Higher dietary glycemic index and glycemic load values increase the risk of osteoporotic fracture in the PREvención con DIeta MEDiterránea (PREDIMED)-Reus trial

Jesús Francisco García-Gavilán,[1,2] Mònica Bulló,[1,2] Lucia Camacho-Barcia,[1,2] Nuria Rosique-Esteban,[1,2] Pablo Hernández-Alonso,[1,2] Josep Basora,[1] Miguel Angel Martínez-González,[2,3] Ramón Estruch,[2,4] Montserrat Fitó,[2,5] and Jordi Salas-Salvadó[1,2]

[1]Human Nutrition Unit, Biochemistry and Biotechnology Department, Faculty of Medicine and Health Sciences, University Hospital of Sant Joan de Reus, IISPV, Universitat Rovira i Virgili, Reus, Spain; [2]CIBER Physiopathology of Obesity and Nutrition, Instituto de Salud Carlos III, Madrid, Spain; [3]Medical School, Department of Preventive Medicine and Public Health, University of Navarra, Pamplona, Spain; [4]Department of Internal Medicine, August Pi i Sunyer Institute of Biomedical Research (IDIBAPS), Hospital Clinic, University of Barcelona, Barcelona, Spain; and [5]Cardiovascular Risk and Nutrition (Regicor Study Group), Hospital del Mar Medical Research Institute, Barcelona, Spain

ABSTRACT

Background: High glucose and insulin concentrations seem to have a negative impact on bone health. However, the relation between the dietary glycemic index (DGI) and the dietary glycemic load (DGL), which has proved to be effective at modulating blood glucose concentrations after carbohydrate consumption, has yet to be explored in relation to bone health.

Objective: The aim of the study was to examine the associations between the DGI or DGL and the risk of osteoporotic-related fractures in an elderly Mediterranean population.

Design: The study was conducted in 870 subjects aged 55–80 y at high cardiovascular risk participating in the PREvención con DIeta MEDiterránea (PREDIMED)-Reus study. The DGI and DGL were estimated from validated food frequency questionnaires with the use of the international glycemic index and glycemic load values, with glucose as reference. Data on osteoporotic fractures were acquired from a systematic review of medical records. We used Cox proportional hazard models to assess the risk of osteoporotic fracture according to tertiles of average DGI and DGL.

Results: A total of 114 new cases of osteoporotic-related fractures were documented after a mean follow-up of 8.9 y. Participants in the highest tertile of DGI and DGL had a significantly higher risk of osteoporotic fractures than those in the lowest tertile after adjusting for potential confounders (HR: 1.80; 95% CI: 1.03, 3.15 and HR: 3.20; 95% CI: 1.25, 8.18, respectively).

Conclusions: A high DGI and DGL are associated with a higher risk of osteoporosis-related fractures in an elderly Mediterranean population at high cardiovascular risk. This trial was registered at isrctn.com as ISRCTN35739639. *Am J Clin Nutr* 2018;107:1–8.

Keywords: glycemic index, glycemic load, fractures, bone, Mediterranean population

INTRODUCTION

Osteoporosis is a disease characterized by compromised bone strength and increased risk of fracture. During aging, bone mineral density decreases and the risk of osteoporotic fractures increases. Other factors such as genetics, specific diseases, medication, and lifestyle habits also help increase the risk of fracture. Many studies have shown that diabetes, controlled and uncontrolled, has a negative impact on risk of fractures although contradictory results on its impact on bone mineral density are found in subjects with type 2 diabetes (T2D) compared with control subjects (1–3). This suggests a complex pathophysiology in diabetes-related bone disease that includes bone microarchitecture and compromised repair mechanisms (3) that could be partially mediated by the associated hyperglycemia (1, 2, 4). Indeed, high blood glucose concentrations impact bone health in different ways, including the induction of inflammation and oxidative stress (5, 6). Hyperglycemia

Supported in part by the Spanish Ministry of Health (ISCIII), PI1001407, Thematic Network G03/140, RD06/0045, FEDER (Fondo Europeo de Desarrollo Regional), and the Centre Català de la Nutrició de l'Institut d'Estudis Catalans.

No funding sources played any role in the design, collection, analysis, or interpretation of the data or in the decision to submit the manuscript for publication. Consorcio Ciber M.P. (CIBERobn) is an initiative of ISCIII, Spain.

Supplemental Table 1 and Supplemental Figure 1 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn.

Address correspondence to MB (e-mail: monica.bullo@urv.cat).

Abbreviations used: DGI, dietary glycemic index; DGL, dietary glycemic load; FFQ, food frequency questionnaire; GI, glycemic index; ICD-CM, International Classification of Diseases, Clinical Modification; MedDiet, Mediterranean diet; PREDIMED, PREvención con DIeta MEDiterránea; T2D, type 2 diabetes mellitus.

Received October 19, 2017. Accepted for publication February 16, 2018.

First published online 0, 2018; doi: https://doi.org/10.1093/ajcn/nqy043.

reduces osteoblast activity (6), inhibits genes involved in osteoblast differentiation and maturation (6), and induces acidosis, which enhances bone resorption (7). Moreover, hyperglycemia can induce hypocalcemia by increasing the excretion of urinary calcium, and it also interferes with the parathyroid hormone and vitamin D receptors (8). In this regard, diets that lower postprandial hyperglycemia may have an important impact on bone health and decrease the risk of osteoporotic fractures.

Some clinical trials have reported a significant increase of blood glucose and insulin concentrations after the intake of diets high in dietary glycemic index (DGI) and dietary glycemic load (DGL) (9, 10). Low–glycemic index (GI) diets have also proved to be postprandially effective at attenuating any increase in blood glucose after carbohydrate intake (11). Similarly, low-DGI diets are associated with a healthier inflammatory and oxidative profile, and lowered glycated hemoglobin (11–13). Nevertheless, the effect of DGI and DGL on the risk of osteoporosis-related fractures has not been studied.

Our aim was to explore the association of the DGI and DGL with the risk of osteoporotic fractures in a subsample of middle-aged and elderly Mediterranean participants of the PREvención con DIeta MEDiterránea (PREDIMED) trial. We hypothesized that higher DGI and DGL increase the risk of osteoporotic fractures.

METHODS

Study design and subjects

This analysis was nested in the PREDIMED study, a multicenter and controlled parallel group trial conducted from 2003 to 2010. It includes all participants recruited at the PREDIMED-Reus center ($n = 870$) with a follow-up to assess outcome that lasted until August 2015. The study participants were women aged 60–80 y and men aged 55–80 y at high cardiovascular risk, with either T2D or ≥3 of the following criteria: hypertension (systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg, or antihypertensive drugs), hypercholesterolemia (high LDL cholesterol ≥160 mg/dL or medication), low HDL cholesterol (≤50 mg/dL in women or ≤40 mg/dL in men), overweight or obesity [BMI (in kg/m^2) ≥25], current smoking, or family history of premature coronary heart disease. They were allocated to 1 of the 3 intervention groups: a Mediterranean diet (MedDiet) supplemented with extra-virgin olive oil (50 mL/d), a MedDiet supplemented with mixed nuts (30 g/d), or advice on a low-fat diet (LFD group). Participants with a BMI >40, severe chronic illness, drug or alcohol addiction, or a low predicted likelihood of changing dietary habits according to Prochaska and DiClemente's stages-of-change model (14) were excluded from the study.

The protocol was approved by the institutional review boards of the respective centers, and all participants provided written informed consent to participate. The PREDIMED trial was registered at http://www.isrctn.com/ as ISRCTN35739639. A detailed protocol has been published elsewhere (15) and it is available at http://www.predimed.es.

Bone fracture assessment

Osteoporotic fractures were adjudicated according to the criteria defined by Warriner and coworkers. Sex, age, and race were all taken into account and scores ranged from 1 to 9, with the higher scores representing those fractures most likely due to osteoporosis (16). In accordance with the International Classification of Diseases, Clinical Modification (ICD-CM), we excluded open clavicle (ICD-CM 810.1–810.3), phalange (ICD-CM 816.1–816.13 and 826.0–826.1), tarsal/metatarsal (ICD-CM 825.0–825.39), scapula (ICD-CM 811.0–811.19), and skull/facial (ICD-CM 800.00–804.99) fractures (16). Incident cases of osteoporotic fractures were initially identified from a systematic, comprehensive, and standardized annual review of all the outpatient and inpatient medical records for each participant. Information on osteoporotic fractures was updated yearly through the use of medical records. An independent researcher, also blinded to the intervention group, confirmed all fracture events.

Measurements

Data regarding lifestyle habits, medical conditions, and medication use were recorded yearly from baseline to the end of the intervention. Weight and height were measured with the use of calibrated scales and a wall-mounted stadiometer and waist circumference was measured midway between the lowest rib and the iliac crest. Blood pressure was measured with the use of a validated oscillometer (Omron HEM705CP; Hoofddorp, The Netherlands). Fasting blood samples for subsequent biochemical analysis were taken and frozen until use at –80°C. The validated Spanish version of the Minnesota Leisure-Time Physical Activity questionnaire was administered at baseline and yearly (17).

Dietary assessment

A semi-quantitative validated food frequency questionnaire (FFQ) with 137 items was given to all participants at baseline and yearly till the end of the intervention (18). The reported frequencies of food consumption were converted into number of intakes per day and multiplied by the weight of the portion size. Energy and all nutrient intake were estimated via multiplying each FFQ item by its nutritional composition according to the Spanish food composition tables (19, 20). GI values for each food were extracted from international GI and glycemic load values (21) with glucose as a reference scale. For foods that were not in the tables, the mean was calculated for similar foods that were present in the FFQ.

The total DGL of each meal was determined by multiplying the total carbohydrates of a specified serving size of the food, the total number of food portions consumed per day, and its specific GI. DGI was calculated by dividing glycemic load by total available carbohydrate intake and multiplying the result by 100 (22, 23). Reproducibility and relative validity, explored via the intraclass correlation coefficient, were 0.321 and 0.244, respectively, for DGI ($P < 0.001$), and 0.846 and 0.525, respectively, for DGL ($P < 0.001$) and similar to those of other studies (22) although the relative validity for DGI was somewhat low. A validated 14-item MedDiet screening tool was administered to assess the degree of adherence to the MedDiet (24).

Statistical analysis

The baseline characteristics of participants are shown as means ± SD and percentages (n). All food consumption data over the

study period (DGI, DGL, alcohol, total energy, SFAs, MUFAs, PUFAs, fiber, protein, calcium, and vitamin D) were estimated as the cumulative average from the baseline to the end of the intervention period or to the last follow-up FFQ before the occurrence of fractures. No significant interaction was found between the exposure variables and T2D.

Participants were divided into tertiles of cumulative DGI and DGL via the mean value of all the FFQs available from the baseline visit to the last FFQ before the fracture event, participant death (for any reason) or the end of follow-up when participants had not suffered a fracture. Follow-up time was estimated as the interval from the baseline visit to the date of the fracture event, death, or end of follow-up. Multivariate Cox regression analysis was used to assess the relations between cumulative DGI or DGL in tertiles and the risk of osteoporotic fractures. To assess the quadratic trend (*P*-q trend), we assigned the median intake within each tertile and included the variables into the model as continuous using a polynomial analysis of the multivariate Cox regression models. In addition, we estimated the risk of osteoporotic fracture using the cumulative DGI and DGL as continuous variables. Results are expressed as HRs and 95% CIs. Covariates were selected based on medical and biological plausibility. Model 1 was adjusted for age (years), sex, BMI, educational level (illiterate or primary education, secondary education, or academic or graduate), leisure time physical activity (Metabolic Equivalent of Task-minutes per day), the intervention group, and smoking status (never, former, or current smoker). Model 2 was also adjusted for the prevalence of T2D (yes or no), prevalence of previous fractures (yes or no), use of insulin (yes or no), use of oral antidiabetic drugs (yes or no), diuretic drugs (yes or no), glucocorticoids (yes or no), osteoporotic drugs (yes or no), use of vitamin D supplements (yes or no), and use of estrogens (yes or no). Model 3 was also adjusted for the yearly updated measurements of intake of alcohol (grams per day) (continuous, adding a quadratic term), total energy (kilocalories per day), SFAs (grams per day), MUFAs (grams per day), PUFAs (grams per day), fiber (grams per day), protein (grams per day), calcium (milligrams per day), and vitamin D (micrograms per day). In addition, we conducted 2 different sensitivity analyses, first excluding the early cases of incident osteoporosis (accounted during the first year of the study) and second by quartiles. All statistical significance levels were set at $P < 0.05$ for all tests for bilateral contrast. Statistical analyses were carried out with SPSS 21.0 for Windows (IBM, Chicago, IL).

RESULTS

We documented 114 incident cases of osteoporosis-related fractures during a median follow-up of 8.9 y (**Supplemental Figure 1**). **Tables 1** and **2** show the anthropometric and dietary

TABLE 1
Baseline anthropometric characteristics of study participants according to tertiles of cumulative average glycemic index and glycemic load[1]

	Glycemic index				Glycemic load			
Variable	T1 ($n = 290$)	T2 ($n = 290$)	T3 ($n = 290$)	*P* value	T1 ($n = 290$)	T2 ($n = 290$)	T3 ($n = 290$)	*P* value
Age, y	68 ± 6	68 ± 6	67 ± 6	0.273	68 ± 6	68 ± 6	67 ± 6	0.115
Women, % (*n*)	66.6 (193)	56.9 (165)	42.1 (122)	<0.001	69.7 (202)	59.3 (172)	36.6 (106)	<0.001
Body weight, kg	74.9 ± 10.9	75.0 ± 10.5	77.6 ± 10.6	0.003	75.1 ± 10.8	75.5 ± 10.8	77.0 ± 10.6	0.072
BMI, kg/m^2	29.8 ± 3.5	29.4 ± 3.3	29.7 ± 3.1	0.214	30.0 ± 3.4	29.7 ± 3.4	29.3 ± 3.0	0.035
Waist circumference, cm	100.4 ± 8.7	100.6 ± 8.8	102.6 ± 8.8	0.003	100.8 ± 8.7	101.2 ± 9.1	101.6 ± 8.8	0.617
Leisure-time energy expenditure in physical activity, MET min/d	258.9 ± 229.5	273.4 ± 294.6	253.2 ± 261.9	0.634	229.1 ± 213.0	257.8 ± 251.0	298.7 ± 312.2	0.006
Smoking status, % (never, current, former)	69.0, 7.6, 23.4	63.4, 12.8, 23.8	53.4, 15.9, 30.7	0.002	70.3, 8.3, 21.4	67.9, 9.0, 23.1	47.6, 19.0, 33.5	<0.001
Educational level, % (*n*)				0.781				0.412
Primary education	76.2 (221)	75.9 (220)	76.2 (221)		77.6 (225)	77.6 (225)	73.1 (212)	
Secondary education	16.6 (48)	17.6 (51)	18.6 (54)		15.5 (45)	16.2 (47)	21.0 (61)	
Academic/graduate	7.2 (21)	6.5 (19)	5.2 (15)		6.9 (20)	6.2 (18)	5.9 (17)	
History of osteoporotic fractures, % (*n*)	16.6 (48)	14.1 (41)	21.4 (62)	0.064	16.9 (49)	17.2 (50)	17.9 (52)	0.945
Diabetes, % (*n*)	68.3 (198)	57.2 (166)	30.3 (88)	<0.001	63.8 (185)	56.9 (165)	35.2 (102)	<0.001
Medication use, % (*n*)								
Diuretics	27.6 (80)	21.4 (62)	25.5 (74)	0.212	28.3 (82)	24.5 (71)	21.7 (63)	0.186
Insulin	9.0 (26)	6.9 (20)	1.7 (5)	0.001	9.0 (26)	6.2 (18)	2.4 (7)	0.003
Oral glucocorticoids	1.4 (4)	1.0 (3)	1.7 (5)	0.776	1.7 (5)	0.3 (1)	2.1 (6)	0.170
Osteoporosis drugs	14.8 (43)	10.3 (30)	8.6 (25)	0.051	13.1 (38)	14.1 (41)	6.6 (19)	0.007
Oral antidiabetic drugs	49.3 (143)	36.9 (107)	17.6 (51)	<0.001	43.8 (127)	38.3 (111)	21.7 (63)	<0.001
Estrogens	3.4 (10)	2.8 (8)	0.7 (2)	0.256	3.4 (10)	2.1 (6)	1.4 (4)	0.580
Vitamin D supplement	18.4 (45)	10.7 (31)	10.7 (31)	0.124	13.8 (40)	14.8 (43)	8.3 (24)	0.036
Intervention group, % (*n*)				0.678				0.486
MedDiet + EVOO	33.8 (98)	34.1 (99)	32.4 (94)		32.8 (95)	36.9 (107)	30.7 (89)	
MedDiet + nuts	35.9 (104)	31.8 (92)	32.1 (93)		32.1 (93)	31.4 (91)	36.2 (105)	
Control diet	30.3 (88)	34.1 (99)	35.5 (103)		35.1 (102)	31.7 (92)	33.1 (96)	

[1]Data are expressed as means ± SD or % (*n*) unless otherwise indicated. *P* value was calculated with ANOVA test (quantitative variables) and chi-square test (qualitative variables). EVOO, extra virgin olive oil; MedDiet, Mediterranean Diet; MET, Metabolic Equivalent of Task; T, tertile.

TABLE 2
Baseline dietary characteristics of study participants according to tertiles of glycemic index and glycemic load[1]

	Glycemic index				Glycemic load			
Variable	T1 ($n = 290$)	T2 ($n = 290$)	T3 ($n = 290$)	*P* value	T1 ($n = 290$)	T2 ($n = 290$)	T3 ($n = 290$)	*P* value
Nutrients								
Total energy intake, kcal/d	2167 ± 515	2355 ± 591	2410 ± 637	<0.001	1947 ± 427	2294 ± 443	2692 ± 631	<0.001
Proteins, g/d	94.7 ± 20.9	97.7 ± 22.7	92.2 ± 22.9	0.011	85.7 ± 19.4	95.3 ± 20.2	103.6 ± 23.4	<0.001
Carbohydrates, g/d	210.3 ± 63.0	235.8 ± 72.6	248.2 ± 80.2	<0.001	182.1 ± 49.0	227.6 ± 51.2	285.4 ± 77.7	<0.001
Total fat, g/d	100.5 ± 28.5	107.6 ± 30.6	106.4 ± 31.7	0.012	92.5 ± 24.6	105.9 ± 27.9	116.1 ± 33.4	<0.001
SFAs, g/d	26.2 ± 8.8	28.4 ± 9.7	27.6 ± 9.5	0.015	23.5 ± 6.9	27.7 ± 8.6	31.0 ± 10.6	<0.001
MUFAs, g/d	50.3 ± 15.6	53.6 ± 16.0	53.5 ± 17.1	0.022	47.4 ± 14.3	52.8 ± 15.3	57.2 ± 17.6	<0.001
PUFAs, g/d	15.7 ± 5.8	16.9 ± 6.4	17.0 ± 6.5	0.022	14.0 ± 4.8	16.8 ± 6.0	18.7 ± 6.9	<0.001
Fiber, g/d	23.2 ± 7.4	23.8 ± 7.9	23.4 ± 7.9	0.597	20.9 ± 6.8	23.6 ± 7.1	25.8 ± 8.5	<0.001
Alcohol, g/d	6.2 ± 11.8	7.5 ± 12.2	12.7 ± 16.0	<0.001	6.4 ± 12.3	7.0 ± 11.4	13.0 ± 16.0	<0.001
Vitamin D, μg/d	5.9 ± 3.4	6.0 ± 3.6	5.8 ± 3.2	0.783	5.3 ± 3.1	5.9 ± 3.5	6.4 ± 3.5	<0.001
Calcium, mg/d	1100.7 ± 362.0	1055.1 ± 339.2	932.4 ± 349.2	<0.001	955.1 ± 333.3	1020.6 ± 360.1	1112.5 ± 360.5	<0.001
Dietary glycemic index	44.6 ± 3.2	47.7 ± 2.9	51.1 ± 3.6	<0.001	45.7 ± 4.0	47.6 ± 3.4	50.1 ± 3.9	<0.001
Dietary glycemic load	94.5 ± 31.6	113.0 ± 37.7	127.5 ± 43.1	<0.001	83.4 ± 24.6	108.4 ± 26.2	143.2 ± 41.5	<0.001
Food								
Olive oil, g/d	40.5 ± 17.2	41.4 ± 17.3	41.8 ± 18.4	0.648	40.3 ± 17.2	41.1 ± 17.5	42.3 ± 18.3	0.399
Legumes, g/d	18.2 ± 8.4	18.4 ± 9.5	16.7 ± 7.6	0.035	15.6 ± 7.6	18.4 ± 8.4	19.2 ± 9.3	<0.001
Vegetables, g/d	325.2 ± 144.0	313.3 ± 128.4	281.4 ± 114.1	<0.001	307.5 ± 144.0	305.8 ± 126.5	306.8 ± 120.6	0.987
Cereals, g/d	213.6 ± 83.1	257.2 ± 92.9	277.9 ± 103.1	<0.001	195.2 ± 68.1	248.5 ± 76.1	305.1 ± 108.8	<0.001
Fruit, g/d	340.1 ± 187.2	299.8 ± 148.9	294.6 ± 175.2	0.002	292.7 ± 163.0	319.0 ± 166.3	322.8 ± 185.4	0.072
Dairy, g/d	432.1 ± 219.0	379.9 ± 196.0	292.2 ± 191.1	<0.001	359.3 ± 205.7	360.1 ± 206.2	384.9 ± 218.3	0.249
Meat, g/d	142.4 ± 56.1	151.6 ± 59.9	141.7 ± 60.2	0.074	133.5 ± 55.9	147.6 ± 53.4	154.7 ± 64.9	<0.001
Fish, g/d	106.0 ± 43.9	103.3 ± 44.2	97.2 ± 41.9	0.044	101.1 ± 42.8	102.0 ± 43.0	103.3 ± 44.7	0.824
Nuts, g/d	12.7 ± 13.8	13.2 ± 15.3	12.7 ± 14.3	0.882	10.6 ± 12.8	13.9 ± 15.2	14.2 ± 15.2	0.004
MedDiet score, 14-point score	8.4 ± 1.9	8.2 ± 2.0	8.1 ± 1.8	0.041	8.2 ± 1.7	8.3 ± 1.9	8.2 ± 2.1	0.795

[1]Data are expressed as means ± SDs. *P* value was calculated with ANOVA test. MedDiet score, Mediterranean Diet score; T, tertile.

TABLE 3
Risk of osteoporotic fracture according to quality and quantity of carbohydrate consumption[1]

	T1 ($n = 290$)	T2 ($n = 290$)	T3 ($n = 290$)	P q-trend	1-point increase
Mean dietary glycemic index	44.85 ± 1.33	47.91 ± 0.81	51.54 ± 2.03		
Fracture event, % (n)	16.20 (47)	9.70 (28)	13.40 (39)		
Crude model	1 (Ref.)	0.57 (0.36, 0.92)	0.83 (0.54, 1.27)	0.066	0.97 (0.91, 1.03)
Multivariable model[2]	1 (Ref.)	0.65 (0.40, 1.04)	1.06 (0.69, 1.64)	0.101	1.00 (0.94, 1.07)
Multivariable model[3]	1 (Ref.)	0.69 (0.43, 1.10)	1.14 (0.70, 1.83)	0.132	1.01 (0.94, 1.09)
Multivariable model[4]	1 (Ref.)	0.84 (0.51, 1.40)	1.80 (1.03, 3.15)	0.016	1.10 (1.01, 1.20)
Mean glycemic load	81.18 ± 9.55	104.60 ± 5.91	140.07 ± 20.52		
Fracture event, % (n)	16.60 (48)	11.70 (34)	11.00 (32)		
Crude model	1 (Ref.)	0.69 (0.44, 1.07)	0.67 (0.43, 1.04)	0.122	0.99 (0.98, 1.00)
Multivariable model[2]	1 (Ref.)	0.75 (0.48, 1.16)	0.94 (0.59, 1.50)	0.411	1.00 (0.99, 1.00)
Multivariable model[3]	1 (Ref.)	0.77 (0.50, 1.21)	1.04 (0.63, 1.70)	0.418	1.00 (0.99, 1.00)
Multivariable model[4]	1 (Ref.)	1.26 (0.70, 2.29)	3.20 (1.25, 8.18)	0.022	1.03 (0.99, 1.07)

[1]Cox regression models were used to evaluate the risk of osteoporotic fracture event by tertiles of the dietary glycemic index and tertiles of glycemic load. Results are expressed as HRs (95% CIs), means ± SDs, or % (n). Ref., reference; T, tertile.

[2]Model: Adjusted for age (years), sex, BMI (kg/m²), educational level (illiterate/primary education, secondary education, academic/graduate), leisure time physical activity [Metabolic Equivalent of Task-minutes per day], the intervention group, and smoking status (never, former, or current smoker).

[3]Model: In addition, adjusted for prevalence of diabetes (yes or no), prevalence of previous fractures (yes or no), use of insulin (yes or no), use of oral antidiabetic drugs (yes or no), use of diuretic drugs (yes or no), use of glucocorticoid drugs (yes or no), use of osteoporotic drugs (yes or no), use of vitamin D supplements (yes or no), and use of estrogens (yes or no).

[4]Model: In addition, adjusted for alcohol intake (grams per day) (continuous, adding a quadratic term), total energy intake (kilocalories per day), SFA intake (grams per day), MUFA intake (grams per day), PUFA intake (grams per day), fiber intake (grams per day), protein intake (grams per day), calcium intake (milligrams per day), and vitamin D intake (micrograms per day).

characteristics of the study participants at baseline, according to tertiles of DGI and DGL. Participants in the lowest tertile of DGI were likely to be women and nonsmokers, and had a lower waist circumference, body weight, and higher prevalence of T2D compared with the participants in the highest tertile. There were no significant differences in age, BMI, physical activity, intervention group, education level, medication (except for insulin, which was significantly lower in the third tertile than in the other tertiles), previous fractures, fiber, or vitamin D intake between tertiles of DGI. Likewise, there were no significant differences in age, waist circumference, intervention group, education level, previous fractures, or dairy product intake between tertiles of DGL.

In **Table 3** we show the HRs and 95% CIs for the association between DGI or DGL and osteoporotic fractures. We observed that the risk of osteoporosis-related fractures was higher in the highest tertile of DGI and DGL than in the reference tertile (DGI HR: 1.80; 95% CI: 1.03, 3.15; DGL HR: 3.20; 95% CI: 1.25, 8.18) after adjusting for anthropometric, demographic, and medical variables such as sex, age, BMI, physical activity, prevalence of T2D, previous fractures, use of insulin, glucocorticoids, or osteoporotic drugs, and nutritional variables such as total energy intake, consumption of alcohol, protein, fiber, calcium, or vitamin D. The marked amplification of the effect of DGL in the risk of bone fractures observed in the fully adjusted model was mainly driven by total energy intake. When analyzed as a continuous variable, a significant increased risk of osteoporotic fractures was observed per 1-point increase in DGI (HR: 1.10; 95% CI: 1.01, 1.20, P value = 0.033), showing a trend to statistical significance for DGL (HR: 1.03; 95% CI: 0.99, 1.07, P value = 0.076). Similarly, when we included fasting glucose concentrations into the full adjusted model instead of the presence of T2D, the association between DGI and the risk of osteoporotic fracture remained significant (HR: 1.10; 95% CI: 1.01, 1.20, P value = 0.032).

The results of several sensitivity analyses were consistent with the findings of the primary analysis. When the events observed in the first year were excluded, a trend to a higher risk of osteoporotic fractures was observed for both higher DGI and DGL (DGI HR: 1.62; 95% CI: 0.90, 2.93; DGL HR: 2.56; 95% CI: 0.98, 6.71). When participants were analyzed by quartiles of exposure variables, results did not reach statistical significance (**Supplemental Table 1**).

DISCUSSION

In this prospective study conducted in elderly participants at high cardiovascular risk, we demonstrated for the first time, to our knowledge, that higher DGI and DGL are associated with an increased risk of osteoporotic fractures, even after adjusting for potential confounders.

The concept of DGI was introduced by Jenkins et al. (25) in 1981 to measure the quality of carbohydrates in relation to the availability of glucose after food ingestion. DGL was later defined as the mathematical product of the DGI and the carbohydrate content of the specific food and is considered the overall indicator of the glucose response and insulin demand induced by a serving of food (23). Since then, several epidemiologic and clinical trials have analyzed the relation between DGI or DGL and several chronic diseases. A meta-analysis of 37 observational studies concluded that diets with high DGI or DGL increased the risk of T2D, heart disease, and several types of cancer (26). Because bone remodeling processes have mechanisms in common with other chronic diseases such as T2D or cardiovascular diseases (3), the inverse association between low DGI or DGL and the risk of osteoporotic fractures observed in our study is not surprising.

T2D is commonly associated with an increased risk of osteoporosis-related fractures (3, 27) and many studies have

demonstrated a detrimental role of hyperglycemia uncontrolled in bone metabolism (4, 28). In this regard, animal and in vitro studies have demonstrated that high glucose concentrations modify the osteoblast and osteoclast function by overstimulating the insulin signaling pathways and have an inhibitory effect on osteoblast cells that leads to abnormal bone growth and higher bone fragility (5, 29–32). Hyperglycemia also stimulates the production of advanced glycation end products which increase the production of cross-links between collagens and the fragility of the human bone (33), and induce cellular apoptosis and inflammation (34–37). Inflammation and oxidation, both of which are involved in the physiopathology of cardiometabolic disorders, have a deleterious effect on bone metabolism. High serum IL-6 concentrations have been associated with osteoporosis (38, 39) and an increased incidence of nontraumatic fractures in older white women (38, 40, 41). Similarly, in vitro studies have demonstrated that IL-6 has a stimulatory effect on osteoclasts, thereby increasing the rates of bone remodeling and bone loss (42, 43). TNFα was also related to an increased risk of fracture (44), stimulating bone resorption and inhibiting bone formation in vitro (45). Other cytokines, commonly recognized as a key regulator of bone metabolism, such as the osteoprotegerin/receptor activator of NF-κB/receptor activator of NF-κB ligand (OPG/RANK/RANKL) triad and osteocalcin, have recently been related to insulin resistance and glucose metabolism (46–49). The modulatory effect on hyperglycemia, insulin resistance, inflammation, and oxidative stress attributed to low-DGI and -DGL diets could explain our findings (11, 22). We found that participants in the highest tertile of DGI and DGL had an increased risk of osteoporotic fracture, independent of other potential confounders including sex or T2D. Similarly, the continuous model showed a 10% increased risk of fractures per 1-point increase in DGI. Since the association remained significant even after adjusting for fasting plasma glucose, our results support the contention that other factors beyond glucose per se may play a significant role in bone health.

Some limitations of our study need to be mentioned. First, the lack of information regarding inflammation and oxidative stress in our study population does not enable us to propose any potential mechanisms to explain our findings. Second, since the study was conducted in elderly subjects at high risk of cardiovascular disease from a Mediterranean region following a diet rich in vegetables and fruit in only one recruiting center from the PREDIMED study, our results could not be generalized to other populations. In a similar manner, DGI and DGL are lower than those in other populations and differences across tertiles are modest. These differences could be probably explained by the fact that MedDiet is a plant-based dietary pattern rich in low–glycemic index food such as whole grains, vegetables, nuts, legumes, and olive oil. Other populations following high-DGI and -DGL diets would gain much more benefit in reducing their DGI and DGL to decrease their risk of osteoporotic fractures. Third, since the study was observational, we cannot discard some residual confounding, although the analysis was adjusted for potential confounders. Fourth, some dietary measurement errors derived from collecting the dietary data via validated FFQs that were not designed to estimate DGI and DGL. Finally, estimations of DGI values obtained from international DGI tables could be misrepresented because food properties may fluctuate across countries. The major strengths of the study are the long follow-up period and the use of repeated dietary measurements, which allow us to reduce the random measurement error produced by within-person variation and dietary changes during follow-up; the control for many potential confounding variables; the use of an objective score to identify fractures although the weakness of this system is that it is based on fracture groups which were identified by diagnostic codes; and the inclusion of sensitivity analyses with similar trends.

In conclusion, our results suggest that high DGI and high DGL are associated with a major risk of osteoporosis-related fractures in an elderly Mediterranean population at high cardiovascular risk. Further studies are needed to confirm this potential deleterious effect of high DGI and DGL diets on bone metabolism and osteoporotic fractures and to determine the potential mechanisms underlying these findings.

We thank the PREDIMED staff, including all the primary center personnel, for their fantastic and animated collaboration in the PREDIMED study. Also, we thank all support contributed by Sant Joan Hospital, Rovira i Virgili University (URV), and Pere Virgili Institute (IISPV). The Fundación Patrimonio Comunal Olivarero and Hojiblanca SA (Málaga, Spain), California Walnut Commission (Sacramento, CA), Borges SA (Reus, Spain), and Morella Nuts SA (Reus, Spain) donated the olive oil, walnuts, almonds, and hazelnuts, respectively, used in the study. We thank them for their contribution and collaboration.

The authors' contributions were as follows—MB, MAM-G, RE, MF, JB, and JS-S: contributed to the conception, design, and implementation of the project; JFG-G, LC-B, NR-E, PH-A, and MB: contributed to data collection and analytic procedures, conducted the statistical analysis, interpreted data, and wrote the manuscript; and authors: read, reviewed, and approved the final version of the manuscript. JSS reports grants from RTIC G03/140 ISCIII, Spain, grants from CIBER obn ISCIII, Spain, other from California Walnut Commission, Sacramento, CA, other from Patrimonio Comunal Olivarero, Spain, other from La Morella Nuts, Spain, other from Borges SA, Spain, other from Nut and Dried Fruit Foundation, and personal fees from Nuts for Life, during the conduct of the study; personal fees from Danone SA, personal fees from Font Vella Lanjaron, personal fees from Eroski Distributors, and personal fees from Instituto Danone, outside the submitted work. RE reports grants from Spanish Institute of Health "Carlos III", other from Patrimonio Comunal Olivarero, Spain, other from California Walnut Commission, Spain, other from Borges SA, Spain, and nonfinancial support from Fundacion Bosch i Gimpera, Spain, during the conduct of the study; grants from Bicentury, SA, Spain, grants from Grand Fountaine, Spain, grants from Novartis Farmaceutica, SA, personal fees from Brewers of Europe, Belgium, personal fees from Instituto Cervantes, Albuquerque, NM, personal fees from Instituto Cervantes, Milan, Italy, personal fees from Lilly Laboratories, personal fees from PRODECA—Generalitat de Catalunya, and personal fees from Wine and Culinary International International Forum, outside the submitted work. None of the other authors had a personal or financial conflict of interest.

REFERENCES

1. Starup-Linde J, Frost M, Vestergaard P, Abrahamsen B. Epidemiology of fractures in diabetes. Calcif Tissue Int 2017;100:109–21.
2. Vestergaard P. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes—a meta-analysis. Osteoporos Int 2007;18:427–44.
3. Oei L, Rivadeneira F, Zillikens MC, Oei EHG. Diabetes, diabetic complications, and fracture risk. Curr Osteoporos Rep 2015;13:106–15.
4. Muka T, Trajanoska K, Kiefte-de Jong JC, Oei L, Uitterlinden AG, Hofman A, Dehghan A, Zillikens MC, Franco OH, Rivadeneira F. The association between metabolic syndrome, bone mineral density, hip bone geometry and fracture risk: the Rotterdam Study. PLoS One 2015;10:e0129116.
5. Ghodsi M, Larijani B, Keshtkar AA, Nasli-Esfahani E, Alatab S, Mohajeri-Tehrani MR. Mechanisms involved in altered bone metabolism in diabetes: a narrative review. J Diabetes Metab Disord 2016;15:52.

6. Pramojanee SN, Phimphilai M, Chattipakorn N, Chattipakorn SC. Possible roles of insulin signaling in osteoblasts. Endocr Res 2014;39:144–51.
7. Napoli N, Strollo R, Paladini A, Briganti SI, Pozzilli P, Epstein S. The alliance of mesenchymal stem cells, bone, and diabetes. Int J Endocrinol 2014;2014:690783.
8. Jiao H, Xiao E, Graves DT. Diabetes and its effect on bone and fracture healing. Curr Osteoporos Rep 2015;13:327–35.
9. Murakami K, Sasaki S, Takahashi Y, Okubo H, Hosoi Y, Horiguchi H, Oguma E, Kayama F. Dietary glycemic index and load in relation to metabolic risk factors in Japanese female farmers with traditional dietary habits. Am J Clin Nutr 2006;83:1161–9.
10. van Aerde MA, Witte DR, Jeppesen C, Soedamah-Muthu SS, Bjerregaard P, Jørgensen ME. Glycemic index and glycemic load in relation to glucose intolerance among Greenland's Inuit population. Diabetes Res Clin Pract 2012;97:298–305.
11. Juanola-Falgarona M, Salas-Salvadó J, Ibarrola-Jurado N, Rabassa-Soler A, Díaz-López A, Guasch-Ferré M, Hernández-Alonso P, Balanza R, Bulló M. Effect of the glycemic index of the diet on weight loss, modulation of satiety, inflammation, and other metabolic risk factors: a randomized controlled trial. Am J Clin Nutr 2014;100:27–35.
12. Wang Q, Xia W, Zhao Z, Zhang H. Effects comparison between low glycemic index diets and high glycemic index diets on HbA1c and fructosamine for patients with diabetes: a systematic review and meta-analysis. Prim Care Diabetes 2015;9:362–9.
13. Thomas D, Elliott EJ. Low glycaemic index, or low glycaemic load, diets for diabetes mellitus. In: D Thomas, editor. Cochrane database of systematic reviews. Chichester, UK: John Wiley & Sons; 2009. p. CD006296.
14. Nigg CR, Burbank PM, Padula C, Dufresne R, Rossi JS, Velicer WF, Laforge RG, Prochaska JO. Stages of change across ten health risk behaviors for older adults. Gerontologist 1999;39:473–82.
15. Martínez-González MÁ, Corella D, Salas-Salvadó J, Ros E, Covas MI, Fiol M, Wärnberg J, Arós F, Ruíz-Gutiérrez V, Lamuela-Raventós RM et al. Cohort profile: design and methods of the PREDIMED study. Int J Epidemiol 2012;41:377–85.
16. Warriner AH, Patkar NM, Curtis JR, Delzell E, Gary L, Kilgore M, Saag K, Thomas-John M, Codd MB, Manne S et al. Which fractures are most attributable to osteoporosis? J Clin Epidemiol 2011;64:46–53.
17. Elosua R, Marrugat J, Molina L, Pons S, Pujol E. Validation of the Minnesota Leisure Time Physical Activity questionnaire in Spanish men. The MARATHOM Investigators. Am J Epidemiol 1994;139:1197–209.
18. Fernández-Ballart JD, Piñol JL, Zazpe I, Corella D, Carrasco P, Toledo E, Perez-Bauer M, Martínez-González MA, Salas-Salvadó J, Martín-Moreno JM. Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. Br J Nutr 2010;103:1808–16.
19. Mataix J. Tablas de composición de alimentos. 4th ed. Granada, Spain: Universidad de Granada; 2003.
20. Moreiras O, Carvajal A, Cabrera L, editors. Tablas de composición de alimentos [Food composition tables]. 9th ed. Madrid, Spain: Ediciones Pirámide; 2005.
21. Atkinson FS, Foster-Powell K, Brand-Miller JC. International tables of glycemic index and glycemic load values: 2008. Diabetes Care 2008;31:2281–3.
22. Juanola-Falgarona M, Salas-Salvadó J, Buil-Cosiales P, Corella D, Estruch R, Ros E, Fitó M, Recondo J, Gómez-Gracia E, Fiol M et al. Dietary glycemic index and glycemic load are positively associated with risk of developing metabolic syndrome in middle-aged and elderly adults. J Am Geriatr Soc 2015;63:1991–2000.
23. Salmerón J, Manson JE, Stampfer MJ, Colditz GA, Wing AL, Willett WC. Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women. JAMA 1997;277:472–7.
24. Schröder H, Fitó M, Estruch R, Martínez-González MA, Corella D, Salas-Salvadó J, Lamuela-Raventós R, Ros E, Salaverría I, Fiol M et al. A short screener is valid for assessing Mediterranean diet adherence among older Spanish men and women. J Nutr 2011;141:1140–5.
25. Jenkins DJ, Wolever TM, Taylor RH, Barker H, Fielden H, Baldwin JM, Bowling AC, Newman HC, Jenkins AL, Goff DV. Glycemic index of foods: a physiological basis for carbohydrate exchange. Am J Clin Nutr 1981;34:362–6.
26. Barclay AW, Petocz P, McMillan-Price J, Flood VM, Prvan T, Mitchell P, Brand-Miller JC. Glycemic index, glycemic load, and chronic disease risk—a meta-analysis of observational studies. Am J Clin Nutr 2008;87:627–37.
27. Johnston SS, Conner C, Aagren M, Ruiz K, Bouchard J. Association between hypoglycaemic events and fall-related fractures in Medicare-covered patients with type 2 diabetes. Diabetes Obes Metab 2012;14:634–43.
28. Oei L, Zillikens MC, Dehghan A, Buitendijk GHS, Castano-Betancourt MC, Estrada K, Stolk L, Oei EHG, van Meurs JBJ, Janssen JAMJL et al. High bone mineral density and fracture risk in type 2 diabetes as skeletal complications of inadequate glucose control: the Rotterdam Study. Diabetes Care 2013;36:1619–28.
29. Cunha JS, Ferreira VM, Maquigussa E, Naves MA, Boim MA. Effects of high glucose and high insulin concentrations on osteoblast function in vitro. Cell Tissue Res 2014;358:249–56.
30. Pramojanee SN, Phimphilai M, Kumphune S, Chattipakorn N, Chattipakorn SC. Decreased jaw bone density and osteoblastic insulin signaling in a model of obesity. J Dent Res 2013;92:560–5.
31. Ogata N, Chikazu D, Kubota N, Terauchi Y, Tobe K, Azuma Y, Ohta T, Kadowaki T, Nakamura K, Kawaguchi H. Insulin receptor substrate-1 in osteoblast is indispensable for maintaining bone turnover. J Clin Invest 2000;105:935–43.
32. Ferron M, Wei J, Yoshizawa T, Del Fattore A, DePinho RA, Teti A, Ducy P, Karsenty G. Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism. Cell 2010;142:296–308.
33. Saito M, Marumo K. Collagen cross-links as a determinant of bone quality: a possible explanation for bone fragility in aging, osteoporosis, and diabetes mellitus. Osteoporos Int 2010;21:195–214.
34. Sanguineti R, Puddu A, Mach F, Montecucco F, Viviani GL. Advanced glycation end products play adverse proinflammatory activities in osteoporosis. Mediators Inflamm 2014;2014:975872.
35. Franke S, Rüster C, Pester J, Hofmann G, Oelzner P, Wolf G. Advanced glycation end products affect growth and function of osteoblasts. Clin Exp Rheumatol 2011;29:650–60.
36. Weinberg E, Maymon T, Weinreb M. AGEs induce caspase-mediated apoptosis of rat BMSCs via TNF production and oxidative stress. J Mol Endocrinol 2013;52:67–76.
37. Miranda C, Giner M, Montoya MJ, Vázquez MA, Miranda MJ, Pérez-Cano R. Influence of high glucose and advanced glycation end-products (ages) levels in human osteoblast-like cells gene expression. BMC Musculoskelet Disord 2016;17:377.
38. Pino AM, Ríos S, Astudillo P, Fernández M, Figueroa P, Seitz G, Rodríguez JP. Concentration of adipogenic and proinflammatory cytokines in the bone marrow supernatant fluid of osteoporotic women. J Bone Miner Res 2010;25:492–8.
39. Weitzmann MN, Pacifici R. Estrogen deficiency and bone loss: an inflammatory tale. J Clin Invest 2006;116:1186–94.
40. Barbour KE, Lui L-Y, Ensrud KE, Hillier TA, LeBlanc ES, Ing SW, Hochberg MC, Cauley JA, Study of Osteoporotic Fractures (SOF) Research Group. Inflammatory markers and risk of hip fracture in older white women: the study of osteoporotic fractures. J Bone Miner Res 2014;29:2057–64.
41. Abrahamsen B, Bonnevie-Nielsen V, Ebbesen EN, Gram J, Beck-Nielsen H. Cytokines and bone loss in a 5-year longitudinal study—hormone replacement therapy suppresses serum soluble interleukin-6 receptor and increases interleukin-1-receptor antagonist: the Danish Osteoporosis Prevention Study. J Bone Miner Res 2000;15:1545–54.
42. Feng W, Liu H, Luo T, Liu D, Du J, Sun J, Wang W, Han X, Yang K, Guo J et al. Combination of IL-6 and sIL-6R differentially regulate varying levels of RANKL-induced osteoclastogenesis through NF-κB, ERK and JNK signaling pathways. Sci Rep 2017;7:41411.
43. Jilka RL, Hangoc G, Girasole G, Passeri G, Williams DC, Abrams JS, Boyce B, Broxmeyer H, Manolagas SC. Increased osteoclast development after estrogen loss: mediation by interleukin-6. Science 1992;257:88–91.
44. Cauley JA, Danielson ME, Boudreau RM, Forrest KYZ, Zmuda JM, Pahor M, Tylavsky FA, Cummings SR, Harris TB, Newman AB. Inflammatory markers and incident fracture risk in older men and women: the Health Aging and Body Composition Study. J Bone Miner Res 2007;22:1088–95.
45. Bertolini DR, Nedwin GE, Bringman TS, Smith DD, Mundy GR. Stimulation of bone resorption and inhibition of bone formation in vitro by human tumour necrosis factors. Nature 1986;319:516–18.

46. Díaz-López A, Bulló M, Juanola-Falgarona M, Martínez-González MA, Estruch R, Covas M-I, Arós F, Salas-Salvadó J. Reduced serum concentrations of carboxylated and undercarboxylated osteocalcin are associated with risk of developing type 2 diabetes mellitus in a high cardiovascular risk population: a nested case-control study. J Clin Endocrinol Metab 2013;98:4524–31.
47. Bulló M, Moreno-Navarrete JM, Fernández-Real JM, Salas-Salvadó J. Total and undercarboxylated osteocalcin predict changes in insulin sensitivity and β cell function in elderly men at high cardiovascular risk. Am J Clin Nutr 2012;95:249–55.
48. Duan P, Yang M, Wei M, Liu J, Tu P. Serum osteoprotegerin is a potential biomarker of insulin resistance in Chinese postmenopausal women with prediabetes and type 2 diabetes. Int J Endocrinol 2017;2017:8724869.
49. Pérez de Ciriza C, Lawrie A, Varo N. Osteoprotegerin in cardiometabolic disorders. Int J Endocrinol 2015;2015:564934.