1).

Among the controls, adherence to the cholesterol-lowering diet was significantly higher in women than in men ($p = 0.02$; Table 3) and in never-smokers compared to current and former smokers ($p = 0.05$), while no significant differences emerged for other socio-demographic characteristics or lifestyle factors.

The heterogeneity of the ORs was also investigated across strata of selected socio-demographic characteristics and lifestyle factors (Figure 1). Overall, an increment of one point in the score was associated with a significantly reduced risk of pancreatic cancer (OR = 0.72; 95% CI: 0.63–0.83). No significant heterogeneity emerged across strata of sex, age, education, and year of interview. The inverse association between pancreatic cancer risk and cholesterol-lowering diet score was confirmed in people with low/medium physical activity (OR = 0.62; 95% CI: 0.51–0.75), but not in those with high physical activity (OR = 0.94; 95% CI: 0.74–1.20; p for heterogeneity < 0.01). Similarly, the inverse association seems restricted to current alcohol users (OR = 0.66; 95% CI: 0.56–0.78), though the ORs were not significantly heterogeneous ($p = 0.07$).

Table 1. Distribution of 258 cases of pancreatic cancer and 551 controls according to study center, socio-demographic characteristics, and lifestyle factors. Italy, 1991–2008.

Variable	Cases		Controls	
	<i>n</i>	(%)	<i>n</i>	(%)
Study center				
Pordenone	138	(53.5)	303	(55.0)
Milan	120	(46.5)	248	(45.0)
Sex				
Men	140	(54.3)	304	(55.2)
Women	118	(45.7)	247	(44.8)
Age (years)				
<60	102	(39.5)	212	(38.5)
60 to 69	93	(36.0)	191	(34.7)
≥70	63	(24.4)	148	(26.9)
Year of interview				
1991–1999	130	(39.9)	395	(71.7)
2000–2008	155	(60.1)	156	(28.3)
Education (years) ^a				
<7	128	(49.6)	295	(53.5)
7 to 11	70	(27.1)	165	(29.9)
≥12	58	(22.5)	89	(16.2)
Physical activity ^a				
Low/medium	143	(55.4)	342	(62.1)
High	115	(44.6)	207	(37.6)
Diabetes mellitus ^a				
No	213	(82.6)	518	(94.0)
Yes	44	(17.1)	33	(6.0)
Family history of pancreatic cancer				
No	249	(96.5)	537	(97.5)
Yes	9	(3.5)	14	(2.5)
Smoking habits ^a				
Never	110	(42.6)	271	(49.2)
Former	69	(26.7)	167	(30.3)
Current < 20 cigarettes/day	41	(15.9)	68	(12.3)
Current ≥ 20 cigarettes/day	37	(14.3)	43	(7.8)
Alcohol drinking habits ^a				
Never	34	(13.2)	89	(16.2)
Former	26	(10.1)	48	(8.7)
Current	196	(76.0)	414	(75.1)

^a The sum does not add up to the total because of some missing values.

Table 2. Adjusted odds ratios (ORs) and corresponding 95% confidence intervals (CIs) for pancreatic cancer risk according to dietary component ^a and cholesterol-lowering diet score. Italy, 1991–2008.

Dietary Component		Score	Cases		Controls		OR (95% CI) ^b	OR (95% CI) ^c
Women	Men		<i>n</i>	(%)	<i>n</i>	(%)		
Non-cellulosic polysaccharide soluble fibers (g/day), as a proxy of viscose fibers								
<6.9	<7.3	0	124	(48.1)	206	(37.4)	Ref.	Ref.
≥6.9	≥7.3	1	134	(51.9)	345	(62.6)	0.42 (0.28–0.62)	0.43 (0.29–0.65)
Monounsaturated fatty acids (% of total energy intake)								
<11.5	<12.0	0	47	(18.2)	153	(27.8)	Ref.	Ref.
≥11.5	≥12.0	1	211	(81.8)	398	(72.2)	1.43 (0.96–2.12)	1.46 (0.97–2.20)
Legumes (servings/week)								
<1	0	0	112	(43.4)	198	(35.9)	Ref.	Ref.
≥1	>0	1	146	(56.6)	353	(64.1)	0.74 (0.52–1.05)	0.80 (0.55–1.15)
Saturated fatty acids (% of total energy intake)								
≥11.2	≥9.7	0	143	(55.4)	280	(50.8)	Ref.	Ref.
<11.2	<9.7	1	115	(44.6)	271	(49.2)	0.76 (0.55–1.04)	0.80 (0.57–1.12)
Seeds or corn oil (g/day per 2000 kcal), as a proxy of phytosterol								
<3.1	<2.7	0	159	(61.6)	285	(51.7)	Ref.	Ref.
≥3.1	≥2.7	1	99	(38.4)	266	(48.3)	0.77 (0.54–1.08)	0.83 (0.58–1.18)
Dietary cholesterol (mg/day)								
≥303.7	≥235.1	0	161	(62.4)	290	(52.6)	Ref.	Ref.
<303.7	<235.1	1	97	(37.6)	261	(47.4)	0.59 (0.37–0.93)	0.62 (0.39–1.00)
Glycemic index ^d								
≥71.0	≥70.9	0	206	(79.8)	353	(64.1)	Ref.	Ref.
<71.0	<70.9	1	52	(20.2)	198	(35.9)	0.43 (0.30–0.62)	0.40 (0.27–0.59)
Cholesterol-lowering diet score								
0 to 2			66	(25.6)	67	(12.2)	Ref.	Ref.
3 to 4			148	(57.4)	334	(60.6)	0.39 (0.26–0.60)	0.40 (0.26–0.63)
5 to 7			44	(17.1)	150	(27.2)	0.27 (0.16–0.45)	0.30 (0.18–0.52)

^a Cut-offs for each dietary component were selected by means of a receiving operating characteristic (ROC) analysis for men and women separately. ^b Estimated by means of logistic regression models conditioned on sex and adjusted for study center, age, year of interview, education, and energy intake. ^c Estimated by means of logistic regression models conditioned on sex and adjusted for study center, age, year of interview, education, physical activity, diabetes mellitus, family history of pancreatic cancer, smoking habits, drinking habits, and energy intake. ^d White bread scale.

Table 3. Distribution of 551 controls according to cholesterol-lowering diet score and socio-demographic characteristics and lifestyle factors. Italy, 1991–2008.

Variable	Cholesterol-Lowering Diet Score						χ^2 Test
	0 to 2		3 to 4		5 to 7		
	<i>n</i>	(%)	<i>n</i>	(%)	<i>n</i>	(%)	
Total	67	(12.2)	334	(60.6)	150	(27.2)	
Study center							
Pordenone	31	(10.2)	187	(61.7)	85	(28.1)	<i>p</i> = 0.31
Milan	36	(14.5)	147	(59.3)	65	(26.2)	
Sex							
Men	40	(13.2)	196	(64.5)	68	(22.4)	<i>p</i> = 0.02
Women	27	(10.9)	138	(55.9)	82	(33.2)	
Age (years)							
<65	35	(11.2)	191	(61.0)	87	(27.8)	<i>p</i> = 0.71
≥65	32	(13.4)	143	(60.1)	63	(26.5)	
Year of interview							
1991–1999	49	(12.4)	242	(61.3)	104	(26.3)	<i>p</i> = 0.75
2000–2008	18	(11.5)	92	(59.0)	46	(29.5)	
Education (years) ^a							
<7	37	(12.5)	169	(57.3)	89	(30.2)	<i>p</i> = 0.39
7 to 11	21	(12.7)	102	(61.8)	42	(25.5)	
≥12	9	(10.1)	61	(68.5)	19	(21.3)	
Physical activity ^a							
Low/medium	48	(14.0)	198	(57.9)	96	(28.1)	<i>p</i> = 0.16
High	19	(9.2)	134	(64.7)	54	(26.1)	
Diabetes mellitus ^a							
No	64	(12.4)	308	(59.5)	146	(28.2)	<i>p</i> = 0.08
Yes	3	(9.1)	26	(78.8)	4	(12.1)	
Family history of pancreatic cancer							
No	64	(11.9)	327	(60.9)	146	(27.2)	<i>p</i> = 0.52
Yes	3	(21.4)	7	(50.0)	4	(28.6)	
Smoking habits ^a							
Never	29	(10.7)	153	(56.5)	89	(32.8)	<i>p</i> = 0.05
Former	21	(12.6)	105	(62.9)	41	(24.6)	
Current < 20 cigarettes/day	10	(14.7)	42	(61.8)	16	(23.5)	
Current ≥ 20 cigarettes/day	6	(14.0)	33	(76.7)	4	(9.3)	
Alcohol drinking habits ^a							
Never/Former	20	(14.6)	83	(60.6)	34	(24.8)	<i>p</i> = 0.53
Current	47	(11.4)	251	(60.6)	116	(28.0)	

^a The sum does not add up to the total because of some missing values.

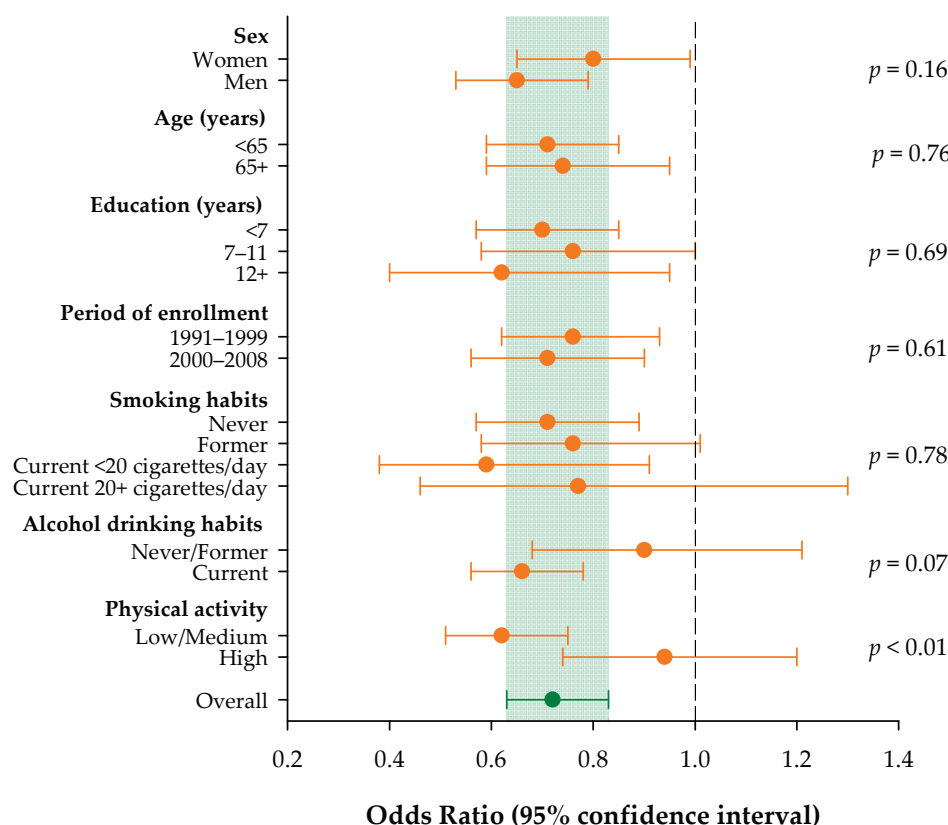


Figure 1. Odds ratios and corresponding 95% confidence intervals for pancreatic cancer risk according to a 1-point increment in the adherence to the cholesterol-lowering diet score and across strata of selected socio-demographic characteristics and lifestyle factors. Italy, 1991–2008.

4. Discussion

The present study reported an inverse association between the risk of pancreatic cancer and the cholesterol-lowering diet score. People with high adherence showed a reduction of 67% in pancreatic cancer risk compared to those with low adherence.

The reported association is strong and possibly overestimated. Nonetheless, it is likely to be genuine. The association between serum cholesterol and pancreatic cancer risk has been reported to vary according to the time to diagnosis [14,15]. Close to pancreatic cancer diagnosis (i.e., within three years), inverse associations have been found for high levels of serum cholesterol [15]. This is probably due to reverse causation: pancreatic cancer may reduce caloric intake and exert a “cholesterol-lowering effect” when it accelerates the cholesterol metabolism to build new membranes and maintain active signaling [30]. In contrast, high serum cholesterol levels were associated with an increased risk of pancreatic cancer when blood samples were taken more than four years prior to diagnosis [15]. This couples with the ability of cholesterol to promote pancreatic cancer cell proliferation, migration, and invasion [31]. Therefore, it is plausible that a diet that lowers circulating cholesterol levels may exert a protective effect towards pancreatic cancer onset. In the present study, we assessed the diet in the two years preceding enrollment, and this was representative of a longer period before diagnosis since the dietary habits were quite stable in the Italian population.

The results of the present study are consistent with previous investigations on similar a posteriori dietary patterns based on food consumption. A recent meta-analysis of six case-control studies and six cohorts reported a 24% increase in pancreatic cancer risk in people adhering to a Western-type dietary pattern [32], which is characterized by elevated intakes of red and processed meat, high-fat products, refined grains, high dietary GI, and low intakes of fruits and vegetables. These findings are supported by a diet modification

intervention to reduce total fat to 20% of the daily energy intake in the Women's Health Initiative, which showed a reduction of 29% in pancreatic cancer risk in overweight women in comparison to those who maintained their usual diet [5].

Hypercholesterolemia causes both innate and adaptive inflammatory responses [33], which may enhance the risk of pancreatic cancer [34]. This is in connection with the increased risk of pancreatic cancer in patients with type 2 diabetes mellitus. Similarly, acute pancreatitis, a medical inflammatory condition enhancing the risk of pancreatic cancer, has been linked to high serum cholesterol [35]. Interestingly, a cohort study found that some of the dietary components used in our cholesterol-lowering diet score (namely, low dietary cholesterol intake, low percentage of total calories from SFAs, and high intake of fiber and legumes) were associated with reduced risk of acute pancreatitis [36].

Beside dietary cholesterol, two other dietary components were significantly associated with reduced pancreatic cancer risk: low GI and high non-cellulosic polysaccharide soluble fibers. The association between GI and pancreatic cancer risk is however controversial since cohort studies thus far have generally failed to find any association [37]. Nevertheless, the beneficial effects of a low dietary GI were mostly seen in metabolically challenged compared to non-metabolically challenged subjects [38]. Indeed, a cohort study found a significant association between a dietary GI-related measure (i.e., the insulin load) and pancreatic cancer risk in people with pre-existing insulin resistance but not in insulin-sensitive subjects [39]. A recent prospective analysis of the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Clinical Trial on the effect of selected screening examinations found an inverse association between a low dietary carbohydrate score and the risk of pancreatic cancer, which remained significant only in people ≥ 65 years of age (hazard ratio = 0.49, 95% CI: 0.34, 0.72) but not in younger people (hazard ratio = 1.03, 95% CI: 0.59, 1.81). These results suggest that insulin resistance is an effect modifier. Similarly, in our study, the association between the cholesterol-lowering diet score and pancreatic cancer risk was significant only in people leading a sedentary lifestyle (i.e., a risk factor for insulin resistance and obesity) but not in physically active subjects. Perhaps a significant effect of high GI has been seen in a recent publication on the association of GI in large cohorts with diabetes-related cancers, of which pancreatic cancer was one of the seven diabetes-related cancers [40]. Furthermore, a systemic review of randomized trials found that low GI lowers the level of low-density lipoprotein (LDL) cholesterol [27] through a reduced stimulation by insulin of HMG-CoA reductase, a rate-limiting enzyme of hepatic cholesterol synthesis. Intake of soluble fibers has also been inversely associated with the risk of pancreatic cancer in observational studies [41]. Viscous fibers—such as β -glucans, pectins, and gums—have been shown to hamper the movement of bile acids into the enteric micelles and the subsequent uptake of micelles into the enterocytes [42]. Consequently, there is a greater fecal bile acid loss that leads to a reduction in the enterohepatic bile acid circulation and an increased hepatic cholesterol utilization to replenish the liver bile acid pool.

The inverse association between adherence to a cholesterol-lowering diet and the risk of pancreatic cancer is consistent across strata of major risk factors, except for physical activity. In the present study population, physical activity was not associated with adherence to the cholesterol-lowering diet score; nonetheless, it could be an effect modifier. Indeed, physical activity may impact the lipid profile by reducing the total cholesterol level and increasing high-density lipoprotein (HDL) cholesterol [43]. Therefore, subjects reporting high physical activity may already have a favorable lipid profile and, thus, be less sensitive to the reduction in cancer risk due to the adherence to the cholesterol-lowering diet.

We acknowledge some potential study limitations. Information bias is possible due to several potential sources of bias. The cases may have recalled their dietary habits differently from controls, and the precision in reporting food consumption might depend on the frequency of consumption itself. Furthermore, recall bias is possible, considering that the usual diet was estimated over the two years prior to enrollment. To reduce these potential sources of bias, the questionnaire was administered to cases and controls by the same

interviewers under similar conditions during their hospital stay. In addition, awareness of any hypotheses on dietary habits in the etiology of pancreatic cancer was limited in the Italian population at the time of this study. Furthermore, the FFQ was successfully tested for validity and reproducibility [21,22] in assessing dietary habits. Nevertheless, we cannot exclude the possibility of residual recall bias. This study enrolled cases and controls from 1991 to 2008, and some dietary habits may have changed in this 18-year period. However, dramatic dietary changes were unlike in the study period, since diet habits were quite stable in the Italian population of the same age as the study population. Nonetheless, if diet modifications occurred, they were within the Mediterranean diet style since the influence of ethnic diets was very limited at the time this study was conducted. Finally, the score was created by assigning one point when the dietary requirement was met and zero otherwise; this may have introduced misclassification since it did not weight each dietary indicator according to its impact on pancreatic cancer risk. However, differently from clinical predictive and prognostic scores, the intent of the cholesterol-lowering diet score was to evaluate associations with outcomes at a group level rather than at an individual level; therefore, potential classification bias is negligible. Notably, this approach is quite common and widely accepted in etiological and observational studies where the adherence to specific *a priori* dietary patterns was evaluated [44–49].

Although the interviewers were instructed not to include people with weight loss prior to cancer diagnosis, abdominal obesity was reported in 18.6% of the cases and 39.7% of the controls, suggesting possible misclassification; therefore, the analyses including obesity were prone to bias and the results could not be adjusted for or stratified by obesity. However, logistic regression models were adjusted for physical activity, which is often a good proxy for body weight. The similarity of results from the minimally and fully adjusted models further reassures us against any relevant role of residual confounding. Selection bias was also possible, though the cases and controls were enrolled from the same hospital catchment areas, and careful attention was paid to exclude from the control group subjects admitted for any condition associated with a long-term modification of dietary habits. Finally, the present score might merely mirror other scores of healthy dietary habits, such as the Mediterranean diet [50]. The Spearman correlation coefficient between the cholesterol-lowering diet score and the Mediterranean diet score was 0.35 (95% CI: 0.29–0.41), indicating a modest correlation. This indicates that the cholesterol-lowering diet score identifies a specific dietary pattern, which is different from other healthy diet patterns. With reference to confounding, we were able to allow for several potential confounders, including physical activity and diabetes.

A number of factors have to be considered in regard to the external validity of results. Firstly, this study was conducted in a geographic area where the Mediterranean diet was widespread, potentially limiting the external validity of the study findings to population eating according to different dietary styles. Secondly, food fortification with phytosterols was not considered since it was not in place at the time of diet assessment. However, food fortification is more widespread nowadays, and this aspect should be considered in nutrient estimations. Finally, although the cholesterol-lowering diet score is an *a priori* dietary pattern, its optimal cut-offs were determined on data and may be different in other contexts. However, the cut-offs in the present study were comparable to those of a similar study on prostate cancer [18]; the distribution of the cholesterol-lowering diet score among the control population of these studies was also similar, showing a stable dietary behavior.

The results of the present study are strengthened by a previous validation of the cholesterol-lowering diet score [19]. In a previous study on women with breast cancer (the DEDiCa trial), we showed an inverse association between the cholesterol-lowering diet score and serum levels of LDL cholesterol and total cholesterol. Notably, the proportion of patients within the recommended ranges of serum total cholesterol (i.e., <200 mg/dL), LDL cholesterol (i.e., <116 mg/dL), and triglycerides (i.e., <150 mg/dL) declined with increasing adherence to the cholesterol-lowering diet. Although the score was not validated on men yet, the DEDiCa study provided reassurance that, among women, the cholesterol-lowering

diet score is associated with the lipid profile. Furthermore, the results are strengthened by the collection of extensive dietary information through a reproducible and valid FFQ [21,22], which was administered by trained interviewers through a direct interview.

5. Conclusions

In conclusion, adherence to a plant-based cholesterol-lowering diet is associated with decreased risk of pancreatic cancer, adding evidence on the beneficial effects of plant-based diets in reducing cancer risk. Physical activity is a potential effect modifier of this association, since the inverse association held true only among subjects with low/medium physical activity. The confirmation of this study's findings in cohort studies would further support the favorable effect of plant-based diet in preventing pancreatic cancer.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/nu16152508/s1>: Table S1: Adjusted odds ratios (ORs) and corresponding 95% confidence intervals (CIs) for pancreatic cancer risk according to dietary component and the cholesterol-lowering diet score, including patients with treated hypercholesterolemia. Italy, 1991–2008.

Author Contributions: Conceptualization: M.D.M., L.S.A.A., D.J.A.J. and J.P.; methodology: M.D.M., L.S.A.A. and J.P.; formal analysis: M.D.M.; data curation: A.C., C.L.V., E.N., F.T. and J.P.; writing—original draft preparation: M.D.M., L.S.A.A. and J.P.; writing—review and editing: D.J.A.J., A.C., F.T., E.N., C.L.V. and M.F.; supervision: M.F. and C.L.V. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: In each of the participating center, the local Institutional authority reviewed the protocol and cleared the conduction of the study, in accordance with the Italian legislation for observational studies in force at the time the study was conceived. The study was conducted in accordance with the Declaration of Helsinki, and approval by the Ethics Committee of the National Cancer Institute, Milan was reconfirmed on 29 March 2001 (INT 32/09).

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

Data Availability Statement: The data presented in this study are available for research purposes upon request from Jerry Polesel.

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Article

Trends in Plant-Based Diets and the Associated Health Characteristics among Canadians

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Abstract: In Canada, unhealthy dietary patterns comprise diets with poor nutrient density and are associated with chronic conditions. Plant-based diets have gained popularity due to their ability to provide a nutritionally adequate healthy diet. This study aims to compare sociodemographic, socioeconomic, and health characteristics, and diet quality between Canadian adults following plant-based and omnivore diets as well as assess the extent to which key nutrient intakes are of public health concern among Canadians following plant-based diets. The study used nationally representative nutritional data from the 2015 Canadian Community Health Survey and descriptive statistics were computed. The analysis determined that Canadians following strict plant-based diets (1% of total population) were significantly more likely to be an immigrant to Canada, less likely to meet national physical activity guidelines, and less likely to be overweight, compared to Canadians following omnivore diets. Compared to omnivore diets, plant-based diets were nutritionally superior according to the Nutrient-Rich Food index. Continued knowledge translation on what comprises healthy plant-based diets, public guidance on the intersection between diet and health, and the completion of prospective cohort studies are needed. To conclude, the research suggests well-planned plant-based diets, in comparison to omnivore diets, offer a nutrient-dense diet.

Keywords: plant-based diet; dietary pattern; diet quality; Nutrient-Rich Food index; chronic conditions; public health; Canada

1. Introduction

Food systems and individual dietary patterns have the potential to nurture human health and support environmental sustainability; however, this is often not attained [1]. Unhealthy diets pose a great risk towards morbidity and mortality in humans—a risk greater than the combined impact of unsafe sex and the use of alcohol, drugs, and tobacco [1]. Environmentally, unsustainable agriculture and food production can negatively impact freshwater use, land use, and greenhouse gas emissions, and negatively impact coastal waters and wetlands through a process called eutrophication, where plant and algae growth increases by mediating the factors needed in the natural photosynthesis process, and, as a result, have a myriad of detrimental impacts on the ecosystem [2,3]. Therefore, what is the solution to healthier dietary patterns and sustainable food production? Plant-based diets have entered the spotlight as a dietary pattern that may help support both human and environmental health, or planetary health [2–4]. Through an environmental lens, the adoption of plant-based diets has the potential to significantly reduce agricultural land use

by 76%, freshwater use by 21% (blue water [water in rivers, lakes, and glaciers] and groundwater), eutrophication by 49%, and greenhouse gas emissions by 49% [2]. Additionally, raising animals for human consumption is responsible for 67% of deforestation, threatening our ecosystems and biodiversity [2]. Through a human health lens, the adoption of well-planned plant-based diets has the potential to improve cholesterol levels, glycemic control, blood pressure, and weight management while reducing the incidence of cardiovascular diseases, type 2 diabetes (T2D), and obesity [2–4]. However, in the context of human health, the distinction of a well-planned or a ‘healthy’ plant-based diet should be made, as opposed to a plant-based diet high in sugar, salt, saturated or trans-fat, or highly processed foods. As with any dietary regimen, there is the possibility to over- or under-consume essential nutrients or nutrients to limit (i.e., saturated fats, sugar, and sodium) [2–4].

In 2019, both the EAT-Lancet Commission and Health Canada independently defined new optimal diets, both of which emphasized plant-based foods and proteins [5,6]. The EAT-Lancet Commission defined a diet with the intent to support optimal planetary health, which consists largely of a diversity of plant-based foods, low amounts of animal sourced foods, primarily unsaturated fats instead of saturated, and limited amounts of refined grains, processed foods, and added sugars [5]. More specifically, the planetary health diet is made up of 50% vegetables and fruits (by volume) or about 300–900 g/day (78 kcal/day vegetables, 126 kcal/day fruits) including other major contributing food groups of whole grains (232 g/day or 811 kcal/day), plant-based proteins (0–175 g/day or 575 kcal/day), and unsaturated oils (20–80 g/day or 354 kcal/day) [5]. Similarly, the 2019 Canadian Food Guide dedicates half of the plate to vegetables and fruits, with whole grains and protein foods each making up one-quarter of the plate [6]. Although much less quantifiable, the 2019 Canadian Food Guide emphasizes plant-based proteins and recommends Canadians choose protein foods from plants every day [6]. These two plant-focused dietary guidelines highlight a Canadian and global movement towards plant-based eating for both human and environmental health.

In the Canadian context, our population is experiencing epidemic proportions of chronic conditions, with significant impacts on population health, the economy, and our society [4]. Based on 2018 Canadian Community Health Survey (CCHS) data, more than half of our population was classified as overweight or obese (36.3% overweight, and 26.8% obese) [7]. Evidently, carrying excess weight predisposes individuals to increased health risks, including the increased risk of several chronic conditions such as T2D, hypertension, cardiovascular diseases, and metabolic syndrome [8]. The number of Canadians impacted by chronic conditions will continue to rise due to our aging population, sedentary lifestyles, and unhealthy dietary patterns [4]. Fortunately, our dietary patterns are a modifiable risk factor that influences our health and wellness. Dietary patterns and specific foods have been linked to several chronic conditions such as cardiovascular diseases, T2D, hypertension, metabolic syndrome, some cancers, and, as mentioned above, obesity [4,9–11]. Red and processed meats have been classified as ‘probably carcinogenic to humans’ and ‘carcinogenic to humans’, respectively, since 2015 by the International Agency for Research on Cancer, and new evidence continues to support these classifications [12]. However, to have a comprehensive understanding on diet and chronic conditions, researchers have recently focused more on overall dietary patterns, rather than individual foods. Several studies have investigated the role of plant-based dietary patterns on chronic condition outcomes and have identified the health-promoting characteristics of these dietary patterns. An analysis of the second American Adventist Health Study found an association between plant-based dietary patterns and reduced odds of developing T2D, hypertension, high cholesterol, and obesity [13,14]. Further, a Canadian randomized controlled trial investigating the differences between types of plant-based diets found that consuming a low-carbohydrate vegan diet over 6 months resulted in reductions in body weight and in low-density lipoprotein (LDL) cholesterol, compared to those consuming a high-carbohydrate lacto-ovo vegetarian diet [15]. Investigating overall dietary patterns provides a more practical and realistic population health perspective of how our diets relate to chronic conditions; however, specific

nutrients of public health concern still need to be acknowledged and investigated. For instance, there is a well-established relationship between vitamin D and calcium on bone health and it is imperative to ensure that one's diet has a sufficient intake of these important vitamins and minerals [11]. Additionally, within a population health lens, understanding the distribution of social determinants of health (e.g., household income, food security status, education level, immigration status, etc.) among a population helps provide a comprehensive understanding of the additional variables at play that also contribute to one's health status [16]. To date, there is a lack of relevant Canadian population-based research assessing the impact of plant-based eating on diet quality, nutrients of public health concern, and the prevalence of chronic conditions, as well as the distribution of plant-based eating across sociodemographic and socioeconomic factors.

Therefore, it is imperative that we investigate the population impact of following plant-based and omnivore dietary patterns on diet quality and nutrient intake metrics among Canadians. The adoption of healthy plant-based diets may address the significant need to improve the health of Canadians [4]. Thus, we set out to examine the trends of Canadians practicing plant-based dietary patterns and the resulting impact on nutrient density. Our specific objectives were: (i) to compare the sociodemographic, socioeconomic, and health characteristics between Canadian adults following plant-based diets to those following an omnivore diet; (ii) to compare diet quality between Canadian adults following plant-based diets to those following an omnivore diet; and (iii) to assess the extent to which key nutrient intakes are of public health concern among Canadians following plant-based diets.

2. Materials and Methods

2.1. Survey Design

This study is based on nutritional data from the 2015 CCHS, a nationally representative cross-sectional survey. The CCHS is a national cross-sectional survey that relied on voluntary responses from the Canadian population and executed a three-stage stratified data collection process to allow for inferences at a national level [17]. The CCHS 2015 was selected as it contains the most recent focused surveys on nutrition, the CCHS-Nutrition, which include 24 h dietary recalls.

2.2. Study Population

The CCHS study population represented the Canadian population aged 1 year and older living in 1 of the 10 provinces, while excluding individuals living on reserves or other Indigenous settlements, full-time members of the Canadian Forces, or the institutionalized population [17]. At the national level, Statistics Canada desired a sample size of 24,000 respondents; therefore, after accounting for non-response and out-of-scope dwellings, a total of 37,694 households were selected, and the overall response rate at the national level was estimated to be 62% [17].

A subset of the CCHS-Nutrition study population was used for the purposes of this study and is referred to as the analytical sample. The analytical sample excluded individuals if they were less than 19 years of age, pregnant, lactating, did not report any food intake in the 24 h dietary recall, or reported a daily calorie intake outside the range of 200–8000 kilocalories. These criteria were selected to focus on Canada's adult population and minimize potential confounders (i.e., those who are pregnant or lactating) and minimize outliers (i.e., those who are consuming less than 200 kcal/day or more than 8000 kcal/day). With consideration of sample weights, bootstrap weights, and the exclusion criteria, the analytical sample based on the weighted sample represent 17,388,314 Canadian adults living in 10 provinces (Figure 1).

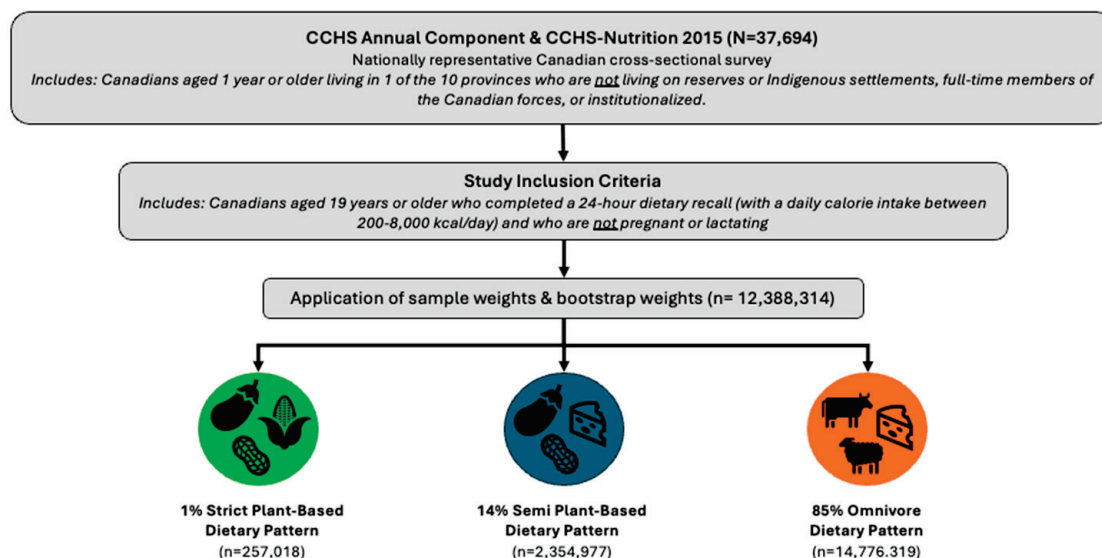


Figure 1. Overview of study inclusion/exclusion criteria and sample sizes.

2.3. Variable Definitions

The CCHS Annual Component and the focused CCHS-Nutrition module collected information on a variety of population health measures, such as household food security, disease and health conditions, lifestyle and social conditions, healthcare services, mental health, and wellbeing [18]. For this study, variables related to sociodemographics, socioeconomic, nutrient intakes, and status of several chronic conditions were accessed, such as T2D, hypertension, heart disease, cancer, or osteoporosis. Overweight and obesity status was classified using body mass index (BMI), such that individuals with a BMI of >18.5 were classified as underweight, individuals with a BMI of $18.5\text{--}24.9$ were normal weight, individuals with a BMI of $25.0\text{--}29.9$ were overweight, and individuals with a BMI ≥ 30 were classified as obese. Physical activity was assessed across leisure, work, housework, and transportation where all respondents reported the number of times and the duration of moderate or vigorous physical activity in the previous seven days [17]. These data were used alongside the national physical activity recommendation to classify individuals as to whether or not they participated in 150 min, or more, of moderate or vigorous physical activity per week [17]. Household food security status was assessed using the 18-item Household Food Security Survey Module. For the purposes of this study, household food security was reported as two levels: food-secure and food-insecure (includes both moderate and severe food insecurity) [17,19]. Nutritional supplementation was assessed by both the type and volume of supplements consumed and the frequency of supplement consumption in the previous month [17]. CCHS respondents were asked to report on supplement brand, ingredients, concentration, and, when available, the Natural Product Number [17]. For the purposes of this study, nutritional supplementation was classified into respondents who reported consumption in the past month and respondents who did not. Furthermore, three variables were derived to help guide the research process—the priori dietary pattern variable, the Nutrient-Rich Food (NRF) index variable, and a total chronic conditions variable.

The dietary pattern variable was derived using participants' 24 h dietary recall data that included food type, amount, eating occasions and locations, and supplement intake. CCHS-Nutrition respondents completed one 24 h dietary recall, and a random subset of approximately 35% of the sample was invited for a second 24 h dietary recall. In most cases, the first 24 h dietary recall was completed in-person; however, some were completed over the phone, and all recalls were completed using the Automated Multiple-Pass Method [17]. For the purposes of this study, only the first 24 h dietary recall was used in analysis. One 24 h dietary recall provides sufficient dietary data to provide population

level estimates of mean intake and is not statistically different than the mean of two 24 h dietary recalls. The derived dietary pattern variable stratified respondents into three unique diet groups—omnivore, semi-plant-based (lacto-ovo vegetarian), or strict plant-based (vegan), following the commonly accepted dietary patterns [20]. A respondent's diet was classified as omnivore if they reported consumption of one or more servings of any animal products (i.e., beef, pork, veal, lamb, game meat, organ meat, processed meat, poultry, fish, and shellfish) during the first 24 h dietary recall. A respondent's diet was classified as semi-plant-based if they reported no consumption, or zero servings, of animal products but reported consumption of at least one serving of animal by-products (i.e., milk, cheese, yogurt, and eggs) and at least one serving of fruits/vegetables during the first 24 h dietary recall. A respondent's diet was classified as strict plant-based if they reported no consumption, or zero servings, of animal products or by-products but reported consumption of at least one serving of fruits/vegetables during the first 24 h dietary recall.

The NRF index variable was computed from an adapted version of the validated NRF 9.3 index, which is a formal metric used to assess overall diet quality, with a high score associated with positive health outcomes [21,22]. The NRF index variable was derived by subtracting the sum percentage of the daily values of nine nutrients to encourage (protein, fibre, vitamin A, vitamin C, vitamin D, calcium, iron, magnesium, and potassium) with the sum percentage of the maximum recommended values for three nutrients to limit (saturated fat, total sugar, and sodium). Higher NRF index scores (maximum 900) indicate a nutrient-rich diet [21,22]. NRF index tertiles were derived and categorized as low score (tertile 1, $\text{NRF} \leq 465.97$), medium score (tertile 2, $\text{NRF} > 465.97$ and < 578.42), or high score (tertile 3, $\text{NRF} \geq 578.42$). The NRF tertiles represent three equal groups making up the entire sample population; however, when the sample population is stratified into dietary patterns, the distribution of the population in each tertile is not equal.

The total chronic conditions variable was developed to complete a broader analysis on the relationship between dietary patterns and the occurrence of at least one of the following chronic conditions—T2D, hypertension, heart disease, cancer, or osteoporosis. The total chronic condition variable was defined by whether the respondent either answered 'yes' to at least one of the indicated chronic condition status questions or answered 'no' to all of the indicated chronic conditions.

2.4. Statistical Analyses

Statistical analyses were completed using SAS (Version 9.4, SAS Institute, Toronto, ON, Canada) software at the Saskatchewan Research Data Centre located at the University of Saskatchewan. To extrapolate results to the national level and produce population level estimates, survey weights and bootstrap weights provided by Statistics Canada were applied to all analyses [18]. A stepwise process was used throughout analysis such that (i) data was cleaned and processed by merging datasets by respondent ID, adding bootstrap and sample weights, developing of variable levels, derivation of variables, and incorporation of exclusion criteria, followed by (ii) computation of descriptive statistics at both the sample level and with recommended bootstrap and sample weights. For descriptive statistics, all categorical variables were computed as weighted frequencies whereas continuous variables were computed as weighted means. For all variables, statistics were reported with the standard error and 95% confidence interval per each dietary pattern classification. Statistical significance was determined via the confidence interval approach, recommended by Statistics Canada for analyses on CCHS data, such that, if the 95% confidence intervals overlapped, the parameters were statistically equivalent, or, alternatively, if the 95% confidence intervals did not overlap, the parameters were determined to be statistically different.

2.5. Ethical Considerations

Ethical review and approval were waived for this study as research pertained to secondary analysis of the CCHS data. In Canada, ethical review and approval are waived when the research conducted relies exclusively on information that is publicly available

through a mechanism set out by legislation or regulation and that is protected by law, or in the public domain, and the individuals to whom the information refers have no reasonable expectation of privacy.

3. Results

This study included a weighted sample size of 17,388,314 Canadians and explored the population level trends of dietary patterns (omnivore, semi-plant-based, and strict plant-based) associated with the sociodemographic/socioeconomic factors, health characteristics, diet quality, and nutrients of public health concern. Approximately, 84.98% of the national population follows an omnivore diet, 13.54% follows a semi-plant-based diet, and 1.48% follows a strict plant-based diet (Figure 2).

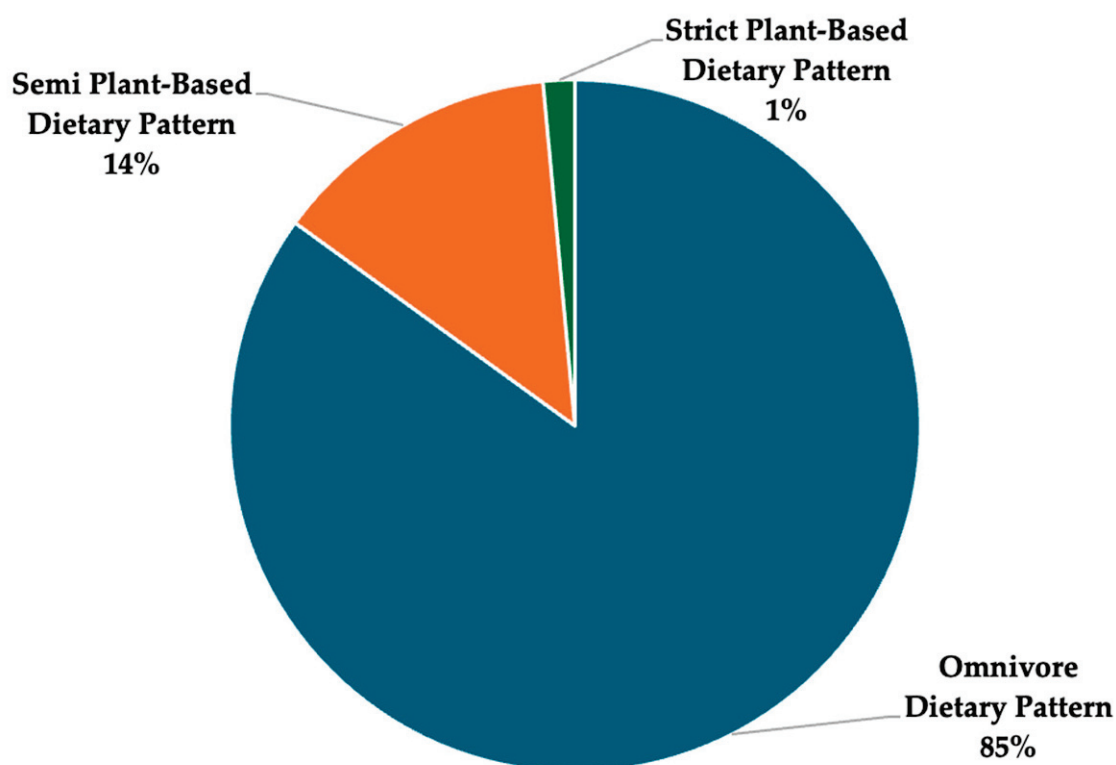


Figure 2. Distribution of dietary patterns among Canadians.

3.1. Dietary Patterns in Canada

Table 1 presents the sociodemographic, socioeconomic, and health characteristics of adults following an omnivore diet, a semi-plant-based diet, and a strict plant-based diet. There were significant differences between the dietary pattern groups associated with sex, immigration status, physical activity, and BMI. For instance, Canadians following a semi-plant-based diet were significantly less likely to be male compared to those following an omnivore diet. Further, compared to Canadians following an omnivore diet, those following a strict plant-based diet were significantly more likely to be an immigrant to Canada, less likely to meet national physical activity recommendations, and less likely to be overweight. There were no significant findings related to chronic condition status, aside from BMI, across the three dietary patterns. However, Canadians following a strict plant-based diet had a higher, yet insignificant, prevalence of total chronic conditions compared to semi-plant-based or omnivore Canadians.

Table 1. Sociodemographic, socioeconomic, and health characteristics of Canadians across three dietary patterns ¹.

Characteristic	Diet (95% Confidence Interval)		
	Omnivore ² (n = 14,776,319)	Semi-Plant-Based ³ (n = 2,354,977)	Strict Plant-Based ⁴ (n = 257,018)
Mean age, years	48.22 ± 0.29 (47.66–48.78)	49.82 ± 0.83 (48.20–51.45)	45.80 ± 2.02 (41.83–49.76)
Sex, % male	50.06 * ± 0.79 (48.51–51.61)	42.82 * ± 2.67 (37.58–48.06)	54.92 ± 8.25 (38.71–71.13)
Education, % post-secondary	77.09 ± 0.90 (75.31–78.85)	75.52 ± 2.21 (71.18–79.86)	86.35 ± 4.46 (77.58–95.12)
Marital status, % married	65.3 ± 1.14 (63.07–67.53)	64.16 ± 2.55 (59.16–69.18)	61.47 ± 8.40 (44.96–77.98)
Food secure ⁵ , % yes	97.78 ± 0.60 (91.60–93.96)	93.44 ± 1.32 (90.84–96.04)	89.72 ± 4.46 (80.97–98.47)
Immigrant, % yes	26.66 * ± 1.21 (24.28–29.04)	31.03 ± 2.66 (25.80–36.25)	48.36 * ± 8.38 (31.90–64.82)
Recent immigrant ⁶ , % yes	27.61 ± 2.05 (25.59–31.64)	31.83 ± 4.67 (22.66–41.00)	33.36 ± 11.75 (10.28–56.44)
Ethnicity, % white	74.87 ± 1.14 (72.64–77.11)	72.60 ± 2.69 (67.31–77.88)	– ⁸
Household income, % distribution decile 5 + 6	19.61 ± 0.83 (17.98–21.23)	21.83 ± 2.03 (17.83–25.82)	18.96 ± 6.02 (7.12–30.80)
Nutritional supplement consumption, % yes	42.26 ± 0.92 (44.46–48.05)	50.01 ± 2.17 (45.75–54.28)	44.01 ± 6.97 (43.29–69.69)
Physical activity, % met national recommendation ⁷	45.44 * ± 1.11 (43.27–47.62)	42.20 ± 2.60 (37.09–47.32)	26.74 * ± 6.52 (13.94–39.55)
Body mass index, % overweight	36.54 * ± 1.15 (34.28–38.80)	32.83 ± 2.41 (28.08–37.58)	20.64 * ± 6.08 (8.69–32.59)
Type 2 diabetes, % with diabetes	6.39 ± 0.45 (5.51–7.28)	6.48 ± 0.93 (4.65–8.30)	7.74 ± 3.68 (0.50–14.97)
Hypertension, % with hypertension	19.34 ± 0.82 (17.73–20.94)	17.63 ± 1.70 (14.30–20.97)	20.31 ± 5.47 (9.56–31.06)
Heart disease, % with heart disease	4.01 ± 0.38 (3.24–4.75)	5.19 ± 0.91 (3.41–6.97)	2.86 ± 1.61 (0.00–6.03)
Cancer, % with cancer	1.60 ± 0.20 (1.21–2.00)	1.02 ± 0.33 (0.38–1.66)	– ⁷
Osteoporosis, % with osteoporosis	4.18 ± 0.37 (3.45–4.91)	5.58 ± 0.94 (3.73–7.43)	1.31 ± 1.30 (0.00–3.87)
Total chronic condition, % with at least one condition	53.05 ± 1.48 (50.13–55.96)	52.07 ± 3.47 (45.26–58.87)	64.93 ± 8.22 (48.77–81.09)

Data source: 2015 Canadian Community Health Survey—Nutrition. * in bold Represents statistically significant difference as determined by 95% confidence interval approach. ¹ All data are weighed and bootstrapped to obtained population level estimates. ² Omnivore diet defined by the study population who reported consumption of any animal products. ³ Semi-plant-based diet defined by the study population who reported no consumption of animal products and reported consumption of animal by-products and at least one serving of fruits/vegetables. ⁴ Strict plant-based diet defined by the study population who reported no consumption of animal products or animal by-products but reported consumption of at least one serving of fruits/vegetables. ⁵ Food security was calculated using the 18-item US Household Food Security Survey Module. ⁶ Recent immigrant defined by less than 10 years in Canada. ⁷ National physical activity recommendation is 150 min of vigorous activity per week. ⁸ Low sample size (data were not released to adhere to Statistics Canada's vetting guidelines).

3.2. Diet Quality of Canadian Dietary Patterns

Table 2 outlines the distribution of the NRF index scores in each dietary pattern, as well as the mean NRF index score for each dietary pattern. Canadians following a semi-plant-based diet were significantly less likely to be classified in the low NRF index score group (lower nutrient density) and significantly more likely to be classified in the high NRF index score group (higher nutrient density) than their omnivore counterparts. Moreover, Canadians following a semi-plant-based diet had a significantly higher mean NRF index score, representing a more nutrient-dense diet, compared to their omnivore counterparts.

Table 2. Nutrient-Rich Food index scores, as determined by the NRF index, of Canadians across three dietary patterns ¹.

Nutrient-Rich Food Index Score ²	Diet (95% Confidence Interval)		
	Omnivore ³ (n = 14,776,319)	Semi-Plant-Based ⁴ (n = 2,354,977)	Strict Plant-Based ⁵ (n = 257,018)
% In Low NRF Index Score	35.41 * ± 1.15 (33.15–37.67)	25.70 * ± 2.43 (20.92–30.48)	35.70 ± 8.69 (18.62–52.77)
% In Medium NRF Index Score	32.77 ± 1.12 (30.56–43.97)	30.67 ± 2.36 (26.02–35.32)	35.22 ± 7.81 (19.87–50.56)
% In High NRF Index Score	31.82 * ± 1.04 (29.79–33.86)	43.63 * ± 2.63 (38.47–48.79)	29.09 ± 7.72 (13.91–44.26)
Mean NRF index score	517.13 * ± 2.86 (511.69–522.94)	544.03 * ± 6.79 (530.69–557.37)	521.50 ± 25.15 (472.10–750.91)

Data source: 2015 Canadian Community Health Survey—Nutrition. * in bold Represents statistically significant difference as determined by 95% confidence interval approach. ¹ All data are weighed and bootstrapped to obtained population level estimates. ² Nutrient-Rich Food index score categories as based on tertiles. ³ Omnivore diet defined by the study population who reported consumption of any animal products. ⁴ Semi-plant-based diet defined by the study population who reported no consumption of animal products and reported consumption of animal by-products and at least one serving of fruits/vegetables. ⁵ Strict plant-based diet defined by the study population who reported no consumption of animal products or animal by-products but reported consumption of at least one serving of fruits/vegetables.

3.3. Key Nutrient Intakes in Canadian Dietary Patterns

Key nutrients, including nutrients of public health concern, are summarized in Tables 3 and 4 along with their associated standard error and 95% confidence intervals for each dietary pattern. Numerous mean nutrient intakes were found to be statistically different between dietary groups; however, the most notable differences were found between Canadians consuming an omnivore diet and Canadians consuming either a semi- or strict plant-based diet. Regarding micronutrients, those practicing a strict plant-based diet had significantly lower intakes of vitamin A, vitamin B, vitamin B2, vitamin D, calcium, cholesterol, mono-unsaturated fatty acids (MUFAs), saturated fatty acids, iron, niacin, phosphorus, riboflavin, sodium, thiamin, and zinc, compared to Canadians practicing an omnivore diet. Micronutrient intakes associated with the semi-plant-based diet tended to fall somewhere between the mean nutrient intakes of the omnivore dietary pattern and the strict plant-based dietary pattern. Nevertheless, Canadians following a semi-plant-based diet had significantly higher mean intakes of folate and dietary fibre, compared to Canadians practicing an omnivore diet. Regarding macronutrients, Canadians practicing either form of the plant-based diet (semi- or strict plant-based) had significantly lower mean intakes of total fat, protein, and energy, compared to their omnivore counterparts.

Table 3. Mean micronutrient intakes of Canadians across three dietary patterns ¹.

Nutrient (Unit)	Diet (95% Confidence Interval)		
	Omnivore ² (n = 14,776,319)	Semi-Plant-Based ³ (n = 2,354,977)	Strict Plant-Based ⁴ (n = 257,018)
Vitamin A (mcg)	666.42 ^a ± 14.66 (637.62–695.22)	671.42 ^{a,b} ± 25.99 (620.36–722.49)	449.50 ^b ± 90.98 (270.75–628.25)
Vitamin B6 (mg)	1.78 ^a ± 0.02 (1.74–1.82)	1.53 ^b ± 0.06 (1.41–1.65)	1.42 ^b ± 0.13 (1.17–1.67)
Vitamin B12 (mcg)	4.45 ^a ± 0.09 (4.27–4.63)	2.93 ^b ± 0.16 (2.61–3.25)	0.65 ^c ± 0.15 (0.35–0.94)
Vitamin C (mcg)	101.76 ^a ± 2.28 (97.27–106.25)	103.67 ^a ± 4.86 (94.12–113.22)	119.98 ^a ± 19.72 (81.24–158.71)
Vitamin D (mcg)	4.92 ^a ± 0.12 (4.69–5.16)	4.41 ^a ± 0.18 (4.05–4.77)	1.30 ^b ± 0.33 (0.66–1.95)
Calcium (mg)	799.59 ^a ± 12.23 (775.56–823.61)	872.92 ^a ± 28.11 (817.70–928.14)	418.90 ^b ± 53.41 (313.97–523.84)
Cholesterol (mg)	291.09 ^a ± 6.65 (278.02–304.17)	235.03 ^b ± 13.66 (208.19–261.88)	47.36 ^c ± 6.86 (33.87–60.84)
MUFAs ⁵ (g)	27.32 ^a ± 0.38 (26.57–28.06)	25.64 ^a ± 0.85 (23.98–27.31)	16.72 ^b ± 1.76 (13.26–20.19)
PUFAs ⁶ (g)	15.17 ^a ± 0.23 (14.72–15.62)	15.07 ^a ± 0.56 (13.97–16.16)	14.23 ^a ± 1.60 (11.09–17.38)
Saturated Fatty Acids (g)	23.75 ^a ± 0.35 ^a (23.06–24.44)	22.78 ^a ± 0.82 (21.17–24.40)	11.78 ^b ± 1.28 (9.07–14.49)
Dietary Fibre (g)	17.27 ^a ± 0.21 (16.85–17.69)	20.36 ^b ± 0.61 (19.5 1- 21.56)	20.42 ^{a,b} ± 3.00 (14.51–26.32)
Folate (mcg)	215.69 ^a ± 2.96 (209.88–221.50)	245.86 ^b ± 8.85 (228.47–263.26)	241.74 ^{a,b} ± 24.29 (194.28–289.47)
Iron (mg)	12.86 ^a ± 0.13 (12.06–13.13)	12.16 ^{a,b} ± 0.36 (11.46–12.86)	10.48 ^b ± 1.07 (8.37–12.59)
Magnesium (mg)	315.60 ^a ± 3.06 (309.58–321.63)	323.20 ^a ± 9.74 (9304.05–324.34)	285.33 ^a ± 32.03 (222.40–348.25)
Niacin (mg)	42.37 ^a ± 0.45 (41.48–43.25)	30.26 ^b ± 1.00 (28.30–32.23)	22.13 ^c ± 1.61 (18.97–25.29)
Phosphorus (mg)	1336.73 ^a ± 13.61 (1309.99–1363.47)	1224.64 ^b ± 39.25 (1127.52–1301.76)	851.39 ^c ± 87.80 (678.89–1023.90)
Potassium (mg)	2794.49 ^a ± 25.68 (2744.04–2844.94)	2674.41 ^a ± 70.59 (2535.72–2813.10)	2317.59 ^a ± 238.63 (1848.74–2786.44)
Riboflavin (mg)	1.99 ^a ± 0.02 (1.95–2.03)	1.92 ^a ± 0.06 (1.81–2.04)	1.05 ^b ± 0.09 (0.86–1.23)
Sodium (mg)	2845.16 ^a ± 35.63 (2775.15–2915.16)	2630.72 ^a ± 88.83 (2456.18–2805.25)	1695.07 ^b ± 175.46 (1250.33–2039.81)
Thiamin (mg)	1.64 ^a ± 0.02 (1.60–1.68)	1.62 ^{a,b} ± 0.05 (1.51–1.73)	1.27 ^b ± 0.15 (0.97–1.57)
Zinc (mg)	11.31 ^a ± 0.14 (11.03–11.59)	8.18 ^b ± 0.28 (7.63–8.73)	6.03 ^c ± 0.66 (4.73–7.33)

Data source: 2015 Canadian Community Health Survey—Nutrition. ^{a,b,c} in bold Represents statistically significant difference, as determined by 95% confidence interval approach, between indicated dietary groups, such that means with different superscripts are statistically different. ¹ All data are weighed and bootstrapped to obtained population level estimates. ² Omnivore diet defined by the study population who reported consumption of any animal products. ³ Semi-plant-based diet defined by the study population who reported no consumption of animal products and reported consumption of animal by-products and at least one serving of fruits/vegetables. ⁴ Strict plant-based diet defined by the study population who reported no consumption of animal products or animal by-products but reported consumption of at least one serving of fruits/vegetables. ⁵ Mono-unsaturated fatty acids. ⁶ Poly-unsaturated fatty acids.

Table 4. Mean macronutrient intakes of Canadians across three dietary patterns ¹.

Nutrient (Unit)	Diet (95% Confidence Interval)		
	Omnivore ² (n = 14,776,319)	Semi-Plant-Based ³ (n = 2,354,977)	Strict Plant-Based ⁴ (n = 257,018)
Total Carbohydrate (g)	227.58 ^a ± 2.18 (223.30–231.86)	238.98 ^a ± 5.90 (227.39–250.58)	215.03 ^a ± 23.10 (169.64–260.41)
Total Fat (g)	72.79 ^a ± 0.94 (70.94–74.63)	69.80 ^a ± 2.21 (65.47–74.13)	46.84 ^b ± 3.66 (39.65–54.03)
Total Protein (g)	85.95 ^a ± 0.90 (84.17–87.72)	61.05 ^b ± 1.84 (57.44–64.66)	40.73 ^c ± 3.09 (34.66–46.80)
Total Sugar (g)	88.67 ^a ± 1.17 (86.37–90.97)	93.41 ^a ± 3.07 (87.38–99.43)	77.88 ^a ± 10.58 (57.10–98.66)
Total Energy (kcal)	1952.98 ^a ± 18.07 (1917.48–1988.48)	1849.29 ^a ± 44.71 (1761.45–1937.12)	1421.57 ^b ± 121.12 (1183.61–1659.54)

Data source: 2015 Canadian Community Health Survey—Nutrition. ^{a,b,c} in bold Represents statistically significant difference, as determined by 95% confidence interval approach, between indicated dietary groups, such that means with different superscripts are statistically different. ¹ All data are weighed and bootstrapped to obtained population level estimates. ² Omnivore diet defined by the study population who reported consumption of any animal products. ³ Semi-plant-based diet defined by the study population who reported no consumption of animal products and reported consumption of animal by-products and at least one serving of fruits/vegetables. ⁴ Strict plant-based diet defined by the study population who reported no consumption of animal products or animal by-products but reported consumption of at least one serving of fruits/vegetables.

4. Discussion

The results presented provide a novel overview on the distribution of omnivore and plant-based diets in Canada and the trends related to sociodemographics, health characteristics, diet quality, and potential nutrients of concern. The analysis determined that Canadians consuming some form of plant-based dietary pattern had certain sociodemographic, socioeconomic, and health trends; for instance, sex, immigration status, physical activity, and body weight were found to be significant. Furthermore, Canadians consuming a semi-plant-based diet were found to have the highest NRF index score and, upon individual nutrient analysis, were found to have sufficient nutrient intakes of common public health nutrients of concern.

4.1. Sociodemographic, Socioeconomic, and Health Trends in Plant-Based Eating

Approximately 15% of the Canadian adult population practice some form of plant-based eating. The present analysis found that Canadians consuming either plant-based diet (semi-plant-based or strict plant-based) were significantly more likely to be female, an immigrant to Canada, not meet national physical activity recommendations, and not be overweight, compared to their omnivore counterparts. Studies have commonly reported that females are more likely to report the consumption of ‘healthy’ dietary patterns or to practice plant-based dietary patterns compared to males [15,23–28]. Similarly, other research often finds individuals practicing plant-based diets to have healthy weights, or fall within normal BMI ranges [23–26]. However, a comparable Canadian study which also analyzed the CCHS-N 2015 but included a wider age range of participants (greater than 2 years of age) found that only 5% of the population reported following some form of plant-based diet [26]. The discrepancy between Valdes et al.’s study and the present study is likely due to the inclusion of young children. A recent 2021 American survey reported that only about 5% of children aged 5 to 17 in the U.S. describe their dietary pattern as vegetarian, or plant-based [29]. Plant-based eating is generally common among younger adult populations, but this trend does not typically include children [29].

Compared to Canadian-born individuals, immigrants to Canada were significantly more likely to practice a strict plant-based diet and less likely to practice an omnivore diet; however, this trend did not remain significant when controlling for the length of

time in Canada. Reasons for this observed trend may include the focus on plant-based eating in many non-Western cultures or the higher prevalence of food insecurity among recent immigrants [28,30]. Many cultures, religious groups, and geographical regions have deep connections to plant-based eating, such as Hindus, Buddhists, and Jains, as well as populations living in specific regions such as Okinawa, Japan. Religious-inspired vegetarianism, or plant-based eating, involves limiting one's dietary intake with the aim of attaining spiritual enlightenment or self-fulfillment, or as a mark of respect for the regulations set by one's religious community [31,32]. For example, due to the dietary guidelines outlined in the Hindu faith, which permit the consumption of only plant-based foods and dairy products, Hindus have traditionally been recognized as lacto-vegetarians [33]. Furthermore, the geo-spatial influence of a community, or cultural group's region, can impact dietary patterns such as in the local population of Okinawa, Japan, who traditionally adhere to a predominantly plant-based dietary pattern characterized by a high consumption of phytochemicals and antioxidants [34]. Their plant-based diet is rich in sweet potatoes, green leafy vegetables, and soy products with minimal amounts of fat (less than 6%) [34]. Okinawa, Japan has been referred to as one of the world's 'Blue Zones', which reflects a demographic and geographic area with a high population longevity, exemplifying the role of healthful plant-based eating on one's health and wellbeing [34].

The second possible rationale for the higher prevalence of immigrants to Canada practicing strict plant-based diets could be due to social determinants of health, namely, food security status. Food insecurity can disproportionately affect newcomers to Canada and plant-based eating, specifically traditional plant-based proteins (i.e., nuts, seeds, and legumes), can offer inexpensive alternatives to omnivore diets and animal-based products [30,35]. For instance, a recent modeling study found that plant-based diets which focused on legumes and whole grains in place of animal products represented the most affordable dietary pattern [35]. Springmann et al. (2021) [35] found that, in high- and upper-middle-income countries, meat and meat products accounted for the greatest proportion of food costs, approximately 32–34%. At a regional level, many community-based organizations promote plant-based proteins as inexpensive alternatives to animal-based proteins. For example, a six-week cooking program involving the provision of plant-based food hampers and cooking classes using plant-based recipes resulted in participants cooking more plant-based meals, having reduced BMIs, and reduced food insecurity from baseline [36]. At an international level, transitioning from animal-based diets to plant-based diets can support achieving global food security and environmental sustainability goals, primarily by shifting major crops away from livestock feed and biofuels to crops for human consumption, such as soy and grain [37]. However, it should not go without mention that the cost of plant-based dietary patterns, or any dietary pattern, is highly dependent on one's geographical location (i.e., rural, remote, and northern) and access to grocery stores or arable land. As a result, plant-based dietary patterns including nuts, seeds, and legumes may not be the most economical choice in some locations.

Our analysis revealed that 45.44% of individuals following an omnivore diet met the national physical activity recommendations, whereas individuals following a strict plant-based diets were significantly less likely to meet recommendations at 26.74% (Table 1). This observed difference between physical activity levels may be influenced by confounding factors such as sex or age. For instance, our analysis found that a significantly higher proportion of males followed an omnivore diet, compared to a semi-plant-based diet. However, although non-significant, Canadians following a strict plant-based diet were younger and more likely to be male, compared to their omnivore counterparts. Irrespective of this finding, the consumption of a nutritionally balanced plant-based diet has not been shown to have a detrimental effect on performance outcomes and may even improve aerobic capacity in athletes [38]. There is a common misconception that plant-based diets, specifically vegan or semi-plant-based diets, are low in protein or lack complete protein sources; however, this is not the case if the plant-based diet is well-balanced and protein intake is considered a priority along with other micro- and macro-nutrients [39].

Previous research has also determined that individuals who follow an omnivore diet do not exhibit any distinct benefit or drawback in terms of strength, anaerobic, or aerobic exercise performance in comparison with people who follow vegetarian or vegan diets [40]. Considering the high carbohydrate content, antioxidants, and phytochemicals present, plant-based diets can provide both health advantages and potential performance-enhancing effects for exercise [41]. Further mixed-methods research should be completed to better understand the potential association between plant-based diets and physical activity and delve into why these relationships may exist.

4.2. Sufficient Diet Quality in Plant-Based Eating

Our analysis found Canadians practicing the semi-plant-based diet had the highest mean NRF 9.3 index score and the largest proportion in the high NRF index score group (higher nutrient density), compared to Canadians practicing an omnivore diet. This statistically significant finding indicates that Canadians following a semi-plant-based diet have a superior diet quality compared to their omnivore counterparts. This finding is comparable to an American study, based on a cross-sectional nationally representative sample, which found American adults following a semi-plant-based diet had a more nutrient-dense diet compared to their omnivore counterparts [42]. Further, a Swedish study found that individuals following a strict plant-based diet, or vegan diet, had the highest Healthy Eating Index (HEI) score, and all plant-based diets under study had significantly higher HEI scores compared to an omnivore diet [43]. A systematic review by Parker and Vadiveloo (2019) [44] reported that vegetarians scored 4.5 to 16.4 points higher on the HEI than non-vegetarians, out of a possible 100 points. Policy documents build on these scientific findings, such as the 2019 Canadian Food Guide, which supports a transition to the increased consumption of plant-based proteins and reduced consumption of animal products compared to previous Canadian Food Guides [6]. According to a recent study by Asher, Doucet, and Luke (2021) [45], Canadian dietitians support the 2019 Canadian Food Guide, believe it was informed by evidence-based information, and have adapted to recommending plant-based foods more often in their practices. There is a consensus among the scientific literature that plant-based diets can provide a diet of high nutritional quality.

Regardless of the significant evidence supporting the transition to plant-based eating patterns, there is wide nutritional variation within various plant-based diets. Plant-based eating generally focuses on a diet that is high in fruits, vegetables, whole grains, and legumes, with the exclusion, to various degrees, of animal products. However, a plant-based diet can also include various unhealthy food options such as refined grains or sugar. Research has shown that plant-based dietary patterns that prioritize less nutritious plant foods like sugary beverages, refined grains, fried foods, and sweets are associated with increased risks of cardiovascular disease, T2D, metabolic syndrome, and overall mortality [46]. Consumers see the term ‘plant-based’ on products and assume that all of these products are automatically healthy, while plant-based ultra-processed foods have the potential to contain elevated levels of undesirable nutrients like saturated fat, sugar, and salt [47]. Therefore, plant-based diets are not a blanket term that consistently means the same thing. A deeper understanding of healthy or unhealthy plant-based diets in the Canadian context is warranted.

4.3. Sufficient Nutrient Intakes in Plant-Based Eating

Numerous nutrients were consumed in statistically lower amounts among Canadians practicing either of the plant-based dietary patterns, compared to those following an omnivore diet. Most notably, Canadians practicing a semi-plant-based diet had significantly lower mean intakes of vitamin B6, vitamin B12, cholesterol, niacin, zinc, and total protein, but higher mean intakes of dietary fibre and folate, compared to Canadians practicing an omnivore diet. Canadians practicing a strict plant-based diet had significantly lower mean intakes of vitamin A, vitamin B6, vitamin B12, vitamin D, calcium, cholesterol,

MUFAs, SFAs, iron, niacin, phosphorus, riboflavin, sodium, thiamin, zinc, total fat, total protein, and total calories compared to Canadians practicing an omnivore diet. Some of these significant differences associated with consuming a plant-based diet can be viewed in a positive manner, such as the reduced intake of cholesterol, SFAs, and sodium, as well as higher intakes of folate and dietary fibre. However, some of these significant differences may indicate concerns, such as the reduced intake of vitamin B6, vitamin B12, niacin, zinc, vitamin D, calcium, and iron. Previous research on nutrient intakes among individuals consuming plant-based diets have found similar concerns [21,42,43,48–53]. The conventional nutrients of concern in plant-based eating seem to be generally well-agreed-upon, including sufficient vitamin B12, iron, zinc, calcium, vitamin D, and omega-3 fatty acids intakes [53]. Another common concern of plant-based nutrition is the adequate intake and quality of protein sources [43,48,51,52,54]. Animal-based proteins are often designated as ‘complete’ proteins as they contain all nine essential amino acids, whereas plant-based proteins are often ‘incomplete’ as they frequently fall short of containing all nine essential amino acids [43,48,51,52,54]. However, not all plant-based proteins fall short of the same amino acids; therefore, multiple plant-based proteins sources can be eaten together to create a ‘complete’ protein source [48,51,54]. As such, plant-based diets should be well-planned to ensure the adequate consumption of these nutrients [21,42,43,48–52]. A well-planned plant-based diet considers a variety of foods with an emphasis on fruits, vegetables, whole grains, and conventional plant-based proteins such as soy, beans, and nuts.

Furthermore, the present study found a significant difference in mean nutrient intakes between the semi- and strict plant-based diets, meaning that not all plant-based diets are equivalent. Canadians practicing a semi-plant-based diet had significantly higher intakes of vitamin B12, vitamin D, calcium, cholesterol, MUFAs, SFAs, niacin, phosphorus, riboflavin, sodium, zinc, total fat, total protein, and total calories compared to Canadians practicing a strict plant-based diet, indicating the consumption of animal by-products makes an important contribution to nutrient intakes. Not all plant-based diets are equivalent—there is an imperative difference between healthy and unhealthy plant-based foods. For instance, a case study by Tso and Forde (2021) [54] modelling the difference in diets focused on traditional plant-based foods (i.e., legumes, nuts, and soy) and diets focused on novel plant-based meat alternatives (i.e., prepared burgers, nuggets, and dairy alternatives) found the traditional plant-based food diets met daily nutritional requirements for potential nutrients of concern (i.e., calcium, iron, zinc, and vitamin B12) and had lower amounts of SFAs, sodium, and sugar compared to the reference omnivore diet. In contrast, the diet focused on novel plant-based meat alternatives did not meet daily nutritional requirements for potential nutrients of concern (i.e., calcium, zinc, and vitamin B12) and had increased intakes of SFAs, sodium, and sugar compared to the reference diet [54]. A cross-sectional analysis on the CCHS 2015 study population found that only 14.5% of Canadians reported consuming traditional plant-based meat alternatives such as legumes, nuts, and seeds [52]. Interestingly, when the authors modelled the nutritional impact of reducing processed meat consumption by 50% and doubling traditional plant-based meat alternatives, there were statistically significance increases in the dietary intake of fibre, PUFAs, magnesium, and folate equivalents paired with significant decreases in protein, cholesterol, zinc, and vitamin B12 [52]. These findings highlight the importance of the type and quality of plant-based foods consumed, as well as emphasize that a well-planned plant-based diet can be nutritionally adequate and beneficial to health.

4.4. Strengths and Limitations

This analysis provides valuable information on the trends and nutritional quality of plant-based eating in Canada and fills a significant knowledge gap based on the most recent nationally representative nutrition and health survey in Canada. The use of the CCHS-Nutrition data should be considered a strength as the survey data were well-suited to this type of nutritional analysis. CCHS-Nutrition data provided nationally representative information on nutrient and food intakes as well as sociodemographic, socioeconomic,

diet, health, and lifestyle factors that can be extrapolated to the Canadian population. Irrespective of the strengths, the limitations of our analysis should be acknowledged. First, dietary pattern classifications were derived based on one self-reported 24 h dietary recall, which may not accurately represent the totality of a participant's usual diet. Dietary groups were defined at the population level based on the inclusion or exclusion of animal products and by-products, and the consumption of at least one serving of fruits and vegetables to be placed in the plant-based dietary patterns groups. As this method is population-based and not specifically focused on healthful or nutritionally balanced plant-based diets, it offers a realistic comparison between the inclusion and exclusion of animal products and by-products based on the group mean intake. Nevertheless, much of the published literature defines dietary groups in different manners, and, thus, caution must be taken when comparing research results. Second, the 95% confidence interval approach to determining statistical significance is a conservative approach and, as a result, although it increases the rigor of the analyses, it is plausible that the results may include false negatives. Third, as a result of the data distribution, small sample sizes occurred in some variables resulting in the need to combine or merge some variables (i.e., ethnicity, household income, BMI, food security, and marital status) to meet Statistics Canada reporting requirements. Special considerations were made when combining variable levels; however the variables with combined levels may not accurately represent the diversity of the variable in question. Finally, there are various limitations that should be declared due to the nature of the CCHS-Nutrition study design. Large cross-sectional studies measure all factors simultaneously, which restricts calculations of incidence and determination of temporal relationships, and introduces the potential of reverse causation. Non-response bias and recall bias could also figure prominently in cross-sectional studies; however, Statistics Canada took precautions to limit these biases and obtain a high response rate. Due to the nature of the CCHS-Nutrition study population parameters, an Indigenous population was included in the analysis, but excluded individuals living on First Nation reserves; the statistical analysis presented does not represent the entirety of the Canadian Indigenous population; however, this is minor as it includes urban Indigenous populations. Further, the data used in the analysis were gathered in 2015 and may not accurately represent the current trends of Canadian dietary patterns as the social acceptance and popularity of plant-based eating has increased within the past five years.

5. Conclusions

The present study based on the CCHS-Nutrition data indicates the superior diet quality of plant-based diets. In Canada, scientific evidence and social support of plant-based eating is present; however, further research is needed to entirely understand its role in improving population health. Further research should include longitudinal studies that assess the usual dietary intake over time more to confidently determine the relationship between dietary patterns with health and disease status. With an increasing body of scientific evidence, Canadian nutrition policies and practices are transitioning to the promotion of well-planned plant-based diets, such as the most recent iteration of the Canadian Food Guide. However, additional effective communication on the benefits of plant-based eating should continue. The promotion of plant-based diets should be incorporated within health promotion endeavours as plant-based diets can be a beneficial modifiable risk factor for many chronic conditions and support general health. Leadership and guidance should emphasize the beneficial connection between well-planned plant-based eating and health. The notion that not all plant-based diets are created equal needs to be effectively communicated to the Canadian population. The market for plant-based meat alternatives is rapidly growing and these novel products are in high demand due to their social popularity, easy preparation, and satisfying taste. However, knowledge translation on the healthfulness of these products is lacking; therefore, awareness that these highly processed plant-based meat alternatives may be high in sugar, fat, and/or salt and are detrimental to our health should be enhanced. As with any dietary pattern, special attention should be paid to ensure

nutrients of concern are consumed in sufficient quantities when adopting a plant-based diet. Furthermore, prospective research studies should be conducted to better understand the relationships between plant-based diets, health, and the incidence of chronic conditions on a Canadian population. A well-planned plant-based diet can offer nutrient-dense diets which confer health benefits and may mitigate the risk of chronic conditions.

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Evaluating the Screening Capability of the SarQoL Questionnaire in Sarcopenic Obesity: A Comparison Study Between Spanish and Belgian Community-Dwelling Older Adults

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Abstract: Background/Objectives: This study aims to evaluate the potential of the SarQoL questionnaire as a screening tool for sarcopenic obesity by comparing its effectiveness in Spanish and Belgian community-dwelling older people. This research seeks to address the primary question of whether the SarQoL can reliably differentiate quality of life impacts between these groups. Methods: A cross-sectional study was conducted involving community-dwelling older adults from Valencia (Spain) and Liège (Belgium). Participants were assessed using the SarQoL questionnaire, which measures health-related quality of life specifically for sarcopenia, and a sarcopenic obesity diagnostic method based on a combination of the EWGSOP2 criteria for sarcopenia and of body mass index for obesity. The sample included diverse demographic and clinical characteristics to ensure comprehensive analysis. Statistical methods were employed to compare the outcomes between the two populations. Results: The study highlighted a significant relationship between quality-of-life scores and the prevalence of sarcopenic obesity in the Spanish and Belgian populations. The SarQoL questionnaire effectively identified lower quality of life in individuals with sarcopenic obesity, demonstrating its potential as a reliable screening tool across different populations. In conclusion, the SarQoL questionnaire proved to be an effective tool for evaluating quality of life and for screening individuals with sarcopenic obesity. Conclusions: Future research should prioritize longitudinal studies to determine the SarQoL questionnaire's predictive value and investigate interventions to alleviate the adverse effects of sarcopenic obesity. Our results highlight the critical need to include quality of life assessments in managing sarcopenic obesity, advocating for a comprehensive approach to patient care.

Keywords: sarcopenic obesity; older adults; SarQoL; quality of life; screening method

1. Introduction

Sarcopenic obesity (SO) is a condition characterized by the coexistence of obesity and the loss of muscle mass and function [1,2]. This pathology presents a growing public health concern worldwide, particularly affecting the ageing population [3]. As the population ages and modern lifestyles promote physical inactivity and unhealthy diets, the risk of developing this complex clinical entity increases significantly. SO not only poses a clinical

challenge in terms of its diagnosis, management, and monitoring by healthcare professionals, it is also associated with a range of negative consequences for affected individuals, including a significant reduction in their quality of life (QoL) [4–7].

QoL is a multidimensional concept encompassing an individual's subjective perception of their physical, psychological, and social well-being [8,9]. In the context of SO, assessing QoL becomes crucial, as this clinical condition often includes reduced mobility and strength, and increased comorbidities, such as metabolic syndrome, which can significantly impact various aspects of daily life for those affected [10]. Despite this apparent direct relationship, the association between the prevalence of SO and QoL has not yet been thoroughly explored, highlighting an urgent need to investigate this link in different populations to better understand its clinical and epidemiological implications.

Our publication employed the sarcopenia quality of life (SarQoL) questionnaire, a validated and widely used tool for assessing QoL specifically in patients with muscle mass deficits [11–14]. This questionnaire is designed to capture various aspects of QoL, including physical function, independence, mobility, pain, and mental health, among others [15]. Using this standardized instrument provides a more comprehensive and detailed view of how SO affects QoL in study populations.

In addition to assessing QoL through the SarQoL questionnaire, anthropometric and clinical data were also collected to determine the prevalence of SO in both populations using a previously utilized and published diagnostic algorithm for this pathology. This algorithm includes the EWGSOP2 definition for sarcopenia [4] and the determination of three variables (body mass index (BMI), body fat percentage (%BF), and waist circumference (WC)) for obesity [16].

The determination of SO was conducted in a community-dwelling population, which was relevant due to the likely underdiagnosis of the pathology in this setting, as observed in other publications [17–20]. Conceptual ignorance of SO in the community can lead to an underestimation of its true prevalence and, therefore, a lack of early and appropriate interventions by which to mitigate its adverse effects on the QoL of affected individuals.

The objectives of this study were to analyze the relationship between the prevalence of SO and QoL in two distinct populations: one in Belgium (Liège) and other in Spain (Valencia). These two countries were selected for comparison due to the significant differences in socioeconomic, cultural, and lifestyle factors that could influence how SO impacts the QoL of their respective populations. Additionally, the potential discriminative capability of the SarQoL questionnaire as a diagnostic screening tool for SO was evaluated in both populations collectively.

2. Materials and Methods

2.1. Study Design

The sample for this study was obtained from two different populations. First from Valencia (Spain), comprising subjects attending an activity center for community-dwelling older people, and the second from Liège (Belgium), comprising subjects attending an outpatient clinic; both strata consisted of community-dwelling older adults. The total number of participants from both populations was 498 subjects, with 165 (33.13%) men and 333 (66.87%) women.

The inclusion criteria for study participation were being over 65 years old, attending the centers where the study-defining tests were conducted, having completed and signed the informed consent, and having completed all study tests. Regarding exclusion criteria, subjects presenting any pathology that could directly affect muscle mass or fat tissue and which suggested secondary SO (severe cognitive impairment, cancer, muscular dystrophy, stroke, myasthenia gravis, Parkinson's, Alzheimer's, amputated limb) were excluded.

Our research complied with the standards set by the Helsinki Declaration and received approval from the Bioethics Committee of the University of Valencia (number 1139186) and the Ethics Committee of the University Teaching Hospital of Liège (number 2013/6).

2.2. Sarcopenic Obesity

SO was determined by a combination of sarcopenia, diagnosed using the latest algorithm published by EWGSOP in 2019 [4], and obesity, based on body mass index (BMI) [16].

A quantification of obesity that derived solely from BMI was decided upon due to its good discriminative capacity for the pathology and its easy clinical application in the study population, as observed in other publications [20–23]. The algorithm for diagnosing SO in this publication can be found in Figure 1.

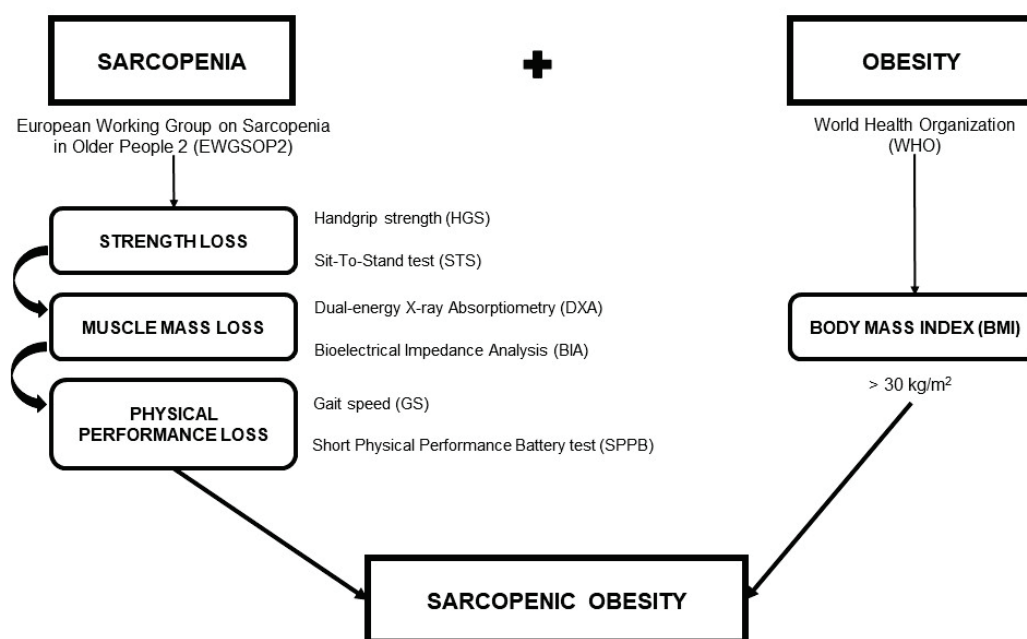


Figure 1. Algorithm for sarcopenic obesity diagnosis.

2.2.1. Strength Determination: Handgrip Strength and Sit-to-Stand Test

To assess the strength of study participants, upper body (handgrip strength) and lower body (sit-to-stand (STS) test) measurements were used in both populations, according to the standards proposed by EWGSOP2 for sarcopenia diagnosis [4].

For upper body strength determined by handgrip strength [4,24–26], a Jamar 5030J1 (Sammons Preston Rolyan, Nottinghamshire, UK) handheld dynamometer, with a measurement scale of 0–90 kg/f and an accuracy of ± 2 kg, was used in the Spanish population, while a Saehan 405108-010113 dynamometer (Saehan, Bongamgongdan, Republic of Korea), with the same measurement scale and accuracy, was used in the Belgian population. Hand-grip strength cut-off points were established according to EWGSOP2 [4], with scores below 16 kg in women and 27 kg in men being indicative of sarcopenia.

For lower body strength determined by the STS test [4,27], subjects from both populations sat and stood from a chair five times, keeping their backs straight, feet approximately shoulder-width apart, and arms crossed over their chests. The cut-off point for this diagnostic test was established following EWGSOP2 criteria [4], with a duration > 15 s for five repetitions considered definitive for sarcopenia.

According to EWGSOP2 criteria [4], low muscle strength in subjects was diagnosed when hand-grip strength and/or STS test showed pathological results (below the cut-off points).

2.2.2. Muscle Mass Determination: Appendicular Skeletal Muscle Mass/Height²

In Spanish participants, appendicular muscle mass was determined using the formula proposed by Kyle et al. [28], based on data obtained through electrical bioimpedance analysis (BIA) with the TANITA DC430MA-S scale (Tokyo, Japan), with an accuracy of 0.05 kg. BIA was implemented following the latest recommendations for more precise measurements [29].

On the other hand, in the Belgian sample, ASMM was determined using dual-energy X-ray absorptiometry (DXA). Cut-off points for sarcopenic pathology determination based on muscle mass were established at $<7 \text{ kg/m}^2$ in men and $<5.5 \text{ kg/m}^2$ in women for the assessment of ASMM adjusted by height (ASSM/h^2), following the EWGSOP2 algorithm [27,30,31].

2.2.3. Physical Performance Determination: Gait Speed and SPPB Test

To determine the physical performance of study participants, the Spanish and Belgian subjects underwent two diagnostic tests: a 4 m gait speed test [4,32] and the short physical performance battery test (SPPB test) [4,33–35]. Cut-off points suggested by EWGSOP2 [4] were used for both tests, being $\leq 0.8 \text{ m/s}$ for gait speed and ≤ 8 points for the SPPB test.

2.2.4. Obesity Determination: BMI

Obesity was determined using body mass index (BMI), a widely accepted method within scientific literature [22,23,36] and the field of SO. As such, the World Health Organization cut-off point was used, diagnosing subjects with a $\text{BMI} > 30 \text{ kg/m}^2$ as obese [16].

2.3. Quality of Life

QoL of participating subjects was assessed using the sarcopenia quality of life (SarQoL) questionnaire, validated for Spanish and Belgian populations [13,14]. This tool for quantifying QoL in subjects with muscle mass alterations was first validated in 2017 [14] and is the primary assessment tool for sarcopenic pathology within scientific literature [11,13,15,37,38].

The SarQoL questionnaire features a Likert scale for most questions, excluding those numbered 7, 14, and 22. It delves into seven distinct domains, evaluating various aspects of life quality, as follows: physical and mental health (D1), mobility (D2), body composition (D3), functional ability (D4), activities of daily living (D5), leisure pursuits (D6), and anxieties (D7). Scores for each domain, as well as the overall questionnaire score, range from 0 to 100, with higher scores indicating better QoL.

Researchers conducted the questionnaire sessions, providing assistance and clarifications as needed. Detailed results were obtained using the official platform provided by the creators (www.SarQoL.org (accessed on 12 January 2024)). The questionnaire has demonstrated strong internal consistency, corroborated by several publications [13,15,39,40].

2.4. Statistical Analysis

The statistical analysis utilized IBM SPSS Statistics v. 24 software for Windows (IBM Corp., Armonk, NY, USA). The normality of the data was assessed using the Shapiro–Wilk test. Descriptive statistics were computed for the SarQoL questionnaire [13] and its domains, as well as for variables concerning physical performance, obesity, and the diagnosis of SO. Differences between groups with and without SO were examined using the Student's *t*-test and the Mann–Whitney U test, with significance set at $p \leq 0.05$. Bonferroni correction was applied to avoid type 1 errors when conducting multiple comparisons. Pearson's correlation coefficient was employed for normally distributed data, while Spearman's correlation coefficient was used for non-normally distributed data. These tests evaluated relationships between variables contributing to SO diagnosis and results from the SarQoL questionnaire [13] and its domains. Variables showing significant differences underwent further analysis, with multiple linear regression used to assess their impact on domain scores and total SarQoL score.

The association between SO diagnosis and SarQoL questionnaire scores, both total and by domain, was examined using logistic regression. Additionally, an ROC curve analysis evaluated the ability of the SarQoL total score and its three most influential domains to discriminate SO. By establishing specificity and sensitivity thresholds for sarcopenia diagnosis based on SarQoL scores, researchers outlined potential risk zones, offering the prospect of using this test for diagnostic and preventive purposes.

3. Results

The total sample of this study comprised subjects from two European populations, Belgium and Spain. The Belgian cohort consisted of 296 individuals, of which 127 (43%) were men, and 15 of these (12%) were diagnosed with SO according to the criteria established by the researchers. In terms of women, they represented 57% of the Belgian sample (169 subjects), with 28 (17%) suffering from SO.

Conversely, the Spanish cohort included 202 individuals. The male group constituted 18% of the sample, with 38 participants, 5 of whom (13%) had SO. Women represented 82% of the Spanish sample (164 participants), with 20 (12%) affected by the condition.

Combining both samples, the total study population included 498 subjects, with 165 men (33%) and 333 women (67%), showing an overall SO prevalence of 13.6%. Dividing the total sample by sex, the male group presented an SO prevalence of 12.6% (20 men), which was similar to that observed in the female group, where the condition affected 14% of the participants (48 women).

The prevalence of SO was higher in women than in men in the Belgian population and the combined total sample. In contrast, in the Spanish cohort, its prevalence was slightly higher in men. The authors found significant differences between body mass index (BMI) and SO in both groups, except in Belgian women, with the SO group showing a higher BMI. Additionally, a greater total amount of appendicular skeletal muscle mass (ASMM) was observed in individuals with SO, regardless of sex, across the entire sample. The data related to the prevalence of SO according to the study populations can be consulted in Table 1.

Regarding the SarQoL questionnaire, which quantified the QoL of the participants, significant differences were observed when relating the obtained scores to the prevalence of SO in most groups, with the exception of the Belgian female group. In the analysis of the total sample, significant relationships were found between the prevalence of SO and the results obtained through the domains and the total score of the SarQoL questionnaire, with the only exception being the results for domain 3 (body composition). Thus, a large group of SO subjects in the study presented lower scores in most domains and the total SarQoL score.

The analysis of the results allowed the authors to find a significant correlation between the total score obtained on the SarQoL questionnaire and the main variables related to the diagnosis of SO, except for ASMM adjusted for height, independent of sex, and the total amount of ASMM in men. Therefore, most participants presented worse results in the diagnostic variables for SO as their scores on the SarQoL questionnaire and its domains decreased. The total SarQoL score and domains 2 (locomotion), 4 (functionality), and 5 (activities of daily living) individually showed a stronger relationship with the prevalence of SO, a fact supported by the finding of more significant correlations between the determinant variables of the pathology and the QoL results of the participants. The analysis of correlations between the main variables used for the diagnosis of SO and the SarQoL scores, as well as the scores obtained in each of its domains according to the sex of the participants, can be consulted in Table 2.

Table 1. Study sample characteristics based on the presence of sarcopenic obesity and perceived QoL, comparing data obtained in two European populations.

	Belgium Sample				Spanish Sample				Total Sample						
	Men		Women		Total n = 296	Men		Women		Total n = 202	Men		Women		Total n = 498
	WSO ^a (n = 112)	SO ^b (n = 15)	WSO ^a (n = 141)	SO ^b (n = 28)		WSO ^a (n = 33)	SO ^b (n = 9)	WSO ^a (n = 144)	SO ^b (n = 20)		WSO ^a (n = 145)	SO ^b (n = 20)	WSO ^a (n = 285)	SO ^b (n = 48)	
Age (years)	74 ± 6	76 ± 5	74 ± 6	74 ± 5	74.2 ± 6.1	72 ± 4	75 ± 6	73 ± 5	72 ± 5	72.8 ± 5	74 ± 6	76 ± 5	74 ± 6	73 ± 5	73.6 ± 5.7
Body mass index (kg/m ²)	28 ± 5	31.4 ± 2.5 *	24.8 ± 3.7	32.3 ± 2 *	27.1 ± 4.9	27.6 ± 4.2	33.5 ± 2 *	26.8 ± 4.1	31.2 ± 2.4 *	27.5 ± 4.2	27.9 ± 4.8	32 ± 2.5 *	25.8 ± 4	31.8 ± 3.4 *	27.3 ± 4.6
Overall quality of life	69.1 ± 15.2 *	56.3 ± 14.4	67.3 ± 14.3	55.9 ± 14.9	66.4 ± 15.3	82.0 ± 10.2 *	63 ± 14.4	75.7 ± 9.2 *	67 ± 8.6	75.3 ± 10.1	71.6 ± 15 *	58 ± 14.3	71.6 ± 12.7 *	60.6 ± 13.7	70 ± 14.1
Physical and mental health	65.8 ± 16.5 *	56 ± 14.2	65.8 ± 16.1	58.2 ± 15	64.6 ± 16.3	81.4 ± 13.2 *	64 ± 16.4	75.6 ± 13.6	69.8 ± 12.6	75.7 ± 13.9	69.3 ± 17.1 *	58 ± 14.7	70.8 ± 15.7 *	63 ± 15	69.1 ± 16.3
Locomotion	65.7 ± 21.3	45.7 ± 18.3	65.3 ± 21.8	53 ± 22.3	63.3 ± 22.1	84.9 ± 15.7 *	67.2 ± 20.3	81.8 ± 14.8 *	68.2 ± 14	80.6 ± 15.7	70.1 ± 21.7 *	51.1 ± 20.6	73.6 ± 20.3 *	59.3 ± 20.5	70.3 ± 21.5
Body composition	61.4 ± 18	54.6 ± 15.6	60.8 ± 16.2	56.8 ± 17.2	60.4 ± 17	75.8 ± 14.2 *	56.7 ± 10.9	64.2 ± 14.3	62.5 ± 15.1	65.7 ± 14.9	64.7 ± 18.2 *	55.1 ± 14.3	62.5 ± 15.4	59.2 ± 16.4	62.5 ± 16.4
Functionality	73 ± 16 *	64 ± 14	74 ± 15	59 ± 16	71.7 ± 15.9	79 ± 15 *	61 ± 16	77 ± 12 *	64 ± 11	75.3 ± 13.4	74 ± 16 *	63 ± 14	75 ± 13 *	61 ± 14	73.2 ± 15
Activities of daily living	69.3 ± 19.1 *	54.1 ± 18.8	62.4 ± 17.5	50 ± 17.5	63.4 ± 19.1	76.7 ± 10.6 *	59.8 ± 18	72.6 ± 9.3 *	67.2 ± 10.2	72.4 ± 10.3	71 ± 17.8 *	55.6 ± 18.3	67.5 ± 14.9 *	57.2 ± 17.1	67.1 ± 16.7
Leisure activities	54.8 ± 18.4 *	44.4 ± 13.7	57 ± 17.5	46.3 ± 20.8	54.5 ± 18.3	71.5 ± 19.3	59.9 ± 14.8	67.3 ± 20.5	68.2 ± 19.4	67.9 ± 20	58.6 ± 19.8 *	48.3 ± 15.2	62.2 ± 19.7 *	55.4 ± 22.8	59.9 ± 20.1
Fears	91 ± 9 *	83 ± 13	90 ± 10	87 ± 14	90 ± 10.6	95 ± 13	90 ± 14	89 ± 17 *	74 ± 22	88.9 ± 17.5	92 ± 10 *	84 ± 13	90 ± 14 *	82 ± 18	89.5 ± 13.8
Physical activity parameters															
ASMM ^c (kg)	55.7 ± 9	60.6 ± 5.5 *	36.7 ± 4.7	43.7 ± 6.3 *	45.8 ± 11.6	23 ± 2.8	23.8 ± 4.1	16.3 ± 2.4	18 ± 2.3 *	17.8 ± 3.6	48.3 ± 15.9	51.4 ± 17.1	26.4 ± 10.9	33 ± 13.7 *	34.4 ± 16.6
ASMM/h ^c d	7.91 ± 1.1	8.3 ± 0.7	5.9 ± 0.7	6.7 ± 0.7 *	6.9 ± 1.3	8.3 ± 0.9	8.6 ± 0.6	6.6 ± 0.8	7.4 ± 0.7 *	7 ± 1	8 ± 1.1	8.4 ± 0.7	6.3 ± 0.9	7 ± 0.8 *	6.9 ± 1.2
Handgrip strength (kg)	39 ± 9 *	37 ± 8	21 ± 6	18 ± 7	28.3 ± 11.6	35 ± 7	29 ± 7	20 ± 4 *	16 ± 5	22.4 ± 7.7	38 ± 9	35 ± 9	21 ± 5 *	17 ± 6	25.9 ± 10.6
Gait speed (m/s)	1.2 ± 0.3 *	0.9 ± 0.2	1.1 ± 0.3 *	0.9 ± 0.3	1.1 ± 0.3	1 ± 0.2	0.9 ± 0.3	1 ± 0.2 *	0.9 ± 0.2	1 ± 0.22	1.2 ± 0.3 *	0.9 ± 0.2	1.1 ± 0.3 *	0.9 ± 0.2	1.1 ± 0.3
Sit to stand test (s)	13.2 ± 4.3	19.4 ± 4.5 *	13.1 ± 3.4	18.7 ± 5.2 *	14 ± 4.5	11.9 ± 3.4	18 ± 5.2 *	11.3 ± 3	14.4 ± 4.9 *	11.9 ± 3.6	12.9 ± 4.2	19 ± 4.6 *	12.2 ± 3.3	16.8 ± 5.5 *	13.1 ± 4.3

Data are mean ± SD. * $p < 0.05$ (Bonferroni correction). ^a Without sarcopenic obesity. ^b Sarcopenic obesity. ^c Appendicular skeletal muscle mass. ^d Appendicular skeletal muscle mass adjusted by height.

Given the significant correlational relationship observed between most of the SO determinant variables and the scores obtained on the SarQoL questionnaire and its domains, the authors decided to develop a multiple linear regression analysis to confirm and quantify the relationship between the QoL scores of the participants and the main variables related to SO, including only those that were previously found to be significantly correlated. Thus, Tables 3 and 4 detail and corroborate the relationships between the participants' QoL and the prevalence of SO, once again showing the total SarQoL score and domains 2 (locomotion), 4 (functionality), and 5 (activities of daily living) as the most sensitive elements in quantifying QoL in SO. In order to improve multiple linear regression model analysis, the authors recommend consulting Scheme 1, which visually condenses the principal significant relationships between sarcopenic obesity variables and SarQoL domains and total score, referring to total sample data.

Additionally, to complete the analysis of the relationship between QoL and the prevalence of SO, the authors decided to conduct a logistic regression model that allowed for both univariate and multivariate analyses of the SarQoL scores and the prevalence of the condition. The results were found to continue to show a strong relationship between the prevalence of SO and the scores obtained through the SarQoL questionnaire, with lower QoL being a risk factor for developing the condition. Domain 4 (functionality) was found to be the parameter most strongly related to the analysis of QoL and the prevalence of SO. The detailed analysis of the logistic regression model can be consulted in Table 5, for visual optimization only significant results were presented.

Due to the relationship between parameters used to diagnose SO and the scores obtained through the SarQoL questionnaire in both populations, an ROC curve analysis was conducted to evaluate the discriminative capacity and screening potential of the SarQoL questionnaire and its domains for SO. Total SarQoL score and scores in domains 2 (locomotion), 4 (functionality), and 5 (activities of daily living) presented a significant area under the curve (AUC), i.e., greater than 0.7. Domain 4 showed the highest discriminative capacity for diagnosing SO, with an AUC of 0.75. The specific results of the ROC curve analysis can be consulted in Table 6.

Considering the sensitivity and specificity in the diagnosis of SO for each total SarQoL score, researchers defined three levels of risk regarding the probability of developing SO, regardless of sex. A high risk of developing the condition was established for participants with scores below 53 points, a moderate risk was defined for scores between 54 and 77 points, and participants with scores above 77 points were categorized as low risk (Figure 2).

Table 2. Correlations between principal variables related with Sarcopenic Obesity and SarQoL Domains.

	Physical and Mental Health (D1)		Locomotion (D2)		Body Composition (D3)		Functionality (D4)		Activities of Daily Living (D5)		Leisure Activities (D6)		Fears (D7)		SarQoL Total	
	Men	Women	Men	Women	Men	Women	Men	Women	Men	Women	Men	Women	Men	Women	Men	Women
Age	−0.276	−0.238	−0.285	−0.336	−0.238	−0.257	−0.264	−0.344	−0.281	−0.367	−0.170	−0.317	−0.148	−0.172	−0.312	−0.389
Body Mass Index (BMI)	−0.090	−0.057	−0.226	−0.160	0.021	0.083	−0.215	−0.278	−0.078	−0.066	−0.147	−0.013	−0.131	−0.160	−0.169	−0.150
Appendicular skeletal muscle mass (ASMM)	−0.178	−0.274	−0.258	−0.384	−0.104	−0.068	−0.067	−0.156	−0.001	−0.304	−0.171	−0.102	−0.127	−0.083	−0.148	−0.322
ASMM/h ² ^a	0.122	0.081	0.029	0.053	0.190	0.133	0.001	−0.120	0.137	0.133	0.021	0.068	0.106	−0.064	0.088	0.055
Handgrip strength	0.074	0.245	0.079	0.313	0.006	0.150	0.239	0.355	0.233	0.367	0.061	0.198	0.045	0.128	0.175	0.368
Sit to stand test	−0.405	−0.415	−0.478	−0.487	−0.224	−0.192	−0.501	−0.462	−0.503	−0.456	−0.274	−0.275	−0.351	−0.242	−0.516	−0.530
Gait speed	0.251	0.226	0.370	0.345	0.075	0.255	0.415	0.443	0.419	0.314	0.190	0.211	0.224	0.240	0.386	0.396

Pearson’s correlation coefficient for normally distributed data and Spearman’s correlation coefficient for data that are not normally distributed. *p* < 0.05 results area shown in bold. ^a Appendicular skeletal muscle mass adjusted by height.

TOTAL SAMPLE DATA

✓ = *p* value ≤ 0.05 in multiple linear regression model (significant).
X = *p* value > 0.05 in multiple linear regression model (no significant).

Variables related with Sarcopenic Obesity	SarQoL DOMAINS							SarQoL Total Score
	D1 (Physical and mental health)	D2 (Locomotion)	D3 (Body composition)	D4 (Functionality)	D5 (Activities of Daily Living)	D6 (Leisure Activities)	D7 (Fears)	
Age	✓	✓	✓	✓	✓	✓	✗	✓
Handgrip	✓	✓	✓	✓	✓	✓	✗	✓
Sit-To-Stand Test	✓	✓	✓	✓	✓	✓	✓	✓
ASMM ^a	✓	✓	✓	✓	✓	✓	✗	✓
Gait Speed	✓	✓	✗	✓	✓	✗	✓	✓
BMI ^b	✗	✓	✗	✓	✗	✗	✓	✓

Scheme 1. Visual representation of the significance of the relationships between principal variables involved in the diagnosis of sarcopenic obesity and SarQoL domains/total score. ^a Appendicular skeletal muscle mass. ^b Body mass index.

Table 3. Multiple Linear Regression Analysis with Total and Domain Scores of SarQol as Dependent Variables, stratified by sex.

Independent Variable	Men						Women												
	B	SE	Beta	T	p	R ²	Adjusted R ²	Model Significance	Independent Variable	B	SE	Beta	T	p	R ²	Adjusted R ²	Model Significance		
(1) Age	−0.682	0.215	D1 Physical and mental health	−0.221	−3.175	0.002	0.248	<0.001	(1) Handgrip	0.409	0.152	D1 Physical and mental health	0.148	2.702	0.007	0.237	0.228	<0.001	
(2) STS Test ^a	−1.222	0.258		(2) STS Test ^a	−0.824				0.238	−0.214	−3468		0.000						
(3) ASMM ^b	−0.265	0.074		(3) ASMM ^b	−0.377				0.075	−0.283	−5032		0.000						
				(4) Gait Speed	7.815				3.582	0.128	2.182		0.030						
(1) Age	−0.811	0.256	D2 Locomotion	−0.203	−3162	0.002	0.424	<0.001	(1) Age	−0.610	0.186	D2 Locomotion	−0.168	−3162	0.001	0.397	0.386	<0.001	
(2) STS Test ^a	−1.314	0.349		(2) Handgrip	0.644				0.182	0.177	3.539		0.000						
(3) ASMM ^b	−0.438	0.098		(3) STS Test ^a	−0.674				0.280	−0.133	−2.408		0.017						
(4) Gait Speed	20.012	6.069		(4) ASMM ^b	−0.703				0.089	−0.401	−7.881		0.000						
(5) BMI ^c	−0.841	0.311	D3 Body Composition	−0.181	−2700	0.008	0.123	<0.001	(5) Gait Speed	12.558	4.572	D3 Body Composition	0.156	2.747	0.006	0.111	0.1	<0.001	
(1) Age	−0.666	0.243		(6) BMI ^c	−0.537				0.212	−0.117	−2529		0.012						
(2) STS Test ^a	−0.731	0.292		(1) Age	−0.291				0.162	−0.108	−1792		0.074						
(3) ASMM ^b	−0.194	0.083		(2) ASMM ^b	−0.180				0.071	−0.135	−2523		0.012						
(1) Age	−0.593	0.191	D4 Functionality	−0.211	−3112	0.002	0.357	<0.001	(3) Gait Speed	15.035	3.705	D4 Functionality	0.248	4.058	0.000	0.366	0.354	<0.001	
(2) Handgrip	0.275	0.127		(4) BMI ^c	0.418				0.191	0.120	2.188		0.029						
(3) Gait Speed	−1.436	0.222		(1) Age	−0.349				0.130	−0.140	−2676		0.008						
(4) BMI ^c	−0.920	0.214		(2) Handgrip	0.359				0.128	0.144	2.812		0.005						
(1) Age	−0.491	0.238	D5 Activities Of Daily Living	0.150	1.878	0.062	0.313	<0.001	(3) STS Test ^a	−0.837	0.196	D5 Activities Of Daily Living	−0.241	−4.268	0.000	0.375	0.365	<0.001	
(2) Handgrip	0.321	0.171		(4) ASMM ^b	0.063				0.207	−0.099	−1.901		0.058						
(3) STS Test ^a	−1.012	0.311		(5) Gait Speed	9.965				3.207	0.181	3.108		0.002						
(4) ASMM ^b	−0.223	0.087		(6) BMI ^c	−0.756				0.149	−0.241	−5079		0.000						
(5) Gait Speed	15.397	5.422	D6 Leisure Activities	0.242	2.840	0.005	0.291	<0.001	(1) Age	−0.358	0.134	D6 Leisure Activities	0.135	5.174	0.000	0.18	0.167	<0.001	
(1) Age	−0.579	0.256		(2) Handgrip	0.699				0.135	0.263	5.174		0.000						
(2) STS Test ^a	−0.822	0.308		(3) STS Test ^a	−0.642				0.208	−0.173	−3095		0.002						
(3) ASMM ^b	−0.378	0.088		(4) ASMM ^b	−0.439				0.066	−0.343	−6709		0.000						
(1) STS Test ^a	−0.919	0.170	D7 Fears	−0.388	−5.401	0.000	0.176	<0.001	(5) Gait Speed	7.803	3.286	D7 Fears	0.133	2.375	0.018	0.107	0.099	<0.001	
(2) BMI ^c	−0.438	0.165							(1) Age	−0.867	0.191		−0.240	−4542					0.000
									(2) STS Test ^a	−0.886	0.283		−0.176	−3130					0.002
									(3) ASMM ^b	−0.349	0.094		−0.201	−3705					0.000
(1) STS Test ^a	−0.919	0.170	D7 Fears	−0.388	−5.401	0.000	0.178	<0.001	(1) STS Test ^a	−0.533	0.219	D7 Fears	−0.143	−2436	0.015	0.107	0.099	<0.001	
(2) BMI ^c	−0.438	0.165							(2) Gait Speed	10.869	3.499		0.184	3.106					0.002
									(3) BMI ^c	−0.439	0.182		−0.130	−2417					0.016
(1) Age	−0.565	0.188	SarQol Total	−0.205	−3008	0.003	0.401	<0.001	(1) Age	−0.363	0.112	SarQol Total	−0.159	−3232	0.001	0.444	0.433	<0.001	
(2) Handgrip	0.242	0.134							(2) Handgrip	0.480	0.110		0.210	4.366					0.000
(3) STS Test ^a	−1.120	0.246							(3) STS Test ^a	−0.719	0.169		−0.225	−4254					0.000
(4) ASMM ^b	−0.231	0.073							(4) ASMM ^b	−0.349	0.054		−0.317	−6485					0.000
(5) Gait Speed	8.867	4.346	SarQol Total	0.167	2.040	0.043	0.178	<0.001	(5) Gait Speed	8.972	2.761	SarQol Total	0.177	3.250	0.001	0.444	0.433	<0.001	
(6) BMI ^c	−0.503	0.220							(6) BMI ^c	−0.276	0.128		−0.096	−2157					0.032

p < 0.05 results area shown in bold. ^a Sit-To-Stand Test. ^b Appendicular Skeletal Muscle Mass. ^c Body Mass Index.

Table 4. Multiple Linear Regression Analysis with Total and Domain Scores of SarQol as Dependent Variables.

Independent Variable	Total Sample					R ²	Adjusted R ²	Model Significance
	B	SE	Beta	T	p			
D1 Physical and mental health								
(1) Age	−0.287	0.125	−0.099	−2.295	0.022	0.245	0.238	<0.001
(2) Handgrip	0.328	0.081	0.216	−4.028	0.000			
(3) STS Test ^a	−0.901	0.182	−0.238	−4.964	0.000			
(4) ASMM ^b	−0.348	0.053	−0.362	−6.529	0.000			
(5) Gait Speed	5.360	3.011	0.089	1.780	0.076			
D2 Locomotion								
(1) Age	−0.592	0.149	−0.157	−3.967	0.000	0.401	0.393	<0.001
(2) Handgrip	0.497	0.096	0.249	5.207	0.000			
(3) STS Test ^a	−0.993	0.213	−0.200	−4659	0.000			
(4) ASMM ^b	−0.577	0.065	−0.457	−8937	0.000			
(5) Gait Speed	14.730	3.632	0.186	4.056	0.000			
(6) BMI ^c	−0.610	0.174	−0.134	−3503	0.000			
D3 Body Composition								
(1) Age	−0.515	0.133	−0.177	−3.877	0.000	0.092	0.084	<0.001
(2) Handgrip	0.204	0.090	0.132	2.260	0.024			
(3) STS Test ^a	−0.503	0.180	−0.131	−2.797	0.005			
(4) ASMM ^b	−0.125	0.058	−0.128	−2.176	0.030			
D4 Functionality								
(1) Age	−0.422	0.106	−0.162	−3974	0.000	0.361	0.353	<0.001
(2) Handgrip	0.262	0.068	0.191	3.858	0.000			
(3) STS Test ^a	−1.016	0.152	−0.297	−6.699	0.000			
(4) ASMM ^b	−0.106	0.046	−0.122	−2.319	0.021			
(5) Gait Speed	8.624	2.584	0.159	3.337	0.000			
(6) BMI ^c	−0.766	0.124	−0.244	−6185	0.000			
D5 Activities of Daily Living								
(1) Age	−0.358	0.118	−0.124	−3.038	0.002	0.338	0.332	<0.001
(2) Handgrip	0.559	0.077	0.366	7.278	0.000			
(3) STS Test ^a	−0.884	0.171	−0.232	−5163	0.000			
(4) ASMM ^b	−0.321	0.050	−0.331	−6.385	0.000			
(5) Gait Speed	10.394	2.839	0.172	3.661	0.000			
D6 Leisure Activities								
(1) Age	−0.691	0.155	−0.193	−4461	0.000	0.185	0.178	<0.001
(2) Handgrip	0.244	0.105	0.128	2.312	0.021			
(3) STS Test ^a	−0.770	0.210	−0.163	−3671	0.000			
(4) ASMM ^b	−0.384	0.067	−0.320	−5.728	0.000			
D7 Fears								
(1) STS Test ^a	−0.581	0.156	−0.179	−3723	0.000	0.113	0.107	<0.001
(2) Gait Speed	8.867	2.482	0.172	3.572	0.000			
(3) BMI ^c	−0.384	0.129	−0.129	−2986	0.003			
SarQol Total								
(1) Age	−0.412	0.095	−0.168	−4321	0.000	0.419	0.411	<0.001
(2) Handgrip	0.389	0.061	0.301	6.380	0.000			
(3) STS Test ^a	−0.903	0.136	−0.280	−6.627	0.000			
(4) ASMM ^b	−0.290	0.041	−0.354	−7030	0.000			
(5) Gait Speed	8.799	2.322	0.172	3.789	0.000			
(6) BMI ^c	−0.341	0.111	−0.115	−3066	0.002			

p < 0.05 results area shown in bold. ^a Sit-To-Stand Test. ^b Appendicular Skeletal Muscle Mass. ^c Body Mass Index.

Table 5. Simple and multiple logistic regression.

	Sarcopenic Obesity			
	Odds Ratio (Univariate)		Odds Ratio (Multivariate)	
SarQol				
Overall quality of life	0.942 (0.924–0.961)		<0.001	
Physical and mental health	0.967 (0.951–0.983)		<0.001	
Locomotion	0.967 (0.955–0.979)		<0.001	

Table 5. Cont.

	Sarcopenic Obesity			
	Odds Ratio (Univariate)	p-Value	Odds Ratio (Multivariate)	p-Value
Body composition	0.980 (0.964–0.996)	0.014	0.948 (0.929–0.967)	<0.001
Functionality	0.942 (0.924–0.960)	<0.001		
Activities of daily living	0.960 (0.946–0.975)	<0.001		
Leisure activities	0.980 (0.967–0.994)	0.004		
Fears	0.965 (0.950–0.981)	<0.001		

$p < 0.05$ results area shown in bold.

Table 6. ROC curves diagnostic performance values (domains and total score of SarQoL questionnaire) when its used to discriminate SO.

Analyzed Variables	95% Asymptotic Confidence Interval			
	Area	p-Value	Lower Limit	Upper Limit
Total SarQoL score	0.73 *	0.00	0.67	0.79
Physical and Mental Health Domain (D1)	0.65	0.00	0.59	0.72
Locomotion Domain (D2)	0.70 *	0.00	0.64	0.77
Body Composition Domain (D3)	0.60	0.01	0.52	0.67
Functionality Domain (D4)	0.75 *	0.00	0.69	0.81
Activities Of Daily Living Domain (D5)	0.70 *	0.00	0.63	0.76
Leisure Activities Domain (D6)	0.61	0.00	0.54	0.69
Fears Domain (D7)	0.64	0.00	0.57	0.72

* Area > 0.70 is showed in bold.

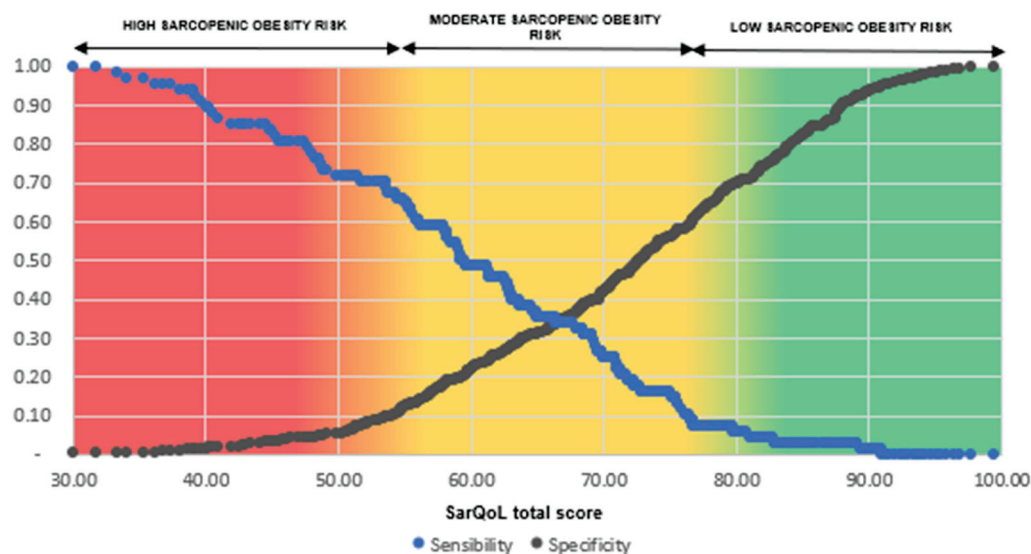


Figure 2. Sarcopenic obesity risk based on SarQoL questionnaire scores, assessing sensibility and specificity for the obtained results.

4. Discussion

The present study compared the prevalence of sarcopenic obesity (SO) in two European populations, Spain and Belgium, while analyzing its relationship with QoL, which was quantified using the SarQoL questionnaire. Results indicate a similar prevalence of SO between the two studied populations, with 12.4% in Spain (Valencia) and 14.5% in Belgium (Liège), yielding a combined total prevalence of 13.6%. These findings are consistent with previous studies conducted in other European regions, reporting similar prevalence ranges [41–43], despite heterogeneity in the diagnostic methods used to assess the pathology. However, interesting differences in gender distribution of the disease were observed: in Belgium, the prevalence of SO was higher in women compared with men (17% vs. 12%, respectively), whereas in Spain, the opposite situation was found (12% vs. 13%, respectively). These differences in gender distribution with regard to the prevalence of SO

have been reported by other authors analyzing similar populations, where women were more prevalent [44,45], and, conversely, where men had a higher prevalence of SO [46,47].

It is notable that the sample origin might have influenced the obtained results; in Belgium, subjects were recruited from their reference primary care center, unlike in Spain, where participants were users of an activity center for community-dwelling older adults. The different recruitment environments might have influenced the observed difference in SO prevalence. Thus, subjects attending the primary care center could present a more deteriorated health profile when compared with Spanish subjects using the activity center for community-dwelling older adults. Despite this, the total difference in SO prevalence between the two populations was approximately 2%, highlighting the silent infiltration of this pathology in society, considering that study participants were autonomous and independent individuals.

The researchers decided to use a combination of the EWGSOP2 algorithm for sarcopenia diagnosis [4] and the BMI criterion for obesity [16]. The use of both diagnostic procedures within the diagnosis of SO has been widely accepted and validated in other literature [4,16]. Although BMI is a useful and practical tool, it is important to consider that it may not fully reflect body composition. Despite this, in the older population range, the excess muscle mass that could result in an increased BMI not mediated by fat tissue is extremely rare. Given its simplicity and ease of use, BMI is a useful tool for epidemiological studies, as well as for quantifying the clinical variable of obesity in subjects with SO, as supported by previous scientific publications [22,23,36].

The SarQoL questionnaire, used by researchers to evaluate the QoL of study participants, proved to be a solid tool for quantifying this element in patients with SO from the Spanish and Belgian populations. This is despite the fact that it was originally intended exclusively for the assessment of QoL mediated through muscle mass deficit, without specifically evaluating obesity in subjects with SO.

Additionally, our study demonstrated the capability of the SarQoL questionnaire [14] as a screening method for SO, a fact that had already been proven previously for sarcopenic pathology alone and for SO through other diagnostic algorithms in smaller populations without an international component. Comparing our results with those obtained by Fonfría-Vivas et al. [19], we found similarities in the study population despite using a different diagnostic algorithm and in the established cut-off points, where we set their limit at ≤ 50 points for the total SarQoL score, without studying the scores of the domains individually. Thus, our research showed a cut-off point for the prevalence of SO of ≤ 53 points when the sample of the two populations under study was used.

Our research presents some limitations and strengths that should be considered. Regarding the limitations, the cross-sectional design of the study prevents the establishment of definitive causal relationships between SO and QoL, and the evaluation of muscle mass and obesity was based on indirect methods (BMI and BIA), which, although validated, may not be as precise as other methods, such as dual-energy X-ray absorptiometry (DXA) [48]. Furthermore, the sample may not be entirely representative of the general population of both countries, which could limit the generalizability of the results.

Regarding the strengths, this study includes populations from two European countries (Spain and Belgium), allowing for an international comparison of results and providing a broader perspective on the prevalence and impact of SO in different socioeconomic and cultural contexts. Thus, the inclusion of participants from different settings (a primary care center in Belgium and a senior activity center in Spain) adds variability to the total sample, improving the generalization of the results. Additionally, the use of standardized criteria for SO diagnosis, such as BMI [16] and the diagnostic algorithm proposed by EWGSOP2 [4] for sarcopenia, ensures consistency and comparability of results with other similar studies. Finally, the implementation of the SarQoL questionnaire [14] to assess QoL provides a specific and validated measure for subjects with sarcopenia, adding rigor and relevance to the study findings.

It is important to mention that this study also highlights the need for greater awareness and education about SO in the community. The lack of knowledge about this condition, as well as the absence of a standardized diagnostic algorithm, can lead to underdiagnosis [49,50] and, consequently, a lack of appropriate interventions. The implementation of educational programs and proper screening, through tools like the one proposed by the authors in this research, can help identify and treat SO at earlier stages, thereby improving the QoL and health of those affected.

5. Conclusions

Our investigation evaluated the potential of the SarQoL questionnaire as a screening tool for SO and its impact on QoL in Belgian and Spanish populations. Our findings have revealed significant differences in SO prevalence, with higher rates in Belgian women and Spanish men, highlighting the role of possible socio-cultural factors. SO was shown to negatively affect QoL, with lower SarQoL scores observed in affected individuals, particularly in domains related to locomotion (D2), physical function (D4) and activities of daily living (D5). These correlations between SarQoL scores and SO diagnostic principal variables validate the questionnaire's effectiveness. The regression model confirmed that SarQoL, especially its physical function domain (D4) and its total score, is a sensitive tool for assessing QoL in SO patients. Additionally, the performed ROC curve analysis demonstrated SarQoL's potential as a screening tool, allowing one to establish cut-off points to evaluate the risk of SO. In conclusion, the SarQoL questionnaire effectively reflects the QoL impairments in SO, advocating for its use in clinical and epidemiological settings. Future research should focus on longitudinal studies to establish its predictive value and explore interventions to mitigate SO's adverse effects. Our findings underscore the importance of incorporating QoL assessments in SO management, emphasizing a holistic approach to patient care.

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Article

Impact of Dietary Niacin on Metabolic Dysfunction-Associated Steatotic Liver Disease in Mediterranean Subjects: A Population-Based Study

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Abstract: Background: The impact of dietary niacin on metabolic dysfunction-associated steatotic liver disease (MASLD) is elusive. This sub-study aimed to investigate the relationship between dietary niacin intake and the presence of MASLD in participants from two Catalan cohorts. Methods: A total of 222 subjects with MASLD were age- and sex-matched to 222 non-MASLD subjects. Dietary nutrients were analyzed using a validated food frequency questionnaire (FFQ). Dietary niacin and other nutrients were adjusted for total energy intake. MASLD was defined by a Fatty Liver Index (FLI) of >60 and by having at least one component of metabolic syndrome. The association between niacin intake (distributed into tertiles) and the presence of MASLD was assessed using multivariate logistic regression. Potential non-linear relationships were also analyzed through restricted cubic spline regression (RCS). Results: Our data revealed that subjects with MASLD had worse metabolic profiles. The dietary intake of niacin did not differ between subjects with and without MASLD. Even after adjusting for different confounding variables, i.e., sociodemographic variables, smoking status, physical activity, and cardiometabolic comorbidities, no significant associations were observed between higher intakes of niacin (tertiles 2 and 3) and the presence of MASLD: odds ratio (95% confidence) second tertile: 0.99 (0.89–1.09); third tertile: 0.98 (0.89–1.10). However, RCS analysis uncovered a significant non-linear dose-response association between dietary niacin intake and odds of MASLD. Specifically, such analysis revealed that MASLD risk was decreased in subjects with niacin intake values of <35 mg/day. Conclusions: Our data showed that dietary niacin intake was associated with lower odds of MASLD in a Mediterranean population; however, our logistic regression analysis failed to reveal significant associations between the intake of niacin and the risk

of MASLD. Further research is warranted to establish a causal relationship between dietary niacin interventions and MASLD.

Keywords: hepatic steatosis; vitamin B3; niacin; tryptophan; fatty liver; case-control study

1. Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) is a dysmetabolic condition of the liver that is frequently linked to excessive caloric intake, overweight/obesity, or impaired insulin signaling in the absence of excessive alcohol consumption, autoimmune factors, infection, and/or other liver disorders [1,2]. Clinically, MASLD is defined by the presence of excessive triglyceride accumulation in hepatocytes (hepatic steatosis) and the presence of at least one cardiometabolic risk factor, i.e., type 2 diabetes mellitus (T2DM), obesity, or one of the components of metabolic syndrome [3,4]. Epidemiologically, its prevalence is steadily increasing worldwide. MASLD affects about one-third of the general population, reaching values closer to 55% in subjects with T2DM [1,5].

MASLD can be a reversible condition [6]. With emerging pharmacological therapies to specifically prevent or regress MASLD still under investigation, current clinical management consists of lifestyle modifications, including healthy diet/nutritional counseling and physical activity recommendations [1,2]. In particular, caloric restriction, limiting the energy intake to 500 to 750 kcal/day in combination with adherence to Mediterranean Diet (MetDiet) patterns, is currently considered the recommended dietary pattern in the treatment of MASLD [6]. In MetDiet, about 35% of calories come from low-glycemic carbohydrates, 45% from monounsaturated fatty acids, and 15–20% from proteins [6,7]. The favorable effects of MetDiet components on hepatic fat accumulation have been assessed in several studies, particularly focusing on the relative proportions of dietary carbohydrates and fats [8–10]. In contrast, the contribution of protein intake, both quantitatively and qualitatively, to MASLD remains poorly studied [11].

Niacin, also known as vitamin B3, encompasses both nicotinamide and nicotinic acid, two forms of the vitamin that contribute to essential metabolic processes in the body. Importantly, tryptophan, an essential amino acid from dietary protein, also contributes to the niacin pool in an organism [12]. Thus, the main dietary sources of niacin are predominantly protein-based, such as beef, poultry, eggs, fish, dairy, legumes, some cereals, and yeast, which are also high in tryptophan [13]. In this respect, the term “niacin equivalents” (NE) is used to estimate niacin intakes and requirements from other macronutrients, particularly proteins, in humans, where 1 mg NE corresponds to 60 mg tryptophan [12–14]. By meeting the daily protein needs of adults, typically achieved by consuming a total of 100 g of protein per day, the daily niacin requirements can by far be fulfilled [13].

Compelling evidence suggests that low levels of the oxidative form of nicotinamide adenine dinucleotide (NAD⁺) are a common feature of MASLD [15–20]. In this regard, the use of NAD⁺ precursors, i.e., niacin derivatives and tryptophan, has been assessed as a NAD⁺-increasing approach to ameliorate experimental MASLD in preclinical models [21–28]. Additionally, niacin may also inhibit hepatic lipogenesis via GPR109A-mediated signaling pathway [29]. Despite this, data on the potential effect of NAD⁺ precursor-based interventions on MASLD in humans are limited and inconsistent [2,30,31]. A few studies have suggested that dietary niacin may help ameliorate MASLD [32,33]. Supporting this, a two-year non-randomized intervention study showed that higher niacin intake reduced liver fat content in 58 subjects with MASLD, of whom 23 reversed their disease [33]. In contrast, a placebo-controlled clinical trial of nicotinamide riboside supplementation (250 mg/day) and pterostilbene (50 mg/day) for 6 months only showed a reduction in signs of liver inflammation but not steatosis in humans with MASLD/MASH [31]. Notably, plasma levels of liver enzymes showed a time-dependent decrease in those subjects receiving the supplementation compared with placebo [31]. Furthermore, in a trial in patients with

diabetes and MASLD, a daily nicotinamide supplementation of 1000 mg daily did not influence liver steatosis or fibrosis [30]. On the other hand, to the best of our knowledge, only one recent cross-sectional study conducted in the United States found that moderate niacin intake was associated with a decrease in the prevalence of MASLD [34].

The present study aimed to assess the association between the intake of niacin and NE and the presence of MASLD in subjects from Catalonia, Northeast Spain. Additionally, because the intake of dietary NE and protein are tightly related, as a secondary objective, we further analyzed whether there was an association between protein intake with the presence of MASLD.

2. Materials and Methods

2.1. Study Design and Settings

This was a case-control analysis using data from two cohorts previously recruited from Catalonia between March 2010 and July 2014 [35–38]. The first study included a cohort of individuals with T2DM that aimed to study microvascular disease in patients with T2DM [37,38]. The second study was a population-based cohort designed to determine the prevalence of prediabetes and risk factors associated with this condition [35,36].

2.2. Participants

For the purpose of this analysis, we screened all of the subjects from the two cohorts [28–30]. We included a total sample of 444 age- and sex-matched subjects: 222 with MASLD and 222 without MASLD. The glucose tolerance status of the study subjects included T2DM, prediabetes, or normal glucose tolerance. The inclusion criteria in the T2DM cohort were age between 40 and 75 and a diagnosis of T2DM. The exclusion criteria were as follows: being a health professional, cognitive impairment (dementia and mental illness), presence of macrovascular complications (heart failure, cerebrovascular disease, ischemic heart disease, and peripheral arterial disease), previous diabetic foot disease, macroalbuminuria (defined as a urine albumin/creatinine ratio greater than 300 mcg/g), renal insufficiency (defined as a glomerular filtration rate < 60 mL/min), and the presence of any other condition requiring specific nutritional therapy (e.g., pregnancy). For the subjects without T2DM, the inclusion criteria were as follows: subjects aged 25 or over attending the Primary Care Centre of the Alt Urgell region of the province of Lleida. Subjects were excluded if they had already been diagnosed with diabetes [type 1 diabetes mellitus (TD1M), T2DM, or other specific subtypes], treatment with oral antidiabetic agents, cardiovascular diseases, history of cancer treated in the last 5 years, non-melanoma skin cancer, renal disease or anemia, hepatitis or other significant liver diseases, gastrointestinal disease, recent abdominal surgery, chronic obstructive pulmonary disease, chronic infectious disease, use glucocorticoids or beta-blockers, and significant psychiatric disorders with psychological symptoms.

Additionally, in this study, we excluded subjects with alcohol consumption exceeding >30 g/day in men and >20 g/day in women [1], individuals with highly unbalanced and/or extreme dietary intake (<1000 kcal/day or $\geq$ 4000 kcal/day), high consumption of niacin (mg/day) > 44, NE (mg/day) > 60 and >2.3 g protein /kg weight/day, those lacking anthropometric measurements and biochemical values (gamma-glutamyl transferase, triglycerides) necessary for Fatty Liver Index (FLI) calculation, and individuals without dietary intake data available.

Both cohorts were approved by the Ethics Committee of the University Hospital of Lleida (CEIC 1079) and Ethics Committee of the Primary Health Care University Research Institute (IDIAP) Jordi Gol (PI12/043). Informed consents were obtained from all participants according to the Declaration of Helsinki [35–38].

2.3. Variables

We estimated the presence of MASLD using the FLI formula, which includes the following clinical parameters: triglycerides (mg/dL); body mass index (BMI; kg/m²);

gamma-glutamyl transferase (GGT; U/L); and waist circumference (WC; cm) [39]. FLI has proven to be a reliable proxy for the diagnosis of hepatic steatosis in current clinical practice and has been proposed as a good predictor of hepatic steatosis in patients diagnosed with MASLD [40,41]. A cut-off point of ≥ 60 was used in this study to enhance the specificity of the diagnostic tool [42]. $FLI \geq 60$, along with one of the three criteria outlined in the new definition consensus for MASLD, was used to define a diagnosis of MASLD, consistent with previous protocols [43]. Variables related to weight, height, blood pressure, BMI, and WC were analyzed [35,37]. Various potential confounding variables were considered a priori, including sociodemographic factors, alcohol intake, current smoking habits, and biochemical measurements.

Physical activity was assessed using the International Physical Activity Questionnaire (IPAQ) validated in Spanish [44], according to the Bernstein et al. [45] method: this was classified as sedentary if less than 10% of daily energy expenditure came from activities requiring at least four METs (metabolic equivalent of tasks, e.g., walking or cycling for over 25 min per day). Regular physical activity was classified if more than 10% of energy expenditure came from such activities. According to the World Health Organization (WHO), one MET is the energy cost of sitting quietly, equivalent to 1 kcal/kg/h [46]. Hypertension and dyslipidemia were considered if patients were on antihypertensive or lipid-lowering medication [36,37]. Prediabetes and diabetes were classified based on the American Diabetes Association (ADA) criteria [47,48].

2.4. Dietary Niacin Consumption

Dietary information was collected using the validated Spanish 101-item semi-quantitative food frequency questionnaire (FFQ) administrated by trained researchers face-to-face [49]. The FFQ allows for the measurement of dietary frequency categorized as monthly, weekly, or daily intake of various food groups and collects data on food consumption during the last year prior to the study visit [49,50]. Nutrient intake food consumption data were obtained from US Department of Agriculture composition tables, as well as other food sources and serving sizes in English and Spanish composition tables [51–53]. Dietary niacin and NE (mg/day) intakes were obtained from the FFQ. NEs were calculated based on both tryptophan and niacin dietary intake, adjusted for the total energy intake [49]. The adjusted niacin and NE intake were evaluated, taking into account the Recommended Daily Allowance (RDA) intake of niacin and NE according to European Food Safety Authority (EFSA) data. The RDA is 5.5 mg NE/1000 kcal per day [13].

2.5. Dietary Macronutrient Consumption

All macronutrients were obtained from the FFQ [49]. All these were adjusted for total energy intake (kcal/day). In particular, protein intake was also adjusted for weight using the formula g/kg/day [54]. An established RDA of 0.8 g/kg/day was used as the reference value [54].

2.6. Statistical Analysis

Categorical and binary variables were summarized using frequency counts and percentages (n, %), while group differences in numeric variables were assessed using the chi-square test or Fisher's test. For the comparison of categorical variables with numerical variables, t-student or the Kruskal–Wallis test was used, depending on the distribution of the data. The intake of niacin, NE and protein was stratified into tertiles (T1 = lowest intake, T3 = highest intake), and the association between the presence of MASLD and intake tertiles was analyzed using multivariate logistic regression, with a total of four models (unadjusted model, model 1, model 2, and model 3). Model 1 was adjusted by age and sex, model 2 by age, sex, physical activity level, smoking status, and total caloric intake, and finally, model 3 was adjusted for all the confounding variables (age, sex, sedentary activity, total caloric intake, smoking, BMI, GFR, hypertension, dyslipidemia, and T2DM). Non-linear relationship analysis between the dietary intake of niacin and MASLD was explored using

restricted cubic spline (RCS) regression with three knots (10th, 50th, and 90th percentiles). In our study, we selected the number of nodes based on previous studies and statistics guidelines [55]. Specifically, a three-node, non-linear association model was used to warrant an adequate balance between model flexibility and the risk of overfitting, hence allowing an effective interpretability and robustness of the results. A conservative type I error rate of 5% was adopted, with statistical significance set at p -value < 0.05 . Confidence intervals (CIs) were examined for the presence of the null value (0 for categorical variables and 1 for continuous variables) to ensure the reliability of the findings. The statistical analysis was performed using R statistical software [56].

3. Results

Out of the 857 participants from both cohorts, 444 were included after applying the selection criteria. Figure S1 summarizes the flowchart of the study.

Compared with the group of subjects without MASLD (non-MASLD group), subjects with MASLD had lower levels of education ($p < 0.001$), higher weight ($p < 0.001$), WC ($p < 0.001$), and BMI ($p < 0.001$). Additionally, they were more sedentary ($p = 0.002$), had a higher frequency of T2DM (52.7%; $p < 0.001$), dyslipidemia (31.5%; $p = 0.005$), and hypertension (25.4%; $p < 0.001$) (Table 1).

Table 1. Clinical and demographic characteristics of study participants.

Characteristics	Non-MASLD (n = 222)	MASLD (n = 222)	<i>p</i> -Value
Age, years	55.1 (11.9)	55.2 (11.9)	0.975
Women, n (%)	113 (50.9)	113 (50.9)	1.000
Caucasian, n (%)	218 (98.2)	211 (95.0)	0.096
Secondary high cycle, n (%)	123 (55.4)	95 (42.8)	0.004
Weight, kg	70.2 (10.8)	89.2 (13.3)	<0.001
Height, cm	166 (9.7)	165 (9.3)	0.080
WC, cm	92.1 (9.7)	110 (10.0)	<0.001
BMI, kg/m ²	25.4 (3.1)	33.0 (5.0)	<0.001
Glucose, mg/dL	102 (30.6)	130 (54.1)	<0.001
HbA1c, %	5.9 (0.9)	6.8 (1.7)	<0.001
sBP, mm/Hg	126 (18.9)	136 (18.4)	<0.001
dBP, mm/Hg	77.0 (9.9)	79.8 (10.9)	0.005
Total cholesterol, mg/dL	203 (34.4)	200 (38.9)	0.360
HDL, mg/dL	59.6 (15.1)	50.5 (12.8)	<0.001
LDL, mg/dL	125 (29.1)	121 (33.1)	0.139
Triglycerides, mg/dL	94.5 (39.9)	163 (134)	<0.001
GGT, U/L	22.6 (30.3)	45.9 (107)	0.002
ALT, U/L	21.3 (25.8)	27.3 (16.6)	0.004
FLI	31.5 (17.1)	81.4 (12.4)	<0.001
GFR	91.0 (13.3)	93.6 (16.2)	0.150
Physically sedentary, n, (%)	76 (34.2)	109 (49.5)	0.002
Smoking, n, (%)	42 (18.9)	54 (24.3)	0.384
Alcohol, g/day	5.25 (6.3)	5.02 (7.1)	0.724
T2DM, n, (%)	50 (22.5)	117 (52.7)	<0.001
Prediabetes, n, (%)	66 (29.7)	48 (21.6)	0.065
Dyslipidemia, n, (%)	43 (19.4)	70 (31.5)	0.005
Hypertension, n, (%)	90 (15.2)	74 (25.4)	<0.001

Data are mean (SD) for continuous variables and number (%) for categorical variables. ALT, alanine amino-transferase; BMI, body mass index; CT, total cholesterol; dBP, diastolic blood pressure; FLI, Fatty Liver Index; GFR, glomerular filtration rate; GGT, gamma-glutamyl transferase; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MASLD, metabolic dysfunction-related steatotic liver disease; sBP: systolic blood; TG, triglyceride; T2DM, type 2 diabetes mellitus; WC, waist circumference.

3.1. Dietary Intake

Dietary niacin and NE consumption did not differ between study groups (p -value = 0.931, p -value = 0.800, respectively) (Table 2). Regarding weight-adjusted protein intake, subjects

with MASLD had a significantly lower intake (~1.3-fold, p -value < 0.001) compared with those without MASLD. There were no differences between the two study groups in the intake of macronutrients (Table 2).

Table 2. Dietary intake of main macronutrients and niacin.

Dietary Intake	Non-MASLD (n = 222)	MASLD (n = 222)	<i>p</i> -Value
Energy intake (kcal/day)	2197.0 (533.0)	2168.0 (543.0)	0.569
Carbohydrates (g/day)	219.0 (35.6)	217.0 (36.8)	0.712
Proteins (g/day)	98.4 (14.6)	99.6 (15.8)	0.400
Protein intake (g/kg/day)	1.4 (0.3)	1.1 (0.2)	<0.001
Total fat (g/day)	89.1 (14.4)	89.2 (15.1)	0.923
Saturated fat (g/day)	25.0 (5.7)	24.8 (4.9)	0.805
Monounsaturated fat (g/day)	42.2 (10.1)	42.8 (10.7)	0.533
Polyunsaturated fat (g/day)	15.1 (4.2)	14.9 (4.4)	0.482
Total fiber (g/day)	24.7 (5.5)	24.4 (5.5)	0.517
Fiber soluble (g/day)	3.8 (1.1)	3.9 (1.2)	0.468
Insoluble fiber (g/day)	14.2 (4.6)	14.2 (4.5)	0.915
Niacin (mg/day)	27.2 (5.13)	27.2 (5.16)	0.931
Niacin Equivalents (mg/day)	43.1 (6.9)	43.3 (6.9)	0.800

Data are mean (SD) for continuous variables. All macronutrients and food groups were adjusted for total caloric intake (kcal/day). Protein intake was further adjusted for the body weight (g/kg/day). MASLD, metabolic dysfunction-related steatotic liver disease.

3.2. Association Analysis Between Dietary Niacin and MASLD

Both dietary niacin intake and odds of MASLD were significantly associated (p -value = 0.035), as revealed by non-linear dose-response relationship analysis using the RCS approach. The distribution of odds of MASLD across dietary niacin intake showed a maximal peak at a dietary niacin intake of 25 mg/day and a plain valley peak at a dietary niacin intake of 32 g/day. Overall, dietary niacin intake conferred protection when niacin intake values were in the range below 35 mg/day (Figure 1), being significantly related to lower odds of MASLD those niacin intake values below 22 g/day and values within the niacin intake range from >25 to <35 mg/day, respectively. In contrast, for dietary niacin intake values beyond 35 mg/day, the area of CI clearly overlapped the neutral OR line, which was defined by the zero value of the log OR. The RCS shape was similar when the dietary NE intake was considered for non-linear association analysis with MASLD (Figure 2). In this case, the distribution of odds of MASLD across dietary niacin intake showed a maximal peak at a dietary niacin intake of 40 mg/day, and a plain valley at a dietary niacin of 50 g/day. The shape of the curve defined by the odds values across dietary NE intake values was very similar to that built when dietary niacin intake was considered. The range of dietary NE intake values showing lower odds of MASLD was established at intake levels below 55 mg/day, being significantly related to lower odds of MASLD in the range of NE intake values below 35 g/day and from >42.5 to <55 mg/day, respectively. Similarly, for dietary NE intake values beyond >55 mg/day, the area of CI clearly overlapped the neutral OR line defined by the zero value of log OR.

Niacin and NE were also considered categorical variables and were analyzed using multivariate logistic regression analysis (Tables 3 and 4; Figures 3 and 4). Descriptive analysis of both variables showed no statistically significant differences in the proportion of subjects in each tertile between the MASLD and non-MASLD group (Tables 3 and 4).

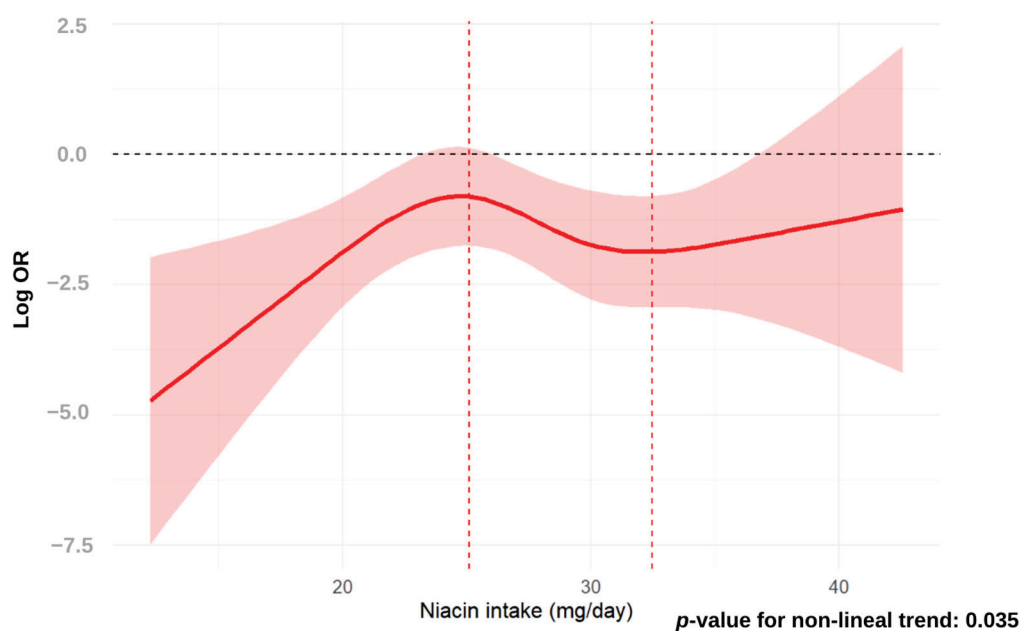


Figure 1. Restricted cubic spline regression analysis of the association between niacin intake (mg/day) and MASLD. RCS regression adjusted for age, sex, total caloric intake, sedentary activity, smoking, GFR, dyslipidemia, hypertension and T2DM. The X-axis represents niacin intake (mg/day) as a continuous variable, and the Y-axis is the Log odds ratio (OR) of the probability of developing MASLD. Greyish red represents the confidence interval (CI). The dashed red lines indicated the inflection points of niacin intake (mg/day).

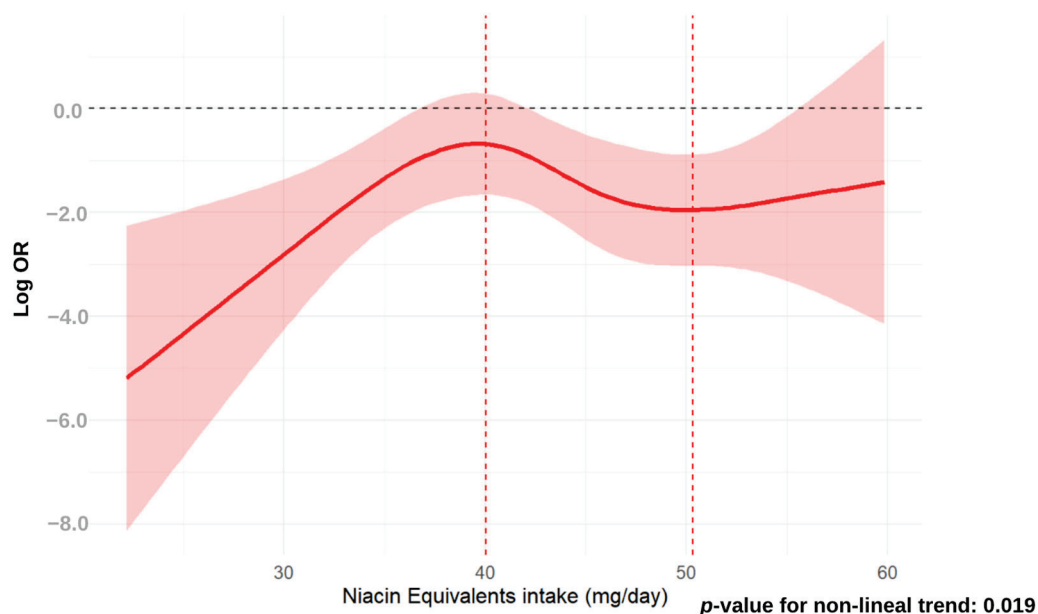


Figure 2. Restricted cubic spline regression analysis of the association between niacin equivalent intake (mg/day) and MASLD. RCS regression adjusted for age, sex, total caloric intake, sedentary activity, smoking, GFR, dyslipidemia, hypertension and T2DM. The X-axis represents the EN intake (mg/day) as a continuous variable, and the Y-axis is the Log ORs (OR) of the probability of developing MASLD. Greyish red represents the confidence interval (CI). The dashed red lines indicated the inflection points of NE intake (mg/day).

Table 3. Niacin consumption (mg/day) in the study groups distributed by tertiles.

	Niacin Intake (mg/day)			<i>p</i> -Value
	Tertile 1 (<25) (n = 151)	Tertile 2 (25–30) (n = 157)	Tertile 3 (>30) (n = 136)	
Non-MASLD	75 (49.7)	80 (51.0)	67 (49.3)	0.954
MASLD	76 (50.3)	77 (49.0)	69 (50.7)	

Data are presented as number (%) for the categorical variable niacin intake (mg/day). Niacin intake levels were categorized into tertiles, being: T1 < 25 mg/day, T2 from 25 to 30 mg/day and T3 > 30 mg/day. MASLD, metabolic dysfunction-related steatotic liver disease.

Table 4. Dietary niacin equivalents (NE) (mg/day) distributed by tertiles.

	Niacin Equivalents Intake (NE) (mg/day)			<i>p</i> -Value
	Tertile 1 (<40) (n = 141)	Tertile 2 (40–46) (n = 154)	Tertile 3 (>46) (n = 149)	
Non-MASLD	73 (51.8)	76 (49.4)	73 (49.0)	0.876
MASLD	68 (48.2)	78 (50.6)	76 (51.0)	

Data are presented as number (%) for the categorical variable NE intake (mg/day). NE intake levels were categorized into tertiles, being: T1 < 40 mg/day, T2 from 40 to 46 mg/day and T3 > 46 mg/day. MASLD, metabolic dysfunction-related steatotic liver disease.

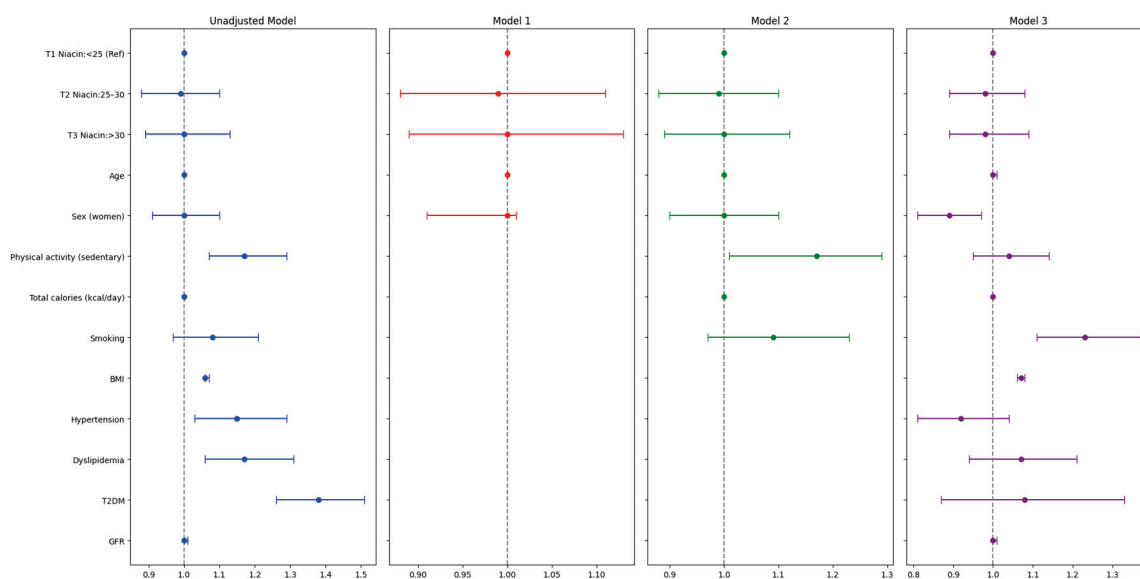


Figure 3. Forest plot of multiple logistic regression of niacin intake (mg/day) in the MASLD group. Data are mean (SD) for continuous variables and number (%) for categorical variables. BMI, body mass index; GFR, glomerular filtration rate; MASLD, metabolic dysfunction-related steatotic liver disease; T2DM, type 2 diabetes. Model 1: adjusted for age and sex. Model 2: adjusted for age and sex, plus physical activity, total calories (kcal/day), and smoking status. Model 3: adjusted for the variables of model 2 plus BMI, hypertension, dyslipidemia, T2DM, and GFR. The blue lines represent the unadjusted model, while the red lines correspond to Model 1, the green lines represent Model 2, and the purple lines correspond to Model 3. The association between MASLD and NE was calculated with the relative effect measure of odds ratios (ORs) and the 95% confidence interval (CI).

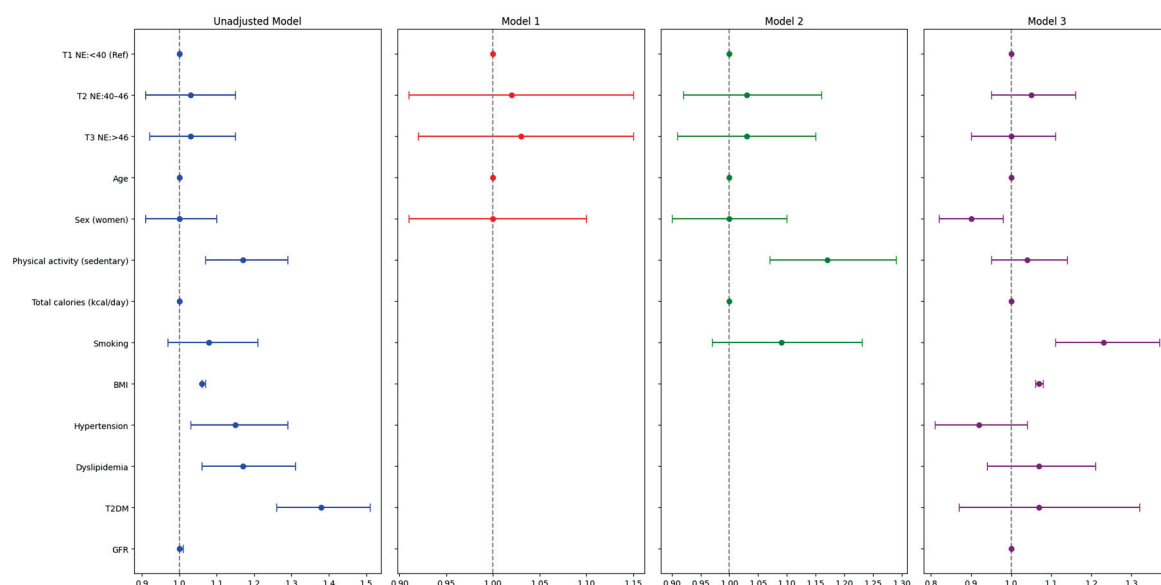


Figure 4. Forest plot of multiple logistic regression of niacin equivalents intake (mg/day) in MASLD group. Data are mean (SD) for continuous variables and number (%) for categorical variables. BMI, body mass index; GFR, glomerular filtration rate; MASLD, metabolic dysfunction-related steatotic liver disease; T2DM, type 2 diabetes. Model 1: adjusted for age and sex. Model 2: adjusted for age and sex, plus physical activity, total calories (kcal/day), and smoking status. Model 3: adjusted for the variables of model 2 plus BMI, hypertension, dyslipidemia, T2DM, and GFR. The blue lines represent the unadjusted model, while the red lines correspond to Model 1, the green lines represent Model 2, and the purple lines correspond to Model 3. The association between MASLD and NE was calculated with the relative effect measure of odds ratios (ORs) and the 95% confidence interval (CI).

The multivariable analysis showed that neither dietary niacin nor NE consumption was associated with MASLD, even after adjusting for confounding variables (Figures 3 and 4; Tables S2 and S3).

Among intrinsic, non-modifiable confounders, the female sex was significantly protective against MASLD, as revealed after adjusting for all relevant confounding variables (Model 3, Figure 3): OR (95% CI) = 0.89 (0.81–0.97) (p -value = 0.009). Aging, which is considered another non-modifiable risk factor for MASLD, did not have any statistically significant influence in any of the models.

Regarding other known modifiable risk factors for MASLD, sedentarism failed to remain associated with MASLD in Model 3 (OR (95% CI) = 1.04 (0.95–1.14) p -value = 0.366). BMI was associated with MASLD in the fully adjusted model (Model 3, OR (95% CI) = 1.07 (1.06–1.08), p -value < 0.001). Smoking was identified as one of the variables positively associated with MASLD (OR (95% CI) = 1.23 (1.11–1.36), p -value < 0.001). Finally, other clinical conditions, such as hypertension, dyslipidemia, T2DM, and GFR, were not associated with MASLD (Figure 3; Table S2). Similar results were reached when considering dietary NE as a variable (Figure 2; Table S3).

Protein intake was also analyzed as a categorical variable according to tertiles of intake. The descriptive analysis showed significant differences, with the group without MASLD having a higher protein intake compared with the non-MASLD group (Table S1). In the multivariable analysis, the second and third tertiles of protein intake showed significant protective effects in the fully adjusted model (Model 3, Figure S2; Table S4). Regarding the non-modifiable factors, neither sex nor age were associated with MASLD in any of the analyses. The detrimental effect of smoking was maintained in Model 2 and Model 3 (OR (95% CI) = 1.15 (1.03–1.27), p = 0.012; 1.23 (1.11–1.37), p < 0.001, respectively). Higher BMI was also associated with a negative effect in Model 3 (OR (95% CI) = 1.07 (1.05–1.08),

$p < 0.001$). Total caloric intake, along with MASLD-related comorbidities (hypertension, dyslipidemia, T2DM, and GFR), had no statistically significant effect in Model 3 (Table S4).

4. Discussion

Our data showed that both dietary niacin and NE did not differ between subjects with or without MASLD. However, the RCS analysis uncovered a significant non-linear dose-response association between dietary niacin intake and odds of MASLD. Specifically, such analysis revealed that MASLD risk was decreased in subjects with niacin intake values of <35 mg/day. In addition, dietary protein intake (expressed as g/kg/day) was significantly lower in the MASLD group; however, it was not related to incident MASLD. Notably, our analysis also revealed that smoking was consistently associated with MASLD in all the models.

Hepatic steatosis in MASLD is closely linked to reduced liver NAD^+ levels [18]. Niacin, a precursor of NAD^+ , replenishes these levels, mitigating oxidative stress, inflammation, and lipotoxicity, as shown in preclinical studies [15–20,23]. Additionally, niacin may also ameliorate MASLD by, on one side, inhibiting hepatic lipogenesis [29] and, on another, by reducing free fatty acid flux to the liver because of the inhibiting effect on adipose tissue lipolysis via the nicotinic acid-specific receptor GPR109A-mediated signaling pathway [29,33]. The relationship between daily dietary niacin intake and MASLD is poorly studied and elusive. To our knowledge, only two large observational, cross-sectional studies have revealed that higher daily dietary niacin intake is associated with a lower likelihood of MASLD [32,34]. Specifically, an independent study using the NHANES cohort found a U-shaped non-linear dose-response relationship between niacin intake and MASLD [34], with the odds of MASLD showing a gradual reduction along with niacin intake increase until reaching the threshold of 23.6 mg/day, where the odds of MASLD was null, while from this, niacin intake value on the odds MASLD progressively increased along with increasingly higher dietary niacin intake values [34]. In contrast with the latter study, in our study, the range of dietary niacin intakes showing potentially protective odds of MASLD was roughly <35 mg/day. Intriguingly, and similar to us, the logistic regression models did not reveal any association between niacin intake and MASLD, despite adjusting for almost the same confounding variables [34]. In support of this, daily niacin intake was not associated with MASLD in two other independent case-control studies [57,58]. In one of these studies, an Iranian case-control study, daily dietary niacin intake in subjects with MASLD did not differ from that estimated in the control group [58]. Furthermore, niacin intake only showed a marginal, non-significant inverse association with MASLD in another independent Indian case-control study, including 160 cases with MASLD and 160 controls [57]. In both previous studies, the estimated dietary niacin values were much lower than those calculated in our study; in the Indian study, 50% of the participants did not meet the RDA for niacin [57,58].

In general, the daily niacin intake reported in all previous studies was much lower than that calculated for our cohort. In our study, niacin intake ranged from the lowest (less than 25 mg) to the highest (greater than 30 mg); the sources considered to estimate dietary niacin intake included dairy products, eggs, meat, fish, poultry, legumes, and nuts, based on the FFQ with 101 items [49]. For instance, in the three NHANES observational studies, the reported daily niacin intake was much lower than the one calculated in our study [32,34,59]. In the first NHANES study, the lower quartile of daily niacin intake established for the participants of the NHANES cohort study group was <16 g/day (1st quartile) compared with the dietary niacin and NE estimated for our lowest tertile (<25 mg/day; <40 mg/day, respectively) [32]. Similar findings were reported in the other independent NHANES cross-sectional sub-study, where participants were distributed into tertiles according to their daily niacin intakes, in which the lowest tertile included participants displaying values of <18 g niacin/day [59]. Of note, in this last NHANES sub-study, the highest tertile of daily dietary niacin intake (i.e., ≥ 26.7 mg niacin/day) was not significantly associated with a lower likelihood of MASLD, but the risk of all-cause mortality was lower

in participants in the MASLD group with the highest intake of niacin [59]. Finally, in the third study of the NHANES cohort, the mean niacin intake was 22.6 mg/day [34]. The reason(s) for the differences in daily niacin consumption between our study and others is unknown; however, possible explanations could include the use of different methods for dietary data collection, along with different dietary patterns due to, e.g., geographical and ethnic differences in the cohorts [32,57–60]. Regarding dietary data collection, a study using the NHANES observational data and the Indian case-control study used two 24-hour recall questionnaires to assess niacin intake [32,34,57,59]. In contrast, the Iranian case-control study utilized a valid and reliable 168-item semi-quantitative FFQ [58]. In our study, we used a 101-item semi-quantitative FFQ validated for the Spanish population [49]. Additionally, in previous studies, only niacin intake and not NE was analyzed [32,57–59], in contrast to our study, which included both. In general, dietary NE values are frequently higher than dietary niacin values, as NE also includes niacin derived from dietary tryptophan, i.e., from protein breakdown. Consistently, in our cohort, dietary protein intake was not associated with incident MASLD after adjusting for other confounders.

Our study identified a direct relationship between smoking and incident MASLD. This relationship remained significant even after discounting other major confounding variables. Consistent with this observation, a recent meta-analysis revealed a significant association between smoking and MASLD [61]. Although the metabolic basis of this relationship is still under investigation, intake of nicotine has been found to enhance hepatic steatosis in an experimental preclinical model of MASLD [62]. In this regard, the increased release of toxins coming from heavy smoking has been recently reported to induce hepatic necroinflammation and worsening liver lesions to much more active forms, such as fibrosis, in chronic liver disease [63]. Taken together, this body of evidence suggests smoking to be a significant risk factor in the development and progression of liver disease.

Another relevant finding from our study was the contribution of sex to MASLD, whereby females were protected against MASLD across the models. Our observation is aligned with the notion that females are less prone to dysmetabolic features compared with males and with epidemiological data showing that MASLD prevalence and severity are significantly increased in men compared with women [64,65]. Furthermore, consistent with previous studies, our analysis revealed that higher BMI was a risk factor for MASLD [66,67].

One of the main strengths of our study was having data from two cohorts collected during the same period in the Mediterranean areas of Catalonia, enhancing the representativeness of the study sample. Furthermore, to our knowledge, this is the first study to consider NE as a dietary parameter for estimating niacin intake and incident MASLD. This approach is more reliable and realistic as it also considers tryptophan sources [40]. Another strength was the use of the FFQ, which has good reproducibility for estimating dietary intake, with data being collected by trained professionals. However, the current study has several limitations, mainly due to its observational case-control design, which does not allow us to establish causal relationships between the study variables. Additionally, FLI, along with at least one trait of metabolic syndrome, was used as a diagnostic proxy for MASLD, so it is a score calculated using cardiometabolic variables; therefore, data should be interpreted with caution. The maximal levels of dietary niacin estimated from FFQ may not be high enough to exert a favorable effect on MASLD incident; therefore, intervention studies with higher doses of niacin than estimated from the FFQ are warranted to directly assess the favorable influence of niacin on MASLD. Finally, the estimation of dietary niacin can be significantly influenced by the methods used to collect dietary data and information on supplements, making it even more challenging to assess the potential benefits of niacin intake on MASLD in human studies. Further research on dietary niacin intake, particularly through interventional studies, is therefore warranted to develop targeted and specific nutritional recommendations for MASLD management.

5. Conclusions

RCS analysis revealed that lower dietary niacin or NE were significantly associated with odds values of MASLD, indicating a negative association with MASLD. However, the fully adjusted logistic regression models did not show any association between dietary intake of niacin or NE and MASLD. Additionally, protein intake was not associated with MASLD after adjusting for the same confounding variables. Instead, our analysis identified the contribution of certain intrinsic characteristics, such as female sex, or modifiable characteristics, such as smoking and BMI, to MASLD. Further clinical studies of interventions with niacin derivatives are warranted to better assess the contribution, if any, to clinical MASLD.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/nu16234178/s1>, Figure S1: Flowchart of inclusion and exclusion criteria of the study; Figure S2: Forest plot of multiple logistic regression of protein intake (g/kg/day) in MASLD group; Table S1: Protein intake (g/kg/day) in the study groups by tertiles categorization; Table S2: Results of multiple logistic regression models for relations between niacin intake and MASLD according to patient sex, age, clinical variables and comorbidities; Table S3: Results of multiple logistic regression models for relations between NE intake and MASLD according to patient sex, age, clinical variables and comorbidities; Table S4: Results of multiple logistic regression models for protein intake (g/kg/day) and MASLD according to patient sex, age, clinical variables and comorbidities.

Author Contributions: Conceptualization, J.J. and D.M.; methodology, J.J. and D.M.; software, P.V.; formal analysis, P.V., M.A., and M.I.R.-L.; investigation, J.J.; resources, M.G.-C., E.C., D.M., and N.A.; data curation, M.G.-C., E.C., and P.V.; writing—original draft preparation, M.A.; writing—review and editing, M.A., M.I.R.-L., J.N.-N., P.V., M.G.-C., I.G., E.M., B.F.-C., B.V., J.R., E.C., E.O., N.A., D.M., and J.J.; supervision, M.I.R.-L., B.V., and J.J.; project administration, D.M., and J.J.; funding acquisition, E.O., N.A., J.J., and D.M. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the local Ethics Committee from the University Hospital of Lleida (CEIC 1079) (Approval Code: CEIC 2075, Approval Date: 29 October 2009 (approval of the amended protocol study on 30 June 2011) and Ethics Committee of the Primary Health Care University Research Institute (IDIAP) Jordi Gol (PI12/043) (Approval Code: P11/11, Approval Date: 3 March 2011).

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

Study of the Association Between Diets Containing Nuts and Seeds and the Degree of Abdominal Aortic Calcification

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Abstract: Background: The association between nuts and seeds (nuts/seeds) consumption and abdominal aortic calcification (AAC) has been studied rarely, if at all. However, AAC is a good marker of CVD risk and premature mortality. Consequently, the present observational study was conducted. It had two primary purposes: (1) to determine the relationship between the consumption of nuts/seeds and AAC, and (2) to examine the effects of nine potential confounding variables on the relationship between nuts/seeds consumption and AAC. **Methods:** The sample included 2611 randomly selected adults representative of the United States population. Data were collected as part of the National Health and Nutrition Examination Survey (NHANES). The outcome measure was AAC, divided into three categories: none, mild, and moderate/severe. Total consumption of nuts/seeds was assessed utilizing the mean of two diet recall assessments. **Results:** There was an inverse dose–response relationship between categories of nuts/seeds consumption and AAC, with age, sex, and race controlled ($F = 6.4$, $p = 0.0233$). After controlling for the demographic variables and physical activity, hypertension status, waist circumference, diabetes status, and smoking, the relationship between nuts/seeds and AAC was minimally affected ($F = 6.0$, $p = 0.0268$). **Conclusions:** In conclusion, nuts/seeds consumption appears to differentiate among adults with different levels of AAC. Control of many covariates had little impact on the associations. The recommendations of the U.S. Dietary Guidelines (2020–2025) that encourage the eating of nuts/seeds are supported by the results of this investigation.

Keywords: dietary guidelines; diet; artery; endothelial; inflammation; NHANES

1. Introduction

Abdominal aortic calcification, or AAC, is defined as calcium deposits that form along the abdominal section of the aorta. When AAC is present, the risk of mortality increases significantly [1,2]. Some research shows that the AAC score outperforms the Framingham Risk Score in forecasting cardiovascular outcomes in men and women, even though the Framingham Risk Score includes six classic predictors of CVD [3].

AAC is a significant risk marker of atherosclerosis, a primary component of cardiovascular disease. AAC tends to increase with age [4] and it is associated with hypertension, diabetes, smoking, obesity, and dyslipidemia [5–7].

Because many organs are served by the abdominal aorta, it is vital that this large artery remains healthy throughout life. Reduced blood flow within the abdominal aorta increases the risk of serious disease. According to a meta-analysis by Goncalves et al. [2], 10 studies were employed to determine the association between AAC and long-term cardiovascular outcomes. Numerous cardiovascular events served as endpoints. Based on thousands of individuals across the 10 investigations, statistical significance was reached for each of the outcomes [2]. Clearly, AAC is closely tied to cardiovascular disease and death. Chronic kidney disease, osteoporosis, and old age are significant risk factors for AAC [4]. However, in a 2024 study, He et al. concluded that some lifestyle risk factors are not significant predictors of AAC after adjusting for potential confounders [6].

Numerous studies show that a variety of dietary variables are good predictors of cardiovascular disease. This has led scientists to explore the link between diet and AAC. For example, recent research by Li and Huang et al. indicated that a plant-based diet pattern, along with protein, fiber, and other nutrients, were inversely related to AAC [8]. On the other hand, they found that caffeine consumption was unfavorably associated with AAC [8]. Hu et al. observed that lycopene was inversely tied to AAC [9], and Xiao et al. noted that saturated and polyunsaturated fats were directly tied to AAC [10].

The U.S. Dietary Guidelines (2020–2025) recommend that nutrient-dense foods be a primary focus of American diets [11]. The Guidelines specifically list nuts and seeds as healthy, nutrient-dense food choices. Moreover, the U.S. Guidelines include nuts and seeds as nutrient-dense protein foods and also as nutrient-dense oils [11]. Clearly, nuts/seeds are valuable components of a healthy dietary pattern.

Several factors make nuts/seeds cardioprotective. They contain unsaturated fatty acids, and a majority include monounsaturated fats [12]. Nuts/seeds also supply significant health-promoting bioactive compounds, including vegetable proteins and fiber, and phytochemicals, including phytosterols and phenolic metabolites [12].

Many nuts contain significant amounts of omega-3 and omega-6 fatty acids. Both of these fats have health benefits [13,14]. Research by Xiao et al. indicates that the consumption of omega-3 and omega-6 fats are favorably and inversely connected with AAC [10]. Additional research indicates that the ratio of omega-6 and omega-3 fatty acids also serves as a meaningful indicator of all-cause, cancer, and cardiovascular mortality [15]. Nuts vary in their omega-6 to omega-3 ratios, with almonds having a high ratio and walnuts having a low ratio [16].

Given these and other cardioprotective nutrients of nuts/seeds, one would expect them to protect against abdominal aortic calcification. However, to date, this gap in the literature has not been addressed. The relationship between the consumption of nuts/seeds and abdominal aortic calcification (AAC) has not been studied. Therefore, the present investigation was conducted. Its primary purpose was to determine the relationship between nuts/seeds intake and abdominal aortic calcification in a sample of 2611 men and women randomly selected from the United States population. An ancillary aim was to ascertain the extent to which potential confounding variables, such as age, sex, race/ethnicity (race), moderate physical activity, vigorous physical activity, diabetes status, waist circumference, smoking, and hypertension, impact the link between nuts/seeds and AAC.

2. Methods

2.1. Study Design and Sample

A significant strength of the National Health and Nutrition Examination Survey (NHANES) was that participants were randomly selected from the population of the United States. Therefore, outcomes can be extended to the non-institutionalized civilian population of the United States. The design of the study was cross-sectional.

For the present study, data collected by NHANES occurred over 2 years, 2013 and 2014. These were the specific years NHANES gathered information on abdominal aortic calcification (AAC). Census data were employed by NHANES to give participants specific sample weights that were used in each statistical analysis.

The United States National Center for Health Statistics authorized the data collection process and posting of the data on the Internet. No identifiable information was included in the online files, and there is no charge for the data. The official code signifying support for the data collected from 2013 to 2014 was Protocol #2011–17 [17]. All subjects gave consent to participate in the survey.

Several factors affected the number of participants in this study. NHANES collected data on individuals as young as a few months old up to 80 years and beyond. NHANES truncated age at 80 years to maximize confidentiality. There were 7574 individuals in the initial 2013–2014 sample, including individuals of all ages. The abdominal aortic

calcification variable was measured only in adults 40 years old and older, decreasing the sample to 2640. Finally, men and women with missing data were not included in the sample, leaving a final sample of 2611.

2.2. Measurement Methods

The relationship between total nuts/seeds intake and abdominal aortic calcification (AAC) was the primary focus of this study. Age, sex, race/ethnicity, diabetes status, moderate physical activity, vigorous physical activity, waist circumference, smoking, and hypertension status were treated as covariates.

Diet. Nuts/seeds consumption and energy intake were each measured utilizing the mean of two 24 h diet recall assessments [18]. The details of these assessments are on the NHANES website [18]. In short, on two different occasions, one in-person and the second over the telephone, everything participants ate and drank during the previous day, from 12:00 AM to 12:00 AM, was recorded via a detailed interview with an NHANES nutrition specialist. Kilocalories for each food were calculated by NHANES utilizing information from the United States Department of Agriculture database [16,18]. Utilizing each participant's food records, particular attention was given to the amount of nuts/seeds eaten, including peanuts. The nuts/seeds variable was reported as grams per 1000 kilocalories (kcal).

The individuals performing the dietary assessments spoke at least two languages. Scripts were used by the interviewers, and a computer afforded a standardized dietary evaluation protocol.

Findings resulting from the diet data have been evaluated, and concurrent validity has been established by numerous investigations [19–21]. A number of investigations have shown that NHANES diet data are closely linked to many diseases [22–25].

Abdominal Aortic Calcification (AAC). NHANES AAC data first became available in September 2018 [26]. DXA examinations were conducted by skilled and licensed radiology technologists to assess the level of calcification along the abdominal aorta. According to NHANES [26], AAC can be identified on lateral spine views used to assess spine fracture risk. Sensitivity and specificity for AAC are good [27,28]. With DXA, the details of lateral spine images are comparable to the resolution of normal radiography, but DXA scans expose individuals to substantially less radiation [27,28]. Pregnant women and individuals over 450 pounds were not scanned.

The lateral spine images were obtained via Hologic Discovery model A densitometers (Hologic, Inc., Marlborough, MA, USA) and the Optasia SpinAnalyzer software [26]. The AAC-8 semi-quantitative methods for scoring were employed to report the AAC scores [29, 30]. Vertebrae from L1 to L4 were used for the AAC scoring. For the present study, the AAC-8 scale included calcification of the front and back aortic walls in front of the L1–L4 vertebrae [26]. The score was reported as zero if no calcification was visible. Participants were given a score of “1” if there was calcification, and the cumulative length of the calcification was equivalent to the height of 1 vertebra or less. When the aggregate length of the calcification was equal to or more than the height of two vertebrae, subjects were given a score of “2”. Those with calcification scores of 0 were labeled “none”. Men and women with scores of 1 were categorized as “mild”, and participants with scores of 2 were categorized as “moderate/severe”.

Professional personnel evaluated the performance of the radiology technologists through in-person visits. Additional training sessions were performed with the technologists at least annually and when needed [26].

Waist Circumference. Trained individuals, who were evaluated regularly, performed the waist circumference measurements and were assisted by a recorder [31]. The reference position for the horizontal placement of the tape was the iliac crest. Mirrors were attached to the walls so that the correct positioning of the tape could be confirmed [31].

Smoking. Participants responded to multiple questions to ascertain if they had ever smoked, if they currently smoke, if they had quit smoking and similar questions. The

information was used to place individuals into one of three categories: current smoker, past smoker (quit a year or more ago), and never smoked.

Diabetes and Hypertension Status: Subjects responded to questions asking if a doctor had ever told them that they had diabetes. They were also asked if they took medication to lower their blood sugar levels. Additionally, subjects were asked if they were taking insulin. If they answered in the affirmative to any of these three questions, they were considered diabetic. Furthermore, participants reported if they had ever been told by a physician or healthcare professional that they had hypertension or high blood pressure. Individuals reporting, yes, were classified as having hypertension or high blood pressure.

Physical Activity. Participants completed a questionnaire to measure physical activity [32]. Separate questions addressed moderate and vigorous activities. Recreational activities that resulted in small heart rate and breathing increases were labeled moderate. Examples of moderate physical activity were casual biking and walking. When large increases occurred in heart or breathing rates, the movement was labeled vigorous. Examples of vigorous activity included jogging, swimming, hard cycling, or walking uphill.

To quantify the time engaged in recreational physical activity, specific questions denoting moderate and vigorous activity were asked separately. Responses about days per week and duration per day were multiplied, and total time engaged in moderate and vigorous recreational activity were reported separately [32].

2.3. Data Analysis

The findings of the present investigation can be extended to the United States adult population because subjects were randomly selected. Consistent with this strategy, each statistical model employed person-level sample weights.

Because the sample size was large ($N = 2611$), most scientists would assume that statistical power was high. However, the degrees of freedom (df) were calculated by taking the number of clusters (30) minus the number of strata (15). Hence, the df were only 15 instead of more than 2600. In short, statistical power was modest.

The primary aim was to ascertain the link between total nuts/seeds consumption and abdominal aortic calcification. Three demographic covariates and 6 lifestyle covariates were controlled statistically to minimize their influence on the connection between total nuts/seeds intake and AAC.

The associations between AAC reported as a categorical measure (None, Mild, and Moderate/Severe) and mean nuts/seeds intake were assessed by utilizing Analysis of Variance (ANOVA). Because the nuts/seeds variable was skewed significantly, it was log-transformed, reducing the skewness index substantially from 6.5 to 1.2. Because the log of 0 cannot be computed, subjects with no intake of nuts/seeds (0 g) were assigned 1 g. The 1 g was converted to 0 g when the log-transformation was performed.

The SAS SurveyReg procedure was employed to test the differences between the nuts/seeds means across the AAC categories, with sampling weights included. Partial correlation was utilized to adjust for the covariates. SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA) was the statistical package used.

3. Results

There were 2611 randomly selected women and men from different parts of the United States included in the sample. Their median age was 56 years, with subjects ranging from 40 to 80 years. The upper quartile of the sample reported approximately 37 min per day of activity categorized as moderate, such as walking and casual bike riding. The mean ($\pm$ SE) intake of nuts/seeds per 1000 kcal was 37.0 ± 3.8 g. On average, subjects consumed $5.5 \pm 0.6\%$ of their dietary energy from nuts/seeds. Information about the continuous variables is displayed in Table 1.

Table 1. Key percentages and distributions of the continuous variables ($n = 2611$).

Variable	Percentile				
	10th	25th	50th	75th	90th
Age (years)	42.4	46.9	55.8	65.5	74.8
Energy intake (kcal)	1131.1	1467.7	1910.5	2399.2	2942.9
Waist (cm)	83.0	90.7	99.3	108.0	117.1
Nuts and seeds (grams/1000 kcal)	0	0	0	26.6	108.6
Moderate PA (min/day)	0	0	0	37.3	58.4
Vigorous PA (min/day)	0	0	0	0	43.5

Note. Less than 10 min of physical activity was considered 0 min by NHANES.

The sample was mostly non-Hispanic White individuals (70.1%) followed by non-Hispanic Blacks (10.4%). Gender was nearly equally split, with 52% females and 48% males. About 12.5% of the sample reported that they had diabetes, and a total of 16% were current smokers. Almost 500 participants were determined to have moderate to severe abdominal aortic calcification (AAC), approximately 18% of the sample. Table 2 shows the traits of the sample according to the categorical measures.

Table 2. Sample information according to the categorical variables ($n = 2611$).

Categorical Variable	N	%	SE
Sex			
Women	1356	51.9	1.50
Men	1255	48.1	1.50
Race/Ethnicity			
Mexican American	184	7.0	1.72
Other Hispanic	202	7.7	1.04
NH White	1828	70.1	3.33
NH Black	272	10.4	1.53
Other Race/Multiracial	125	4.8	0.90
Diabetes			
No	2285	87.5	1.15
Yes	326	12.5	1.15
Smoking Status			
Never Smoked	1481	56.7	1.78
Quit Smoking	707	27.1	1.61
Current Smoker	423	16.2	0.92
High blood pressure			
Yes	1164	44.6	1.83
No	1447	55.4	1.83
* AAC			
None	1888	72.3	2.23
Mild	243	9.3	1.17
Moderate/Severe	480	18.4	1.61

Note: NH is non-Hispanic. SE is the standard error of the percentage. * AAC: abdominal aortic calcification.

Total intake of nuts/seeds per 1000 kcal differed significantly across the participants based on their degree of AAC, as shown in Table 3. The relationship was inverse and dose-response. The lower the intake of nuts/seeds, the greater the severity AAC tended to be. The association was significant after controlling for the demographic covariates and after controlling statistically for all the covariates, which included moderate and vigorous physical activity, waist circumference, smoking, blood pressure status, diabetes status, and the demographic covariates.

Table 3. Mean differences in total nuts/seeds consumption across categories of abdominal aortic calcification after adjusting for the covariates.

Outcome Variable	Abdominal Aortic Calcification (N = 2611)							
	None		Mild		Moderate/Severe			
	Mean	±SE	Mean	±SE	Mean	±SE	F	p
Total Nuts and Seeds Intake (g/1000 kcal)	n = 1893		n = 243		n = 477			
Model 1	1.29 ^a	0.10	1.20 ^{a,b}	0.18	0.91 ^b	0.13	6.4	0.0233
Model 2	1.18 ^a	0.08	1.14 ^{a,b}	0.17	0.88 ^b	0.12	6.0	0.0268

The outcome variable, grams of nuts/seeds eaten per 1000 kcal, was log-transformed to minimize skewness. Non-significant differences were identified by means of the same Model with the same letter. Means were modified statistically based on the covariates. Model 1 included age, sex, and race/ethnicity as covariates. Model 2 included age, sex, and race/ethnicity as covariates, plus minutes of moderate and vigorous physical activity, smoking status, waist circumference, blood pressure status, and diabetes status. The antilog of the means for Model 1 were None: 3.63, Mild: 3.31, and Moderate/Severe: 2.49. The antilog of the means for Model 2 were None: 3.27, Mild: 3.12, and Moderate/Severe: 2.41.

Consumption of nuts/seeds did not differ significantly between those with no AAC intake and mild AAC. However, the mean difference in nuts/seeds intake was substantial between those with no detectable signs of AAC and those with moderate to severe calcification, the two extreme categories. Specifically, those with no detectable aorta calcification consumed about 34% more nuts/seeds than those with moderate/severe calcification after adjusting for all the covariates ($1.18 - 0.88 = 0.3$, $0.3/0.88 = 0.34$). The large mean difference was detected with 15 degrees of freedom.

4. Discussion

This investigation had three key findings: (1) Adults with moderate to severe AAC consumed significantly fewer nuts/seeds per 1000 kcal compared to those with no AAC. (2) The relation between nuts/seeds intake and severity of AAC was inverse and dose-response—the lower the intake of nuts/seeds, the more severe AAC tended to be in participants. (3) After statistically controlling all nine of the potential confounders, the strength of the relationship between nuts/seeds and AAC changed minimally.

Although the connection between eating nuts/seeds and AAC has been studied rarely if at all, several investigations have examined the impact of nuts/seeds on all-cause mortality. All-cause mortality is an excellent single measure of overall health and disease risk. Specifically, Hsieh et al. studied over 20,000 male physicians using a prospective cohort design [33]. After almost 10 years of follow-up, those with a nut intake ≥ 5 servings per week had a 26% lower death rate than those reporting no intake of nuts after controlling for numerous covariates [33]. Bao et al. also examined the risk of death across groups of women and men based on nut intake [34]. With approximately 120,000 participants, results indicated that those who ate nuts had a 7% lower mortality rate than those who consumed nuts < 1 time per week. Adults who ate nuts seven or more times per week had 20% less risk of mortality than non-consumers. Nut intake was also predictive of fewer deaths from the primary killer diseases [34].

According to the Iowa Women's Health Study, Ellsworth et al. reported that in more than 34,000 postmenopausal women with no known cardiovascular disease, there was a 12% decreased risk of overall mortality in women who ate ≥ 2 nut servings/week compared to those who ate < 1 serving per month [35]. Finally, Grosso et al. published a meta-analysis of seven investigations indicating that one nut serving/day was related to a 27% decreased risk of all-cause mortality, and across six investigations, a 39% decreased risk of CVD mortality [1].

Why would eating nuts/seeds be inversely associated with overall mortality? And more relevant to the present investigation, why would eating nuts/seeds be inversely related to reduced abdominal aortic calcification? There are several potential mechanisms.

First, nuts/seeds, including peanuts, may lower the risk of AAC by decreasing inflammation [12]. Inflammation is a critical part of the development of atherogenesis, as well as other chronic diseases. In an investigation by Jiang et al., results from the Multi-Ethnic Study of Atherosclerosis showed that the consumption of nuts/seeds was inversely related to multiple indexes of inflammation, including fibrinogen, interleukin 6, and C-reactive protein [12,36]. According to Kris-Etherton, this is significant because inflammation contributes to all stages in the progression of atherosclerosis, including the preliminary enlistment of circulating leukocytes, the triggering of endothelial dysfunction, and the rupture of atherosclerotic plaque [12].

Another mechanism could be that regular consumption of nuts/seeds might reduce AAC by decreasing unhealthy cholesterol levels. Approximately 30 years ago, Sabate, Fraser, et al. were among the first to examine the influence of diet on serum lipid levels [12]. They employed a randomized cross-over design and found that LDL levels decreased by 18% when 20% of energy intake was from walnuts [37]. Following this investigation, dozens of clinical trials were performed to evaluate the effects of nuts/seeds on cholesterol levels. These investigations have evaluated lipids, lipoproteins, and apolipoproteins [12]. Most of these studies have shown that nuts/seeds not only decrease cholesterol levels but also tend to lower cholesterol levels more than expected, given the composition of the diet [37–40].

Another mechanism that could account for the inverse relationship between nuts/seeds and AAC is the reduction of oxidative stress. Oxidative stress is the imbalance between reactive oxygen species (ROS), which result from cellular metabolism, and the body's capacity to counteract them with antioxidants. ROS damage the endothelium or inner lining of arteries.

Nuts/seeds are good sources of tocopherols and phenolic antioxidants. According to Blomhoff et al., many nuts are among the dietary plants with the most antioxidants [41]. Moreover, because much of the fat in nuts/seeds is monounsaturated, it is not susceptible to oxidation [42]. Multiple investigations have shown that nuts/seeds reduce the oxidation of LDL molecules [40,43]. In studies incorporating nuts with high polyunsaturated content, after controlling for the saturated fat content in the meal, the reduction of oxidative stress has been consistent [39,44,45]. However, some research has failed to find a protective effect [46].

Endothelial function plays a key role in arterial health and cardiac events [47]. Endothelial damage caused by cardiovascular risk factors and atherosclerotic disease often leads to arterial abnormalities and harmful vessel responses [12,47]. Of significant importance, food can have damaging or favorable effects on these critical conditions.

Several investigations indicate that diets loaded with saturated fat tend to damage endothelial function [48,49]. Fortunately, polyunsaturated fats and other nutrients found in nuts/seeds, including antioxidants, tend to counter some of these harmful effects [50]. In a study by Ros et al., endothelial function was improved significantly in participants with high cholesterol levels who were treated with regular walnut intake [44]. The control regime focused on Mediterranean foods with similar energy and saturated fat content. Cortes et al. conducted a similar study adding walnuts to a meal containing high fat and high saturated fat [51]. The control meal was similar; only olive oil was added rather than walnuts. The walnut meal significantly improved the after-meal endothelial function compared to the olive oil meal.

Blood levels of molecules that cause cells to stick together can also serve as an index of endothelial function [47]. Research shows that diets containing meaningful amounts of alpha lipoic acid from walnuts or other foods tend to decrease plasma adhesion molecules [44,52,53]. Likewise, in a study where hamsters were fed a high-fat, atherogenic diet, walnut consumption decreased endothelial-1, a powerful endothelial stimulator [54].

Another mechanism that could contribute to the link between nuts/seeds and AAC is the length of telomeres. Telomeres are the endcaps of chromosomes and are an index of

biological aging. Research by Tucker indicated that the more nuts/seeds that were eaten, the longer telomeres tended to be, signifying decreased cellular aging [21].

The current investigation had multiple limitations. First, it was an observational investigation. There was no treatment or intervention. As a result, cause-and-effect conclusions cannot be inferred. Frequent consumption of nuts/seeds could lead to less AAC. However, variables other than nuts/seeds could explain the differences found in the current study. Likewise, eating nuts/seeds could be a marker of other behaviors that might affect AAC. Many potential confounding variables were controlled, and they had minimal influence on the association between nuts/seeds intake and AAC. However, other factors not measured could account for some of the associations found in this investigation. Lastly, nuts/seeds intake was measured using two dietary recall assessments. Numerous studies have found this strategy to be valid and reliable. That is why it was used in the U.S. government-sponsored NHANES investigation. However, dietary recalls are not without measurement error. However, decreased assessment errors from the two diet recall assessments would have likely strengthened, not weakened, the association between nuts/seeds intake and AAC because measurement error is not a good predictor.

This study also had several strengths. The sample was large, with more than 2600 participants. A random selection of participants was employed so that the findings could be generalized to the adult population of the United States. Five racial/ethnic categories were included, and the subjects were 40–80 years old, so subjects represented a variety of individuals and backgrounds across the country. Intake of nuts/seeds was evaluated utilizing two 24 h diet recall assessments, not just one. Finally, those responsible for collecting the data and performing the measurements were well-trained specialists. They were evaluated frequently to safeguard against measurement errors.

5. Conclusions

In summary, the present study had three primary findings: (1) Adults with moderate to severe AAC consumed substantially fewer nuts/seeds compared to those with no AAC. (2) The association between nuts/seeds consumption and severity of AAC was inverse and dose–response. In short, the lower the intake of nuts/seeds among participants, the more severe AAC tended to be. (3) After adjusting for differences in all the covariates, the strength of the association between nuts/seeds and AAC changed minimally.

In conclusion, there is notable evidence from experimental trials, cross-over studies, and epidemiological investigations indicating that eating nuts/seeds improves health and reduces the risk of disease. It appears that arterial function is also improved by the regular eating of nuts/seeds, and cardiovascular events tend to be reduced significantly. The link between nuts/seeds intake and abdominal aortic calcification has rarely, if ever, been evaluated to date. However, the current investigation revealed that men and women from the United States with moderate to severe levels of AAC consume significantly fewer nuts/seeds than those with no AAC, even after being controlled statistically for many potential confounding factors. The recommendations of the Dietary Guidelines (2020–2025) produced by the United States, which recommend the regular intake of nuts/seeds, are supported by the results of the current investigation.

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Data Availability Statement: Data for this study is posted on the NHANES website. <https://wwwn.cdc.gov/nchs/nhanes/Default.aspx> (accessed 27 November 2024).

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Review

Consumption of Red Versus White Wine and Cancer Risk: A Meta-Analysis of Observational Studies

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Abstract: Background/Objectives: While alcoholic beverage consumption increases cancer risk, red wine has been touted as a healthier option. To address this unexplored question, we conducted a meta-analysis to summarize evidence from observational studies. Methods: A literature search of PubMed and EMBASE through December 2023 identified studies examining wine and cancer risk. A random-effects meta-analysis was performed to estimate relative risks (RRs) and 95% confidence intervals (CIs) for an association between wine intake and overall cancer risk. Results: A total of 20 cohort and 22 case-control studies were included. Wine intake was not associated with overall cancer risk ($n = 95,923$) when comparing the highest vs. lowest levels of consumption, with no differences observed by wine type (red: summary RR = 0.98 [95% CI = 0.87, 1.10], white: 1.00 [0.91, 1.10]; $P_{\text{difference}} = 0.74$). However, white wine intake was significantly associated with an increased risk of cancer among women (white: 1.26 [1.05, 1.52], red: 0.91 [95% CI: 0.72, 1.16], $P_{\text{difference}} = 0.03$) and in analyses restricted to cohort studies (white: 1.12 [1.03, 1.22], red: 1.02 [95% CI: 0.96, 1.09], $P_{\text{difference}} = 0.02$). For individual cancer sites, there was a significant difference in associations between red and white wine intake only in skin cancer risk [6 studies, white: 1.22 (1.14, 1.30), red: 1.02 (0.95, 1.09); $P_{\text{difference}} = 0.0003$]. Conclusions: We found no differences in the association between red or white wine consumption and overall cancer risk, challenging the common belief that red wine is healthier than white wine. Our significant results related to white wine intake in subgroup analyses warrant further investigation.

Keywords: wine; alcohol; cancer; nutrition; meta-analysis

1. Introduction

Alcoholic beverages have been classified as Group 1 carcinogens (i.e., carcinogenic to humans) by the International Agency for Research on Cancer (IARC) based on sufficient evidence in humans for cancers of the oral cavity, pharynx, larynx, esophagus, liver, colorectum, and female breast [1]. According to the IARC Global Cancer Observatory database, 741,300 cancer cases in 2020, which is 4.1% of total global cancer cases for that year, were attributed to alcohol consumption. Ethanol in alcohol metabolizes into acetaldehyde, which readily forms Schiff-base adducts with DNA and cellular proteins [2], resulting in point mutations and harmful DNA-protein and DNA-DNA crosslinks [3]. It has also been found that some alcoholic beverages contain significant levels of preexisting acetaldehyde [4,5]. Despite these findings, the consumption of wine in particular has been rising [6].

It is not clear whether cancer risk differs by consumption of different types of wine, in particular red versus white. Red wine has been considered a healthier option because it contains a higher number of antioxidants, including flavonoids and polymeric tannins, than white wine [7]. In fact, a recent Canadian survey showed that 41% of respondents felt uncertain about whether or not red wine reduced cancer risk, while 54% agreed that alcohol consumption increased cancer risk [8]. Numerous experimental studies found that resveratrol, a polyphenol stilbenoid in red wine, inhibits the proliferation of several different types of cancer cells [9–15]. Epidemiologic studies have primarily examined the relationship between overall alcohol intake and cancer risk [16,17]; however, studies that have separately analyzed red and white wine have reported inconsistent findings. Several studies have observed inverse or no associations between red wine intake and cancers of the breast, skin, prostate, ovaries, colon, and lungs [18–43], while other studies have shown increased risk of aforementioned cancers [19,37,39,44–47]. Similarly, white wine, which generally contains a lower amount of resveratrol than red wine (0–1.09 mg/L vs. red wine: 0.36–1.97 mg/L [48]), has been positively [29,32,33,35,36,41,44], inversely, or not [34,42] associated with cancers of the breast, skin, and prostate. A systematic review and meta-analysis summarized the relationship between wine consumption and cancer risk but did not evaluate red and white wine separately [49]. To our knowledge, there are few studies evaluating the difference in cancer risk by type of wine.

To address this research gap, especially given the vast and often contradictory literature on the carcinogenicity of red and white wine separately, we conducted a meta-analysis to investigate the association between red versus white wine consumption and the risk of all and site-specific cancers. We aimed to clarify the potentially differing carcinogenic effects of red and white wine and contribute to a more nuanced understanding of the role of wine type in cancer risk. Ultimately, this research may provide valuable insights to inform public health guidelines and individual lifestyle decisions regarding wine.

2. Materials and Methods

2.1. Search Strategy

A comprehensive search of PubMed and Embase was conducted for studies published up to December 2023 using the Medical Subject Headings (MeSH) terms or keywords including cancer, tumor, carcinoma, malignant neoplasm, alcohol, wine, red wine, and white wine (Supplementary Table S1). Three independent reviewers (RL, JR, and MH) screened the titles, abstracts, and full texts based on pre-defined eligibility criteria. Any disagreements regarding study inclusion were discussed and resolved by the three investigators (RL, JR, and MH). Additionally, the reference lists of identified articles and relevant meta-analyses or reviews were examined to locate additional relevant studies. The Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines were adhered to in the design, execution, analysis, and reporting of this meta-analysis [50].

2.2. Study Selection and Data Extraction

We included prospective cohort and case-control studies that examined the association between red or white wine and cancer risk. We restricted our analysis to studies providing the type of wine (red or white, rather than wine as a whole) and relevant measures of association, relative risk (RR) or odds ratio (OR) estimates with corresponding 95% confidence intervals (CIs), to perform a meta-analysis. Articles not published in English, literature reviews, abstracts, posters, case reports, and experimental studies were excluded (Figure 1). Studies with more than three categories of wine consumption, along with data on the case and non-case numbers, RR or OR estimates, and 95% CIs for each category, were eligible for inclusion in the dose-response analysis.

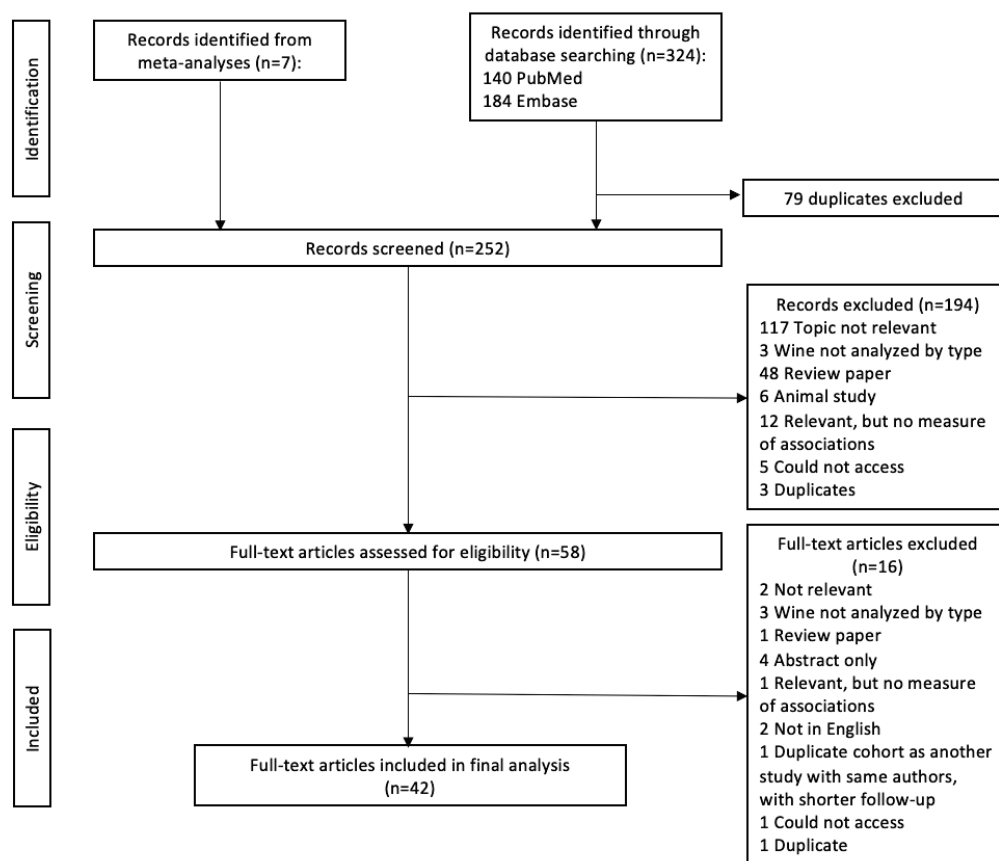


Figure 1. Literature search results for publications related to wine consumption (red or white) and cancer risk.

From each included study, we extracted the following details: author names, publication year, study design (cohort or case–control), type of wine (red or white), number of cases and non-cases, sample size, the most fully adjusted estimates of association and corresponding 95% CIs, study region, measurement of wine consumption (validated or not), measurement of outcomes (confirmed by medical record or not), and any adjustments for potential confounders (Table 1). For the dose–response meta-analysis, we collected category-specific doses of wine consumption (range, median) and the most adjusted RRs or ORs and their corresponding 95% CIs. The lowest wine intake category (nondrinkers) was used as the reference group. For each study, we assigned the midpoint of each wine intake category (range) as the corresponding RR or OR for that category. If the highest category was unbounded, we estimated the same range as the other categories for the highest category (e.g., if categories are <1, 1–3, or >3, we assumed 3–5 for the last category and assigned 4 as the midpoint). Wine intake measurements were all converted to grams of ethanol per day for dose–response analysis. The standard drink definition established by the government of the study’s country of origin was utilized to calculate conversions [51]. All extracted data were independently reviewed and cross-verified by the authors at least twice.

Table 1. Summary table of 42 selected studies in the meta-analysis.

Cancer Type	Author, Year	Country	Study Design	Population	Measurement of Wine Intake (Used Validated Food Frequency Questionnaire)	Number of Cases/Number of Participants	Adjusted Confounders
Skin (BCC, SCC)	Ansems, 2008 [45]	Australia	Cohort (Nambour Skin Cancer Study)	Adult residents of Nambour, Queensland, Australia, average age of 50 years	Yes	394/1360	BCC: age, sex, beta-carotene treatment, sunscreen treatment, elastosis of the neck, occupational sun exposure, leisure time sun exposure, history of skin cancer before 1992 SCC: age, sex, beta-carotene treatment, sunscreen treatment, self-reported skin color, number of pack-years smoked until 1992, elastosis of the neck, leisure time sun exposure, history of skin cancer before 1992
Skin (MM, NM/SC)	Kubo, 2014 [29]	USA	Cohort (Women's Health Initiative)	White postmenopausal women aged 50–79 from across the USA	Yes	10,125 / 59,575	Age, education, BMI, Langley's of exposure, physical activity, history of NM/SC, history of MM, smoking, sunscreen use, current summer sun exposure, childhood sun exposure, skin reaction to the sun, last medical visit within 1 year, having a current care provider, having insurance
Skin (BCC)	Zhang, 2014 [42]	USA	Case–control	Individuals aged <40 in Connecticut diagnosed with BCC and matched controls	No	365 / 747	Age, gender, body site of biopsy, indoor tanning, skin color, education, smoking status, hours spent outdoors in warm months, sunburns, family history of skin cancer, consumption of other types of alcohol
Skin (BCC)	Wu, 2015 [41]	USA	Cohort (Nurses' Health Study; Nurses' Health Study II; Health Professionals Follow-Up Study)	Female registered nurses aged 25–55, male health professionals	Yes	15,681 / 211,462	Age, BMI, smoking status, physical activity, caffeine intake, ethnicity, family history of melanoma, natural hair color, number of moles on arms or legs, skin reaction to sun as a child / adolescent, number of severe sunburns, cumulative UV flux since baseline, Average time spent in direct sunlight in summer months, use of sunscreen in summer months, consumption of other types of alcohol
Skin (SCC)	Siiskonen, 2016 [36]	USA	Cohort (Nurses' Health Study; Nurses' Health Study II; Health Professionals Follow-Up Study)	Female registered nurses aged 25–55, male health professionals	Yes	2938 / 223,138	Age, BMI, smoking status and pack-years smoked, physical activity, caffeine intake, family history of melanoma, tanning ability, lifetime number of severe sunburns, number of moles, natural hair color, average annual UV-B flux at place of residence, total alcohol intake
Skin (MM)	Rivera, 2016 [32]	USA	Cohort (Nurses' Health Study; Nurses' Health Study II; Health Professionals Follow-Up Study)	Female registered nurses aged 25–55, male health professionals	Yes	2209 / 21,052	Age, BMI, smoking status, physical activity, caffeine intake, family history of melanoma, tanning ability, lifetime number of severe sunburns, number of moles on forearms, hair color at age 18, average annual UV-B flux at place of residence, intake of other alcoholic beverages
Ovary	Webb, 2004 [40]	Australia	Case–control	Women aged 18–79 with epithelial ovarian cancer in Australia	Yes	696 / 1482	Age, age squared, level of education, BMI, smoking status, duration of oral contraceptive use, parity, caffeine intake, consumption of other types of alcohol
Ovary	Kelemen, 2004 [26]	USA	Cohort (Iowa Women's Health Study)	Women aged 55–69 randomly selected from Iowa driver's license registry	Yes	147 / 27,205	Age at menopause, physical activity, postmenopausal hormone use, oral contraceptive use, family history of breast cancer, family history of ovarian cancer, known diabetes at baseline, smoking, energy-adjusted intakes of total carotene, vitamin C and vitamin E

Table 1. Cont.

Cancer Type	Author, Year	Country	Study Design	Population	Measurement of Wine Intake (Used Validated Food Frequency Questionnaire)	Number of Cases/Number of Participants	Adjusted Confounders
Ovary	Kelemen, 2013 [27]	USA	Case-control	Pooled data from 12 case-control studies performed in USA, Canada, Europe, and Australia; all of used population-based ascertainment methods for identifying cases and control	Yes	5342/10,358	Age, smoking status, site, race/ethnicity, menopausal status, oral contraceptive use, tubal ligation, endometriosis, hysterectomy, Family history of breast or ovarian cancer in first-degree relatives, parity/age at last birth, interview year, age at menarche, BMI, education
Ovary	Cook, 2016 [23]	Canada	Case-control	Women aged 20–79 diagnosed with ovarian tumors and matched controls	No	1144/3657	Age, oral contraceptive use, parity, current smoking, family history of ovarian or breast cancer
Ovary	L'Espérance, 2023 [52]	Canada	Case-control	Women aged 18–79 years of the Greater Montreal area with ovarian cancer	No	495/1397	Age, number of full-term pregnancies, education, other type of alcoholic beverage intake
Prostate	Schuurma, 1999 [35]	Netherlands	Cohort (Netherlands Cohort Study)	Men aged 55–69	Yes	680/58,279	Age, family history of prostate cancer, socioeconomic status, total alcohol intake
Prostate	Schoonen, 2005 [34]	USA	Case-control	European and African American men aged 40–64 in King County, WA	Yes	753/1456	Age, PSA screening, total lifetime number of female sexual partners, smoking status, consumption of other types of alcohol
Prostate	Velicer, 2006 [38]	USA	Cohort (Vitamins and Lifestyle (VITAL) Cohort)	Men aged 50–76 in Washington State	Yes	816/34,565	Age, PSA test within 2 years of baseline, consumption of other types of alcohol
Prostate	Sutcliffe, 2007 [37]	USA	Cohort (Health Professionals Follow-Up Study)	Male health professionals aged 40–75	Yes	3348/45,433	Age, race/ethnicity, BMI at 21, cumulative family history of prostate cancer through 1996, height, updated cigarette smoking in the past 10 years, baseline intakes of total energy, tomato sauce, red meat, fish, calcium and vitamin E, baseline energy-adjusted intakes of fructose and α -linolenic acid, baseline vigorous physical activity, diabetes mellitus type 2, vasectomy
Prostate	Chao, 2010 [20]	USA	Cohort (California Men's Health Study)	Men aged 45–69 who were members of Kaiser Permanente health plans in California	Yes	1340/65,972	Red wine: age, race/ethnicity, income, BMI, intake of other alcoholic beverages, meat consumption, family history of prostate cancer, person history of PSA testing, STI, BPH, BPH surgery, prostatitis, diabetes mellitus White wine: race, income, BMI, intake of other alcoholic beverage, meat consumption, family history of prostate cancer, personal history of PSA testing, STI, BPH, BPH surgery, prostatitis, diabetes
Prostate	Downer, 2019 [53]	USA	Cohort (Health Professionals Follow-Up Study)	Male health professionals aged 40–75 who were diagnosed with nonmetastatic prostate cancer during follow-up	Yes	5182/47,568	Total energy intake, smoking, BMI, vigorous physical activity, choline, vegetable fat, coffee, lycopene, whole milk, and diabetes, prostate-specific antigen (PSA) screening beginning in 1994, diabetes. Beer, wine, and liquor were mutually adjusted for each other.

Table 1. Cont.

Cancer Type	Author, Year	Country	Study Design	Population	Measurement of Wine Intake (Used Validated Food Frequency Questionnaire)	Number of Cases/Number of Participants	Adjusted Confounders
Pancreatic	Farrow, 1990 [54]	USA	Case-control	Men aged 20–74 diagnosed with pancreatic cancer and matched controls	No	148/336	Age, smoking, race, education
Pancreatic	Bueno de Mesquita, 1992 [55]	Netherlands	Case-control	Individuals aged 35–79 living in the central Netherlands diagnosed with cancer of the exocrine pancreas and matched controls	No	176/663	Age, sex, response status, lifetime smoking of cigarettes, dietary intake of energy and vegetables, lifetime consumption of other alcoholic drinks
Urinary Tract	Andreatta, 2010 [18]	Argentina	Case-control	Patients with urinary tract tumors in Cordoba, Argentina, and controls taken from hospital registries at the time of diagnosis of cases	Yes	168/502	Age, sex, BMI, tobacco smoking, occupational exposure, social status, physical activity
Renal	Nicodemus, 2003 [56]	USA	Cohort (Iowa Women's Health Study)	Women aged 55–69 randomly selected from Iowa driver's license registry	Yes	124/34,637	Age
Renal	Greving, 2007 [57]	Sweden	Case-control	Men and women aged 20–79 diagnosed with renal cancer and born and living in Sweden; controls were randomly selected and matched to cases	No	855/2059	Age, sex, BMI, cigarette smoking, consumption of other types of alcohol
Breast	Viel, 1997 [39]	France	Case-control	Women aged 30–50 diagnosed with breast cancer without previous specific treatment	No	154/308	Total calorie intake, parity
Breast	Hirvonen, 2006 [25]	France	Cohort (French Supplementation en Vitamines et Mine raux Antioxydants Study)	Women aged 35–60 and men 45–60 without a history of cancer	Yes	95/4396	Age, smoking, number of children, use of oral contraception, family history of breast cancer, menopausal status
Breast	Allen, 2009 [44]	United Kingdom	Cohort (Million Women Study)	Women who attended breast cancer screenings in the UK	Yes	28,380/1,280,296	Age, region of residence, socioeconomic status, BMI, smoking status, physical activity, use of oral contraceptives and hormone replacement therapy
Breast	Newcomb, 2009 [30]	USA	Case-control	Women aged 20–69 diagnosed with breast cancer and matched controls	No	6327/13,885	Age, state, family history of breast cancer, age at menarche, age at first birth, parity, menopausal status, age at menopause, postmenopausal hormone use, BMI, education, total alcohol consumption
Breast (ductal, ductal-lobular, lobular)	Baglia, 2017 [19]	USA	Case-control	Women aged 55–74 diagnosed with breast cancer in the Greater Seattle area and matched controls	No	1961/2852	Age, reference year, county of residence

Table 1. Cont.

Cancer Type	Author, Year	Country	Study Design	Population	Measurement of Wine Intake (Used Validated Food Frequency Questionnaire)	Number of Cases/Number of Participants	Adjusted Confounders
Mouth/Pharynx	De Stefani, 2007 [47]	Uruguay	Case-control	Patients and controls recruited from hospitals in Montevideo, Uruguay	No	776/2277	Age, residence, urban/rural status, hospital, year at diagnosis, education, family history of cancer among first-degree relatives, occupation, total vegetables and fruits consumption, maté intake, smoking status, years since quit, cigarettes/day among current smokers, total alcohol intake
Lung	De Stefani, 1993 [46]	Uruguay	Case-control	Patients and controls recruited from hospitals in Montevideo, Uruguay	No	327/677	Age, residence, education, cigarette smoking measured in pack-years
Lung	Ruano-Ravina, 2004 [33]	Spain	Case-control	Lung cancer patients in Santiago University Teaching Hospital and matched controls	No	132/319	Age, sex, occupation, smoking habit, total alcohol intake
Lung	Chao, 2008 [22]	USA	Cohort (California Men's Health Study)	Men aged 45–69 who were members of Kaiser Permanente health plans in California	Yes	210/84,170	Age, ethnicity, household income, BMI, smoking status, cigarettes smoked per day, smoking duration, and history of COPD/emphysema
Lung	Chao, 2011 [21]	USA	Cohort [Vitamins and Lifestyle (VITAL) Study]	Men and women aged 50–76 living in western Washington State covered by SEER registry	Yes	580/66,186	Gender, race, education, household income, BMI, history of COPD/emphysema, cigarette smoking, family history of lung cancer, high-intensity physical activity, fat intake, fruit and vegetable intake
Stomach	Falcao, 1994 [58]	Portugal	Case-control	Patients undergoing gastroscopy	No	74/267	Social class, milk consumption, number of meals/day, rice consumption, vegetable consumption
Colon/rectum	Chao, 2010 [43]	USA	Cohort (California Men's Health Study)	Men aged 45–69 who were members of Kaiser Permanente health plans in California	Yes	176/43,483	Crude
Colon/rectum	Kontou, 2012 [28]	Greece	Case-control	Men and women diagnosed with CRC at a hospital in Athens, Greece	Yes	250/500	Age, BMI, current smoking, physical activity, family history of colorectal cancer
Colon/rectum	Grosso, 2014 [24]	Italy	Case-control	Men and women diagnosed with CRC recruited from the list of patients who visited a hospital in Catania, Southern Italy	No	338/1014	Age, sex, smoking status, family history of CRC, obesity status, having diabetes, physical activity level, alcohol quantity
Rectum	Murtaugh, 2004 [59]	USA	Case-control	Men and women from the Northern California Kaiser Permanente aged 30–79	Yes	952/2157	Energy, fiber and calcium intake, age, physical activity
Colon	Phipps, 2016 [31]	USA	Cohort (Multicenter Phase III drug trial led by North Central Cancer Treatment Group)	Patients with resected stage III colon cancer	No	649/1984 (outcome is colon cancer recurrence or death)	Treatment, sex, BMI, smoking, physical activity, performance score, race

Table 1. Cont.

Cancer Type	Author, Year	Country	Study Design	Population	Measurement of Wine Intake (Used Validated Food Frequency Questionnaire)	Number of Cases/Number of Participants	Adjusted Confounders
Lymphoma (non-Hodgkin lymphoma)	Chiu, 1999 [60]	USA	Cohort (Iowa Women's Health Study)	Women aged 55–69 randomly selected from Iowa driver's license registry	Yes	143/35,156	Age, residence, education, marital status, transfusion history, diabetes history, intake of red meat, intake of fruits, total energy intake
Lymphoma	De Stefani, 2013 [61]	Uruguay	Case-control	Men and women diagnosed with lymphoid cancers in Montevideo, Uruguay, with matched controls	No	697/4303	Age, sex, residence, urban/rural status, education, BMI, smoking intensity in pack-years, total meat, total vegetable, and fruit intake, total meat, total energy
Brain (glioma, meningioma)	Ryan, 1992 [62]	Australia	Case-control	Adults aged 25–74 diagnosed with primary tumors of the brain and meninges in metropolitan Adelaide, Australia	No	170/587	Age, sex
Brain (glioma, glioblastoma)	Cote, 2021 [63]	USA	Cohort (Nurses' Health Study; Nurses' Health Study II; Health Professionals Follow-Up Study)	Female registered nurses aged 25–55, male health professionals	Yes	554/237,505	Age, calendar year, smoking status, BMI, total caloric intake

Abbreviations: BCC, basal cell carcinoma; BMI, body mass index; BPH, benign prostatic hyperplasia; COPD, chronic obstructive pulmonary disease; CRC, colorectal cancer; MM, malignant melanoma; NMSC, non-melanoma skin cancer; PSA, prostate-specific antigen; SCC, squamous cell carcinoma; STI, sexually transmitted infection. Murtaugh et al. [57] only presented effect estimates for women and men separately; we added both estimates and considered them as separate studies. For studies that provided estimates for all sex, women only, and/or men only, we included estimates for all sex. When a study provided results for individual cancer subtypes (instead of overall risk) or different kinds of cancers, we included all estimates and considered them as separate studies (e.g., Baglia et al. [45] (breast cancer); ductal, lobular, and ductal-lobular; Kubo et al. [28] (skin cancer); MM and NMSC; De Stefani et al. [46]; oral and pharyngeal cancer). Andreatta et al. [44], De Stefani et al. [23], De Stefani et al. [64], De Stefani et al. [46], and Chao et al. [42] only provided results of red wine intake.

2.3. Statistical Analysis and Quality Assessment

A meta-analysis for highest versus lowest exposure levels was conducted. The summary RRs and 95% CIs were calculated by using a random-effects model. We performed subgroup analyses by type of wine (red, white) and cancer type for those having more than three studies (skin, colon/rectum, kidney/urinary tract, lung, ovary, female breast, and prostate). Then, meta-regression was used to assess whether the risks of cancer overall or site-specific cancers differ by type of wine (*p*-value for difference). We also conducted analyses restricted to cohort studies, which are less susceptible to recall or selection bias than case-control studies. A random-effects exposure-response meta-analysis was performed to examine a linear relationship between wine intake and cancer risk.

Potential publication bias was assessed through visual inspection of funnel plot asymmetry and by the *p*-value from Egger's tests [64]. Sensitivity analyses were conducted to evaluate the cancer risk, excluding studies that only had one type of wine intake (red or white) [18,43,46,47,61]. Additionally, the analysis was repeated after removing the most influential study with results that significantly deviated from the summary estimates. Between-study heterogeneity was evaluated using Cochran's Q statistic and quantified with Higgins I² statistic and associated 95% CI [65]. Sources of heterogeneity were investigated through meta-regression and subgroup analyses, considering variables such as study design (case-control or cohort), geographic region (US/Canada or other regions), sex, publication year (1990s, 2000s), exposure measurement assessment (validated or not), and the level of confounder adjustment [smoking and body mass index (BMI)] (Table 1).

3. Results

The searching scheme resulted in a total of 252 published articles, and we selected 42 articles (20 cohort [20–22,25,26,29,31,32,35–38,41,43–45,53,56,60,63] and 22 case-control studies [18,19,23,24,27,28,30,33,34,39,40,42,46,47,52,54,55,57–59,61,62]) to conduct a meta-analysis (Figure 1, Table 1). Of the 42 studies selected, 25 used validated methods of dietary assessment [18,20–22,25–30,32,34–38,40,41,43–45,53,59,60,63] (Table 1). A total of thirty-seven studies included measures of association for site-specific cancer incidence and consumption of both red and white wine, and five studies included data for red wine intake only [18,43,46,47,61]. We included cancers of the skin (number of studies = 6) [29,32,36,41,42,45], prostate (*n* = 6) [20,34,35,37,38,53], female breast (*n* = 5) [19,25,30,39,44], colon/rectum (*n* = 5) [24,28,31,43,59], ovary (*n* = 5) [23,26,27,52], lung (*n* = 4) [21,22,33,46], kidney/urinary track (*n* = 3) [18,56,57], pancreas (*n* = 2) [54,55], brain (*n* = 2) [62,63], lymphoma (*n* = 2) [60,61], stomach (*n* = 1) [58], and mouth/pharynx (*n* = 1) [46]. When a study presented effect estimates for multiple cancers (different sites or a subtype) or sex-specific estimates, we included all estimates and considered them as separate studies in our analyses.

The summary RR for the overall cancer risk, comparing the highest versus lowest level of wine intake, was 0.98 (95% CI = 0.87 to 1.10) for red wine and 1.00 (95% CI = 0.91 to 1.10) for white wine (Table 2). We observed no difference between red and white wine consumption on overall cancer risk (*P*_{difference} = 0.74). After excluding five studies [18,43,46,47,61] that only included measurements of association for red wine, we still observed no difference in overall cancer risk between the two types of wine (*P*_{difference} = 0.38). When restricted to cohort studies, the association with white wine intake became stronger and significant (RR: 1.12, 95% CI: 1.03, 1.22), while the null association remained for red wine intake (RR: 1.02, 95% CI: 0.96, 1.09), and the difference became significant (*P*_{difference} = 0.02).

Table 2. Summary RRs and 95% CIs of cancer risk associated with red and white wine, overall and stratified by sex and cancer sites.

	Red Wine			White Wine			P _{difference}
	No. of Studies	No. of Cases /No. of Participants	Summary RR (95% CI)	No. of Studies	No. of Cases /No. of Participants	Summary RR (95% CI)	
All cancer	42	95,923/2,824,425	0.98 (0.87, 1.10)	37	95,747/2,780,942	1.00 (0.91, 1.10)	0.74
Sex							
Men	13	10,300/678,821	1.01 (0.91, 1.12)	10	8906/484,051	1.08 (0.99, 1.18)	0.37
Women	15	61,211/1,723,992	0.91 (0.72, 1.16)	15	61,211/1,723,992	1.26 (1.05, 1.52)	0.03
Cancer sites							
Female breast	5	36,917/1,301,737	1.17 (0.97, 1.42)	5	36,917/1,301,737	1.12 (1.05, 1.20)	0.61
Skin	6	31,712/706,534	1.02 (0.95, 1.09)	6	31,712/706,534	1.22 (1.14, 1.30)	0.0003
Ovary	5	7676/44,099	0.76 (0.46, 1.26)	5	7676/44,099	0.95 (0.81, 1.11)	0.76
Prostate	6	12,119/253,273	0.92 (0.71, 1.18)	6	12,119/253,273	1.08 (0.72, 1.60)	0.51
Lung	4	1249/151,352	0.82 (0.41, 1.63)	3	922/150,675	0.79 (0.52, 1.22)	0.94
Kidney/Urinary tract	3	1147/37,198	0.79 (0.44, 1.42)	2	979/36,696	0.67 (0.49, 0.92)	0.65
Colon/rectum	5	2365/49,138	0.91 (0.62, 1.32)	4	2189/5655	1.01 (0.78, 1.33)	0.64

Abbreviations: CI, confidence interval; RR, relative risk. We included each effect estimate for different subtypes of breast cancer (ductal, ductal-lobular, and lobular) in the study by Baglia et al. [45] We assumed wine intake in De Stefani et al. [46] as red wine intake since the authors mentioned that Uruguayans drink mainly red wine.

The difference between red and white wine consumption on overall cancer risk was not significant in men ($P_{\text{difference}} = 0.37$) but was significant in women ($P_{\text{difference}} = 0.03$). The eligible studies for linear dose–response analyses were three cohort studies [22,25,38] and twelve case–control studies [18,19,23,25,30,34,39,47,52,54,57–59]. Based on these studies, we observed that every additional 10 g of estimated ethanol (e.g., about one glass) from red wine per day was associated with a 5% increase in overall cancer risk (summary RR = 1.05 [1.03, 1.08]); however, this association was null when restricted to cohort studies (1.01 [0.97, 1.04]) [21,24,37]. We found no significant dose–response relationship for white wine (RR = 1.02 [0.98, 1.05]), which was consistent when restricting to cohort studies (RR = 1.00 [0.96, 1.04]).

When we evaluated the association between wine intake and cancer risk by cancer site, we observed a significant difference in skin cancer risk associated with red versus white wine ($P_{\text{difference}} = 0.0003$). White wine intake was associated with a 22% increased risk of skin cancer (RR = 1.22, 95% CI = 1.14 to 1.30, Table 2, Figure 2), while red wine consumption was not associated with skin cancer (RR = 1.02, 95% CI = 0.95 to 1.09). Studies of skin cancer were not eligible to assess the dose–response relationship. An increased risk of breast cancer was observed for both wine types (red: RR = 1.17, 95% CI = 0.97 to 1.42, white: 1.12, 95% CI = 1.05 to 1.20) with no significant difference ($P_{\text{difference}} = 0.61$). Other than skin cancer, we did not observe a significant difference between the associations of red and white wine consumption and the risk of site-specific cancers.

The test of heterogeneity resulted in a moderate level of heterogeneity ($I^2 = 63.9\%$, $P_{\text{heterogeneity}} < 0.0001$) (Table 3). After excluding the most influential study [39], there was no change in directionality and significance of the summary RRs of high vs. low meta-analysis for overall cancer risk (red: 0.96, 95% CI: 0.86, 1.08; white: 1.00, 95% CI: 0.91, 1.10) and no significant difference between red and white wine ($p = 0.63$).

We observed possible publication bias through visual inspection of the funnel plot (Supplementary Figure S1) and Egger’s tests ($p = 0.03$). We used Duval and Tweedie’s trim and fill method [66] to estimate what the summary effect size would be if there was no publication bias. Briefly, this method omits small studies until the funnel plot is symmetrical (“trimming”), uses the trimmed plot to estimate the “true” center of the funnel plot, and then puts back the omitted studies, as well as their theoretical missing “counterparts”, around the new center (“filling”). We observed slightly increased summary RRs for both

red and white wine on overall cancer risk after conducting trim and fill analysis (red: 1.00, 95% CI 0.91, 1.09; white: 1.13, 95% CI 1.03, 1.24) (Supplementary Figure S2).

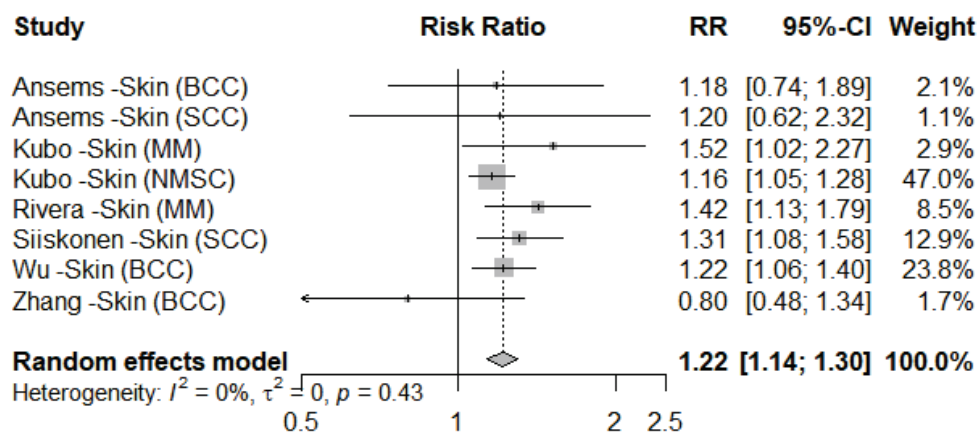


Figure 2. Forest plot of meta-analysis using random effects model for white wine consumption associated with skin cancer risk (comparing highest vs. lowest intake) [29,32,36,41,42,45]. The rectangles represent 95% confidence intervals and the diamond signifies the pooled effect of all included studies. Abbreviations: BCC, basal cell carcinoma; SCC, squamous cell carcinoma; MM, malignant melanoma.

Table 3. Subgroup meta-analyses of wine intake (all types) and overall cancer risk comparing the highest vs. lowest levels.

Subgroups	No. of Studies	No. of Cancer Cases /No. of Participants	Summary RR (95% CI)	I^2	$P_{\text{difference}}$
All cancer	42	95,923 /2,824,425	0.99 (0.91, 1.06)	61.3%	0.10
Study design					
Cohort	20	73,771 /2,772,622	1.06 (1.99, 1.13)	50.5%	
Case-control	22	22,152 /51,803	0.94 (0.83, 1.07)	67.7%	0.39
Study region					
US/Canada	24	56,219 /1,454,778	1.03 (0.97, 1.13)	49.2%	
Other regions	18	39,704 /1,369,647	0.95 (0.80, 1.13)	74.8%	0.93
Sex ^a					
Men	13	10,300 /678,821	1.10 (0.90, 1.33)	62.2%	
Women	15	61,211 /1,723,992	1.09 (1.05, 1.13)	57.5%	0.92
Publication year					
1990s	8	1872 /96,273	0.98 (0.69, 1.37)	75.8%	
2000s	34	94,051 /2,728,152	0.99 (0.93, 1.06)	58.4%	0.28
Exposure validation					
Yes	25	81,283 /2,787,093	1.04 (0.96, 1.12)	56.5%	
No	17	15,975 /37,332	0.96 (0.85, 1.08)	67.8%	0.97
Adjustments for confounders					
Smoking and BMI	3	67,057 /2,407,152	0.98 (0.86, 1.12)	71.3%	
Smoking or BMI	13	12,068 /122,604	1.00 (0.84, 1.19)	64.3%	0.97
No smoking or BMI adjustment	25	18,133 /294,669	0.98 (0.88, 1.09)	58.2%	

Abbreviations: BMI, body mass index; CI, confidence interval; RR, relative risk. ^a Studies that provided sex-specific effect estimates are included.

4. Discussion

We conducted a meta-analysis to investigate the association between red or white wine consumption and cancer risk. Although red wine has been considered healthier compared to white wine [67–70], there was no difference in cancer risk between the consumption of the two types of wine. When we restricted analysis to cohort studies, we observed a significant increase in overall cancer risk with white wine consumption. White wine intake was significantly associated with an increased risk of skin cancer compared to red wine intake.

A recent UK cohort study evaluating alcohol consumption patterns and health outcomes found no significant increase in the overall cancer risk and alcohol-related cancer (colon, rectum, breast, liver, esophagus, and larynx) incidence with white wine consumption compared to that of red wine, which is in line with our findings [71]. Red wine contains resveratrol, a natural stilbene and a non-flavonoid polyphenol, that possesses antioxidant, anti-inflammatory, cardioprotective, and anti-cancer properties [72]. Resveratrol has been investigated extensively as a potential chemopreventive agent because it has been shown to inhibit the proliferation of cancer cells in the breast, colon/rectum, skin, stomach, and kidney [9–14]. Many *in vitro* studies found that resveratrol influences tumor initiation and cancer progression pathways; however, *in vivo* studies have yielded mixed results [73]. For example, resveratrol can promote cell-cycle arrest leading to apoptosis of tumor cells, prevent tumor-derived nitric oxide synthase expression to block tumor growth and migration, as well as act as an antioxidant to prevent DNA damage that can lead to tumor formation [73]. Wu et al. [74] found that resveratrol is more toxic to cancer cell lines than to normal cell lines by comparing the activity of both types of cells following treatment with resveratrol. Additionally, it has been observed that combination therapy of resveratrol and other cancer drugs kills breast cancer cells more dramatically than either treatment in isolation [75]. Although there is some evidence of an anticarcinogenic effect of resveratrol in experimental studies, we did not observe that red wine consumption is associated with a reduced risk of cancer in our meta-analysis. In addition, there was no difference in the association between red vs. white wine and cancer risk, except for skin cancer.

A potential reason why we did not observe a difference in cancer risk between red and white wine may be that resveratrol has a fast metabolism [76]. Although concentrations of resveratrol are greater in red compared to white wine [48,77], studies have demonstrated that resveratrol is metabolized quickly, with nearly 75% excreted via feces and urine [76]. Several clinical studies have shown that the peak levels of resveratrol in plasma were low, given its extensive metabolism and poor bioavailability, after single or repeated oral administration [78]. A study involving 15 healthy participants who were given a single dose of 500 mg resveratrol observed a peak plasma concentration of 71.2 ng/mL [79]. Another study of 40 healthy subjects who received daily oral resveratrol of 500–5000 mg/day for 29 days reported a peak plasma concentration ranging from 44 to 967 ng/mL between days 21 and 28 [80]. Daily consumption of two glasses of red wine (375 mL) is equivalent to a dose of ~27 µg/kg body weight of resveratrol for a 70 kg individual, which leads to detectable concentrations of derivatives but not free resveratrol [81]. These low levels of resveratrol after consuming red wine may not result in bio-effective concentrations that may eventually make differences in health outcomes compared to white wine consumption.

We observed that white wine was associated with an increased risk of skin cancer. It has been hypothesized that alcohol consumption can promote skin carcinogenicity through the intermediate byproducts or metabolites of alcohol, such as acetaldehyde and reactive oxygen species, that have photosensitizing effects [82]. Also, drinking wine may be more common among whites who are more susceptible to skin cancer than other racial groups [68,83]. However, it may not explain why white wine specifically was associated

with skin cancer risk. In addition, heavy wine consumption may be related to high-risk behaviors such as sunburn, indoor tanning, and lack of use of sun protection [84]. In fact, earlier studies have demonstrated an association between alcohol consumption and increased prevalence of severe sunburn [82,85]. Given that all six studies properly adjusted for detailed information on the risk factors of skin cancer, e.g., skin type, sun exposure, and sun protection habits, a positive association we found in the meta-analysis was most likely the true detrimental effect of white wine [29,32,36,41,42,45]. The sample size for skin cancer was the largest among individual cancer sites and potentially provided enough statistical power to detect the difference. Wine intake (both red and white) was associated with increased breast cancer risk, which may suggest that resveratrol is not a significant factor in breast carcinogenesis.

There are limitations to this meta-analysis. First, wine intake was obtained from a self-reported food frequency questionnaire (FFQ). While most of the included cohort studies used validated FFQs (18 out of 19), only six out of twenty-two case-control studies used validated FFQs. However, because the intake information on red and white wine was collected in each study, the misclassification would similarly affect the intake assessment of both wines. Nevertheless, the misclassification of exposure might have occurred randomly regardless of cancer status, which may attenuate our findings. In addition, exposure validation was not a source of heterogeneity in our summary estimates for both red and white wine. Additionally, case-control studies may be more susceptible than cohort studies to recall bias when evaluating wine intake; however, we also presented results restricted to cohort studies. For some cancer sites including the pancreas, lymphatic system, brain, mouth, and pharynx, there was a limited number of studies (<3) to explore the risk difference between red and white wine. In addition, our dose-response analyses were limited to a smaller number of studies, largely case-control studies. Potential regression dilution bias in the included cohort studies might have also played a role in underestimating summary risk estimates [86].

However, we added an important finding to the controversial conversation about whether red wine intake reduces cancer risk. The quality of outcome measurements in all included studies was reliable (from a cancer registry or confirmed by medical professionals). Although we found evidence of potential publication bias, we found similar summary estimates after conducting trim and fill analysis.

5. Conclusions

To our knowledge, we conducted the first meta-analysis and the largest investigation of red versus white wine consumption and cancer risk and found no difference between the two types of wine. However, we observed that white wine had a significantly stronger association with cancer when the analysis was restricted to cohort studies. Furthermore, white wine intake, but not red wine intake, was associated with an increased risk of skin cancer. Our findings provided a critical public health message that drinking red wine may not be any better than drinking white wine in terms of cancer risk.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/nu17030534/s1>, Table S1: Medical Subject Heading (MeSH) terms and title/abstract (tiab) keywords in PubMed and Embase search strategy through December 2023; Figure S1: Funnel plot of meta-analysis on wine intake (highest vs. lowest) and risk of all cancer (A) All wine ($p = 0.03$) (B) Red wine ($p = 0.04$) (C) White wine ($p = 0.04$); Figure S2: Funnel plot of meta-analysis on wine intake (highest vs. lowest) and risk of all cancer after conducting trim-and-fill analysis.

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