OPEN ACCESS Freely available online

PLOS ONE

A High Dietary Glycemic Index Increases Total Mortality in a Mediterranean Population at High Cardiovascular Risk

Itandehui Castro-Quezada[1], Almudena Sánchez-Villegas[1,2], Ramón Estruch[2,3], Jordi Salas-Salvadó[2,4], Dolores Corella[2,5], Helmut Schröder[6,7], Jacqueline Álvarez-Pérez[1,2], María Dolores Ruiz-López[8,9], Reyes Artacho[8], Emilio Ros[2,10], Mónica Bulló[2,4], María-Isabel Covas[2,6], Valentina Ruiz-Gutiérrez[2,11], Miguel Ruiz-Canela[2,12], Pilar Buil-Cosiales[2,12], Enrique Gómez-Gracia[2,13], José Lapetra[2,14], Xavier Pintó[2,15], Fernando Arós[2,16], Miquel Fiol[2,17,19], Rosa María Lamuela-Raventós[2,18], Miguel Ángel Martínez-González[2,12], Lluís Serra-Majem[1,2]*, on behalf of the PREDIMED Study Investigators

1 Research Institute of Biomedical and Health Sciences, University of Las Palmas de Gran Canaria, Las Palmas de Gran Canaria, Spain, **2** Ciber Fisiopatología Obesidad y Nutrición (CIBEROBN, CB06/03), Instituto de Salud Carlos III (ISCIII), Spanish Government, Madrid, Spain, **3** Department of Internal Medicine, Hospital Clinic, Institut d'Investigacions Biomèdiques August Pi Sunyer (IDIBAPS), Barcelona, Spain, **4** Human Nutrition Department, School of Medicine, University Rovira i Virgili, Reus, Tarragona, Spain, **5** Department of Preventive Medicine, School of Medicine, University of Valencia, Valencia, Spain, **6** Cardiovascular Risk and Nutrition Research Group, Institut Municipal d'Investigació Medica (IMIM)-Institut de Recerca del Hospital del Mar, Barcelona, Spain, **7** CIBER Epidemiología y Salud Pública (CIBERESP), Instituto de Salud Carlos III (ISCIII), Spanish Government, Madrid, Spain, **8** Department of Nutrition and Food Science, School of Pharmacy, University of Granada, Granada, Spain, **9** Institute of Nutrition and Food Technologies, University of Granada. Armilla, Granada, Spain, **10** Lipid Clinic, Endocrinology and Nutrition Service, Hospital Clinic, l'Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain, **11** Group of Nutrition and Lipid Metabolism, Instituto de la Grasa (CSIC), Seville, Spain, **12** Department of Preventive Medicine and Public Health, School of Medicine – Clinica Universitaria de Navarra, University of Navarra, Pamplona, Navarra, Spain, **13** Department of Preventive Medicine, School of Medicine, University of Malaga, Malaga, Spain, **14** Department of Family Medicine, Primary Care Division of Sevilla, San Pablo Health Center, Sevilla, Spain, **15** Internal Medicine Service, Hospital of Bellvitge, Barcelona, Spain, **16** Department of Cardiology, Hospital Txagorritxu, Vitoria, Alava, Spain, **17** University Institute for Health Sciences Investigation, University of Balearic Islands, Palma de Mallorca, Spain, **18** Department of Nutrition and Bromatology, School of Pharmacy, University of Barcelona, Barcelona, Spain, **19** Department of Cardiology, Hospital Son Espases, Palma de Mallorca, Spain

Abstract

Objective: Different types of carbohydrates have diverse glycemic response, thus glycemic index (GI) and glycemic load (GL) are used to assess this variation. The impact of dietary GI and GL in all-cause mortality is unknown. The objective of this study was to estimate the association between dietary GI and GL and risk of all-cause mortality in the PREDIMED study.

Material and Methods: The PREDIMED study is a randomized nutritional intervention trial for primary cardiovascular prevention based on community-dwelling men and women at high risk of cardiovascular disease. Dietary information was collected at baseline and yearly using a validated 137-item food frequency questionnaire (FFQ). We assigned GI values of each item by a 5-step methodology, using the International Tables of GI and GL Values. Deaths were ascertained through contact with families and general practitioners, review of medical records and consultation of the National Death Index. Cox regression models were used to estimate multivariable-adjusted hazard ratios (HR) and their 95% CI for mortality, according to quartiles of energy-adjusted dietary GI/GL. To assess repeated measures of exposure, we updated GI and GL intakes from the yearly FFQs and used Cox models with time-dependent exposures.

Results: We followed 3,583 non-diabetic subjects (4.7 years of follow-up, 123 deaths). As compared to participants in the lowest quartile of baseline dietary GI, those in the highest quartile showed an increased risk of all-cause mortality [HR = 2.15 (95% CI: 1.15–4.04); P for trend = 0.012]. In the repeated-measures analyses using as exposure the yearly updated information on GI, we observed a similar association. Dietary GL was associated with all-cause mortality only when subjects were younger than 75 years.

Conclusions: High dietary GI was positively associated with all-cause mortality in elderly population at high cardiovascular risk.

Citation: Castro-Quezada I, Sánchez-Villegas A, Estruch R, Salas-Salvadó J, Corella D, et al. (2014) A High Dietary Glycemic Index Increases Total Mortality in a Mediterranean Population at High Cardiovascular Risk. PLoS ONE 9(9): e107968. doi:10.1371/journal.pone.0107968

Editor: Olga Y. Gorlova, Geisel School of Medicine at Dartmouth College, United States of America

Received March 13, 2014; **Accepted** August 21, 2014; **Published** September 24, 2014

Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper.

Funding: This report has been supported by the official funding agency for biomedical research of the Spanish government, Instituto de Salud Carlos III (ISCIII), through grants provided to research networks specifically developed for the trial [RTIC G03/140, to R E; RTIC RD 06/0045, to MA M-G] and through Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición (CIBERobn); Centro Nacional de Investigaciones Cardiovasculares [CNIC 06/2007]; Fondo de Investigación Sanitaria–Fondo Europeo de Desarrollo Regional [PI04-2239, PI 05/2584, CP06/00100, PI07/0240, PI07/1138, PI07/0954, PI 07/0473, PI10/01407, PI10/02658, PI11/01647, and P11/02505]; Ministerio de Ciencia e Innovación [AGL-2009-13906-C02 and AGL2010-22319-C03]; Fundación Mapfre 2010; Agencia Canaria de Investigación, Innovación y Sociedad de la Información-EU FEDER [PI 2007/050]; Consejería de Salud de la Junta de Andalucía [PI0105/2007]; Public Health Division of the Department of Health of the Autonomous Government of Catalonia; Generalitat Valenciana [ACOMP06109, GVACOMP2010-181, GVACOMP2011-151, CS2010-AP

-111, and CS2011-AP-042]; Regional Government of Navarra [P27/2011] and IC-Q is supported by a scholarship of the Consejo Nacional de Ciencia y Tecnología de México (National Council on Science and Technology of México, CONACYT). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* Email: lluis.serra@ulpgc.es

Introduction

The epidemiological transition and increased prevalence of chronic-degenerative diseases in the elderly population is related to nutritional status. In Spain, 35% of the population aged 65 years or more is obese [1]. Obesity, especially visceral obesity, confers an increased risk of mortality not only from cardiovascular diseases (CVD) but also from other causes such as cancer or diabetes and its complications [2].

Currently, dietary recommendations from FAO and WHO to prevent chronic diseases are aimed to reduce or change the proportion of fats to a healthier profile, while increasing the carbohydrate content of the diet [2–5]. Although these recommendations establish a limit in the intake of free sugars and suggest the ideal amount of fiber consumption, they are not specific in relation to the quality of the dietary carbohydrates [2–5]. New evidence-based recommendations from the WHO are being prepared and probably further adjustments will be made regarding the proportion of fat and sugar intake. However, both the quality and the amount of carbohydrates, generate different responses of insulin secretion and postprandial glucose [6]. Thus, Jenkins et al. [7] introduced the concept of glycemic index (GI). GI is defined as the postprandial blood glucose response of a test food, compared to the response of the same amount of available carbohydrate in a reference food consumed by the same subject. Similarly, glycemic load (GL) evaluates the overall glycemic effect of the diet, multiplying the glycemic index of the diet by the content of total carbohydrates consumed by the individual [8]. Dietary GI and GL were developed to measure the average daily consumption of an individual in terms of quality and amount of available carbohydrates [9].

Current research has evaluated the relationship between dietary GI and GL in the prevention and study of obesity, CVD, cancer and many other health problems, such as macular degeneration [6,9–12].

The literature to confirm the relationship between GI and GL for overall fatal events is scarce. In fact, usually, references are related to specific causes of fatal events such as cancer [13] and cardiovascular disease mortality [14,15]. The association between dietary GI and GL and the risk of total mortality has been investigated in men with established CVD [16] and breast cancer survivors [17], but no significant associations were found between all-cause mortality when comparing the highest intakes of dietary GI and GL vs. the lowest. Furthermore, an association between higher intake of dietary GL and risk for all-cause mortality has been found in diabetic subjects with normal weight [18]. Similarly, Baer et al. [19] found a relationship in women aged 30–55 years: an increase of 41 units of GL, augment by 22% the risk for total mortality.

The association between GI/GL and all-cause mortality is still unclear; therefore, we aimed to evaluate the association of the quality of carbohydrates on all-cause mortality in an elderly population at high cardiovascular risk.

Materials and Methods

Study design

This study was conducted within the frame of the PREDIMED study (PREvención con DIeta MEDiterránea), a randomized, multicenter, parallel group, single-blinded dietary intervention trial conducted in Spain (Trial Registration: clinicaltrials.gov Identifier: ISRCTN35739639) [20]. The main objective of the trial was to analyze the effect of the Mediterranean Diet (MeDiet) on major cardiovascular disease prevention when comparing to a control diet [20]. Participants were randomly assigned to three groups: MeDiet supplemented with extra virgin olive oil (MeDiet + EVOO), MeDiet with nuts (MeDiet + nuts), and the control group. Participants received dietary training without calorie restriction or advice on physical activity [20]. After the completion of the trial, the PREDIMED study provided an opportunity for conducting the long-term follow-up of a large observational cohort of high cardiovascular risk subjects in a Mediterranean setting [21]. The design of the PREDIMED study has been reported in detail elsewhere [21]. The protocol was approved by the institutional review boards of each recruitment center and all participants provided a written informed consent prior to their inclusion in the study.

Study population

The PREDIMED study included 7447 participants, aged between 55 and 80 years. Subjects without prior CVD were selected when they met at least one of the following criteria: presence of type 2 Diabetes Mellitus (T2DM) or the presence of three or more cardiovascular risk factors (current smoking, hypertension, high LDL cholesterol ($\geq$160 mg/dl), low HDL cholesterol ($\leq$40 mg/dL in men and $\leq$50 mg/dL in women), overweight or obesity (BMI$\geq$25 kg/m^2), or family history of premature CVD).

For this analysis, we selected participants without T2DM at baseline (n = 3833). Exclusion criteria were: subjects without follow-up (n = 125), with values of total energy intake outside of predefined limits ($<$800 or$>$4000 kcal/d in men and: $<$500 or$>$ 3500 kcal/d in women) (n = 86), use of anti-diabetic medication (n = 21) and incomplete data in any variable used in the analyses (n = 18). Overall, 3,583 subjects were analyzed in this study. The rationale for exclusion of persons with a baseline history of diabetes mellitus (including type 1 diabetes, T2DM or use of anti-diabetic medication) was potential effect modification and potential changes in the diet as a result of diagnosis and treatment of T2DM.

Dietary assessment

Trained dietitians used a 137–item food frequency questionnaire (FFQ) to assess dietary habits by face-to-face interviews. This FFQ was repeatedly administered each year during follow-up. The FFQ has been validated in a sample of subjects with similar characteristics to the participants of the PREDIMED study [22]. Energy (kcal/day) and nutrient intake (g/day) were calculated as frequency multiplied by nutrient composition of specified portion size where frequencies were measured in nine categories for each food item. Nutrient data bank was updated using the latest available information included in food composition tables for Spain [23]. Alcohol intake was also ascertained through the use of this questionnaire. Carbohydrates, proteins, fat and dietary fiber intakes, GI and GL were adjusted for total energy intake using the residual method proposed by Willet [24].

Estimation of dietary GI and GL

We assigned the GI of each food of the FFQ using Louie et al. protocol [25] with the International Tables of GI and GL values [26] and the Sydney University GI research service [27]. Values were extracted from published studies conducted in normal subjects, using glucose as reference food [26].

After the GI assignment, we estimated dietary GI for each individual summing the GI of each food multiplied by the amount of available carbohydrate consumed (g/day) and divided by the total available carbohydrate amount. For dietary GL, the sum of the GI multiplied by the amount of available carbohydrate consumed was divided by 100 [28]. Energy-adjusted dietary GI and GL intake was finally categorized into quartiles. To assess the repeated measurements of diet, we updated GI and GL values from the yearly FFQs.

Other measurements

Socio-demographic information, medical history and lifestyle habits were collected via specific questionnaires carried out by trained personnel. Height and weight were measured wearing light clothes, barefoot, using a wall-mounted stadiometer and calibrated scales. BMI was estimated as weight (kg) divided by the height (m^2) squared. We estimated energy expenditure using a validated Spanish version of the Minnesota leisure-time physical activity questionnaire [29,30].

Outcome ascertainment

All-cause mortality was determined by review of the End Point adjudication Committee. This panel was blinded to the intervention group. Information on all-cause mortality was updated on a yearly basis. Information on the occurrence of each fatality was initially obtained from the continuous contact with participants and their families that we had during the trial, contact with family physicians, the yearly comprehensive review of all medical records and by yearly consultation of the National Death Index. The analyses included cases confirmed between October 1st, 2003, and December 1st, 2010.

Statistical analysis

Baseline characteristics of the population and dietary intakes were calculated according to quartiles of dietary GI and GL. Follow up time was calculated from the date of recruitment to the date of either death or end of follow-up (the date of the last visit or the last recorded clinical event of participants still alive). Different Cox regression models were used to estimate multivariable-adjusted hazard ratios (HR) and their 95% CI for all-cause mortality according to baseline quartiles of energy adjusted dietary GI and GL. A first model was adjusted for sex, age (years), recruitment center and intervention group (Med Diet + EVOO, Med Diet + Nuts and control diet). A second model was further adjusted for potential confounders including: smoking (never, past, current), education (low, middle, high, no data available), marital status (married, other), physical activity (continuous), BMI (continuous), self-reported history of cancer (yes, no), self-reported history of arterial hypertension (yes, no), self-reported history of dyslipidemia (yes, no), self-reported history of cardiovascular disease (yes, no), total energy intake (continuous), alcohol intake (continuous) and dietary fiber intake (continuous, energy adjusted). The third model was further adjusted for saturated fatty acids (continuous, energy adjusted) and monounsaturated fatty acids (continuous, energy adjusted). In all the analyses, recruitment center was entered into the model through the strata statement. Tests of linear trend across increasing quartiles of GI and GL were conducted by assigning the medians to each quartile and these variables were treated as continuous in the multivariate models. To assess a possible interaction between dietary GI/GL and sex or intervention group, we introduced product terms in the different multivariable models and considered $p<0.05$ in the likelihood ratio test as statistically significant.

To update GI and GL intake with all available longitudinal data, we used Cox regression models with time-dependent exposures. Three models were adjusted using the same covariates used for the models of baseline analysis. For dietary measures, we used the cumulative average of food intakes from baseline to the censoring events. BMI and physical activity were also yearly updated. Because changes in diet after development of diabetes may confound the association between exposure and mortality [31], we stopped updating dietary variables at the time interval during which individuals developed T2DM.

Finally, sensitivity analyses were conducted using quartiles of energy-adjusted GI and GL specific for different groups of the population to observe the effect of changing energy limits (percentiles 1 and 99, percentiles 5 and 95), including population with T2DM, excluding obese subjects (BMI$\geq$30 kg/m^2) and including only obese subjects, excluding participants with more than 6 years of follow-up and exclusion of subjects aged 75 years or more. Statistical analyses were performed using Stata 12.0 (StataCorp, College Station, TX, USA) and the significance level was set at $p<0.05$.

Results

In this study, participants were followed for a median of 4.7 years. Of 3,583 subjects, 123 cases of all-cause mortality were registered. The mean (SD) dietary GI and GL of participants at recruitment was 57.6 (4.7) and 117.6 (24.1) respectively. **Table 1** shows the main baseline characteristics of the population according to energy adjusted dietary GI and GL quartiles. Subjects in the top quartile of dietary GI differed from the individuals in the lowest quartile in that they were younger and more often male, were more likely to be current smokers and more likely to be married. Participants in the highest quartile of dietary GI also had slightly lower BMI (-0.6 kg/m^2), higher consumption of alcohol and carbohydrate but less intake of protein, fat and dietary fiber than the subjects in the bottom quartile of dietary GI. Characteristics according to dietary GL were similar to GI excepting that subjects in the highest quartile of GL were more often female, older, less likely to be current smokers, more likely to have only primary education, less likely to be married and physically active, had lower alcohol consumption, lower energy intake had higher intake of fiber when compared to the lowest quartile. The proportion of prevalent diseases such as cancer or arterial hypertension was similar across quartiles of dietary GI and GL. According to intervention group, mean dietary GI and GL were significantly higher when comparing the highest quartiles vs. the lowest.

We found a significant association between the highest quartile of dietary GI and all-cause mortality (**Table 2**). In the first model, subjects in the top quartile of baseline dietary GI had a 2.2 fold risk of all-cause mortality vs. subjects in the lowest quartile [HR = 2.22 (95% CI: 1.26–3.94); p for trend = 0.002]. After multivariate adjustment, this association was attenuated but remained significant [HR = 2.15 (95% CI: 1.15–4.04); P for trend = 0.012]. Our results revealed a higher risk for all cause mortality and baseline dietary GL in the multivariate analysis although it did not reach statistical significance [HR = 1.95 (95% CI: 0.97–3.90); p for trend = 0.072]. No significant interactions with sex or intervention group were

Table 1. Characteristics of non diabetic subjects in the PREDIMED study assessed at baseline according to quartiles of energy adjusted dietary glycemic index and dietary glycemic load[a, b].

	Energy adjusted dietary glycemic index					Energy adjusted dietary glycemic load				
Variables	**Q1**	**Q2**	**Q3**	**Q4**	**p-value**	**Q1**	**Q2**	**Q3**	**Q4**	**p-value**
	n = 896	**n = 896**	**n = 896**	**n = 895**		**n = 896**	**n = 896**	**n = 896**	**n = 895**	
Demographic characteristics										
Sex (% Female)	75.8	67.1	57.5	48.9	<0.001	56.1	66.6	65.5	61.0	<0.001
Age (years)	66.8±5.9	67.0±6.2	66.9±6.1	66.0±6.1	0.007	65.8±6.0	67.1±5.8	66.7±6.1	67.0±6.4	<0.001
Smoking (%)					<0.001					<0.001
Current	12.7	11.4	16.5	22.0		19.4	14.4	12.8	16.0	
Past	18.2	20.1	22.8	27.2		24.9	21.2	21.7	20.5	
Never	69.1	68.5	60.7	50.8		55.7	64.4	65.5	63.6	
Education (%)					0.547					<0.001
Elementary school	73.9	73.8	73.6	73.5		67.5	73.9	74.0	79.3	
Secondary school	15.6	17.0	16.3	16.4		20.9	16.0	16.7	11.7	
Graduate school	8.4	7.4	7.7	9.1		9.8	8.5	6.7	7.5	
No data available	2.1	1.9	2.5	1.0		1.8	1.7	2.6	1.5	
Marital status (% Married)	72.2	78.0	75.2	78.1	0.010	82.4	76.7	72.5	72.0	<0.001
Physical activity (METs-h/w)	233.3±229.4	227.2±237.1	218.5±217.1	215.3±209.0	0.300	251.1±236.9	201.1±204.6	223.6±224.3	218.4±224.2	<0.001
BMI (kg/m^2)	30.3±3.7	30.1±3.6	30.1±3.6	29.7±3.5	0.013	30.3±3.7	30.1±3.8	29.9±3.6	29.8±3.5	0.032
Cancer (%)	3.6	2.7	3.4	3.5	0.712	3.8	2.6	2.8	3.9	0.703
Arterial hypertension (%)	91.6	92.0	92.0	91.0	0.849	91.0	91.3	91.9	92.4	0.262
Alcohol intake (g/d)	6.0±11.2	7.5±12.7	10.3±16.3	12.1±16.9	<0.001	15.0±20.5	7.6±11.8	7.1±11.7	6.1±10.8	<0.001
Dietary intake										
Total energy intake (kcal/d)	2263±536	2251±525	2297±516	2284±531	0.257	2394±507	2177±485	2177±524	2346±555	<0.001
Carbohydrate intake (g/d)[c]	235.3±39.1	241.8±39.1	245.0±39.6	251.9±40.0	<0.001	199.1±27.2	232.5±16.9	253.5±17.9	288.9±27.8	<0.001
Protein intake (g/d)[c]	95.6±14.0	91.4±12.9	89.3±13.0	86.1±12.1	<0.001	93.9±15.3	90.7±12.6	90.3±12.7	87.6±12.2	<0.001
Fat intake(g/d)[c]	100.8±16.2	98.6±16.8	96.2±15.8	93.1±16.6	<0.001	111.6±14.5	102.4±10.6	93.6±10.7	81.1±12.7	<0.001
Monounsaturated fatty acids (g/d)[c]	49.3±10.7	48.9±11.4	48.2±10.6	47.4±10.6	0.005	56.3±10.1	51.5±8.4	46.2±8.4	39.7±8.7	<0.001
Polyunsaturated fatty acids (g/d)[c]	16.0±5.3	15.8±4.8	15.5±4.7	14.7±4.6	<0.001	17.8±5.4	16.2±4.3	15.1±4.4	12.9±4.2	<0.001
Saturated fatty acids (g/d)[c]	25.9±5.9	25.0±5.6	24.2±5.4	23.2±5.5	<0.001	28.0±5.9	25.8±4.7	23.8±4.3	20.7±5.0	<0.001
Dietary fiber intake (g/d)[c]	27.6±8.2	26.5±7.6	24.9±7.4	22.7±6.9	<0.001	23.1±6.9	24.6±6.4	26.3±7.6	27.8±9.1	<0.001
GI (per day)[c]	51.7±2.2	55.9±0.9	59.0±0.9	63.6±2.4	<0.001					
GL (per day)[c]						88.5±12.2	109.4±4.2	124.0±4.5	148.6±15.0	<0.001
	Mean GI [c] (SD) according to intervention group					**Mean GL[c] (SD) according to intervention group**				
Mediterranean Diet + EVOO	51.4±2.3	55.9±1.0	59.1±0.9	63.6±2.6	<0.001	88.5±11.4	109.3±4.2	124.2±4.5	148.9±16.2	<0.001
Mediterranean Diet + Nuts	51.8±2.2	55.9±0.9	59.0±1.0	63.5±2.1	<0.001	88.1±13.3	109.3±4.4	123.9±4.5	148.3±14.2	<0.001

Table 1. Cont.

Variables	Energy adjusted dietary glycemic index					Energy adjusted dietary glycemic load				
	Q1	Q2	Q3	Q4	p-value	Q1	Q2	Q3	Q4	p-value
	n = 896	n = 896	n = 896	n = 895		n = 896	n = 896	n = 896	n = 895	
Control group	51.8±2.0	56.0±0.9	59.0±1.0	63.8±2.6	<0.001	89.0±11.5	109.7±3.9	124.0±4.6	148.6±14.3	<0.001

[a] Data are presented as mean ± SD for quantitative variables and as percentages for qualitative variables, n = 3604.
[b] Abbreviations: n, number of subjects; Q, quartile; MET, metabolic equivalent; BMI, body mass index; GI, dietary glycemic index; GL, dietary glycemic load.
[c] Values are adjusted for energy using the residuals method.
doi:10.1371/journal.pone.0107968.t001

observed. In the repeated measurement analyses using as exposure the yearly updated information on GI, we found a significant association with all-cause mortality [HR = 2.25 (95% CI: 1.16–4.36) for the highest versus the lowest quartile; p for trend = 0.014]. Regarding updated dietary GL, we observed that subjects in the top quartile had around 75% higher risk for mortality when compared to those in the bottom quartile, however, this association did not achieve statistical significance.

Results from sensitivity analyses comparing the risk of mortality between the upper quartiles (quartile 3 and quartile 4) of GI and GL with reference to the lowest quartile are shown in **Table 3**. We also found positive associations between the upper quartile of dietary GI with all-cause mortality when considering energy limits as percentiles 1 and 99 or percentiles 5 and 95, and in subjects younger than 75 years. Finally, obese subjects in the top quartiles of dietary GI presented a 3-fold increased risk for all-cause mortality with a statistically significant positive trend (p for trend = 0.044). Dietary GL was associated with all-cause mortality when we excluded participants with total energy intake lower than percentile 1 or higher than percentile 99 [HR = 2.10 (95% CI: 1.03–4.27) for the highest versus the lowest quartile; p for trend = 0.042] and when we restricted the analysis to subjects younger than 75 years [HR = 3.16 (95% CI: 1.32–7.54); p for trend = 0.019].

Discussion

We found that higher intake of baseline dietary GI was associated with an increased risk of all-cause mortality in 3,583 non-diabetic elderly subjects. This association remained significant in the repeated measurement analysis. With respect of dietary GL, we observed a higher risk of all-cause mortality only in subjects at high risk of CVD younger than 75 years.

Our results differ from a cohort of Swedish men, aged from 45 to 79 years with prior cardiovascular disease, where no association was found between dietary GI nor GL and all-cause mortality [16]. We did not find a relationship for all-cause mortality and dietary GL for the original sample evaluated; however, the association was statistically significant for subjects younger than 75 years. This effect is similar to the Nurses' Health Study, where GL was identified as a risk factor for all-cause mortality among 50,112 women aged 30–55 years [19]. It is of interest to note that in the Nurses' Health study a significant association was found with "other causes" but not with CHD and cancer mortality. Therefore, the differences could be explained because our study was conducted in aged population at high cardiovascular risk and our endpoint incorporated all causes of death, including CHD and cancer.

Our results also agree with a previous study where Oba et al. reported an association of similar magnitude between dietary GI and mortality from stroke risk in women. No associations were observed between dietary GL and the risk of total stroke or death from ischemic stroke [15]. A positive trend was found for GL and death from hemorrhagic stroke in women. In our study, a significant trend was found in the sensitivity analysis only in subjects younger than 75 years. When individuals over 75 years were analyzed, no associations were found between dietary GI nor GL with all cause mortality (data not shown). This fact reflects that the oldest subjects in our sample could be attenuating our results due to age-related influences, such as deterioration in glucose metabolism. Pancreatic, insulin receptor, and post-receptor changes associated with aging are critical components of the endocrinology of aging. Apart from decreased (relative) insulin secretion by the β cells, peripheral insulin resistance related to

Table 2. Hazard Ratios (95% CI) for total mortality by quartiles of energy adjusted dietary glycemic index and dietary glycemic load assessed at baseline in non-diabetic subjects[a].

	Baseline energy adjusted dietary glycemic index					Baseline energy adjusted dietary glycemic load				
	Q1	Q2	Q3	Q4	p-trend	Q1	Q2	Q3	Q4	p-trend
Median	52.1	55.9	59.1	63.1		91.9	109.6	123.9	144.4	
Cases	17	23	32	51		33	28	22	40	
Person-years	3797	3830	3925	4003		4010	3948	3862	3735	
HR[b] (95% CI)	1 (Ref.)	1.17 (0.62–2.19)	1.47 (0.81–2.67)	2.22 (1.26–3.94)	0.002	1 (Ref.)	0.97 (0.59–1.58)	0.80 (0.47–1.36)	1.43 (0.88–2.32)	0.210
HR[c] (95% CI)	1 (Ref.)	1.30 (0.68–2.49)	1.40 (0.75–2.61)	2.00 (1.08–3.70)	0.019	1 (Ref.)	1.04 (0.61–1.77)	0.90 (0.49–1.65)	1.75 (1.00–3.06)	0.070
HR[d] (95% CI)	1 (Ref.)	1.36 (0.72–2.58)	1.46 (0.79–2.70)	2.15 (1.15–4.04)	0.012	1 (Ref.)	1.09 (0.63–1.88)	0.96 (0.51–1.82)	1.95 (0.97–3.90)	0.072
	Updated energy adjusted dietary glycemic index					Updated energy adjusted dietary glycemic load				
HR[b] (95% CI)	1 (Ref.)	1.32 (0.70–2.48)	1.63 (0.88–3.02)	2.69 (1.50–4.84)	0.001	1 (Ref.)	1.49 (0.91–2.44)	0.91 (0.53–1.59)	1.41 (0.86–2.30)	0.381
HR[e] (95% CI)	1 (Ref.)	1.26 (0.66–2.41)	1.52 (0.80–2.91)	2.18 (1.13–4.20)	0.015	1 (Ref.)	1.67 (1.01–2.77)	1.09 (0.61–1.95)	1.73 (0.98–3.05)	0.212
HR[f] (95% CI)	1 (Ref.)	1.27 (0.67–2.43)	1.55 (0.81–2.96)	2.25 (1.16–4.36)	0.014	1 (Ref.)	1.69 (0.98–2.91)	1.10 (0.59–2.08)	1.76 (0.88–3.54)	0.326

[a] Abbreviations: Q, quartile; HR, hazard ratio; CI, confidence interval.

[b] Adjusted for sex, age (years), recruitment center, intervention group (Med Diet with EVOO, Med Diet with Nuts, Low fat diet).

[c] Adjusted for sex, age (years), recruitment center, intervention group (Med Diet + EVOO, Med Diet + Nuts, Low fat diet), smoking (never, past, current), education (low, middle, high, no data available), marital status (married, other), physical activity (continuous), BMI (continuous), self-reported history of cancer (yes, no), self-reported history of arterial hypertension (yes, no), total energy intake (continuous), alcohol intake (continuous) and dietary fiber intake (continuous, energy-adjusted).

[d] Multivariate model with additional adjustment for intake of saturated fatty acids (continuous, energy-adjusted) and monounsaturated fatty acids (continuous, energy-adjusted).

[e] Multivariate model with yearly updated measures of physical activity (continuous), BMI (continuous), total energy intake (continuous), alcohol intake (continuous), dietary fiber intake (continuous, energy-adjusted).

[f] Multivariate model with yearly updated measures with additional adjustment for saturated fatty acids intake (continuous, energy-adjusted) and monounsaturated fatty acids intake (continuous, energy-adjusted).

doi:10.1371/journal.pone.0107968.t002

Table 3. Sensitivity analysis. Hazard Ratios (95% CI) for total mortality according to baseline quartiles of energy adjusted dietary glycemic index and dietary glycemic load[a].

		Energy adjusted dietary glycemic index[b]			Energy adjusted dietary glycemic load[b]		
	n	Q3	Q4	p-trend	Q3	Q4	p-trend
Energy limits percentiles 1 and 99							
HR[b] (95% CI)	3606	1.47 (0.80–2.71)	2.08 (1.12–3.87)	0.015	1.14 (0.60–2.16)	2.10 (1.03–4.27)	0.042
Energy limits percentiles 5 and 95							
HR[b] (95% CI)	3333	1.46 (0.76–2.80)	2.39 (1.23–4.63)	0.010	0.86 (0.43–1.71)	1.96 (0.94–4.06)	0.109
Sample with diabetic population							
HR[b] (95% CI)	7013	0.94 (0.68–1.30)	1.21 (0.86–1.71)	0.272	1.02 (0.70–1.49)	1.16 (0.76–1.78)	0.657
Excluding obese subjects (BMI≥30 kg/m^2)							
HR[b] (95% CI)	1888	0.76 (0.35–1.65)	1.79 (0.83–3.86)	0.221	0.79 (0.33–1.90)	1.94 (0.76–4.97)	0.214
Including only obese subjects (BMI≥30 kg/m^2)							
HR[b] (95% CI)	1695	3.75 (1.11–12.64)	3.24 (0.96–10.86)	0.044	1.25 (0.46–3.42)	2.10 (0.69–6.38)	0.183
Excluding subjects>6 y of follow-up							
HR[b] (95% CI)	2935	1.13 (0.61–2.08)	1.66 (0.89–3.09)	0.052	0.81 (0.42–1.56)	1.75 (0.88–3.45)	0.160
Excluding subjects ≥75 y							
HR[b] (95% CI)	3158	1.46 (0.67–3.18)	2.35 (1.04–5.33)	0.026	1.45 (0.66–3.19)	3.16 (1.32–7.54)	0.019

[a] Abbreviations: Q, quartile; HR, hazard ratio; CI, confidence interval; BMI, Body Mass Index.

[b] Model adjusted for sex, age (years), recruitment center, intervention group (Med Diet + EVOO, Med Diet + Nuts, Low fat diet), smoking (never, past, current), education (low, middle, high, no data available), marital status (married, other), physical activity (continuous), BMI (continuous), self-reported history of cancer (yes, no), self-reported history of arterial hypertension (yes, no), total energy intake (continuous), alcohol (continuous), dietary fiber intake (continuous, energy-adjusted), saturated fatty acids intake (continuous, energy-adjusted) and monounsaturated fatty acids intake (continuous, energy-adjusted).

[b] Dietary GI and GL quartiles were estimated for each specific population group.

doi:10.1371/journal.pone.0107968.t003

poor diet, physical inactivity, increased abdominal fat mass, and decreased lean body mass contribute to the deterioration of glucose metabolism [32]. Furthermore, this age-effect is similar to previous findings, where the association between obesity and mortality was attenuated with advancing age [33]. However the age-related weaker associations may reflect confounding from cohort variation in mortality risk, healthy participant effects (i.e., biases introduced by survey selection of healthy respondents) or duration of exposition to the risk factor [34]. Therefore, we consider that it can be not concluded whether dietary GL is associated with higher risk of mortality in older subjects and further studies with a larger sample size and follow-up are needed to confirm the association between dietary GL and all-cause mortality. Our results alternatively, may indicate that, in this population at high cardiovascular risk, the quality of carbohydrates (represented by dietary GI) could be more important than its quantity (partially represented by dietary GL) in predicting all-cause mortality [15].

We identified an association between the highest quartiles of dietary GI and risk of all-cause mortality in obese subjects. Two recent meta-analyses have shown that the effect of dietary GL on the risk of coronary heart disease appeared more evident in subjects with higher BMI, although the authors recommended treating this information with caution because of the limited evidence and diversity among the BMI cut-off points used across studies [35,36]. Nevertheless, the individual response to a given carbohydrate load is influenced by the degree of insulin resistance, which is, firstly determined by the degree of adiposity, and also by physical activity, genetics, and other aspects of diet. Thus, it might be expected that the adverse metabolic effects of high-GI foods would be exacerbated in sedentary, overweight, or genetically susceptible persons [37].

Furthermore, higher values of dietary GI or GL could increase the risk of all-cause mortality by raising the risk of chronic diseases. Recent meta-analyses have shown a relationship between dietary GI, GL and increased risk of CVD in women but not in men [35,38,39], greater risk of T2DM [6,40,41], and risk for certain types of cancer such as colorectal or endometrial ones [11,42]. Higher dietary GI has also been associated with an increased risk of breast cancer [6,11,43] although these results are contradictory [44–46].

After a high-GI meal, blood glucose concentration increases at least twice that after a low-Gl meal with the same nutrients and energy. This hyperglycemia stimulates insulin release and inhibits glucagon liberation. High insulin-to-glucagon ratio affects normal anabolic responses such as uptake of nutrients by insulin-responsive tissues, glycogenesis, lipogenesis, and inhibits gluconeogenesis and lipolysis. From 2 to 4 hours after a high-GI meal, the absorption from the gastrointestinal tract declines, but the effects of hiperinsulinemia and low glucagon remain. Blood glucose drops to lower hypoglycemic range and release of free fatty acid is more suppressed than compared with a low-GI meal. In the late postprandial period, the low circulating concentration of metabolic fuel activates the hormone response that restores euglicemia and elevates free fatty acids to higher levels than observed after low-GI meals [47].

Eventually these postprandial responses may contribute to insulin resistance and obesity [48]. In a meta-analysis, Livesey et al. found that reduction in GL was associated with a decrease in body weight and vice versa [49]. When comparing high GI foods, such as white bread, Bautista-Castaño et al. found a dose-response relationship between the increase in white bread consumption and weight or waist circumference gain [50]. However, these results are inconsistent. A systematic review of 14 randomized controlled trials did not find differences in the effect of low GI/GL vs. high GI/GL diets on anthropometric data, but decreases in C-reactive protein and fasting insulin were significant in the low GI/GL groups [51].

Additionally, higher dietary GI has been associated with small increases in C-reactive protein [52] which has been related to all-cause mortality [53]. Another European study conducted in overweight subjects, showed that following a low GI diet after a weight loss intervention, had a greater decrease of high sensitivity C-reactive protein blood levels than the high GI groups [54]. Moreover, in the PREDIMED study, top quartiles of dietary GI were associated with higher plasma levels of TNF and IL-6 than those in the lowest quartiles [55].

A study conducted in healthy elderly Europeans found that a posteriori plant-based dietary pattern was associated with lower all-cause mortality. This plant-based pattern with high intakes of vegetables, vegetable oils, fruit, legumes and pasta/rice/other grains and low intakes of potatoes, margarine and non-alcoholic beverages, was correlated with the MeDiet pattern [56]. A previous study has shown that greater adherence to the MeDiet is inversely associated with dietary GI and GL [57]. A high degree of adherence to the MeDiet has been associated with a reduction in total mortality [58–62], obesity [63–65], T2DM [66,67], major cardiovascular events [21,68] and their risk factors [23]. Thus, Estruch et al. [23] assessed the effects of a 3-month intervention with MeDiet on changes in cardiovascular risk factors within the PREDIMED trial. MeDiet supplemented with EVOO, reduced C-reactive protein levels. Moreover, other inflammation biomarkers such as interleukin-6, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) decreased in subjects following a MeDiet supplemented either with EVOO or nuts [23]. Even more, after one year of intervention with MeDiet, the prevalence of metabolic syndrome was reduced by 13.7% when compared with control group [69]. However the number of randomized controlled-feeding trials that compare low vs. high GI/GL diets and that include measures of glucose homeostasis, blood lipids or inflammation is limited [70].

The limitations of this study are mainly methodological. Firstly, due to the scarcity of GI values for Spanish foods, we used as reference GI data from other countries. This fact could be a source of bias, because GI values may differ wide ranges, depending on variety, processing and cooking [71]. Secondly, the FFQ was not designed to evaluate dietary GI and dietary GL. Thirdly, we conducted the study in elderly subjects at high cardiovascular risk. Therefore, our results cannot be generalized to other populations. Finally, our study was conducted in a cohort that went through a nutritional intervention, which may have had an effect on dietary GI and GL. However, in order to address this issue, we adjusted all analyses by intervention group to minimize the effect. Moreover, because updated dietary information during the follow-up was used in our analyses, we accounted for changes in dietary habits over time. To our knowledge, this is the first large cohort study assessing GI and GL with yearly repeated measurements of diet. Repeated measurements of diet capture changes in dietary exposure, but also contribute to overcome, at least partially, the potential problems of measurement errors in nutritional epidemiology. Our study also has other strengths, such as the large sample size that allowed us to adjust for all possible potential confounders in the multivariate analyses. Other strengths are the use of a comprehensive and validated FFQ and the assignment of GI values trough an established protocol.

Conclusions

In summary, this study provides evidence that high GI diets are related to increased risk of all-cause mortality in non-diabetic

elderly subjects at high cardiovascular risk. In the sensitivity analyses this association was statistically significant when subjects were younger than 75 years or obese. Nevertheless, more evidence is necessary to evaluate these associations in different population groups and further studies that elucidate the mechanisms supporting the inclusion of GI and GL in dietary recommendations.

Acknowledgments

The authors want to thank the participants of the study for their collaboration and the PREDIMED personnel for their excellent assistance with all aspects of the trial.

Author Contributions

Conceived and designed the experiments: LSM ICQ ASV RE JSS DC HS JAP MDRL RA ER MB MIC VRG MRC PBC EGG JL XP FA MF RMLR MAMG. Performed the experiments: ICQ ASV. Analyzed the data: ICQ ASV. Wrote the paper: ICQ ASV. Critical revision of the manuscript: RA MDRL MAMG LSM. Primary responsibility for the final content: LSM. Provided input and feedback on manuscript: LSM ICQ ASV RE JSS DC HS JAP MDRL RA ER MB MIC VRG MRC PBC EGG JL XP FA MF RMLR MAMG. Read and approved the final version of the manuscript: LSM ICQ ASV RE JSS DC HS JAP MDRL RA ER MB MIC VRG MRC PBC EGG JL XP FA MF RMLR MAMG.

References

1. Gutiérrez-Fisac JL, Guallar-Castillón P, León-Muñoz LM, Graciani A, Banegas JR, et al. (2012) Prevalence of general and abdominal obesity in the adult population of Spain, 2008-2010: the ENRICA study. Obes Rev 13: 388–92.
2. WHO (2003) Diet, Nutrition and the Prevention of Chronic Diseases. Report of a Joint WHO/FAO Expert Consultation. WHO Technical Report Series No. 916. Geneva: World Health Organization. pp. 1–149.
3. Aston LM (2006) Glycaemic index and metabolic disease risk. Proc Nutr Soc 65: 125–34.
4. WHO (2009) Fats and fatty acids in human nutrition. Proceedings of the Joint FAO/WHO Expert Consultation. November 10–14, 2008. Geneva, Switzerland Ann Nutr Metab 55: 5–300.
5. FAO (2010) Fats and fatty acids in human nutrition. Report of an expert consultation. FAO Food Nutr Pap 91: 1–166.
6. Barclay AW, Petocz P, McMillan-Price J, Flood VM, Prvan T, et al. (2008) Glycemic index, glycemic load, and chronic disease risk—a meta-analysis of observational studies. Am J Clin Nutr 87: 627–37.
7. Jenkins DJ, Wolever TM, Taylor RH, Barker H, Fielden H, et al. (1981) Glycemic index of foods: a physiological basis for carbohydrate exchange. Am J Clin Nutr 34: 362–6.
8. Jenkins DJ, Kendall CW, Augustin LS, Franceschi S, Hamidi M, et al. (2002) Glycemic index: overview of implications in health and disease. Am J Clin Nutr 76: 266S–73S.
9. Du H, van der A DL, van Bakel MM, van der Kallen CJ, Blaak EE, et al. (2008) Glycemic index and glycemic load in relation to food and nutrient intake and metabolic risk factors in a Dutch population. Am J Clin Nutr 87: 655–61.
10. Larsen TM, Dalskov SM, van Baak M, Jebb SA, Papadaki A, et al. (2010) Diets with high or low protein content and glycemic index for weight-loss maintenance. N Engl J Med 363: 2102–13.
11. Choi Y, Giovannucci E, Lee JE (2012) Glycaemic index and glycaemic load in relation to risk of diabetes-related cancers: a meta-analysis. Br J Nutr 108: 1934–47.
12. Kaushik S, Wang JJ, Flood V, Tan JS, Barclay AW, et al. (2008) Dietary glycemic index and the risk of age-related macular degeneration. Am J Clin Nutr 88: 1104–10.
13. Nimptsch K, Kenfield S, Jensen MK, Stampfer MJ, Franz M, et al. (2011) Dietary glycemic index, glycemic load, insulin index, fiber and whole-grain intake in relation to risk of prostate cancer. Cancer Causes Control 22: 51–61.
14. Burger KN, Beulens JW, Boer JM, Spijkerman AM, van der A DL (2011) Dietary glycemic load and glycemic index and risk of coronary heart disease and stroke in Dutch men and women: the EPIC-MORGEN study. PLoS One 6: e25955.
15. Oba S, Nagata C, Nakamura K, Fujii K, Kawachi T, et al. (2010) Dietary glycemic index, glycemic load, and intake of carbohydrate and rice in relation to risk of mortality from stroke and its subtypes in Japanese men and women. Metabolism 59: 1574–82.
16. Levitan EB, Mittleman MA, Wolk A (2009) Dietary glycemic index, dietary glycemic load and mortality among men with established cardiovascular disease. Eur J Clin Nutr 63: 552–7.
17. Belle FN, Kampman E, McTiernan A, Bernstein L, Baumgartner K, et al. (2011) Dietary fiber, carbohydrates, glycemic index, and glycemic load in relation to breast cancer prognosis in the HEAL cohort. Cancer Epidemiol Biomarkers Prev 20: 890–9.
18. Burger KN, Beulens JW, van der Schouw YT, Sluijs I, Spijkerman AM, et al. (2012) Dietary fiber, carbohydrate quality and quantity, and mortality risk of individuals with diabetes mellitus. PLoS One 7: e43127.
19. Baer HJ, Glynn RJ, Hu FB, Hankinson SE, Willett WC, et al. (2011) Risk factors for mortality in the nurses' health study: a competing risks analysis. Am J Epidemiol 173: 319–29.
20. Estruch R, Ros E, Salas-Salvadó J, Covas MI, Corella D, et al. (2013) Primary prevention of cardiovascular disease with a Mediterranean diet. N Engl J Med 368: 1279–90.
21. Martínez-González MÁ, Corella D, Salas-Salvadó J, Ros E, Covas MI, et al. (2012) Cohort profile: design and methods of the PREDIMED study. Int J Epidemiol 41: 377–85.
22. Fernández-Ballart JD, Piñol JL, Zazpe I, Corella D, Carrasco P, et al. (2010) Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. Br J Nutr 103: 1808–16.
23. Estruch R, Martínez-González MA, Corella D, Salas-Salvadó J, Ruiz-Gutiérrez V, et al. (2006) Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. Ann Intern Med 145: 1–11.
24. Willett WC, Howe GR, Kushi LH (1997) Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr 65: 1120–8.
25. Louie JC, Flood V, Turner N, Everingham C, Gwynn J (2011) Methodology for adding glycemic index values to 24-hour recalls. Nutrition 27: 59–64.
26. Atkinson FS, Foster-Powell K, Brand-Miller JC (2008) International Tables of Glycemic Index and Glycemic Load Values: 2008. Diab Care 31: 2281–2283.
27. Sydney University Glycemic Index Research Service. The University of Sydney. Available: http://www.glycemicindex.com. Accessed 2013 Nov 20.
28. Du H, van der A DL, van Bakel MM, van der Kallen CJ, Blaak EE, et al. (2008) Glycemic index and glycemic load in relation to food and nutrient intake and metabolic risk factors in a Dutch population. Am J Clin Nutr 87: 655–61.
29. Elosua R, Garcia M, Aguilar A, Molina L, Covas MI, et al. (2000) Validation of the Minnesota Leisure Time Physical Activity Questionnaire In Spanish Women. Investigators of the MARATDON Group. Med Sci Sports Exerc 32: 1431–7.
30. Elosua R, Marrugat J, Molina L, Pons S, Pujol E (1994) Validation of the Minnesota Leisure Time Physical Activity Questionnaire in Spanish men. The MARATHOM Investigators. Am J Epidemiol 139: 1197–209.
31. Shekelle RB, Stamler J, Paul O, Shryock AM, Liu S, et al. (1982) Dietary lipids and serum cholesterol level: change in diet confounds the cross-sectional association. Am J Epidemiol 115: 506–514.
32. Lamberts SW, van den Beld AW, van der Lely AJ (1997) The endocrinology of aging. Science 278: 419–424.
33. Flegal KM, Kit BK, Orpana H, Graubard BI (2013) Association of all-cause mortality with overweight and obesity using standard body mass index categories: a systematic review and meta-analysis. JAMA 309: 71–82.
34. Masters RK, Reither EN, Powers DA, Yang YC, Burger AE, et al. (2013) The impact of obesity on US mortality levels: the importance of age and cohort factors in population estimates. Am J Public Health 103: 1895–901.
35. Dong JY, Zhang YH, Wang P, Qin LQ (2012) Meta-analysis of dietary glycemic load and glycemic index in relation to risk of coronary heart disease. Am J Cardiol 109: 1608–13.
36. Fan J, Song Y, Wang Y, Hui R, Zhang W (2012) Dietary glycemic index, glycemic load, and risk of coronary heart disease, stroke, and stroke mortality: a systematic review with meta-analysis. PLoS One 7: e52182.
37. Willett W, Manson J, Liu S (2002) Glycemic index, glycemic load, and risk of type 2 diabetes. Am J Clin Nutr 76: 274S–80S.
38. Ma XY, Liu JP, Song ZY (2012) Glycemic load, glycemic index and risk of cardiovascular diseases: meta-analyses of prospective studies. Atherosclerosis 223: 491–6.
39. Mirrahimi A, de Souza RJ, Chiavaroli L, Sievenpiper JL, Beyene J, et al. (2012) Associations of glycemic index and load with coronary heart disease events: a systematic review and meta-analysis of prospective cohorts. J Am Heart Assoc 1: e000752.
40. Dong JY, Zhang L, Zhang YH, Qin LQ (2011) Dietary glycaemic index and glycaemic load in relation to the risk of type 2 diabetes: a meta-analysis of prospective cohort studies. Br J Nutr 106: 1649–54.
41. Livesey G, Taylor R, Livesey H, Liu S (2013) Is there a dose-response relation of dietary glycemic load to risk of type 2 diabetes? Meta-analysis of prospective cohort studies. Am J Clin Nutr 97: 584–96.
42. Gnagnarella P, Gandini S, La Vecchia C, Maisonneuve P (2008) Glycemic index, glycemic load, and cancer risk: a meta-analysis. Am J Clin Nutr 87: 1793–801.
43. Dong JY, Qin LQ (2011) Dietary glycemic index, glycemic load, and risk of breast cancer: meta-analysis of prospective cohort studies. Breast Cancer Res Treat 126: 287–94.
44. Sluijs I, Beulens JW, van der Schouw YT, van der A DL, Buckland G, et al. (2013) Dietary glycemic index, glycemic load, and digestible carbohydrate intake

are not associated with risk of type 2 diabetes in eight European countries. J Nutr 143: 93–9.
45. Mulholland HG, Murray LJ, Cardwell CR, Cantwell MM (2009) Glycemic index, glycemic load, and risk of digestive tract neoplasms: a systematic review and meta-analysis. Am J Clin Nutr 89: 568–76.
46. Mulholland HG, Murray LJ, Cardwell CR, Cantwell MM (2008) Dietary glycaemic index, glycaemic load and breast cancer risk: a systematic review and meta-analysis. Br J Cancer 99: 1170–5.
47. Ludwig DS (2002) The glycemic index: physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. JAMA 287: 2414–23.
48. Huffman FG, Zarini GG, Cooper V (2010) Dietary glycemic index and load in relation to cardiovascular disease risk factors in Cuban American population. Int J Food Sci Nutr 61: 690–701.
49. Livesey G, Taylor R, Hulshof T, Howlett J (2008) Glycemic response and health—a systematic review and meta-analysis: relations between dietary glycemic properties and health outcomes. Am J Clin Nutr 87: 258S–268S.
50. Bautista-Castaño I, Sánchez-Villegas A, Estruch R, Martínez-González MA, Corella D, et al. (2013) Changes in bread consumption and 4-year changes in adiposity in Spanish subjects at high cardiovascular risk. Br J Nutr 110: 337–46.
51. Schwingshackl L, Hoffmann G (2013) Long-term effects of low glycemic index/load vs. high glycemic index/load diets on parameters of obesity and obesity-associated risks: a systematic review and meta-analysis. Nutr Metab Cardiovasc Dis 23: 699–706.
52. Levitan EB, Cook NR, Stampfer MJ, Ridker PM, Rexrode KM, et al. (2008) Dietary glycemic index, dietary glycemic load, blood lipids, and C-reactive protein. Metabolism 57: 437–43.
53. Mendall MA, Strachan DP, Butland BK, Ballam L, Morris J, et al. (2000) C-reactive protein: relation to total mortality, cardiovascular mortality and cardiovascular risk factors in men. Eur Heart J 21: 1584–90.
54. Gögebakan O, Kohl A, Osterhoff MA, van Baak MA, Jebb SA, et al. (2011) Effects of weight loss and long-term weight maintenance with diets varying in protein and glycemic index on cardiovascular risk factors: the diet, obesity, and genes (DiOGenes) study: a randomized, controlled trial. Circulation 124: 2829–38.
55. Bulló M, Casas R, Portillo MP, Basora J, Estruch R, et al. (2013) Dietary glycemic index/load and peripheral adipokines and inflammatory markers in elderly subjects at high cardiovascular risk. Nutr Metab Cardiovasc Dis 23: 443–50.
56. Bamia C, Trichopoulos D, Ferrari P, Overvad K, Bjerregaard L, et al. (2007) Dietary patterns and survival of older Europeans: the EPIC-Elderly Study (European Prospective Investigation into Cancer and Nutrition). Public Health Nutr 10: 590–8.
57. Rodríguez-Rejón AI, Castro-Quezada I, Ruano-Rodríguez C, Ruiz-López MD, Sánchez-Villegas A, et al. (2013) Effect of a Mediterranean diet intervention on dietary glycemic index and dietary glycemic load: the PREDIMED study (abstract). Ann Nutr Metab (suppl 1): 387.
58. Sofi F, Abbate R, Gensini GF, Casini A (2010) Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. Am J Clin Nutr 92: 1189–96.
59. Sofi F, Macchi C, Abbate R, Gensini GF, Casini A (2013) Mediterranean diet and health. Biofactors 39: 335–42.
60. Knoops KT, de Groot LC, Kromhout D, Perrin AE, Moreiras-Varela O, et al. (2004) Mediterranean diet, lifestyle factors, and 10-year mortality in elderly European men and women: the HALE project. JAMA 292: 1433–9.
61. Trichopoulou A, Orfanos P, Norat T, Bueno-de-Mesquita B, Ocké MC, et al. (2005) Modified Mediterranean diet and survival: EPIC-elderly prospective cohort study. BMJ 330: 991.
62. Mitrou PN, Kipnis V, Thiébaut AC, Reedy J, Subar AF, et al. (2007) Mediterranean dietary pattern and prediction of all-cause mortality in a US population: results from the NIH-AARP Diet and Health Study. Arch Intern Med 167: 2461–8.
63. Martínez-González MA, García-Arellano A, Toledo E, Salas-Salvadó J, Buil-Cosiales P, et al. (2012) A 14-item Mediterranean diet assessment tool and obesity indexes among high-risk subjects: the PREDIMED trial. PLoS One 7: e43134.
64. García-Calzón S, Gea A, Razquin C, Corella D, Lamuela-Raventós RM, et al. (2014) Longitudinal association of telomere length and obesity indices in an intervention study with a Mediterranean diet: the PREDIMED-NAVARRA trial. Int J Obes (Lond) 38: 177–82.
65. Bulló M, Garcia-Aloy M, Martínez-González MA, Corella D, Fernández-Ballart JD, et al. (2011) Association between a healthy lifestyle and general obesity and abdominal obesity in an elderly population at high cardiovascular risk. Prev Med 53: 155–61.
66. Salas-Salvadó J, Bulló M, Babio N, Martínez-González MÁ, Ibarrola-Jurado N, et al. (2011) Reduction in the incidence of type 2 diabetes with the Mediterranean diet: results of the PREDIMED-Reus nutrition intervention randomized trial. Diabetes Care 34: 14–9.
67. Rossi M, Turati F, Lagiou P, Trichopoulos D, Augustin LS, et al. (2013) Mediterranean diet and glycaemic load in relation to incidence of type 2 diabetes: results from the Greek cohort of the population-based European Prospective Investigation into Cancer and Nutrition (EPIC). Diabetologia 56: 2405–13.
68. Serra-Majem L, Roman B, Estruch R (2006) Scientific evidence of interventions using the Mediterranean diet: a systematic review. Nutr Rev 64: S27–47.
69. Salas-Salvadó J, Fernández-Ballart J, Ros E, Martínez-González MA, Fitó M, et al. (2008) Effect of a Mediterranean diet supplemented with nuts on metabolic syndrome status: one-year results of the PREDIMED randomized trial. Arch Intern Med 168: 2449–58.
70. Kristo AS, Matthan NR, Lichtenstein AH (2013) Effect of diets differing in glycemic index and glycemic load on cardiovascular risk factors: review of randomized controlled-feeding trials. Nutrients 5: 1071–80.
71. Wolever TMS (2006) Physiological mechanisms and observed health impacts related to the glycaemic index: some observations. Int J Obes 30: S72–S78.