The association of vitamin D status and dietary calcium intake with individual components of the metabolic syndrome: a population-based study in Victoria, Australia

Poonam K. Pannu^a, Mario J. Soares^a, Leonard S. Piers^c, Yun Zhao^b and Zahid Ansari^c

Objective This study examined the associations between 25-hydroxyvitamin D (25-OHD), dietary calcium (Ca) intake, and individual components of the metabolic syndrome (MetS).

Methods We analyzed a population-based sample of 18–75-year-old adults ($n = 3387$) from the Victorian Health Monitor survey.

Results After adjustment for sociodemographic, physical, and dietary factors, as well as other MetS components, every 10 nmol/l increment in 25-OHD was associated with reduced adjusted odds ratio (AOR) of elevated triglycerides (TG) [AOR: 0.79, 95% confidence interval (CI): 0.74–0.84, $P < 0.001$], and higher fasting plasma glucose (AOR: 0.91, 95% CI: 0.86–0.96, $P = 0.002$). After adjustment for confounders, every 500 mg/day increment in dietary Ca intake significantly reduced the odds of elevated diastolic blood pressure (AOR: 0.80, 95% CI: 0.66–0.99, $P = 0.038$). When nine combinations of 25-OHD and Ca tertiles were examined, certain combinations were associated with reduced AOR for elevated TG ($P < 0.001$), when referenced against the combination of low 25-OHD (median: 33 nmol/l)

and low Ca (median: 579 mg/day). At low 25-OHD, increasing Ca intake decreased the AOR for low high-density lipoprotein cholesterol in a dose-dependent manner, but at high 25-OHD; such effects of Ca were blunted.

Conclusion Higher vitamin D status and Ca intake or their combination were associated with reduced odds for a number of individual MetS components. *Cardiovasc Endocrinol* 6:136–144 Copyright © 2017 Wolters Kluwer Health, Inc. All rights reserved.

Cardiovascular Endocrinology 2017, 6:136–144

Keywords: calcium, metabolic syndrome, vitamin D

^aFood, Nutrition and Health, School of Public Health, Curtin Health Innovation Research Institute, Curtin University, ^bOccupation and the Environment, School of Public Health, Faculty of Health Sciences, Curtin University, Perth, Western Australia and ^cDepartment of Health, Health Intelligence Unit, System Intelligence and Analytics Branch, Health Strategy, Productivity and Analytics Division, Melbourne, Victoria, Australia

Correspondence to Mario J. Soares, PhD, MBBS, Food, Nutrition and Health, School of Public Health, Curtin Health Innovation Research Institute, Curtin University, Perth, Western Australia 6102, Australia
Fax: + 61 892 662 958, e-mail: m.soares@curtin.edu.au

Received 23 May 2017 Accepted 26 July 2017

Introduction

The metabolic syndrome (MetS) is the clustering of five inter-related biomedical components including central adiposity, hypertension, hypertriglyceridemia, low high-density lipoprotein cholesterol (HDL-C), and altered glucose homeostasis [1]. It is a significant health issue, with ~25% of the global population being diagnosed with the condition [2]. The presence of MetS predicts an increased risk of type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) [3]. Furthermore, the risk of T2DM appears to increase in line with increasing number of MetS components with risk of 11.9, 31.2, and 40.8% in those with zero, three, and four or more MetS components, respectively [4]. However, each MetS component is an independent risk factor that clinicians will treat if diagnosed in their patients.

Vitamin D deficiency is a worldwide health issue, and an estimated one billion individuals are classified as having insufficient vitamin D status [5,6]. Coupled with this are lower intakes of calcium (Ca)-rich foods, despite

increasing production and availability of dairy products [7]. 25-Hydroxyvitamin D (25-OHD) has been implicated in the mechanisms underlying a number of the MetS components [8]. The majority of studies have found an inverse relationship between 25-OHD and glucose homeostasis, lipids, obesity, and blood pressure (BP) [8,9]. However, a few studies have also shown an inverse relationship with Ca intake and MetS [10,11], with limited evidence supporting an association between Ca intake and individual MetS components. Vitamin D and Ca are physiologically tightly interlinked and it is possible that their combination may have a greater potential to interactively influence components of MetS [11,12]. We have previously found that, in individuals within low to medium 25-OHD tertiles, increasing Ca intake tertiles had a trend to lower adjusted odds ratio (AOR) of MetS. However, in the high 25-OHD tertile, this trend was no longer present [12]. The aim of this study was to explore the potential combined effect of vitamin D status and dietary Ca intake on individual components of MetS.

Methods

This cross-sectional study was based on the analysis of the Victorian Health Monitor (VHM) survey, which is a population representative survey of Victorian adults aged 18–75 years. Details about the VHM, and the collection and analysis of the physical, dietary, and biomedical variables, can be found in previous publications [12–14].

Sample

In this sample, participants were excluded if there was missing information on HbA1c data ($n=31$), those with HbA1c of at least 6.5% as they were classified as having T2DM according to the American Diabetes Association cutoffs ($n=39$) [15], those with diagnosed T2DM ($n=140$), those with type 1 diabetes mellitus ($n=9$), those on diabetic medications ($n=25$), and those with missing data on metabolic components ($n=22$). Thus, out of 3653 participants recruited in the VHM survey, this study sample consisted of a total of 3387 participants. The three independent variables of interest were serum 25-OHD concentration, dietary Ca intake, and the combination of 25-OHD and dietary Ca.

Biomedical analyses

Venous blood was collected by venepuncture, processed, refrigerated, and transported daily to a single accredited laboratory in Melbourne for storage (at -80°C). All analyses and reporting were completed within two weeks of collection. Blood samples were analyzed for fasting plasma glucose (FPG) using the hexokinase method; HDL-C using elimination/catalase method; and triglycerides (TG) using GPO Trinder reagent set with serum blank. Blood pressure measurements were collected (GE Dinamap 8100 Vital Sign Monitor; Critikon, Tampa, Florida, USA) after a 5-min rest [14].

Serum 25-OHD was measured using the DiaSorin Corporation Liaison 25-OHD total assay, an automated direct competitive chemiluminescent immunoassay that measures D2 and D3 to provide a total value for circulating vitamin D in nmol/l. The detection limit was 10 nmol/l. The precision of the LIAISON assay [(DiaSorin S.p.A. Via Crescentino 13040 Saluggia (VC) – Italy)] was determined by using human serum-based quality controls, spanning a 25-OHD range of 35–180 nmol/l. Each control sample was assayed in 2–4 replicates per run for 3–4 runs; however, within-run and total coefficient of variations were not reported. The laboratory was not part of the vitamin D standardization programs at the time of fieldwork for the study (2009–2010). The all laboratory trimmed mean was not computed by the laboratory, nor were results compared with a target value assigned by the National Institute of Standards and Technology reference measurement procedure.

Dietary calcium intake

Dietary data were collected by a five-pass 24-h diet recall using computer-assisted telephone interviews. The

FoodWorks nutrition software [(Xyris Software Pvt Ltd., Kenmore Hills, Australia)] were used in conducting the three dietary recalls [13]. This provided data on energy, macronutrient, micronutrient, and Ca intake. Dietary Ca intake was included as a continuous variable for every 500 mg/day increment.

The combination of 25-OHD concentration and dietary calcium intake

25-OHD concentration was categorized into tertiles: low 25-OHD tertile (range: 10–44 nmol/l; median: 33 nmol/l), medium 25-OHD tertile (range: 45–65 nmol/l; median: 54 nmol/l), and high 25-OHD tertile (range: 65–204 nmol/l; median: 77 nmol/l). Dietary Ca intake was also classified into tertiles, which were low Ca tertile (range: 72–719 mg/day; median: 579 mg/day), medium Ca tertile (range: 720–1009 mg/day; median: 858 mg/day), and high Ca tertile (range: 1010–3726 mg/day; median: 1233 mg/day).

To assess the combined effect of 25-OHD and Ca tertiles, a nine-level variable was generated based on the combination of these tertiles, which were as follows: (i) low 25-OHD and low Ca; (ii) low 25-OHD and medium Ca; (iii) low 25-OHD and high Ca; (iv) medium 25-OHD and low Ca; (v) medium 25-OHD and medium Ca; (vi) medium 25-OHD and high Ca; (vii) high 25-OHD and low Ca; (viii) high 25-OHD and medium Ca; (ix) and high 25-OHD and high Ca.

MetS components

Information on the collection of the physical and anthropometric measurements can be found in our previous publications [12,14]. MetS components were the dependent variables, and were categorized as per the cutoffs defined by the joint interim statement of key organizations [1]. The criteria for the MetS components were as follows: (i) elevated TG of at least 1.7 mmol/l (≥ 150 mg/dl) (yes/no); (ii) reduced HDL-C of less than 1.0 mmol/l (< 40 mg/dl) in males and less than 1.3 mmol/l (< 50 mg/dl) in females (yes/no), or on lipid-lowering therapy; (iii) elevated systolic BP (SBP) of at least 130 mmHg or on antihypertensive medications (yes/no); (iv) elevated diastolic BP (DBP) of at least 85 mmHg or on antihypertensive medications (yes/no); (v) elevated FPG of at least 5.6 mmol/l (≥ 100 mg/dl) (yes/no); and (vi) elevated waist circumference (WC) of at least 94 cm for males (≥ 90 cm for Aboriginal and Torres Strait Islander, Asian, and South American males) and at least 80 cm for females (yes/no).

Confounders

We adjusted for the following confounders in our analysis on the basis of our previous findings [12] and as reported by others [16]. The demographic factors included were age (years), sex, country of birth (born in Australia, born overseas), BMI (kg/m^2), physical activity level (insufficient physical activity < 150 min/week, sufficient physical

activity ≥ 150 min/week), smoking status (current smoker, nonsmoker), income ($< \$30,000$, $\$30,001$ – $\$70,000$, $\geq \$70,001$, do not know/refused), and education (high school or less, TAFE/diploma/certificate, tertiary education). Season was categorized as summer, autumn, winter, and spring. The dietary variables were all entered as continuous variables and were energy intake (kJ/day), dietary fiber (g/day), alcohol (g/day), magnesium (mg/day), zinc (mg/day), Ca (25-OHD model only), and 25-OHD (Ca model only).

Statistical analysis

The analysis targeted the following questions:

- (1) Was there any association between MetS components and increments of 10 nmol/l of 25-OHD?
- (2) Was there any association between MetS components and increments of 500 mg/day of dietary Ca intake?
- (3) Did combinations of 25-OHD and Ca tertiles have a greater influence on any of the MetS components?

The analysis was conducted according to the following steps:

Step 1: Demographic/descriptive statistics by the MetS components were obtained and differences between groups were tested by χ^2 test for categorical variables of interest.

Step 2: Logistic regression analysis was used, and the following regression models were developed to examine whether there was any association between MetS components and (i) increments in 25-OHD concentration, (ii) increments in dietary Ca intake, and (iii) the combination of 25-OHD and Ca tertiles, and unadjusted OR, AOR, and 95% CI were reported.

Model 1: An unadjusted logistic regression model was fitted for MetS components, including (1) 25-OHD concentration, (2) Ca intake, and (3) the combination of 25-OHD and Ca tertiles. The combination of 'low 25-OHD and low Ca' combination tertile served as the reference group.

Model 2: We adjusted the model 1 for confounders such as age, sex, country of birth, smoking status, physical activity, income, education, BMI, season, energy intake, dietary fiber, alcohol, magnesium, zinc, Ca (25-OHD model only), and 25-OHD (Ca model only).

Model 3: We explored whether any of the associations between the combination of 25-OHD and Ca tertiles and individual MetS components were independent of the other components of MetS. Thus, in model 3, we additionally adjusted model 2 for those MetS components (reduced HDL-C, elevated WC, elevated TG, elevated SBP, elevated DBP, or elevated FPG), which were not the dependent factors.

Analyses were conducted using the IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, New York, USA) 'Complex Samples' module to eliminate sampling bias arising from the multistage cluster sampling method used in the VHM survey.

Results

The mean age was 49 years, with a higher proportion of subjects with normal levels of HDL-C (86%), TG (79%), SBP (58%), DBP (73%), and FPG (84%) compared with abnormal levels (Supplementary Table 1, Supplemental digital content 1, <http://links.lww.com/CAEN/A16>).

Association between 25-OHD concentration and MetS components

The unadjusted model found a significant association between every 10 nmol/l increment in 25-OHD concentration and a reduced odds of elevated WC ($P=0.014$), TG ($P<0.001$), DBP ($P=0.006$), FPG ($P=0.027$), and reduced HDL-C ($P=0.017$). There was no significant association between 25-OHD concentration and elevated SBP ($P=0.189$). In model 2, after adjustment for all confounders (age, sex, country of birth, smoking, physical activity, income, education, BMI, season, Ca, energy, fiber, alcohol, magnesium, zinc), and model 3, after further adjustment for respective MetS components, every 10 nmol/l increment in 25-OHD reduced the odds of elevated TG by 21%, and elevated FPG by 9% (Table 1). There was no significant association between 25-OHD concentration and elevated WC ($P=0.707$), elevated SBP ($P=0.525$), and reduced HDL-C ($P=0.433$).

Association between dietary calcium intake and MetS components

The unadjusted model found a significant association between every 500 mg/day increment in dietary Ca intake and reduced odds of elevated WC ($P=0.008$), elevated DBP ($P<0.001$), and reduced HDL-C ($P<0.001$). There were no significant associations between dietary Ca intake and elevated TG ($P=0.540$), elevated SBP ($P=0.208$), and elevated FPG ($P=0.142$). After adjustment for confounders in models 2 and 3, every 500 mg/day increment in dietary Ca intake reduced the odds of elevated DBP by 20% (Table 1). There were no significant associations between increments of 500 mg/day in dietary Ca intake and elevated WC ($P=0.709$), elevated TG ($P=0.635$), and elevated FPG ($P=0.907$).

Association between the combination of 25-OHD concentration and dietary calcium intake and MetS components

Models 1 and 2 revealed a significant association between the combination of 25-OHD concentration and Ca intake and reduced HDL-C, elevated TG, and elevated DBP. After further adjustment for respective MetS components

Table 1 The odds ratio of having MetS components for every 10 nmol/l increment in 25-OHD concentration, and every 500 mg/day increment in dietary Ca intake

	25-OHD concentration (10 nmol/l increment) (model 1)			25-OHD concentration (10 nmol/l increment) (model 2)			25-OHD concentration (10 nmol/l increment) (model 3)		
	AOR	95% CI	P value	AOR	95% CI	P value	AOR	95% CI	P value
Elevated WC	0.93 ^a	0.88–0.99	0.014	0.97	0.90–1.06	0.521	0.98	0.91–1.07	0.707
Reduced HDL-C	0.93 ^a	0.88–0.99	0.017	0.93 ^a	0.87–0.99	0.023	0.97	0.90–1.05	0.433
Elevated TG	0.82 ^a	0.77–0.87	< 0.001	0.79 ^a	0.74–0.84	< 0.001	0.79 ^a	0.74–0.84	< 0.001
Elevated SBP	0.97	0.93–1.01	0.189	1.01	0.96–1.05	0.961	1.02	0.97–1.07	0.525
Elevated DBP	0.94 ^a	0.89–0.98	0.006	0.96	0.92–1.00	0.050	0.97	0.92–1.01	0.147
Elevated FPG	0.92 ^a	0.86–0.99	0.027	0.91 ^a	0.86–0.97	0.003	0.91 ^a	0.86–0.96	0.002

	Dietary Ca intake (500 mg/day increment) (model 1)			Dietary Ca intake (500 mg/day increment) (model 2)			Dietary Ca intake (500 mg/day increment) (model 3)		
	AOR	95% CI	P value	AOR	95% CI	P value	AOR	95% CI	P value
Elevated WC	0.83 ^a	0.72–0.95	0.008	0.94	0.70–1.26	0.672	0.95	0.70–1.28	0.709
Reduced HDL-C	0.64 ^a	0.50–0.80	< 0.001	0.82	0.61–1.10	0.174	0.80	0.61–1.05	0.104
Elevated TG	0.95	0.79–1.14	0.540	1.02	0.82–1.26	0.870	1.05	0.84–1.31	0.635
Elevated SBP	0.91	0.80–1.05	0.208	0.86	0.71–1.04	0.113	0.86	0.72–1.03	0.099
Elevated DBP	0.76 ^a	0.65–0.88	< 0.001	0.81 ^a	0.66–0.99	0.044	0.80 ^a	0.66–0.99	0.038
Elevated FPG	0.87	0.72–1.05	0.142	0.97	0.76–1.25	0.824	0.99	0.77–1.26	0.907

Model 1 unadjusted model.

Model 2 adjusted for age, sex, country of birth, smoking, physical activity level, income, education, BMI, season, Ca (25-OHD model only), 25-OHD (Ca model only) energy, fiber, alcohol, magnesium, zinc.

Model 3 adjusted for all variables in model 2 and remaining MetS components.

AOR, adjusted odds ratio; Ca, calcium; CI, confidence interval; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure; TG, triglycerides; WC, waist circumference.

^aSignificant as compared with the reference category.

in model 3, the significant association remained between the combination of 25-OHD concentration and Ca intake and reduced HDL-C and elevated TG. There was no significant association between combined 25-OHD and Ca intake and elevated DBP, WC, SBP, and FPG (Table 2).

The combined effects of 25-OHD concentration and dietary calcium intake on reduced HDL-C

In all models, the significant association persisted between the combination of 25-OHD concentration and Ca intake and reduced HDL-C. In all models, and all combinations of 25-OHD concentration and Ca intake, there appeared to be a downward trend, with a lower AOR of reduced HDL-C, as Ca intake increased. There appeared to be a dose–response effect of Ca at low 25-OHD on reduced HDL-C, but at higher 25-OHD these effects of Ca were blunted. In model 3, the following four combinations appeared to lower the odds of reduced HDL-C by 47% in low 25-OHD and medium Ca, 68% in medium 25-OHD and high Ca, 57% in medium 25-OHD and low Ca, and 68% in medium 25-OHD and high Ca (Fig. 1 and Table 2).

The combined effects of 25-OHD concentration and dietary calcium intake on elevated TG

In all models the significant association persisted between the combination of 25-OHD concentration and Ca intake and elevated TG. The combination of medium

25-OHD and low and medium Ca significantly reduced the odds of having elevated TG by 43 and 41%, respectively ($P < 0.001$). The combination of high 25-OHD and low, medium, and high Ca significantly reduced the odds of having elevated TG by 83, 62, and 67%, respectively ($P < 0.001$) (Fig. 2 and Table 2).

Discussion

In this study, we investigated the associations between the individual MetS components and (i) 25-OHD concentration, (ii) dietary Ca intake, and (iii) the combination of 25-OHD concentration and dietary Ca intake, as they are commonly recommended together for optimal bone health. After adjustment for demographic, physical, dietary factors, and MetS components, every 10 nmol/l increment in 25-OHD reduced the odds of elevated TG and elevated FPG. Every 500 mg/day increment in dietary Ca intake reduced the odds of elevated DBP. The combination of 25-OHD concentration and Ca intake lowered the odds of having low HDL-C and elevated TG.

Previous studies of 25-OHD and MetS components

We found that increments of 10 nmol/l in 25-OHD lowered the odds of having elevated TG, and having elevated FPG (Table 1). However, there appears to be inconsistent evidence between 25-OHD concentration and MetS components. A large British study ($n = 7198$) of middle-aged (45 years) Caucasian adults found an inverse

Table 2 Model 3: adjusted odds ratio of having MetS components by combination of tertiles of 25-OHD and tertiles of dietary Ca intake

	Low 25-OHD (33 nmol/l) ^a		Medium 25-OHD (54 nmol/l) ^a		High 25-OHD (77 nmol/l) ^a		<i>P</i> value
	AOR	95% CI	AOR	95% CI	AOR	95% CI	
Low Ca (579 mg) ^a							
Elevated WC	1.0	–	1.16	0.47–2.87	1.21	0.52–2.79	0.999
Reduced HDL-C	1.0	–	0.43 ^b	0.27–0.69	0.62	0.33–1.15	0.035
Elevated TG	1.0	–	0.57 ^b	0.39–0.84	0.17 ^b	0.10–0.27	< 0.001
Elevated SBP	1.0	–	1.22	0.84–1.79	0.93	0.56–1.54	0.133
Elevated DBP	1.0	–	0.95	0.50–1.80	1.06	0.63–1.78	0.080
Elevated FPG	1.0	–	1.20	0.57–2.54	0.55	0.28–1.06	0.105
Medium Ca (858 mg) ^a							
Elevated WC	1.09	0.59–2.01	1.05	0.60–1.85	0.95	0.43–2.09	0.999
Reduced HDL-C	0.53 ^b	0.35–0.82	0.57	0.31–1.06	0.58	0.30–1.13	0.035
Elevated TG	0.84	0.47–1.51	0.59 ^b	0.37–0.94	0.38 ^b	0.22–0.64	< 0.001
Elevated SBP	0.96	0.54–1.70	0.90	0.52–1.57	1.14	0.66–1.98	0.133
Elevated DBP	0.99	0.56–1.76	0.83	0.47–1.48	0.57	0.39–0.85	0.080
Elevated FPG	1.08	0.65–1.79	0.72	0.40–1.30	0.78	0.40–1.52	0.105
High Ca (1233 mg) ^a							
Elevated WC	1.06	0.50–2.24	1.09	0.49–2.45	0.97	0.50–1.89	0.999
Reduced HDL-C	0.36 ^b	0.16–0.79	0.32 ^b	0.16–0.63	0.51	0.21–1.24	0.035
Elevated TG	0.69	0.36–1.32	0.66	0.42–1.04	0.33 ^b	0.17–0.61	< 0.001
Elevated SBP	0.75	0.41–1.39	0.80	0.48–1.33	0.69	0.37–1.28	0.133
Elevated DBP	0.90	0.48–1.71	0.81	0.49–1.35	0.67	0.34–1.33	0.080
Elevated FPG	1.18	0.56–2.51	0.71	0.37–1.39	0.58	0.28–1.20	0.105

Model adjusted for age, sex, country of birth, smoking, physical activity level, income, education, BMI, season, energy, fiber, alcohol, magnesium, zinc, MetS components. AOR, adjusted odds ratio; Ca, calcium; CI, confidence interval; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure; TG, triglycerides; WC, waist circumference.

^aMedian 25-OHD concentration and dietary calcium intake in each tertile.

^bSignificant as compared with the reference category.

Fig. 1

Model 3: The AOR of the combination of 25-OHD concentration and dietary calcium intake tertiles on reduced HDL-C. Adjusted for age, sex, country of birth, smoking, physical activity, income, education, BMI, season, energy, fiber, alcohol, magnesium, zinc, and MetS components. *Significant as compared with the reference category. AOR, adjusted odds ratio; HDL-C, high-density lipoprotein cholesterol.

association between 25-OHD concentration and high WC, high BP, high HbA1c, and high TG but not with low HDL-C [17]. We too found an association between 25-OHD and elevated TG and FPG; however, there were no associations with other MetS components. An American study ($n=8421$) found an inverse association between 25-OHD and high WC, TG, and FPG [18]; however, we found no association with WC. In an older

cohort, 25-OHD concentration was inversely associated with high WC, TG, FPG, and reduced HDL-C; however, no association was seen with BP [16]. A large prospective study from Australia ($n=4164$) found an inverse association between 25-OHD and the incidence of WC, TG, FPG, but not with HDL-C and BP [19]. The variation in results among previous studies and our findings may be owing to a few reasons, such as the use of different MetS

Fig. 2

Model 3: The AOR of the combination of 25-OHD concentration and dietary calcium intake tertiles on elevated TG. Adjusted for age, sex, country of birth, smoking, physical activity, income, education, BMI, season, energy, fiber, alcohol, magnesium, zinc, and MetS components. *Significant as compared with the reference category. AOR, adjusted odds ratio; TG, triglycerides.

criteria and cutoff points for 25-OHD concentration. The majority of these associations were found in middle-aged or older population; however, our study covered adults aged 18–75 years [16,20,21]. Older populations may be at higher risk of vitamin D deficiency, owing to certain physiological impairments that occur with age; thus, the results from older adults may not be applicable to the general population [22]. Evidence drawn from specific cultural groups [17,20,21], sex [20], and the use of differing confounders may also partially explain the variation in results.

Previous studies of dietary calcium intake and MetS components

There are limited studies examining the association between Ca and MetS components. In a large cohort study ($n=6375$) of adults aged older than or equal to 40 years, the highest quartile of Ca intake (median: 530.8 mg/day) was inversely associated with high WC and high blood glucose in women and hypertriglyceridemia in men [23]. A second large study ($n=10\,066$) in women found a lower prevalence of lower WC, high BP, and high HDL-C in the highest quintile of dietary Ca intake [11]. However, we found an association between 500 mg/day increments in dietary Ca intake and reduced odds of elevated DBP. We found no significant association between Ca intake and any other MetS components, after controlling for demographic, physical, dietary factors, and MetS components. Results may vary owing to the investigation of one sex [11], older age group (≥ 40 vs. ≥ 45 vs. 30–65 years) [11,23,24] versus a broad age range of 18–75 years in our study, and the adjustment for different confounders. In addition, these studies did not mutually adjust for MetS components in their analysis,

and thus the associations between MetS components and dietary Ca intake may be confounded by the presence of other MetS components. The association between dietary Ca intake and MetS components has not been well investigated, and requires further research.

The combined effect of 25-OHD concentration and dietary calcium intake on MetS components

Calcium intake and vitamin D play an interdependent role in bone metabolism and skeletal health. It was therefore appropriate to examine their combined role in extraskelatal health. The combined effect of 25-OHD concentration and Ca intake tertiles lowered the odds of having reduced HDL-C and elevated TG, and the reduction in AOR of having these components was greater than that obtained from the independent effects of 25-OHD concentration or dietary Ca intake.

Independently, every 10 nmol/l increment in 25-OHD reduced the odds of elevated TG by 21%. However, the combination of medium 25-OHD tertile and low and medium Ca intake appeared to reduce the odds of elevated TG further by 43 and 41%, respectively (Fig. 2). There also appeared to be a dose-response effect of Ca intake at low 25-OHD concentration, although the combinations were not significant. The odds of having elevated TG were approximately halved between medium 25-OHD and high 25-OHD.

Increments in 25-OHD and Ca intake did not significantly reduce the odds of elevated HDL-C. However, in combination, there appeared to be a strong effect of Ca at low 25-OHD on reduced HDL-C. The combination of low 25-OHD and medium and high Ca intake lowered the odds of having reduced HDL-C by 47 and 64%,

respectively (Fig. 1). The combination of 25-OHD concentration and Ca intake appeared to further reduce the odds of having reduced HDL-C than when 25-OHD concentration was taken into account alone. However, at high 25-OHD concentration, the effects of Ca appear to be blunted.

Independently, 500 mg/day increment in dietary Ca intake appeared to reduce the odds of elevated DBP by 22%, with a marginal association between 25-OHD concentration and elevated DBP. The combined effect of high 25-OHD and medium Ca tertiles revealed 43% lower odds of having elevated DBP, which was marginally significant ($P=0.080$) (Table 2).

We are unaware of other studies that have investigated the combined effect of 25-OHD concentration and Ca intake on MetS components. From our results it appears that there may be a combined effect of Ca to 25-OHD in reducing the odds of having reduced HDL-C and elevated TG. There appeared to be a strong effect of Ca at low 25-OHD on reduced HDL-C, whereas there appeared to be a stronger effect of Ca at high 25-OHD on elevated TG. The potential mechanisms are explored below.

Potential mechanisms

25-OHD concentration and MetS components

The pathophysiological mechanisms linking vitamin D to the MetS components are not yet confirmed. One potential mechanism may be through the renin-angiotensin system (RAS). The RAS is the key regulatory system of cardiovascular function, particularly for BP [25]; however, it has regained attention for its potential action in aspects of MetS [26]. Vitamin D may play a role in modulating BP through regulating the RAS, and renin production (the enzyme involved in maintaining BP) [27]. Vitamin D deficiency has been found to increase RAS activity [28,29] and stimulate renin synthesis. Increased RAS and renin activity can result in hypertension and insulin resistance (IR), which are characteristics of MetS [27,30]. Second, IR may also be related to RAS whereby vitamin D deficiency may increase renin-angiotensin II expression, which may induce IR [31–33]. In addition, the vitamin D pathway is involved in insulin secretion through the regulation of intracellular Ca^{2+} concentration [34], and lower levels of vitamin D are linked with increased IR [35,36]. Third, 25-OHD modulates enzyme activity directly related to lipoprotein lipase [37]. Vitamin D and its metabolites may upregulate lipoprotein lipase and in turn increase HDL-C and reduce TG [37]. Fourth, vitamin D may reduce inflammation and as a result reduce IR, leading to improved lipid metabolism [38, 39]. Overall, vitamin D deficiency may increase RAS activity, which is linked with the pathogenesis of hypertension, reduced insulin secretion, and IR [40].

Dietary calcium intake and MetS components

The positive effect of Ca on the MetS components may occur through different pathways. Those with lower Ca

intakes (<600 mg/day) tend to have higher body weight, BMI, % fat mass, fat mass, WC, and abdominal adiposity [41]. Dietary Ca intake may increase fat oxidation, which may in turn lower lipid levels [42,43]. Zemel [44] suggested that low Ca intake may affect hormones associated with bone growth. An increase in parathyroid hormone (PTH) and 1,25- OH_2D may result in a rise in intracellular Ca^{2+} concentrations influencing lipogenesis [44,45]. Higher intakes of Ca of ~ 1200 mg/day from dairy sources may increase fecal fat excretion by ~ 5 g/day [46]. A meta-analysis of randomized controlled trials found that Ca supplementation of ~ 1000 mg/day reduced SBP and DBP; however, those with lower Ca intake had a greater decrease in BP [47]. Interestingly, those who were Ca deficient and receiving Ca supplements tended to have a stronger effect on BP compared with those with higher habitual intakes.

From our results, it appears that the additive effect of 25-OHD and Ca is more beneficial than 25-OHD alone, in reducing the odds of having reduced HDL-C and elevated TG. There appears to be a dose-response effect of Ca at low 25-OHD on reduced HDL-C, but at higher 25-OHD the effects of Ca were blunted (Fig. 1). However, high 25-OHD appeared to lower the odds of elevated TG, irrespective of Ca intake (Fig. 2). This indicates that there are a multitude of mechanisms at play for each component constituting a complex relationship between 25-OHD, Ca intake, and MetS components.

Strengths

There are several strengths to our study. We draw our findings from a large population-based sample of adults aged 18–75 years. We adjusted for a range of demographic and dietary variables as compared with other studies [11,16, 17,20,21,23,24]. In our study, 25-OHD concentration was measured at one laboratory rather than multiple laboratories, which may limit variability in 25-OHD results [48]. In contrast to other studies [23,24,49], we adjusted for the effect of other MetS components in the final model. This allowed us to see whether the association between 25-OHD and Ca tertiles and MetS components was independent from the rest of the components.

Limitations

The cross-sectional study design is good to show an association between two variables; however, causality and National Health and Medical Research Council level 2 evidence can be obtained from longitudinal cohort studies [50]. Supplement usage was not collected in the VHM survey, thus teasing out the role of Ca from food versus a pharmacological source would be important to public health recommendations. Furthermore, Ca consumed as part of a food or food matrix has varying absorbability and hence metabolic effects. Future studies may wish to explore the differences between dietary Ca versus Ca as part of a food matrix for any metabolic differences [51].

The database lacked information on sun exposure and vitamin D supplement use; thus, the contribution of each could not be investigated. The lack of data on markers of IR, such as HOMA-IR, limits our understanding of the effects of 25-OHD and Ca intake on glucose homeostasis IR [52]. However, unlike some studies, we have controlled for other individual components of MetS, which would be strongly related to IR. PTH is crucial for 25-OHD concentration and Ca metabolism; however, this was not measured in our participants. A raised PTH can increase MetS components [53,54] and controlling for such a confounder would be important.

Conclusion

Our findings have indicated that increments of 10 nmol/l of 25-OHD concentration may lower the likelihood of having elevated TG and FPG, and 500 mg/day increments in dietary Ca intake may reduce the odds of elevated DBP. The combination of 25-OHD and Ca tertiles appears to further reduce the odds of reduced HDL-C and elevated TG. This warrants further investigation of the optimal level of the combination of 25-OHD and dietary Ca intake in reducing the risk of MetS components. Future prospective longitudinal studies and high-quality randomized controlled trials of vitamin D and/or calcium supplementation are needed to evaluate whether there is a causal relationship between 25-OHD concentration, dietary Ca intake, and individual MetS components.

Acknowledgements

P.K.P. analyzed the data and wrote the first draft. M.J.S. generated the idea, planned the analysis, and co-wrote the manuscript. Y.Z. cross-checked the analysis and co-wrote the manuscript. L.S.P. and Z.A. critically reviewed all aspects of the manuscript.

M.J.S. acknowledges the Victorian Department of Health and Human Services for use of the Victorian Health Monitor survey dataset. P.K.P. is the recipient of an Australian Postgraduate Award.

Conflicts of interest

There are no conflicts of interest.

References

- Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, *et al.* Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 2009; **120**:1640–1645.
- Cameron AJ, Shaw JE, Zimmet PZ. The metabolic syndrome: prevalence in worldwide populations. *Endocrinol Metab Clin North Am* 2004; **33**:351–375.
- Eckel RH, Alberti K, Grundy SM, Zimmet PZ. The metabolic syndrome. *The Lancet* 2010; **375**:181–183.
- Wannamethee SG, Shaper AG, Lennon L, Morris RW. Metabolic syndrome vs Framingham risk score for prediction of coronary heart disease, stroke, and type 2 diabetes mellitus. *Arch Intern Med* 2005; **165**:2644–2650.
- Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr* 2008; **87**:1080S–1086SS.
- Lips P. Worldwide status of vitamin D nutrition. *J Steroid Biochem Mol Biol* 2010; **121**:297–300.
- Wang Y, Li S. Worldwide trends in dairy production and consumption and calcium intake: Is promoting consumption of dairy products a sustainable solution for inadequate calcium intake? *Food Nutr Bull* 2008; **29**:172–185.
- Holick MF. Vitamin D deficiency. *N Engl J Med* 2007; **357**:266–281.
- Martini LA, Wood RJ. Vitamin D status and the metabolic syndrome. *Nutr Rev* 2006; **64**:479–486.
- Cho GJ, Park HT, Shin JH, Hur JY, Kim YT, Kim SH, *et al.* Calcium intake is inversely associated with metabolic syndrome in postmenopausal women: Korea National Health and Nutrition Survey, 2001 and 2005. *Menopause* 2009; **16**:992–997.
- Liu S, Song Y, Ford ES, Manson JE, Buring JE, Ridker PM. Dietary calcium, vitamin D, and the prevalence of metabolic syndrome in middle-aged and older U.S. women. *Diabetes Care* 2005; **28**:2926–2932.
- Pannu PK, Zhao Y, Soares MJ, Piers LS, Ansari Z. The associations of vitamin D status and dietary calcium with the metabolic syndrome: an analysis of the Victorian Health Monitor survey. *Public Health Nutr* 2016; **24**:1–12.
- Department of Health. *The Victorian Health Monitor Food and Nutrition Report*. Melbourne, Victoria: 2012.
- Department of Health. *The Victorian Health Monitor*. Melbourne, Victoria: 2012.
- American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2010; **33** (Suppl 1): S62–S69.
- Vitezova A, Zillikens MC, van Herpt TT, Sijbrands EJ, Hofman A, Uitterlinden AG, *et al.* Vitamin D status and metabolic syndrome in the elderly: the Rotterdam Study. *Eur J Endocrinol* 2015; **172**:327–335.
- Hypponen E, Boucher BJ, Berry DJ, Power C. 25-hydroxyvitamin D IGF-1 and metabolic syndrome at 45 years of age. *Diabetes* 2008; **57**:298–305.
- Ford ES, Ajani UA, McGuire LC, Liu S. Concentrations of serum vitamin D and the metabolic syndrome among U.S. adults. *Diabetes Care* 2005; **28**:1228–1230.
- Gagnon C, Lu ZX, Magliano DJ, Dunstan DW, Shaw JE, Zimmet PZ, *et al.* Low serum 25-hydroxyvitamin D is associated with increased risk of the development of the metabolic syndrome at five years: results from a national, population-based prospective study (The Australian Diabetes, Obesity and Lifestyle Study: AusDiab). *J Clin Endocrinol Metab* 2012; **97**:1953–1961.
- Lee DM, Rutter MK, O'Neill TW, Boonen S, Vanderschueren D, Bouillon R, *et al.* Vitamin D, parathyroid hormone and the metabolic syndrome in middle-aged and older European men. *Eur J Endocrinol* 2009; **161**:947–954.
- Yin X, Sun Q, Zhang X, Lu Y, Sun C, Cui Y, Wang S. Serum 25(OH)D is inversely associated with metabolic syndrome risk profile among urban middle-aged Chinese population. *Nutr J* 2012; **11**:68.
- Timpi A, Pini L, Tantucci C, Cossi S, Grassi V. Vitamin D and health status in elderly. *Intern Emerg Med* 2011; **6**:11–21.
- Shin SK, Kim MK, Lee YH, Shin DH, Shin MH, Chun BY, Choi BY. The cross-sectional relationship between dietary calcium intake and metabolic syndrome among men and women aged 40 or older in rural areas of Korea. *Nutr Res Pract* 2015; **9**:328–335.
- Drouillet P, Balkau B, Charles MA, Vol S, Bedouet M, Ducimetière P. The DESIR Study Group. Calcium consumption and insulin resistance syndrome parameters. Data from the Epidemiological Study on the Insulin Resistance Syndrome (DESIR). *Nutr Metab Cardiovasc Dis* 2007; **17**:486–492.
- Schmieder RE, Hilgers KF, Schlaich MP, Schmidt BMW. Renin-angiotensin system and cardiovascular risk. *The Lancet* 2007; **369**:1208–1219.
- de Kloet AD, Krause EG, Woods SC. The renin angiotensin system and the metabolic syndrome. *Physiol Behav* 2010; **100**:525–534.
- Li YC, Qiao G, Uskokovic M, Xiang W, Zheng W, Kong J. Vitamin D: a negative endocrine regulator of the renin-angiotensin system and blood pressure. *J Steroid Biochem* 2004; **89**:387–392.
- Dluhy RG, Williams GH. Aldosterone-villain or bystander? *N Engl J Med* 2004; **351**:8–10.
- Vaidya A, Forman JP, Williams JS. Vitamin D and the vascular sensitivity to angiotensin II in obese Caucasians with hypertension. *J Hum Hypertens* 2011; **25**:672–678.
- Engeli S, Schling P, Gorzelnik K, Boschmann M, Janke J, Ailhaud G, *et al.* The adipose-tissue renin-angiotensin-aldosterone system: role in the metabolic syndrome? *Int J Biochem Cell Biol* 2003; **35**:807–825.
- Leiter LA, Lewanczuk RZ. Of the renin-angiotensin system and reactive oxygen species type 2 diabetes and angiotensin II inhibition. *Am J Hypertens* 2005; **18**:121–128.

- 32 Wei Y, Sowers JR, Clark SE, Li W, Ferrario CM, Stump CS. Angiotensin II-induced skeletal muscle insulin resistance mediated by NF- κ B activation via NADPH oxidase. *Am J Physiol Endocrinol Metab* 2008; **294**:E345–E351.
- 33 Rammos G, Tseke P, Ziakka S. Vitamin D, the renin-angiotensin system, and insulin resistance. *Int Urol Nephrol* 2008; **40**:419–426.
- 34 Sooy K, Schermerhorn T, Noda M, Surana M, Rhoten WB, Meyer M, et al. Calbindin-D(28 K) controls [Ca(2+)] (i) and insulin release. Evidence obtained from calbindin-d(28 K) knockout mice and beta cell lines. *J Biol Chem* 1999; **274**:34344–34349.
- 35 Chiu KC, Chu A, Go VL, Saad MF. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. *Am J Clin Nutr* 2004; **79**:820–825.
- 36 Chonchol M, Scragg R. 25-Hydroxyvitamin D, insulin resistance, and kidney function in the Third National Health and Nutrition Examination Survey. *Kidney Int* 2007; **71**:134–139.
- 37 Querfeld U, Hoffmann MM, Klaus G, Eifinger F, Ackerschott M, Michalk D, Kern PA. Antagonistic effects of vitamin D and parathyroid hormone on lipoprotein lipase in cultured adipocytes. *J Am Soc Nephrol* 1999; **10**:2158–2164.
- 38 Chagas CE, Borges MC, Martini LA, Rogero MM. Focus on vitamin D, inflammation and type 2 diabetes. *Nutrients* 2012; **4**:52–67.
- 39 Hewison M. An update on vitamin D and human immunity. *Clin Endocrinol (Oxf)* 2012; **76**:315–325.
- 40 Vaidya A, Williams JS. The relationship between vitamin D and the renin-angiotensin system in the pathophysiology of hypertension, kidney disease, and diabetes. *Metabolism* 2012; **61**:450–458.
- 41 Jacqmain M, Doucet E, Després J-P, Bouchard C, Tremblay A. Calcium intake, body composition, and lipoprotein-lipid concentrations in adults. *Am J Clin Nutr* 2003; **77**:1448–1452.
- 42 Soares MJ, Chan She Ping-Delfos W, Ghanbari MH. Calcium and vitamin D for obesity: a review of randomized controlled trials. *Eur J Clin Nutr* 2011; **65**:994–1004.
- 43 Soares MJ, Murhadi LL, Kurpad AV, Chan She Ping-Delfos WL, Piers LS. Mechanistic roles for calcium and vitamin D in the regulation of body weight. *Obes Rev* 2012; **13**:592–605.
- 44 Zemel MB. Nutritional and endocrine modulation of intracellular calcium: implications in obesity, insulin resistance and hypertension. *Mol Cell Biomech* 1998; **188**:129–136.
- 45 Zemel MB, Shi H, Greer B, Dirienzo D, Zemel PC. Regulation of adiposity by dietary calcium. *FASEB J* 2000; **14**:1132–1138.
- 46 Christensen R, Lorenzen JK, Svith CR, Bartels EM, Melanson EL, Saris WH, et al. Effect of calcium from dairy and dietary supplements on faecal fat excretion: a meta-analysis of randomized controlled trials. *Obes Rev* 2009; **10**:475–486.
- 47 van Mierlo LAJ, Arends LR, Streppel MT, Zeegers MPA, Kok FJ, Grobbee DE, Geleijnse JM. Blood pressure response to calcium supplementation: a meta-analysis of randomized controlled trials. *J Hum Hypertens* 2006; **20**:571–580.
- 48 Black LJ, Anderson D, Clarke MW, Ponsonby AL, Lucas RM. Ausimmune Investigator Group. Analytical bias in the measurement of serum 25-hydroxyvitamin D concentrations impairs assessment of vitamin D status in clinical and research settings. *PLoS One* 2015; **10**:e0135478.
- 49 Liu E, Meigs JB, Pittas AG, McKeown NM, Economos CD, Booth SL, Jacques PF. Plasma 25-hydroxyvitamin D is associated with markers of the insulin resistant phenotype in nondiabetic adults. *J Nutr* 2009; **139**:329–334.
- 50 National Health and Medical Research Council. *How to review the evidence: systematic identification and review of the scientific literature*. Canberra: ACT; 1999.
- 51 Zemel MB. Proposed role of calcium and dairy food components in weight management and metabolic health. *Phys Sports Med* 2009; **37**:29–39.
- 52 Singh B, Saxena A. Surrogate markers of insulin resistance: a review. *World J Diabetes* 2010; **1**:36–47.
- 53 Ahlström T, Hagström E, Larsson A, Rudberg C, Lind L, Hellman P. Correlation between plasma calcium, parathyroid hormone (PTH) and the metabolic syndrome (MetS) in a community-based cohort of men and women. *Clin Endocrinol (Oxf)* 2009; **71**:673–678.
- 54 Reis JP, von Muhlen D, Miller ER 3rd. Relation of 25-hydroxyvitamin D and parathyroid hormone levels with metabolic syndrome among US adults. *Eur J Endocrinol* 2008; **159**:41–48.