Risk of Myocardial Infarction Among New Users of Calcium Supplements Alone or Combined With Vitamin D: A Population-Based Case-Control Study

Sara Rodríguez-Martín[1,2,3], Diana González-Bermejo[4], Antonio Rodríguez-Miguel[1,2,3], Diana Barreira[1,2,3], Alberto García-Lledó[5,6], Miguel Gil[4] and Francisco J. de Abajo[1,2,3,*]

A population-based case-control study was conducted to evaluate the risk of acute myocardial infarction among new users of calcium supplements either in monotherapy (CaM) or in combination with vitamin D (CaD). A total of 23,025 cases and 114,851 controls randomly sampled from the underlying cohort and matched with cases by age, sex, and index date were included. New users of CaM and CaD were categorized as current users, recent users, past users, and nonusers. We computed adjusted odds ratios (AORs) and their 95% confidence intervals (CIs) among current users as compared with nonusers through a conditional logistic regression. No increased risk was associated with CaM overall (59 cases (0.26%) and 273 controls (0.24%); AOR = 0.80; 95% CI 0.59–1.09), nor was it found in any of the conditions examined. Instead, the use of CaD was associated with a decreased risk (275 cases (1.19%) and 1,160 controls (1.45%); AOR = 0.78; 95% CI 0.67–0.90), dose and duration-dependent, and particularly evident in patients with a high cardiovascular risk (AOR = 0.59; 95% CI 0.43–0.81).

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Calcium supplements alone or combined with vitamin D have been reported to increase the risk of acute myocardial infarction (AMI) and, as a consequence, their use in the prevention of osteoporotic fractures has been put into question.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ Is there an increase in the risk of AMI associated to the use of calcium supplements, either alone or combined with vitamin D?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ The results do not confirm the hypothesis that calcium supplements increase the risk of AMI. Instead, calcium supplements containing high-dose vitamin D appear to reduce the risk of AMI, particularly in patients at high cardiovascular risk.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ The available data on cardiovascular safety of calcium supplements combined with vitamin D are reassuring and to abandon the recommendation of using them as first-line therapy in the prevention of osteoporotic fractures is not justified.

Calcium is an essential micronutrient for bone health and regulation of critical physiological functions.[1] The American National Osteoporosis Foundation[2] recommends for adults a total daily intake of 1,000 mg for women under 51 years old and men under 71 years old, and 1,200 mg for everyone else. Despite calcium being ubiquitous in foods, the recommended daily amounts are often not met making its supplementation a common practice, in particular among women as first-line therapy to prevent osteoporotic fractures, in whom clear benefits have been shown.[2] However, the benefit-risk ratio of this practice has been questioned[3] after

[1]Clinical Pharmacology Unit, University Hospital Príncipe de Asturias, Alcalá de Henares, Madrid, Spain; [2]Department of Biomedical Sciences (Pharmacology Sector), University of Alcalá, Alcalá de Henares, Madrid, Spain; [3]Pharmacoepidemiology Research Group, Institute for Health Research IRYCIS, Madrid, Spain; [4]Division of Pharmacoepidemiology and Pharmacovigilance, Spanish Agency for Medicines and Medical Devices, Madrid, Spain; [5]Cardiology Department, University Hospital Príncipe de Asturias, Alcalá de Henares, Madrid, Spain; [6]Department of Medicine, University of Alcalá, Alcalá de Henares, Madrid, Spain. *Correspondence: Francisco J. de Abajo (francisco.abajo@uah.es)

Preliminary results of this work were presented at the XXX Congress of the Spanish Society of Clinical Pharmacology, Santander, Spain, October 3–5, 2018.

Received February 12, 2019; accepted September 12, 2019. doi:10.1002/cpt.1636

the publication of two meta-analyses, which showed an increased risk of acute myocardial infarction (AMI) associated with the use of calcium supplements either in monotherapy (CaM)[4] or in combination with vitamin D (CaD).[5] Yet, recent meta-analyses did not replicate those findings[6,7] and the issue is a matter of controversy.[8–11] A criticism of those meta-analyses is that the randomized clinical trials included were not designed to assess atherothrombotic end points.[11] Although a large randomized clinical trial could put this controversy to an end, it seems a difficult endeavor. In this context, new analytic studies specifically designed to test the cardiovascular risk hypothesis are deemed necessary. It would also be important to gauge whether there is a differential effect between CaM and CaD on cardiovascular outcomes. In a recent meta-analysis,[12] no increased or decreased risk of AMI was reported in association with the use of vitamin D supplements in primary prevention, but the number of AMI cases was relatively small (n = 2,550). In addition, we examined whether risk estimates were dependent on dose or duration and whether they could be modified by sex, age, and background cardiovascular risk. These were the main aims of the present epidemiological study, which followed a new user design to improve validity.[13]

RESULTS

The primary research cohort was composed of 3.7 million patients. From this cohort, we identified 24,155 valid AMI cases and 120,775 controls. After excluding prevalent users of CaM and/or CaD, a total of 23,025 cases and 114,851 controls were considered in the analysis (**Figure 1**).

Cases had a higher prevalence of cardiovascular risk factors, such as history of angina pectoris, heart failure, diabetes, hyperlipidemia, peripheral artery disease, hypertension, smoking, or high body mass index (BMI). They also presented a higher use of cardiovascular drugs (**Table 1**).

Fifty-nine cases (0.26%) and 273 controls (0.24%) were current users of CaM supplements, yielding an adjusted odds ratio (AOR) of 0.80 (95% confidence interval (CI): 0.59–1.09). High daily doses (≥ 1,000 mg) or long durations (over 1 year) were not associated with an increased risk either (**Table 2**). No interaction was observed with sex, age (< 70 or ≥ 70 years old), or background cardiovascular risk (**Figure S1**).

Regarding CaD supplements, 275 cases (1.19%) and 1,160 controls (1.45%) were current users, resulting in an AOR of 0.78 (0.67–0.90). The risk reduction seemed to persist during the first year after discontinuation (AOR in recent users = 0.80; 0.68–0.95) and then totally disappeared afterward (AOR in past users = 1.07; 0.94–1.22). The association with a decreased risk was only statistically significant at high doses (≥ 800 UI of vitamin D; AOR = 0.68; 0.54–0.86), with prolonged durations (> 1 year; AOR = 0.77; 0.62–0.95; **Table 3**), as well as in women (AOR = 0.74; 0.62–0.89), and patients with 70 years or older (AOR = 0.77; 0.65–0.92). The decreased risk associated with CaD was particularly strong among patients at high background cardiovascular risk (AOR = 0.59; 0.43–0.81; test for interaction P = 0.0767; as compared with low-intermediate; **Figure 2**). Furthermore, among patients presenting with any cardiovascular risk factor, current use of CaD had an AOR of 0.74 (0.63–0.87).

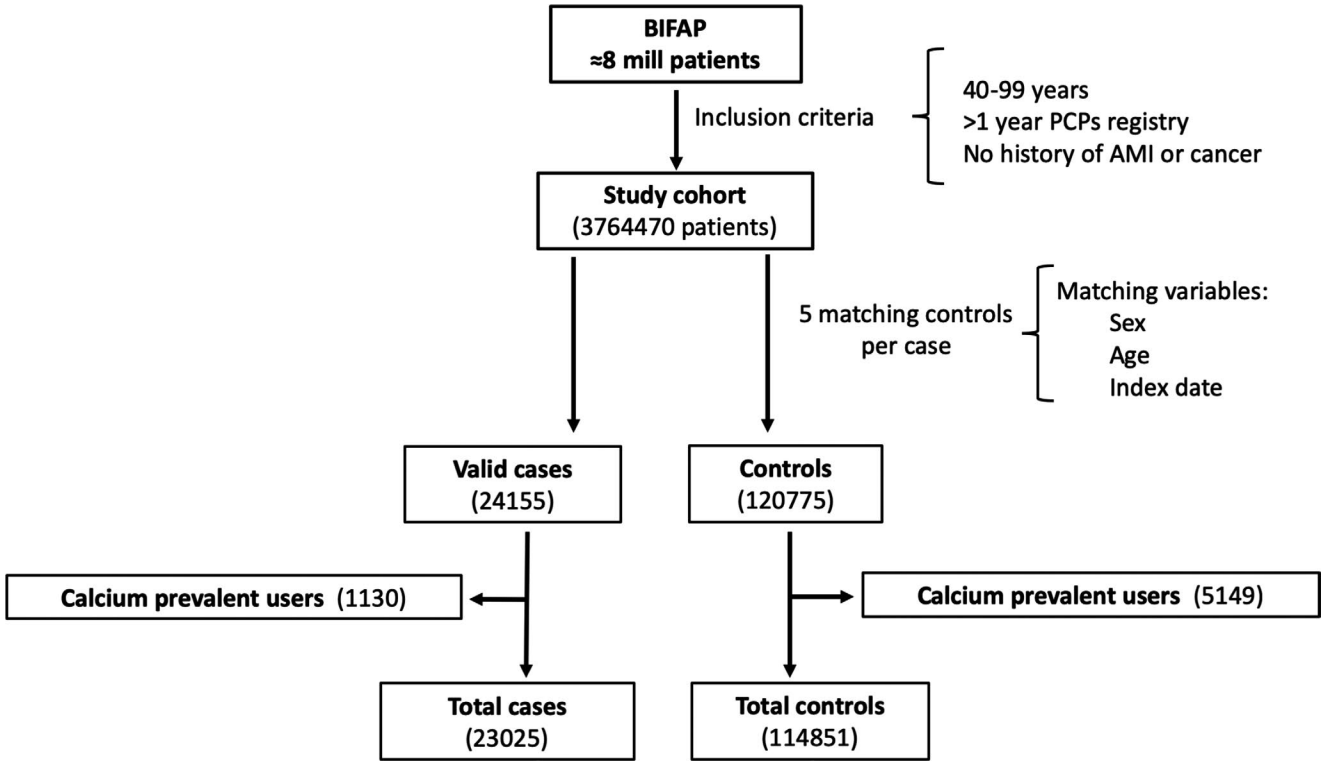

Figure 1 Flowchart of selection of cases and controls. AMI, acute myocardial infarction; BIFAP, Base de Datos para la Investigación Farmacoepidemiológica en Atención Primaria, version 2016; PCPs, primary care providers.

Table 1 Characteristics of cases and controls

	Cases (%) *n* = 23,025	Controls (%) *n* = 114,851	Nonadjusted OR[a] (95% CI)
Age mean at index date, year (SD)	66.6 (13.4)	66.6 (13.4)	
Men	16,988 (73.8)	84,828 (73.9)	
Visits (last 12 months), n			
< 6	6,783 (29.5)	44,166 (38.0)	1 (ref.)
6–15	8,618 (37.4)	41,355 (36.0)	1.44 (1.39–1.50)
16–24	4,207 (18.3)	17,012 (14.8)	1.81 (1.72–1.89)
> 24	3,417 (14.8)	12,368 (10.8)	2.10 (2.00–2.21)
CVA	1,516 (6.6)	5,993 (5.2)	1.29 (1.22–1.37)
Ischemic CVA	570 (2.5)	2,091 (1.82)	1.40 (1.27–1.54)
Hemorrhagic CVA	85 (0.37)	340 (0.30)	1.30 (1.02–1.65)
Unspecified CVA	404 (1.75)	1,698 (1.48)	1.21 (1.09–1.36)
TIA	457 (1.98)	1,864 (1.62)	1.25 (1.12–1.38)
Venous thromboembolic disease	307 (1.33)	1,349 (1.17)	1.14 (1.00–1.29)
Heart failure	815 (3.5)	2,839 (2.5)	1.46 (1.35–1.59)
Angina pectoris[b]	2,562 (11.1)	4,869 (4.2)	2.94 (2.80–3.10)
Atrial fibrillation	1,290 (5.6)	6,345 (5.5)	1.02 (0.96–1.08)
PAD	1,050 (4.6)	2,369 (2.1)	2.31 (2.14–2.49)
COPD	1,914 (8.3)	7,641 (6.7)	1.29 (1.22–1.36)
Hypertension	11,762 (51.1)	48,966 (42.6)	1.49 (1.44–1.53)
Diabetes[c]	6,235 (27.1)	18,992 (16.5)	1.92 (1.85–1.98)
Hyperlipidemia[d]	10,736 (46.6)	40,071 (34.9)	1.68 (1.63–1.73)
Hyperuricemia (not gout)	1,706 (7.4)	7,544 (6.6)	1.15 (1.09–1.21)
Gout	1,110 (4.8)	4,973 (4.3)	1.12 (1.05–1.20)
Rheumatoid arthritis	173 (0.75)	554 (0.48)	1.56 (1.31–1.85)
Osteoarthritis	1,973 (8.6)	9,323 (8.1)	1.06 (1.01–1.12)
Chronic kidney failure	851 (3.7)	2,658 (2.3)	1.66 (1.53–1.80)
Alcohol abuse	655 (2.71)	3,011 (2.49)	1.09 (1.00–1.19)
BMI, kg/m^2			
< 25	2,522 (11.0)	13,544 (11.8)	1 (ref.)
25–29	6,614 (28.7)	32,146 (28.0)	1.11 (1.05–1.17)
30–34	3,944 (17.1)	17,829 (15.5)	1.19 (1.13–1.26)
35–49	1,075 (4.7)	4,242 (3.7)	1.38 (1.28–1.50)
≥ 40	316 (1.37)	1,082 (0.94)	1.60 (1.40–1.83)
Unknown	8,554 (37.2)	49,008 (40.0)	0.99 (0.95–1.04)
Smoking			
Never smoker	5,041 (21.9)	29,771 (25.9)	1 (ref.)
Current smoker	6,345 (27.6)	19,662 (17.1)	2.04 (1.95–2.13)
Past smoker	1,247 (5.4)	6,862 (6.0)	1.11 (1.04–1.19)
Unknown	10,392 (45.1)	58,556 (51.0)	1.07 (1.03–1,11)
Current use of			
Low-dose aspirin	3,616 (15.7)	11,039 (9.6)	1.97 (1.89–2.06)
Nonaspirin antiplatelet	1,398 (6.1)	3,017 (2.6)	2.53 (2.36–2.70)
Oral anticoagulants	855 (3.7)	4,707 (4.1)	0.91 (0.84–0.98)
NSAIDs	2,248 (9.8)	10,109 (8.8)	1.20 (1.14–1.26)
Paracetamol	2,430 (10.6)	10,979 (9.6)	1.20 (1.14–1.26)

(Continued)

Table 1 (Continued)

	Cases (%) *n* = 23,025	Controls (%) *n* = 114,851	Nonadjusted OR[a] (95% CI)
Metamizole	855 (3.7)	3,111 (2.7)	1.49 (1.37–1.61)
Corticosteroids	414 (1.80)	1,435 (1.25)	1.47 (1.31–1.64)
ACE inhibitors	3,961 (17.2)	16,312 (14.2)	1.37 (1.31–1.42)
Angiotensin II receptor blockers	3,529 (15.3)	13,635 (11.9)	1.43 (1.37–1.49)
Calcium channel blockers	3,109 (13.5)	10,712 (9.3)	1.64 (1.56–1.71)
β-Blockers	2,507 (10.9)	7,144 (6.2)	1.93 (1.83–2.02)
α-Blockers	584 (2.5)	2,380 (2.1)	1.24 (1.13–1.36)
Diuretics, high ceiling	1,883 (8.2)	6,412 (5.6)	1.63 (1.54–1.73)
Diuretics, low ceiling	609 (2.6)	3,024 (2.6)	1.02 (0.93–1.11)
Diuretics, K sparing	329 (1.43)	994 (0.87)	1.66 (1.46–1.89)
Hormonal replacement therapy			
All	35 (0.15)	115 (0.10)	1.55 (1.05–2.29)
Estrogens	12 (0.05)	51 (0.04)	1.18 (0.62–2.25)
Tibolone	11 (0.05)	35 (0.03)	1.73 (0.86–3.49)
Progestogens	6 (0.03)	30 (0.03)	0.99 (0.41–2.40)
Combination estrogen + progestogen	7 (0.03)	18 (0.02)	1.97 (0.81–4.75)
Selective estrogen receptor modulators	22 (0.10)	188 (0.16)	0.56 (0.36–0.88)
Strontium ranelate	17 (0.07)	86 (0.07)	0.92 (0.54–1.55)
Bisphosphonates	288 (1.25)	1,596 (1.39)	0.88 (0.77–1.00)
Calcitonin	16 (0.07)	73 (0.06)	1.09 (0.63–1.88)
Denosumab	8 (0.03)	30 (0.03)	1.25 (0.56–2.78)
Teriparatide	5 (0.02)	31 (0.03)	0.74 (0.29–1.91)
Vitamin D (active forms)			
Alfacalcidol	0 (0.00)	2 (0.00)	–
Calcifediol	62 (0.27)	287 (0.25)	1.12 (0.85–1.48)
Calcitriol	31 (0.13)	72 (0.06)	2.11 (1.38–3.23)

Percentages ≥ 2 have been rounded to the first decimal place. Odds ratios and percentages < 2 have been rounded to the second decimal.
ACE, angiotensin converting enzyme; BMI, body max index; CI, confidence interval; COPD, chronic obstructive pulmonary disease; CVA, cerebrovascular accident; NSAIDs, nonsteroidal anti-inflammatory drugs; OR, odds ratio; PAD, peripheral artery disease; TIA, transient ischemic accident.
[a]Adjusted only for the matching factors (age, sex, and calendar year).[b]Recorded as such or when patients were using nitrates.[c]Recorded as such or when patients were using glucose-lowering drugs.[d]Recorded as such or when patients were using lipid-lowering drugs.

Multiple imputations by chained equations and complete data analysis yielded virtually identical results (**Tables S1 and S2**, respectively).

The trends over the study period of the proportion of users of calcium supplements in controls are shown in **Figure 3**. In women, the use of CaM showed a progressive decrease since the start of the study period, whereas the use of CaD steadily increased reaching a plateau around 9.5% in 2008–2011 and went down thereafter to around 6.5% in 2015. In men, CaD showed a more stable pattern. The use of calcium supplements increased by age up to 80 years old and then considerably decreased (**Figure S2**).

DISCUSSION

Our data do not support the hypothesis that the use of calcium supplements alone or combined with vitamin D increases the risk of AMI, as suggested by Bolland *et al.*[4,5] and several epidemiological studies[14–16] and add evidence to the reassuring results reported by two more recent meta-analyses performed by Lewis *et al.*[7] and Mao *et al.*[6] It is important to note, however, that in these two meta-analyses a quasi-significant increased risk associated with CaM was found (risk ratio (RR) = 1.37, 0.98–1.92, and RR = 1.28, 0.97–1.68, respectively), but not with CaD (RR = 1.07, 0.90–1.26, and RR = 1.06, 0.92–1.21). Such results point to a differential effect between the two forms of calcium supplements. Recently, our group reported an increased risk of ischemic stroke associated with CaM at a daily dose of 1,000 mg or higher but not when used at lower doses or when combined with vitamin D,[17] a result which supports such a differential effect. In the present study, however, we did not find an increased risk of AMI associated with CaM at high doses or in any of the conditions of use examined.

Table 2 Risk of AMI associated with the use of CaM and the effect of daily dose and duration of treatment

CaM	Cases (%) *n* = 23,025	Controls (%) *n* = 114,851	Nonadjusted OR[b] (95% CI)	AOR[c] (95% CI)
Current	59 (0.26)	273 (0.24)	1.04 (0.78–1.38)	0.80 (0.59–1.09)
Recent	41 (0.18)	183 (0.16)	1.13 (0.80–1.59)	0.93 (0.65–1.34)
Past	111 (0.48)	543 (0.47)	1.04 (0.85–1.29)	0.96 (0.77–1.20)
Daily dose[a]				
< 1,000 mg	30 (0.12)	135 (0.12)	1.03 (0.69–1.54)	0.94 (0.62–1.44)
1,000 + mg	21 (0.09)	91 (0.08)	1.17 (0.72–1.89)	0.63 (0.37–1.06)
Unknown	8 (0.03)	47 (0.04)	0.80 (0.38–1.71)	0.82 (0.38–1.77)
Continuous duration[a]				
< 12 months	37 (0.16)	179 (0.16)	1.00 (0.70–1.44)	0.75 (0.51–1.10)
12 + months	22 (0.10)	94 (0.08)	1.10 (0.69–1.75)	0.92 (0.56–1.52)

AMI, acute myocardial infarction; AOR, adjusted odds ratio; CaM, calcium supplements in monotherapy; CI, confidence interval; OR, odds ratio.
[a]Among current users. [b]Adjusted only for matching factors (age, sex, and calendar year). [c]Adjusted for covariates shown in **Table 1.**

Table 3 Risk of AMI associated with the use of CaD and the effect of daily dose and duration of treatment

CaD	Cases (%) *n* = 23,025	Controls (%) *n* = 114,851	Nonadjusted OR[b] (95% CI)	AOR[c] (95% CI)
Current	275 (1.19)	1,660 (1.45)	0.81 (0.71–0.92)	0.78 (0.67–0.90)
Recent	202 (0.88)	1,097 (0.96)	0.89 (0.76–1.04)	0.80 (0.68–0.95)
Past	375 (1.63)	1,679 (1.46)	1.12 (1.00–1.26)	1.07 (0.94–1.22)
Daily dose[a]				
< 800 IU	145 (0.63)	779 (0.68)	0.92 (0.77–1.10)	0.89 (0.73–1.09)
800 + IU	100 (0.43)	668 (0.58)	0.72 (0.58–0.90)	0.68 (0.54–0.86)
Unknown	30 (0.13)	213 (0.19)	0.68 (0.46–1.00)	0.64 (0.43–0.96)
Continuous duration[a]				
< 3 months	68 (0.30)	396 (0.34)	0.84 (0.64–1.08)	0.79 (0.60–1.04)
3–11.9 months	87 (0.38)	541 (0.47)	0.79 (0.63–0.99)	0.78 (0.61–1.00)
12 + months	120 (0.52)	723 (0.63)	0.81 (0.66–0.98)	0.77 (0.62–0.95)

AMI, acute myocardial infarction; AOR, adjusted odds ratio; CaD, calcium supplements in combination with vitamin D; CI, confidence interval; OR, odds ratio.
[a]Among current users. [b]Adjusted only for matching factors (age, sex, and calendar year). [c]Adjusted for covariates shown in **Table 1.**

The results of our study are especially reassuring for the combination of calcium with vitamin D. Actually, we found a decreased risk of AMI, which was dose-dependent and duration-dependent and particularly relevant in women, older subjects, and in patients at high background cardiovascular risk. These findings are compatible with the hypothesis of a cardioprotective effect of vitamin D, which has been greatly debated in recent years.[18] Although this hypothesis was not predefined *a priori* in our study, several pieces of information support it. On the one hand, many studies have shown that the deficiency of vitamin D is associated with several cardiovascular risk factors[18] and metabolic syndrome,[19,20] as well as with an increased risk of AMI,[21,22] ischemic heart disease,[23] and all-cause death.[23,24] On the other hand, in western societies there is a high prevalence of vitamin D deficiency,[25] even in countries like Spain with many hours of sunlight.[26] It is then conceivable that, in populations with a high prevalence of vitamin D deficiency, supplementation can provide cardiovascular benefits. The vitamin D receptors recently found in many cells,[27] in which they may be playing important regulatory functions,[28,29] provide a biological underpinning to this hypothesis.

The cardioprotective effect of vitamin D, however, has not been substantiated in two randomized clinical trials, the ViDa[30] and VITAL[31] trials, and in a recent meta-analysis.[12] They failed to show cardiovascular benefits associated with high doses of vitamin D, but presented some features that might explain such failure. For instance, in the ViDa trial,[30] the cardiovascular outcome was too heterogeneous, including events with different pathophysiological mechanisms (hypertension, arrhythmias, venous thrombosis, and stroke, among others) and the number of AMI cases was rather small (59 cases). Additionally, the researchers used a dose regimen far from the one used in common practice (100,000 IU per month). All of these factors hamper the comparison with our study. In the VITAL trial,[31] researchers used a daily dose of 2,000 IU, over a long period of time and focused on serious cardiovascular events (AMI, stroke, and cardiovascular deaths). No decreased risk was observed as compared with placebo in the composite variable, or in any of the isolated outcomes (in particular AMI). Importantly, an effect was not observed in patients presenting with low serum concentrations of 25-hydroxi-vitamin D. The population included in the VITAL trial,[32] however, had a much lower cardiovascular risk than

Subgroup	Risk factor	Cases (%)	Controls (%)	AOR (95%CI)	Test of interaction p-value
Sex	Men	80 (0.47)	412 (0.49)	0.83 (0.64-1.09)	0.4902
	Women	195 (3.2)	1248 (4.2)	0.74 (0.62-0.89)	
Age	<70	83 (0.64)	471 (0.73)	0.78 (0.59-1.03)	0.9441
	70+	192 (1.91)	1189 (2.40)	0.77 (0.65-0.92)	
CV risk	Low-intermediate	162 (1.15)	1194 (1.37)	0.83 (0.67-1.01)	0.0767
	High	113 (1.27)	466 (1.70)	0.59 (0.43-0.81)	

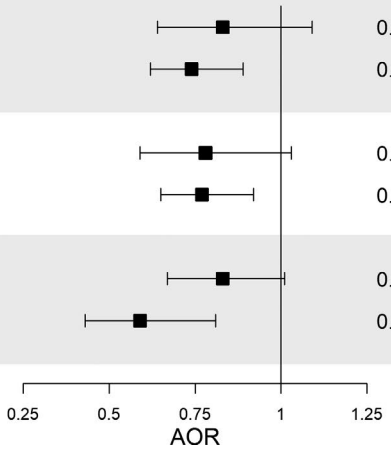

Figure 2 Risk of acute myocardial infarction associated to calcium supplements in combination with vitamin D exposure by sex, age group, and cardiovascular (CV) risk. CI, confidence interval.

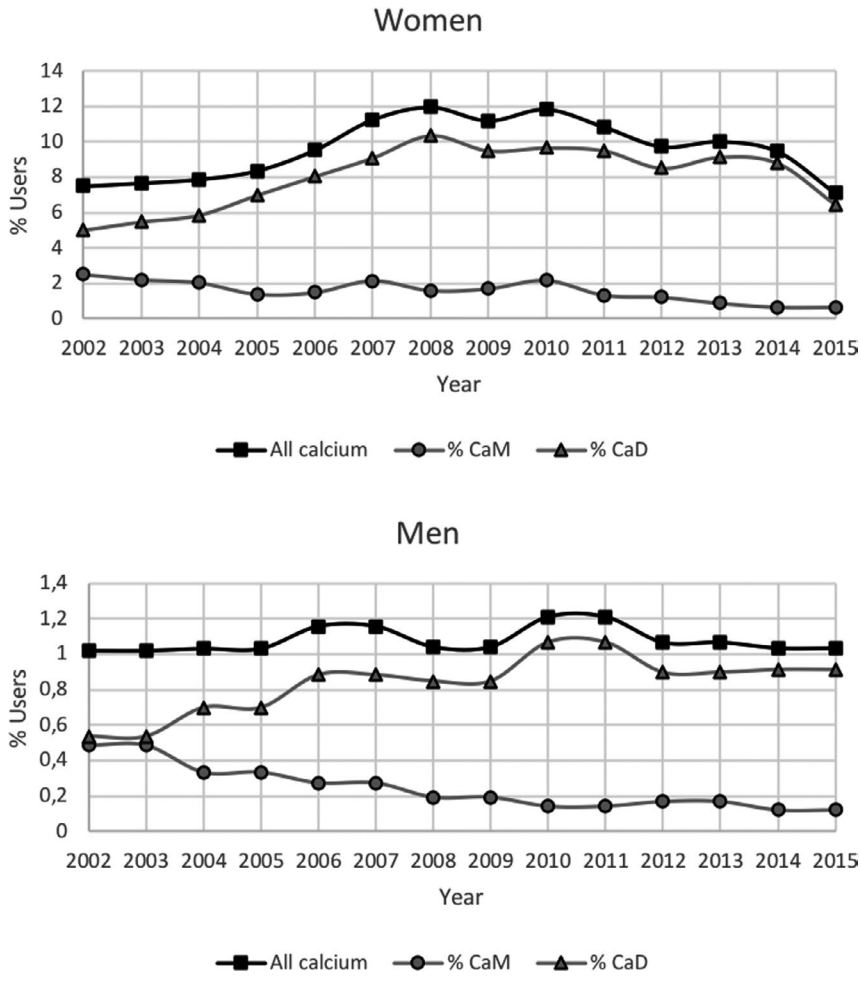

Figure 3 Trends of use prevalence of calcium supplements in monotherapy alone (CaM) or with vitamin D (CaD) by sex (only controls were considered). Note that scales are different.

the patients of our study (no one had cardiovascular, cerebrovascular, or kidney diseases, and the prevalence of smoking and diabetes was much lower). Interestingly, when we analyzed our study according to the selection criteria of the VITAL trial we found an AOR of 0.91 (95% CI: 0.76–1.09) for those patients who would have been included and 0.56 (95% CI: 0.36–0.87) for those who would have been excluded (**Table 4**). Therefore, we postulate that these differences could partly explain the different results. In addition, most randomized clinical trials included in the meta-analysis were not designed to assess cardiovascular events, as recognized by the authors.[12] Also, the total number of AMI cases was 10 times lower than our study and may be underpowered to detect an effect in certain subgroups (e.g., patients at high cardiovascular risk). Of note, in a stratified analysis, the authors found that patients with advanced age showed a significantly reduced risk of major adverse cardiovascular events.[12]

The use of calcium supplements decreased in our study population of women by one third after 2011, probably as a direct consequence of the highly publicized meta-analyses by Bolland *et al.*[4,5] A similar or greater decrease has been observed in other countries.[33] It should be emphasized that most drugs for osteoporosis were licensed in the context of calcium and vitamin D supplementation,[34] and guidelines on osteoporosis keep on recommending its use as first-line therapy, in particular when diet and sunlight exposure are considered insufficient.[2] Our results, along with those of other studies,[6,7,35,36] do not support a change in this recommendation, at least not for the alleged safety reasons.[9,37] On the other hand, the population impact of not using calcium and vitamin D supplements on the incidence of fractures could be important in the long-run.

Our study presents several strengths: (i) It is a large study using real-world data; (ii) controls were randomly selected from the underlying cohort, which prevents a selection bias; (iii) the cases were selected after an exhaustive validation exercise, which assured a high positive predictive value; (iv) the investigators were blinded to drug exposure, thus avoiding a differential misclassification of the cases influenced by the exposure; (v) the information on drug prescriptions in the database is complete as the primary care practitioners (PCPs) filled them through the computer; and (vi) the analysis was restricted to new users, which would eliminate the bias of the prevalent users.[13]

The limitations of the study are as follows: (i) we did not have information on the calcium intake with diet; (ii) nonprescription calcium supplements are not captured in the database, however, such a potential misclassification of the exposure is likely to be nondifferential with respect to the case-control status, as the intake of calcium supplements always precedes the event, and the latter has no way to influence the former; in such a case, it is well-known that a nondifferential misclassification of the exposure distorts the measure to association towards the null value[38]; in other words, the true effect would be higher than the one we observed; (iii) information on serum vitamin D levels was not available in the database, as this test was not routinely performed in the National Health System during the study period, so a confounding by indication for this reason is unlikely; but if patients at high cardiovascular risk were more prone to have this test performed and, as a consequence, they were prescribed vitamin D in a greater proportion than those at low-risk, we should have observed a spurious association of vitamin D with an increase in AMI risk, not a decrease, as we found; (iv) due to low numbers we were unable to evaluate the effect of vitamin D in monotherapy without calcium salts; there were users of active forms of vitamin D, mainly calcifediol and calcitriol, but these are indicated in patients with chronic kidney failure, which is an important cardiovascular risk factor that would lead to unreliable estimates due to a confounding by indication; (v) due to the observational nature of the study, the possibility of a residual confounding by unmeasured factors cannot be ruled out; (vi) it has been reported that observational studies that examine preventive effects may be affected by a "healthy user" bias,[39] which would occur when patients using preventive measures had healthier habits than those not using them; however, the fact that the highest decreased risk was observed among people with the highest background cardiovascular risk is not compatible with such a bias; and (vii) the participation in Base de Datos para la Investigación Farmacoepidemiológica en Atención Primaria, version 2016 (BIFAP) is voluntary, thus despite the

Table 4 Risk of AMI associated with CaD supplements when patients were classified according to VITAL trial eligibility criteria

	Cases (%) *n* = 23,025	Controls (%) *n* = 114,851	Nonadjusted OR (95% CI)	Adjusted OR[b] (95% CI)
Eligible patients[a]	*n* = 15,338	*n* = 84,688		
Current	203 (1.32)	1,261 (1.49)	0.89 (0.76–1.05)	0.91 (0.76–1.09)[c]
Recent	136 (0.89)	860 (1.02)	0.90 (0.73–1.08)	0.87 (0.71–1.07)
Past	238 (1.55)	1,266 (1.49)	1.08 (0.93–1.26)	1.07 (0.91–1.26)
Noneligible patients	*n* = 7,687	*n* = 30,163		
Current	72 (0.94)	399 (1.32)	0.60 (0.41–0.86)	0.56 (0.37–0.86)[c]
Recent	66 (0.86)	237 (0.79)	1.03 (0.69–1.53)	0.82 (0.52–1.31)
Past	137 (1.78)	413 (1.37)	1.21 (0.91–1.61)	1.27 (0.90–1.79)

AMI, acute myocardial infarction; AOR, adjusted odds ratio; CaD, calcium supplements in combination with vitamin D; CI, confidence interval; OR, odds ratio.
[a]VITAL trial eligibility criteria applied: (i) men aged 50 or older or women aged 55 or older; (ii) no history of cancer heart attack, stroke, transient ischemic attack, angina pectoris, coronary artery bypass grafting, or percutaneous coronary intervention; (iii) none of the following safety exclusions: history of renal failure or dialysis and severe liver disease (cirrhosis).[b]See Methods for variables included in the full adjusted model.[c]Altman test of interaction, $P = 0.0385$.

large sample included in this database (16% of the total Spanish population), it is difficult to assure a perfect representativeness of all potential risk factors and this may affect the external validity of the results.

In conclusion, our data are reassuring with respect to the cardiovascular safety of calcium supplements. The decreased risk associated with the combination of calcium supplements and high dose of vitamin D (800 IU or higher), though consistent with the available biological evidence and some epidemiological studies, it is apparently in contradiction with the VITAL trial and should be treated with caution, although the different populations included may be the keystone to reconcile the findings. The greater protection observed in people at high cardiovascular risk, including diabetes, needs confirmation.

PATIENTS AND METHODS

The study was carried out in BIFAP, a database of electronic health records from primary care, representative of the Spanish population with respect to age and sex,[40] validated through multiple studies,[17,41] and successfully compared with other well-known European databases.[42] Over the study period, BIFAP included anonymized information from 7.6 million patients (38.6 million person-years) with a mean follow-up of 5.1 years, from 9 regions (out of 17; regions take part in BIFAP on a voluntary basis). For disease classification, eight regions used the International Classification of Primary Care (ICPC) and one the International Classification of Diseases, version 9 (ICD-9).

Study design

We performed a case-control study nested in a primary cohort selected from BIFAP over the period January 1, 2002, to December 31, 2015. Subjects entered the primary cohort (start date) when they fulfilled all the following criteria: age 40–99 years old, with at least 1-year registry with their PCP, and no record of previous AMI or cancer. We preferred to include only new AMI cases to avoid a potential selection bias, as a previous AMI may condition a different drug exposure; also, we excluded patients with cancer, as cancer may reduce the life expectancy and they usually have hospital-dispensed polypharmacy not recorded in the database. Members of the study cohort (n = 3,764,470) were then followed up until the earliest occurrence of an incident AMI, 100 years old, a diagnosis of cancer, death, or end of the study period.

Selection of cases and controls

The event of interest was AMI. In a first step, we identified all potential cases applying a predefined case-finding algorithm for AMI that includes ICPC code K75 (AMI), ICD-9 code 410.9 (myocardial infarction), and free text search strategies of related terms. Then, we performed an in-depth case validation procedure in a random sample of 600 cases resulting in a positive predictive value of 87.2% (95% CI: 84.1–89.8; online **Supplementary Methods**). The index date was considered the date of the first record. Five controls per case[43] were randomly selected from the underlying cohort following a risk set sampling in which controls were matched to cases by exact age, sex, and index date.[44]

New users

The analysis was performed in new users of calcium supplements.[13] To that end, we excluded, from both cases and controls, all patients with a recorded prescription of either CaM or CaD before the start date of the cohort entry (prevalent users; **Figure 1**).

Exposure definition

Subjects were classified as "current users" when they had a recorded prescription of the drug of interest that ended within 30 days prior to the index date, "recent users" when the recorded prescription ended between 31 and 365 days prior to the index date, "past users" when the recorded prescription ended > 365 days prior to the index date, and "nonusers" when there was no recorded prescription before the index date.

BIFAP only records medicinal products prescribed by general practitioners. Under CaM, we grouped all medicinal products containing any calcium salt as the only ingredient. Under CaD, we grouped all medicinal products containing fixed-dose combinations of calcium salts and vitamin D. Among current users, we evaluated the effect of dose. CaM was categorized as "low-dose" when the daily dose of calcium salts was equivalent to < 1,000 mg of elemental calcium and "high dose" when it was equal or higher than this cutoff value. CaD was classified as "low-dose" when the daily dose of vitamin D was < 800 IU (always associated with calcium salts containing < 1,000 mg of elemental calcium) and "high-dose" when it was equal or higher than 800 IU (always associated with calcium salts containing at least 1,000 mg of elemental calcium).

We also assessed the effect of the continuous duration of treatments among current users. Continuous duration was defined as the sum of all consecutive prescriptions (when there were < 60 days between the end of supply of one prescription and the start of the next one). Two or three categories were used, depending on the number of patients.

Potential confounding factors

The selection of potential confounding variables was driven by expert knowledge avoiding data-driven methods. A record prior to the index date of the following diseases or risk factors was considered a potential confounder: cerebrovascular disease (ischemic, hemorrhagic or nonspecified stroke, and transient ischemic attack), peripheral artery disease, angina pectoris (recorded as such and/or use of nitrates), venous thromboembolic disease, heart failure, atrial fibrillation, chronic obstructive pulmonary disease, hypertension, diabetes (recorded as such and/or use of glucose-lowering drugs), chronic renal failure, dyslipidemia (recorded as such and/or use of lipid-lowering drugs), gout, hyperuricemia (asymptomatic, recorded as such and/or use of uric acid lowering drugs with no record of gout), rheumatoid arthritis, and osteoarthritis. Additionally, we considered the following factors: number of visits to the PCPs in the year prior to the index date, BMI, smoking, alcohol abuse (defined as such by the general practitioner), and the current use of the following drugs: low-dose aspirin, nonaspirin antiplatelet drugs, oral anticoagulants, nonsteroidal anti-inflammatory drugs, paracetamol, metamizole, corticosteroids, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, calcium-channel blockers, beta-blockers, alpha-blockers, diuretics, active forms of vitamin D (calcifediol, calcitriol, and alfacalcidiol), and drugs used for osteoporosis (hormonal replacement therapy, estrogen receptor modulators, strontium ranelate, bisphosphonates, calcitonin, denosumab, and teriparatide).

Statistical analysis

We built a conditional logistic regression model to evaluate the association of CaM or CaD with AMI. Two models were run: (i) a nonadjusted model, including only the independent variables CaM and CaD, and (ii) a fully adjusted model, including CaM, CaD, and all potential confounding factors described above. Unless otherwise specified, only AORs with their 95% CIs are provided. A result was considered statistically significant when $P < 0.05$.

Furthermore, we studied the interaction with age (stratified as < 70 and 70 + years old), sex, and background cardiovascular risk. For the latter, we classified patients into three categories: (i) *high risk*: those with records of peripheral artery disease, angina pectoris, cerebrovascular accident,

or diabetes; (ii) *intermediate risk*: those patients with hypertension or dyslipidemia, or current smoking, or BMI > 30 kg/m^2, or chronic renal failure; and (iii) *low risk*: the remainder. For the statistical evaluation of the interaction, we ran adjusted models across different categories of the interacting variables and computed the AOR associated with current use of the drugs of interest as compared with nonuse by each stratum. The AORs across strata were compared using the test of interaction described by Altman and Bland.[45]

Covariates "smoking" and "BMI" had missing values (45.1% cases, 51.0% controls, and 37.2% cases, 40.0% controls, respectively). We applied for all analyses the missing-indicator method, as the distribution of missing values was similar across the exposure.[46] As a consistency test, we also constructed multiple imputations by chained equations models[47] and performed a complete data analysis.

We conducted all analyses using STATA version 15/SE (StataCorp, College Station, TX).

Trends of supplement use over time

Among controls, we computed each year the proportion of patients who, at the index date, were current users of either CaM or CaD. Due to the random nature of the control series, such proportion was deemed to be an estimate of the use prevalence of calcium supplements per year. The data were stratified by sex allowing for matching.

Ethics review

Access to anonymized data from BIFAP was granted by the BIFAP Scientific Committee (project #04/2016, approval date: May 26, 2016). According to the Spanish law, no specific ethical review was required for studies using fully anonymized data.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

Figure S1. Risk of acute myocardial infarction associated to calcium supplements in monotherapy exposure by sex, age group, and cardiovascular (CV) risk.
Figure S2. Use prevalence of calcium supplements by age group (5 years) and sex (only controls were considered).
Table S1. Main analysis comparing multiple imputations by chained equations (MICE) against missing-indicator method.
Table S2. Risk of acute myocardial infarction associated with the use of calcium supplements in monotherapy or calcium supplements in combination with vitamin D and the effect of daily dose and duration of treatment when only patients with complete data were considered.

ACKNOWLEDGMENTS

The authors would like to thank the excellent collaboration of primary care practitioners participating in Base de Datos para la Investigación Farmacoepidemiológica en Atención Primaria (BIFAP). BIFAP is funded and managed by the Spanish Agency for Medicines and Medical Devices.

CONFLICTS OF INTEREST

The authors declared no competing interests for this work.

FUNDING

This study was supported by a research grant from Instituto de Salud Carlos III – Ministerio de Ciencia e Innovación (# PI16/01353), cofounded by Fondo Europeo de Desarrollo Regional.

AUTHOR CONTRIBUTIONS

F.d.A. and S.R.M. wrote the manuscript. F.d.A., S.R.M., M.G., and A.G.L. designed the research. S.R.M., F.d.A., M.G., D.G.B., A.R.M., and D.B. analyzed the data.

DISCLAIMER

The results, discussion, and conclusions are from the authors and do not necessarily represent the position of the Spanish Agency for Medicines and Medical Devices.

1. Watts, N.B. *et al.* AACE (American association of clinical endocrinologists) medical guidelines for clinical practice for the diagnosis and treatment of postmenopausal osteoporosis. *Endocr. Pract.* **16** (suppl. 3), 1–37 (2010).
2. Cosman, F. *et al*. Clinician's guide to prevention and treatment of osteoporosis. *Osteoporos Int.* **25**, 2359–2381 (2014).
3. Reid, I.R., Bristow, S.M. & Bolland, M.J. Calcium supplements: benefits and risks. *J. Intern. Med.* **278**, 354–368 (2015).
4. Bolland, M.J. *et al*. Effect of calcium supplements on risk of myocardial infarction and cardiovascular events: meta-analysis. *BMJ* **341**, c3691 (2010).
5. Bolland, M.J., Grey, A., Avenell, A., Gamble, G.D. & Reid, I.R. Calcium supplements with or without vitamin D and risk of cardiovascular events: reanalysis of the Women's Health Initiative limited access dataset and meta-analysis. *BMJ* **342**, d2040 (2011).
6. Mao, P.J. *et al*. Effect of calcium or vitamin D supplementation on vascular outcomes: a meta-analysis of randomized controlled trials. *Int. J. Cardiol.* **169**, 106–111 (2013).
7. Lewis, J.R. *et al.* The effects of calcium supplementation on verified coronary heart disease hospitalization and death in postmenopausal women: a collaborative meta-analysis of randomized clinical trials. *J. Bone Miner. Res.* **30**, 165–175 (2015).
8. Prentice, R.L. *et al*. Health risks and benefits from calcium and vitamin D supplementation: Women's Health Initiative clinical trial and cohort study. *Osteoporosis Int.* **24**, 567–580 (2013).
9. Reid, I.R. & Bolland, M.J. Does widespread calcium supplementation pose cardiovascular risk? Yes: the potential risk is a concern. *Am. Fam. Physician* **87**, 1–2 (2013).
10. Bhattacharya, R.K. Does widespread calcium supplementation pose cardiovascular risk? No: concerns are unwarranted. *Am. Family Physician* **87**, 1–2 (2013).
11. Barice, E.J. & Hennekens, C.H. Calcium and vitamin D supplementation: facts and myths. *J. Cardiovasc. Pharmacol. Ther.* **20**, 9–10 (2015).
12. Barbarawi, M. *et al.* Vitamin D supplementation and cardiovascular disease risks in more than 83 000 individuals in 21 randomized. Clinical trials: a meta-analysis. *JAMA Cardiol.* **4**, 765 (2019).
13. Ray, W.A. Evaluating medication effects outside of clinical trials: new-user designs. *Am. J. Epidemiol.* **158**, 915–920 (2003).
14. Pentti, K. *et al.* Use of calcium supplements and the risk of coronary heart disease in 52–62-year-old women: the Kuopio Osteoporosis Risk Factor and Prevention Study. *Maturitas* **63**, 73–78 (2009).
15. Li, K., Kaaks, R., Linseisen, J. & Rohrmann, S. Associations of dietary calcium intake and calcium supplementation with myocardial infarction and stroke risk and overall cardiovascular mortality in the Heidelberg cohort of the European Prospective Investigation into Cancer and Nutrition study (EPIC-Heidelberg). *Heart* **98**, 920–925 (2012).
16. Xiao, Q., Murphy, R.A., Houston, D.K., Harris, T.B., Chow, W.H. & Park, Y. Dietary and supplemental calcium intake and cardiovascular disease mortality: the National Institutes of Health-AARP Diet and Health Study. *JAMA Intern. Med.* **173**, 639–646 (2013).
17. De Abajo, F.J., Rodríguez-Martín, S., Rodríguez-Miguel, A. & Gil, M.J. Risk of ischemic stroke associated with calcium

supplements with or without vitamin D: a nested case-control study. *J. Am. Heart Assoc.* **6**, pii: e005795 (2017).
18. Mirhosseini, N., Rainsbury, J. & Kimball, S.M. Vitamin D supplementation, serum 25(OH)D concentrations and cardiovascular disease risk factors: a systematic review and meta-analysis. *Front. Cardiovasc. Med.* **5**, 87 (2018).
19. Barbalho, S.M. *et al.* Association between vitamin D status and metabolic syndrome risk factors. *Diabetes Metab. Syndr.* **12**, 501–507 (2018).
20. Verrusio, W., Andreozzi, P., Renzi, A., Musumeci, M., Gueli, N. & Cacciafesta, M. Association between serum vitamin D and metabolic syndrome in middle-aged and older adults and role of supplementation therapy with vitamin D. *Ann. Ist. Super. Sanità* **53**, 54–59 (2017).
21. Giovannucci, E., Liu, Y., Hollis, B.W. & Rimm, E.B. 25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. *Arch. Intern. Med.* **168**, 1174–1180 (2008).
22. Roy, A., Lakshmy, R., Tarik, M., Tandon, N., Reddy, K.S. & Prabhakaran, D. Independent association of severe vitamin D deficiency as a risk of acute myocardial infarction in Indians. *Indian Heart J.* **67**, 27–32 (2015).
23. Brøndum-Jacobsen, P., Benn, M., Jensen, G.B. & Nordestgaard, B.G. 25-Hydroxyvitamin d levels and risk of ischemic heart disease, myocardial infarction, and early death: population-based study and meta-analyses of 18 and 17 studies. *Arterioscler. Thromb. Vasc. Biol.* **32**, 2794–2802 (2012).
24. Gaksch, M. *et al.* Vitamin D and mortality: individual participant data meta-analysis of standardized 25-hydroxyvitamin D in 26916 individuals from a European consortium. *PLoS One* **12**, e0170791 (2017).
25. Gorey, S., Canavan, M., Robinson, S., O'Keeffe, S.T. & Mulkerrin, E. A review of vitamin D insufficiency and its management, a lack of evidence and consensus persists. *QJM* **11**, 165–167 (2018).
26. Gradillas-Garcia, A., Alvarez, J., Rubio, J.A. & de Abajo, F.J. Relationship between vitamin D deficiency and metabolic syndrome in adult population of the Community of Madrid [article in Spanish]. *Endocrinol. Nutr.* **62**, 180–187 (2015).
27. Bouillon, R., Carmeliet, G., Verlinden, L., van Etten, E., Verstuyf, A. & Luderer, H.F. Vitamin D and human health: lessons from vitamin D receptor null mice. *Endocr. Rev.* **29**, 726–776 (2008).
28. Pilz, S., Verheyen, N., Grübler, M.R., Tomaschitz, A. & März, W. Vitamin D and cardiovascular disease prevention. *Nat. Rev. Cardiol.* **13**, 404–417 (2016).
29. Vaidya, A., Forman, J.P., Hopkins, P.N., Seely, E.W. & Williams, J.S. 25-Hydroxyvitamin D is associated with plasma renin activity and the pressor response to dietary sodium intake in Caucasians. *J. Renin Angiotensin Aldosterone Syst.* **12**, 311–319 (2011).
30. Scragg, R. *et al*. Effect of monthly high-dose vitamin D supplementation on cardiovascular disease in the vitamin D assessment study: a randomized clinical trial. *JAMA Cardiol.* **2**, 608–616 (2017).
31. Manson, J.E. *et al*. Vitamin D supplements and prevention of cancer and cardiovascular disease. *N. Engl. J. Med.* **380**, 33–44 (2018).
32. ClinicalTrials.gov number: NCT01169259 <https://clinicaltrials.gov/ct2/show/NCT01169259?term=01169259&rank=1>. Accessed March 12, 2018.
33. Bolland, M.J., Grey, A. & Reid, I.R. Translation of research into clinical practice: a case study of calcium supplement prescribing in New Zealand. *N. Z. Med. J.* **127**, 94–101 (2014).
34. Harvey, N.C. *et al.* The role of calcium supplementation in healthy musculoskeletal ageing: an expert consensus meeting of the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO) and the International Foundation for Osteoporosis (IOF). *Osteoporos. Int.* **28**, 447–462 (2017).
35. Harvey, N.C. *et al*. Calcium and vitamin D supplementation are not associated with risk of incident ischemic cardiac events or death: findings from the UK Biobank Cohort. *J. Bone Miner. Res.* **33**, 803–811 (2018).
36. Van Hemelrijck, M., Michaelsson, K., Linseisen, J. & Rohrmann, S. Calcium intake and serum concentration in relation to risk of cardiovascular death in NHANES III. *PLoS One* **8**, e61037 (2013).
37. Bolland, M.J., Grey, A. & Reid, I.R. Calcium supplements and cardiovascular risk: 5 years on. *Ther. Adv. Drug. Saf.* **4**, 199–210 (2013).
38. Szklo, M. & Nieto, F.J. Epidemiology – Beyond the Basics, 3rd edn (Jones & Bartlett Learning, Burlington, 2014, pp: 121–125).
39. Dormuth, C.R. *et al.* Statin adherence and risk of accidents: a cautionary tale. *Circulation* **119**, 2051–2057 (2009).
40. BIFAP: Base de Datos para la Investigación Farmacoepidemiológica en Atención Primaria <http://www.bifap.org>. Accessed June 29, 2018.
41. De Abajo, F.J. *et al.* Risk of nonfatal acute myocardial infarction associated with non-steroidal antiinflammatory drugs, non-narcotic analgesics and other drugs used in osteoarthritis: a nested case-control study. *Pharmacoepidemiol. Drug Saf.* **23**, 1128–1138 (2014).
42. Requena, G. *et al.* Hip/femur fractures associated with the use of benzodiazepines (anxiolytics, hypnotics and related drugs): a methodological approach to assess consistencies across databases from the PROTECT-EU project. *Pharmacoepidemiol. Drug Saf.* **25** (suppl. 1), 66–78 (2016).
43. Hennessy, S., Bilker, W.B., Berlin, J.A. & Strom, B.L. Factors influencing the optimal control-to-case ratio in matched case-control studies. *Am. J. Epidemiol.* **149**, 195–197 (1999).
44. Rothman, K.J., Greenland, S. & Lash, T. Modern Epidemiology, 3rd edn (Lippincott Williams and Wilkins, Philadelphia, PA, 2013).
45. Altman, D.G. & Bland, J.M. Interaction revisited: the difference between two estimates. *BMJ* **326**, 219 (2003).
46. Groenwold, R.H., White, I.R., Donders, A.R., Carpenter, J.R., Altman, D.G. & Moons, K.G. Missing covariate data in clinical research: when and when not to use the missing-indicator method for analysis. *CMAJ* **184**, 1265–1269 (2012).
47. Toh, S., García-Rodríguez, L.A. & Hernán, M.A. Analyzing partially missing confounder information in comparative effectiveness and safety research of therapeutics. *Pharmacoepidemiol. Drug Saf.* **21** (suppl. 2), 13–20 (2012).