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The Metabolic Syndrome and Carotid Intima-Media Thickness in Relation to the Parathyroid Hormone to 25-OH-D_3 Ratio in a General Population

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BACKGROUND
Parathyroid hormone (PTH) and vitamin D interactively regulate calcium fluxes across membranes, and thereby modulate insulin sensitivity, blood pressure, and arterial calcification. We hypothesized that lower calcium intake as reflected by circulating PTH and 25-OH-D_3 might be associated with the metabolic syndrome (MS) and arterial calcification.

METHODS
In a random population sample ($n = 542$; 50.5% women; mean age, 49.8 ± 13.1 years), we measured MS prevalence (International Diabetes Federation (IDF) and American Heart Association (AHA) criteria), PTH and 25-OH-D_3, serum and 24-h urinary calcium, MS components, carotid intima-media thickness (CIMT), and calcium intake from dairy products. We assessed associations in multivariable-adjusted analyses, using linear and logistic regressions.

RESULTS
The prevalence of MS was 21.0% (IDF criteria) and 23.6% (AHA criteria). MS prevalence, blood pressure, waist circumference, body mass index, fasting blood glucose, insulin and triglycerides, and CIMT increased ($P \le 0.042$) across quartiles of the PTH/25-OH-D_3 ratio, whereas serum and 24-h urinary calcium decreased ($P \le 0.029$). Waist circumference and fasting blood glucose decreased across quartiles of habitual calcium intake ($P \le 0.04$). In models that included MS (IDF) and PTH/25-OH-D_3, the regression coefficients relating CIMT to PTH/25-OH-D_3 ratio and MS were +51 µm ($P = 0.013$) and +19 µm ($P = 0.45$), respectively. Multivariable-adjusted analyses were confirmatory.

CONCLUSIONS
MS prevalence and CIMT were positively associated with PTH/25-OH-D_3. CIMT was not associated with MS. Prospective studies and intervention trials should address the causality of these associations.

Keywords: *25-OH-D_3; blood pressure; calcium; hypertension; insulin resistance; intima-media thickness; metabolic syndrome; PTH; vitamin D*

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Active vitamin D_3 (1,25-$(OH)_2$-D_3; calcitriol) and parathyroid hormone (PTH) are calciotropic hormones that interactively control renal calcium excretion and keep circulating calcium levels within physiological limits by balancing calcium fixation and mobilization in bone tissue against gastrointestinal uptake. The precursor of active vitamin D, cholecalciferol, is generated in the skin from 7-deoxy-cholesterol by UV irradiation or is gastrointestinally absorbed from food or medicinal supplements.[1] The 25-hydroxylase activity in the liver converts cholecalciferol to 25-OH-D_3 (calcidiol), the commonly measured biomarker of vitamin D status. The enzymatic 1α-hydroxylase activity, which in the proximal renal tubuli converts circulating 25-OH-D_3 to the hormonally active 1,25-$(OH)_2$-D_3 (calcitriol), is central to the endocrine function of calcitriol and PTH as modulators of calcium homeostasis.[2] The interactive effects of these hormones also regulate calcium fluxes across cell membranes, hereby modulating pancreatic islet cell insulin secretion,[3] target cell insulin sensitivity,[3] blood pressure control,[2] and carotid artery calcification.[4]

Population studies showed positive associations of circulating PTH and negative associations of 25-OH-D_3 with carotid intima-media thickness (CIMT),[4–6] blood pressure,[2,6,7] insulin resistance,[8,9] and fasting blood glucose.[8,9] A high calcium intake lowers PTH[10,11] and decreases the risk of cardiovascular and metabolic diseases.[12] Furthermore, when habitual calcium intake is low, calcium appears to shift from bone into the arterial wall, the so-called "calcium paradox."[13] To our knowledge, the interactive effects of habitual calcium intake, PTH and 25-OH-D_3 on the prevalence of the metabolic syndrome (MS) and its components and on arterial phenotypes have not yet been simultaneously assessed at the level of a general population.

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We hypothesized that an increased PTH/25-OH-D_3 ratio promotes an inward calcium flux in target cells that are involved in glucose metabolism and arterial calcification, and that these effects are more pronounced in individuals with low habitual calcium intake. To address our working hypothesis, we assessed in a general population habitual calcium intake, glucose and calcium metabolism, and CIMT.

METHODS

Study population. The Ethics Committee of the University of Leuven approved the protocol of the FLEMENGHO (Flemish Study on Environment, Genes and Health Outcomes). All subjects gave informed written consent. From August 1985 until November 1990, a random sample of the households living in a geographically defined area of Northern Belgium was invited, with the goal of recruiting equal numbers of participants in each of the six subgroups defined by sex and age (20–39, 40–59, and ≥60 years) that would go through repeated examination cycles. The study population included 2,310 subjects. The participation rate among the subjects contacted was 66.1%. The random subsample for the present analysis, included 550 subjects invited during a specific phase of the study, from which we excluded eight patients with diabetes mellitus. At enrolment, blood pressure was measured five times consecutively by trained observers at each of two home visits at an interval of 3–4 weeks. Thus, the study subjects were familiarized with the settings of the study and the study team, the same observer measuring their blood pressure at the baseline and the follow-up examinations in most cases. The diagnosis of hypertension therefore complied with current guidelines, which recommend blood pressure measurement at repeated occasions to ascertain the diagnosis of an elevated blood pressure.

Clinical measurements. For at least 3 h before being examined, the participants were asked to refrain from heavy exercise, smoking, alcohol, or caffeine-containing beverages. Trained nurses administered a standardized and validated questionnaire to collect information about each subject's medical history, smoking and drinking habits, dietary intake of calcium, and the use of medications. The questionnaire also provided detailed information on the total number of hours spent in sports and occupational activities, including attending school for younger people. With the use of published tables, we estimated the energy spent in physical activity from body weight, time devoted to work and sports, and types of physical activity.[14] We estimated dietary calcium intake from the use of dairy products and the calcium content as listed in the Dutch food tables. For statistical analysis, we expressed alcohol intake and current smoking as a dichotomous variable.

After the subjects had rested for at least 5 min in the sitting position, the nurses measured blood pressure five times consecutively with a standard mercury sphygmomanometer by auscultation of the Korotkoff sounds. For participants with an arm circumference of <32 cm, a standard cuff, with an inflatable bladder with a length of 22 cm and a width of 12 cm, was used to measure blood pressure. In participants with a greater arm circumference, cuffs with a 35 × 15 cm bladder were applied. For analysis, we averaged the five blood pressure readings. Body mass index was weight in kilograms divided by the square of height in meters. The waist-to-hip ratio, determined by means of a tape measure, was the ratio of the smallest circumference at the waist to the largest circumference at the hip. The nurses measured the skinfold at the mid portion of the triceps by means of a Harpenden Caliper (Baty, West Sussex, UK) providing a constant pressure of 0.01 kg/mm^2 (0.098 N/mm^2) at all openings of the 90 mm^2 anvils.

The MS components were as follows: elevated blood pressure, defined as a blood pressure of at least 130 mm Hg systolic or 80 mm Hg diastolic or the use of antihypertensive drugs; central obesity, defined as a waist circumference of ≥88 cm or ≥102 cm in women and men, respectively; serum triglycerides of ≥150 mg/dl; high-density lipoprotein-cholesterol <50 or <40 mg/dl in women and men, respectively; and a fasting blood glucose of ≥100 mg/dl. The American Heart Association (AHA)[15] recommends that the MS be identified as the presence of three or more components. The International Diabetes Federation (IDF)[16] defines the presence of the MS as an elevated waist circumference and the presence of two or more other components.

Biochemical measurements. The participants collected a timed 24-h urine sample for the measurement. Blood samples were collected into chilled EDTA-containing tubes after an overnight fast, centrifuged and divided in aliquots within 30 min after venipuncture. Total and high-density lipoprotein-cholesterol and triglycerides in serum, blood glucose, and serum and urinary creatinine were measured by automated enzymatic methods. Serum insulin was determined by immunoassay, and calcium in serum and urine by a complexometric titration method.[17] We computed the homeostasis model assessment of insulin resistance index as (fasting glucose × fasting insulin)/65. Plasma intact PTH was measured by an immunoradiometric assay and plasma 25-OH-D_3 by a competitive protein binding assay (Medgenix, Fleurus, Belgium). The PTH/25-OH-D_3 ratio was PTH in pg/ml divided by 25-OH-D_3 in ng/ml.

Arterial phenotypes. The same observer (E.J.B.) performed all measurements of CIMT, using a wall-tracking ultrasound system with a 7.5 MHz probe. She recorded IMT at the right common carotid artery 2 cm proximal of the bulb, and recorded blood pressure for off-line calibration of distension curves. The same operator (E.J.B.) measured off-line the distances from the adventitia-media boundary of the near wall to the lumen-intima and media-adventitia interfaces of the far wall. The IMT was computed as the mean difference between these distances measured over three separate intervals of 5.2 s, which on average included 15 heart cycles. The intraobserver intrasession coefficients of variability amounted to (± s.d.) 5.2 ± 1.7%.[18] During the examination and postprocessing of ultrasonographic data, the observer was blinded to the biochemical profiles used to classify subjects according to their MS status.

Statistical analysis. For database management and statistical analysis, we used SAS software, version 9.2 with the JMP add-on, version 7 (SAS Institute, Cary, NC). Measurements with a skewed distribution were normalized by a logarithmic transformation. The geometric mean and the 5th–95th percentile interval represented the central tendency and spread of logarithmically transformed variables. We compared means and proportions by the standard normal *z* test and Fisher's exact test, respectively. We used the PROC GLM procedure, as implemented in the SAS package to compare means across quartiles of the PTH/25-OH-D_3 ratio. We searched for covariables of the outcome variables of interest, using stepwise multiple linear regression, with the *P* value for independent variables to enter and stay in the model set at 0.15. We used logistic regression to evaluate associations of the MS defined according to AHA/IDF criteria with covariables. We expressed effect sizes with 95% confidence interval (CI) in regression analysis for a 1-s.d. increase in the explanatory variable.

RESULTS

Characteristics of the participants

The participants included 272 women and 270 men. There were no differences between women and men in age (mean ± s.d., 49.3 ± 12.8 vs. 50.2 ± 13.3 years, respectively; $P = 0.38$); in the circulating levels of PTH (geometric mean (5th–95th percentile interval), 23.22 pg/ml (12.60–43.59) vs. 22.56 pg/ml (12.70–38.95); $P = 0.39$) and 25-OH-D_3 (29.4 ng/ml (27.8–30.9) vs. 28.6 ng/ml (27.24–29.93); $P = 0.45$), or in the PTH/25-OH-D_3 ratio (0.83 (0.39–1.66) vs. 0.81 (0.41–1.68); $P = 0.79$). Among women, 148 (54.4%) and 124 (45.6%) were pre- or postmenopausal, respectively. Among women and men, 83 (30.7%) and 87 (33.1%) were smokers; 22 women (8.2%) and 91 men (34.6%) reported alcohol intake. In smokers, median tobacco use was 15 cigarettes per day (interquartile range, 10–21). In drinkers, the median alcohol consumption was 20 g per day (interquartile range, 10–30). At the time of questioning, 89 (16.4%) of the participants reported intake of antihypertensive medication.

The MS and its components across quartiles of calcium intake from dairy sources

The median daily intake of calcium from dairy sources was 128 mg/day (interquartile range, 69–252 mg/day). Across increasing quartiles of calcium intake, waist circumference decreased from 90.0 to 85.7 cm and fasting glucose from 92.3 to 87.0 mg/dl (*P* for trend, <0.02 and <0.04, respectively). Lower calcium intake was also associated with increased systolic (Q1–Q4, 128.5–123.6 mm Hg, $P = 0.018$) and diastolic blood pressure (Q1–Q4, 79.8–76.8 mm Hg, $P = 0.024$), but this trend did not reach significance for CIMT (Q1–Q4, 798–841 μm, $P = 0.24$), for fasting insulin (Q1–Q4, 5.98–7.27 μIU/ml, $P = 0.32$) or triglycerides (Q1–Q4, 180.7–169.1 mg/dl, $P = 0.17$). The prevalence of the MS did not decrease significantly across quartiles of calcium intake (Q1–Q4, 22.6–21.2%, $P = 0.88$).

The MS and its components across quartiles of the PTH/25-OH-D_3 ratio

Table 1 lists the demographic characteristics of the participants by quartiles of the PTH/25-OH-D_3 ratio. Across quartiles, there were significant increases in systolic and diastolic blood pressure ($P = 0.0048$ and 0.014, respectively), body mass index ($P = 0.0005$), waist circumference ($P < 0.0001$), waist-to-hip ratio ($P = 0.048$), fasting blood glucose ($P = 0.011$), fasting insulin ($P = 0.0005$), homeostasis model assessment of insulin resistance index ($P = 0.0003$), serum triglycerides ($P = 0.027$), and the CIMT ($P = 0.042$). On the other hand, serum total calcium ($P = 0.0003$) and the 24-h urinary calcium excretion decreased ($P = 0.0292$) across quartiles of the PTH/25-OH-D_3 ratio.

The prevalence of the MS increased across quartiles of the PTH/25-OH-D_3 ratio, irrespective of whether the IDF ($P = 0.0005$) or AHA ($P = 0.0513$) definition was applied (Figure 1a). Of the dichotomized components of the MS, waist circumference ($P = 0.0019$), fasting blood glucose ($P = 0.024$), and serum triglycerides ($P = 0.060$), but not the prevalence of hypertension ($P = 0.30$) increased across the PTH/25-OH-D_3 quartiles (Figure 1b). There was no association between serum high-density lipoprotein-cholesterol and the PTH/25-OH-D_3 ratio ($P = 0.32$).

Odds of the MS in relation to calciotropic hormones

Figure 2 shows the odds ratios (ORs) for the MS associated with 1-s.d. increases (3.02 units) in PTH/25-OH-D_3 and the components of this ratio. The OR for the MS according

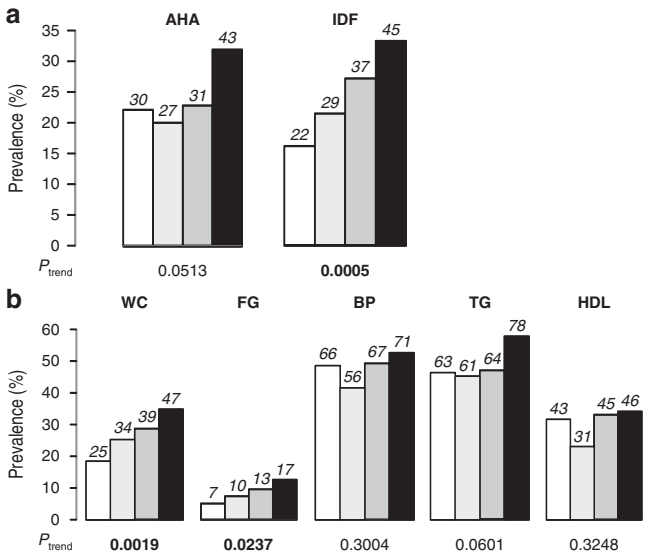

Figure 1 | Prevalence of the (**a**) metabolic syndrome and (**b**) its components across quartiles of the ratio of parathyroid hormone (in pg/ml) to 25-OH-D_3 (in ng/ml) ratio. *P* values are for the trends across quartiles of this ratio. Numbers above the bars refer to the subjects contributing to the numerator to compute the prevalence. (**a**) IDF and AHA refer to the metabolic syndrome according to the International Diabetes Federation[16] and American Heart Association[15] definitions, respectively. (**b**) WC, waist circumference; FG, fasting glucose; BP, blood pressure; TG, serum triglycerides; HDL, high-density lipoprotein. The components of the metabolic syndrome were dichotomized according to IDF as described in the Methods.

Table 1 | Characteristics of participants by quartiles of the PTH/25-OH-D_3 ratio

	Low	Medium-low	Medium-high	High	*P*
Quartile boundaries	0.16–0.56	0.57–0.79	0.80–1.17	1.18–8.66	
Number	136	135	136	135	
Mean ± s.d. of characteristic					
Age (years)	47.1 ± 12.4	49.7 ± 12.3	49.6 ± 13.8	52.7 ± 13.2	0.0007
Systolic pressure (mm Hg)	123.6 ± 16.2	124.7 ± 16.1	127.1 ± 16.9	128.9 ± 18.2	0.0048
Diastolic pressure (mm Hg)	76.9 ± 9.8	78.4 ± 10.1	79.9 ± 10.3	79.5 ± 10.4	0.014
Pulse rate (beats/min)	69.8 ± 9.6	70.6 ± 10.7	70.2 ± 9.5	71.3 ± 10.6	0.29
Body mass index (kg/m^2)	24.7 ± 3.5	26.1 ± 4.4	26.4 ± 4.1	26.4 ± 4.2	0.0005
Triceps skinfold thickness (cm)	1.55 ± 0.68	1.63 ± 0.66	1.71 ± 0.69	1.66 ± 0.82	0.11
Waist circumference (cm)	85.2 ± 11.4	88.3 ± 12.5	88.5 ± 11.8	91.5 ± 12.6	<0.0001
Waist-to-hip ratio	0.85 ± 0.09	0.85 ± 0.09	0.85 ± 0.10	0.87 ± 0.09	0.048
Blood glucose (mg/dl)	88.4 ± 11.3	87.3 ± 9.1	88.9 ± 10.4	92.7 ± 23.4	0.011
HDL-cholesterol (mg/dl)	51.1 ± 12.6	52.0 ± 15.3	50.5 ± 13.5	49.9 ± 15.4	0.34
Serum calcium (mg/dl)	9.40 ± 0.36	9.36 ± 0.40	9.28 ± 0.40	9.24 ± 0.40	0.0003
24-h Urinary calcium (mg)	175 ± 81	179 ± 88	161 ± 79	149 ± 93	0.029
24-h Urinary sodium (g)	4.47 ± 1.79	4.14 ± 1.40	4.18 ± 1.36	4.07 ± 1.41	0.21
24-h Urinary potassium (g)	2.85 ± 1.11	2.69 ± 0.80	2.86 ± 1.12	2.72 ± 1.01	0.55
24-h Na^+/K^+ ratio	1.66 ± 0.60	1.63 ± 0.58	1.58 ± 0.55	1.63 ± 0.61	0.69
Carotid IMT (μm)	814 ± 223	820 ± 183	839 ± 203	880 ± 196	0.042
Geometric mean (5th–95th percentile interval)					
Insulin (μIU/ml)	5.98 (2.48–14.40)	5.98 (2.48–14.40)	7.41 (2.83–19.34)	7.78 (2.85–21.26)	0.0005
Dietary calcium intake (mg/day)[a]	183.6 (151.3–215.8)	183.6 (151.3–215.8)	178.4 (156.8–199.9)	185.2 (155.1–215.4)	0.78
PTH (pg/ml)	14.78 (12.73–18.20)	20.29 (16.84–25.04)	23.86 (19.30–29.60)	31.32 (24.02–41.60)	<0.0001
25-hydroxyvitamin-D_3 (ng/ml)	32.6 (30.7–34.5)	32.6 (30.7–34.5)	27.4 (25.9–29.0)	20.7 (19.2–22.2)	<0.0001
HOMA (units)	1.50 (0.57–3.96)	1.50 (0.57–3.96)	1.74 (0.59–5.17)	1.89 (0.57–6.31)	0.0003
Triglycerides (mg/dl)	145.0 (56.9–390.2)	145.0 (56.9–390.2)	153.4 (44.7–526.2)	173.2 (62.1–483.1)	0.022
Daily energy expenditure (kcal)	1,297 (420–2,605)	1,297 (420–2,605)	1,393 (449–2,873)	1,204 (232–2,637)	0.39
Number (%) with characteristic					
Female sex	66 (48.5)	66 (48.5)	70 (51.5)	69 (51.1)	0.67
Smokers	55 (40.4)	55 (40.4)	40 (29.4)	36 (26.7)	0.018
Drinkers	29 (21.3)	29 (21.3)	28 (20.6)	29 (21.5)	0.95
Diuretics	4 (2.9)	4 (2.9)	17 (12.5)	13 (9.6)	0.0036
β-Blockers	8 (5.9)	8 (5.9)	18 (13.2)	18 (13.3)	0.0051
Other antihypertensive drugs	11 (8.1)	11 (8.1)	11 (8.1)	14 (10.5)	0.48

P values are for trend across quartiles.
HDL, high-density lipoprotein; HOMA, homeostasis model assessment of insulin resistance; IMT, intima-media thickness; PTH, parathyroid hormone.
[a]Intake from dairy products.

to the IDF definition was 0.19 (95% CI, 0.07–0.55; P = 0.0021) for 25-OH-D_3, and 1.78 (CI, 1.15–2.84; P = 0.012) for the PTH/25-OH-D_3 ratio, whereas the OR of 1.40 (CI, 0.84–2.33; P = 0.18) for PTH did not reach statistical significance (Figure 2). The findings for the MS according to the AHA definition were similar, but only the OR for 25-OH-D_3 reached statistical significance (Figure 2). Adjustment of these ORs for lifestyle factors, such as smoking or drinking alcohol, did not materially alter the estimates as shown in Figure 2.

Association of the MS components with calciotropic hormones

Table 2 lists the standardized regression coefficients expressing the changes in the MS components and other biomarkers associated with 1-s.d. increments (3.02 units) in 25-OH-D_3, PTH (with and without mutual adjustment) and the PTH/25-OH-D_3 ratio. Blood pressure, waist circumference, serum triglycerides, fasting insulin, and the homeostasis model assessment of insulin resistance index increased with the PTH/25-OH-D_3 ratio. Serum calcium and the 24-h urinary

Table 2 | Parameter estimates for a 1-s.d. increase in 25-OH-D_3, PTH, and the PTH/25-OH-D_3 ratio

Characteristic	25-OH-D_3	25-OH-D_3 adjusted for PTH	PTH	PTH adjusted for 25-OH-D_3	PTH/25-OH-D_3
Systolic pressure (mm Hg)	−12.19 (−19.51 to −4.86)†	−12.06 (−19.38 to −4.75)†	1.59 (−1.73 to 4.90)	1.37 (−1.92 to 4.65)	3.26 (0.29 to 6.24)*
Diastolic pressure (mm Hg)	−4.17 (−8.61 to 0.28)	−4.05 (−8.49 to 0.38)	1.36 (−0.64 to 3.36)	1.28 (−0.71 to 3.28)	1.78 (−0.02 to 3.57)
Waist circumference (cm)	−10.53 (−15.79 to −5.25)‡	−10.14 (−15.36 to −4.93)‡	4.31 (1.94 to 6.68)‡	4.12 (1.78 to 6.46)	5.19 (3.08 to 7.30)‡
Blood glucose (mg/dl)	−10.05 (−16.45 to −3.65)*	−9.91 (−16.31 to −3.50)*	1.78 (−1.12 to 4.66)	1.59 (−1.28 to 4.46)	3.07 (0.48 to 5.66)†
HDL-cholesterol (mg/dl)	7.87 (1.73 to 14.01)*	7.85 (1.70 to 14.01)*	−0.38 (−3.11 to 1.41)	−0.19 (−2.96 to 2.57)	−1.55 (−4.04 to 0.94)
Serum calcium (mg/dl)	0.16 (−0.02 to 0.33)	0.15 (−0.026 to 0.319)	−0.13 (−0.21 to −0.05)‡	−0.13 (−0.21 to −0.01)‡	−0.13 (−0.20 to −0.06)‡
24-h Urinary calcium (mg)	4.27 (0.12 to 8.42)*	4.11 (−0.03 to 8.24)*	−1.52 (−3.29 to 0.25)	−1.43 (−3.20 to 0.33)	−1.88 (−3.47 to −0.28)*
Triglycerides (mg/dl)	−0.72 (−0.57 to −0.91)†	−0.72 (−0.56 to −0.94)†	1.06 (−0.96 to 1.18)	1.06 (−0.96 to 1.17)	1.11 (1.01 to 1.22)*
Insulin (μIU/ml)	−0.85 (−0.96 to 1.05)	−0.86 (−0.69 to 1.06)	1.16 (1.05 to 1.27)†	1.15 (1.05 to 1.27)†	1.16 (1.06 to 1.26)‡
HOMA index (units)	−0.11 (−0.22 to −0.01)*	−0.11 (−0.21 to 0.01)	0.07 (0.02 to 0.12)†	0.07 (0.02 to 0.12)†	0.08 (0.03 to 0.12)‡
CIMT (μm)	30 (−74 to 134)	33 (−69 to 136)	68 (24 to 112)†	68 (25 to 112)†	52 (12 to 93)†

Estimates express the change (95% confidence interval) in the dependent variable associated with a 1-s.d. increase in the explanatory variable (25-OH-D_3: 11.9 ng/ml; PTH: 7.54 pg/ml). CIMT, carotid intima-media thickness; HDL, high-density lipoprotein; HOMA, homeostasis model assessment of insulin resistance; PTH, parathyroid hormone.
Significance of the effect sizes: *$P \le 0.05$, †$P \le 0.01$, and ‡$P \le 0.001$.

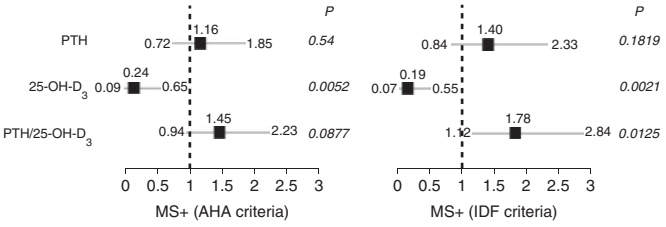

Figure 2 | Odds ratios for the metabolic syndrome (plotted along the horizontal axis) associated with 1-s.d. increases in the ratio of PTH (in pg/ml) to 25-OH-D_3 (in ng/ml). AHA, American Heart Association; IDF, International Diabetes Federation; MS, metabolic syndrome; PTH, parathyroid hormone.

calcium excretion decreased with higher PTH/25-OH-D_3 ratio.

Association of the CIMT with calciotropic hormones and MS

CIMT increased with higher PTH/25-OH-D_3 ratio and higher PTH, but was not significantly associated with 25-OH-D_3 (Table 2). The association between CIMT and MS according to the IDF definition[16] was not significant (24 μm; CI, −23 to 71 μm; P = 0.28). Next, to estimate the independent contributions of the MS and the calciotropic hormones to the variance of CIMT, we constructed models that included the MS according to the IDF definition[16] and either PTH, 25-OH-D_3, or PTH/25-OH-D_3. The partial regression coefficients for the PTH/25-OH-D_3 ratio (51 μm; CI, 11–91 μm; P = 0.013) and PTH (67 μm/pmol/l; CI, 24–111 μm; P = 0.0027) were significant, whereas this was not the case for the regression coefficients for the MS in models including either the PTH/25-OH-D_3 ratio (19 μm; CI, −30 to 68 μm; P = 0.45) or PTH (19 μm; CI, −29 to 69 μm; P = 0.43).

Multivariable-adjusted analyses

Figure 3 illustrates the prevalence of the MS according to the IDF definition (Figure 3a), CIMT (Figure 3b), fasting blood glucose (Figure 3c), and the 24-h urinary calcium excretion (Figure 3d) as a function of the cross-classification by quartiles of PTH and 25-OH-D_3. The analyses were adjusted for sex, age, lifestyle (current smoking and use of alcohol), and intake of antihypertensive medication. With increasing levels of PTH and decreasing levels of 25-OH-D_3, we noted increases in the prevalence of the MS, fasting blood glucose, and CIMT, whereas the opposite was the case for the 24-h urinary calcium excretion.

Sensitivity analyses

Exclusion of postmenopausal women on hormonal replacement therapy (n = 7 of 124; 5.6%) from the analyses, and additional adjustment for ever smoking, physical activity, or 24-h urinary Na, K, and/or the urinary Na/K ratio did not materially alter our results. 25-OH-D_3 levels were not significantly related to the level of physical activity (β = 0.0016; P = 0.16).

DISCUSSION

The key findings of our population-based study are the significant positive associations of the PTH/25-OH-D_3 ratio with the prevalence of the MS and its components and CIMT. Furthermore, in models with CIMT as the dependent variable,

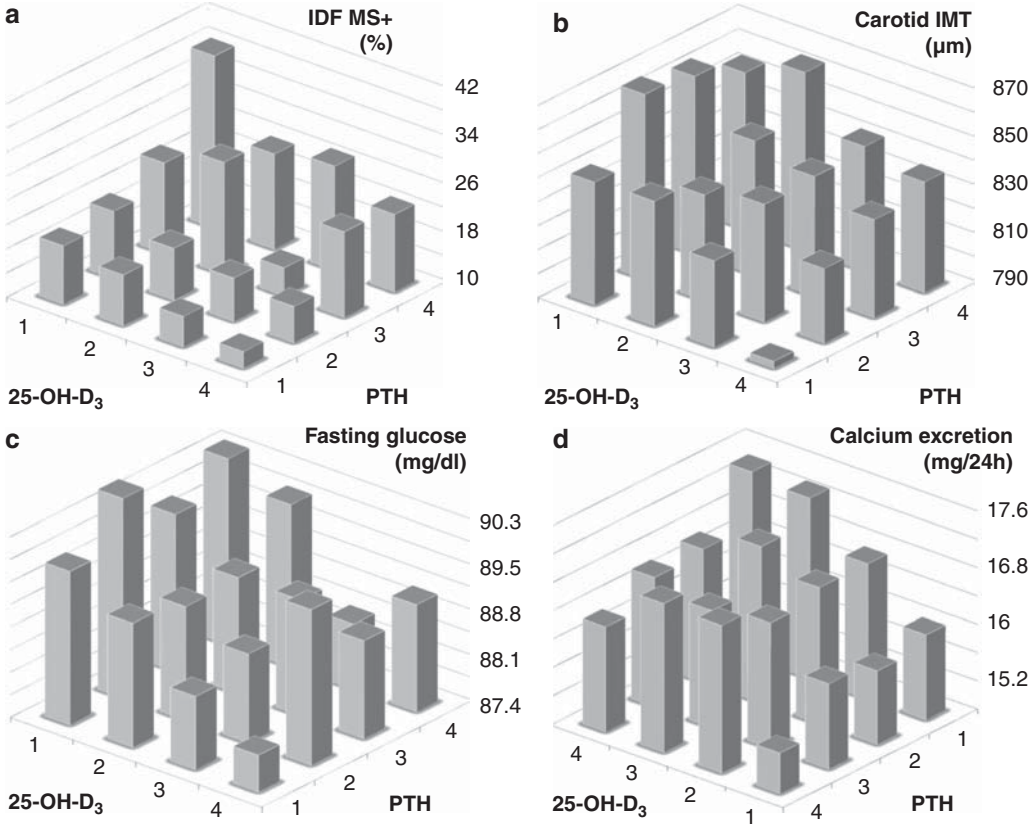

Figure 3 | Prevalence of the (**a**) metabolic syndrome, (**b**) thickness of the carotid intima-media, (**c**) fasting blood glucose, and (**d**) 24-h urinary calcium excretion across quartiles of parathyroid hormone (PTH) and 25-OH-D_3 (VD). The analyses were adjusted for sex, age, body mass index, smoking, alcohol intake, and the use of antihypertensive drugs. IDF, International Diabetes Federation; IMT, intima-media thickness; MS, metabolic syndrome; PTH, parathyroid hormone.

the PTH/25-OH-D_3-ratio remained significant, whereas the presence of the MS did not. Analyses adjusted for sex, age, and lifestyle variables were confirmatory. Furthermore, higher calcium intake was negatively associated with waist circumference, fasting blood glucose, and systolic and diastolic blood pressures. The novel aspect of our study lies in the simultaneous assessment of the PTH/25-OH-D_3 ratio, the prevalence of the MS, its components, and CIMT in a general population. By and large, our current findings emphasize the role of calciotropic hormones and calcium homeostasis in the pathogenesis of the MS and arterial calcification.

Our current findings are in keeping with other epidemiologic studies, in which either PTH,[9,19] 25-OH-D_3,[12] or both[20] were measured. In the National Health Examination and Nutrition Survey (NHANES III), in adjusted analyses, the prevalence of hypertension (OR, 1.30), obesity (OR, 2.29), and elevated serum triglycerides (OR, 1.47) was significantly higher in the bottom than in the top quartile of 25-OH-D_3 ($P < 0.001$ for all).[21] Similarly, in a Dutch population survey, the prevalence of hypertension was higher in the top vs. lowest quartile of PTH (OR, 2.00; 95% CI 1.31–3.06).[22] Our results indicate that the risk of MS, related to the PTH/25-OH-D_3 ratio, was mainly driven by 25-OH-D_3.

In our current population study, lower calcium intake was associated with increased measures of central obesity and fasting blood glucose. This is in line with the NHANES III findings, where the odds of being in the top quartile of body fat content were 0.16 for the top quartile ($P < 0.001$) with the bottom quartile of habitual calcium intake used as reference.[23] Along similar lines, the DASH (Dietary Approaches to Stop Hypertension) diet reduced PTH by 0.26 pmol/l in healthy adults without a change in urinary calcium excretion ($P < 0.05$).[11]

The currently available evidence on the association of cardiovascular risk factors with low 25-OH-D_3,[8,12,24] high PTH levels,[9,19] or both[20] encompasses hypertension,[2,24] obesity,[19] the MS,[9,12,19,20] and diabetes mellitus.[8] Some studies reported an inverse relationship between 25-OH-D_3 and blood pressure after adjustment for PTH,[25,26] whereas others reported no relationship between blood pressure and 25-OH-D_3 after adjustment for PTH.[22,27] Our point of view is that expressing the physiologic interaction between PTH and 25-OH-D_3 as a ratio instead of by mutual adjustment[22,25–27] for both calciotropic hormones sheds more clarity on the associations of these hormones with the MS, its components, and arterial calcification. Furthermore, calciotropic hormone ratios can provide additional information over and beyond their components in inflammatory disease[28] and in other fields.[29]

The multiple correlations between PTH, vitamin D, calcium, obesity, and cardiometabolic and vascular risk markers can

be explained by several mechanisms. Obesity is associated with increased PTH and hypovitaminosis D in the general population,[20] whereas increased habitual calcium intake decreases the risk for obesity and cardiometabolic disease[12,19] and suppresses PTH secretion in healthy individuals.[10] On the other hand, in patients with early hyperparathyroidism devoid of hypertension and renal disease, parathyroidectomy does not significantly change CIMT.[30] Increased renal activation of 25-OH-D_3 to the active hormonal form inhibits the activity of the renin–angiotensin–aldosterone system.[2] Supplementation with cholecalciferol or UV-B exposure increases 25-OH-D_3 and decreases blood pressure.[2] Deficiencies in 25-OH-D_3 are associated with insulin resistance and decreased insulin secretion.[3] In vitamin D–deficient patients, calcium supplementation improves glucose intolerance,[31] whereas vitamin D supplementation does not.[9,32,33] Oral vitamin D supplementation does not have an effect on weight loss in overweight or obese subjects.[34] Production of 25-OH-D_3 from orally absorbed vitamin D precursors, as opposed to endogenously produced precursors in the skin, is not controlled by internal feedback mechanisms, which might partially account for the lack of effect.[2] Abdominal obesity, a principal component in the current definition of the MS,[16] had the strongest association with the PTH/25-OH-D_3 ratio at all ages, whereas measures of subcutaneous fat (triceps skinfold thickness) were not associated with this ratio in our population (data not shown). Fat tissue is considered as a reservoir for vitamin D precursors, the amount and distribution of fat tissue is altered in obese individuals. Indeed, concentrations of 25-OH-D_3 in biopsies of subcutaneous fat tissue from obese adults were inversely related to the degree of overweight, and positively to the concentration of circulating 25-OH-D_3.[35] Furthermore, inflammatory cytokines released from abdominal adipose tissue further decrease the amount of circulating precursors by promoting extrarenal activation of 25-OH-D_3 by tissue macrophages.[36] In obese children, both PTH and 25-OH-D_3 levels normalize after weight loss, in parallel with a decrease in insulin resistance,[37] suggesting reversibility at young age. Furthermore, the extrarenal activation of circulating 25-OH-D_3, by activated macrophages in the vascular wall, opens calcium channels on vascular smooth muscle cells and thus accelerates arterial calcification.[2] This local activation to the active hormonal form partially underlies the lowered circulating concentration of the precursor, 25-OH-D_3.[2]

The results of this study must be interpreted according to its strengths and weaknesses. We did not assess markers of inflammation. Inflammation is often considered to accelerate the pathogenesis of the MS and some of its components. Under inflammatory conditions, tissue macrophages can locally activate circulating 25-OH-D_3 to the active hormone, which opens calcium channels and promotes calcification in vascular smooth muscle cells.[2] The production of vitamin D precursors in the skin declines with age, and is confounded by several factors including geographic location, skin color, habitual solar exposure, use of sunscreen, and dietary intake of animal foodstuffs and supplements.[38–40] In the current study, we did not measure skin color, but adjusting PTH and/or 25-OH-D_3 values for the hours of sun in the month of blood sampling did not substantially change the results (data not shown). We did not measure the excretion of the major metabolite of vitamin D (calcitroic acid), which might better quantify the uncontrolled extrarenal activation of circulating 25-OH-D_3. Furthermore, the cross-sectional approach used in this study does not allow inference of causality. Finally, we did not assess genetic variation in the vitamin D receptor, which is associated with serum total calcium,[41] PTH,[42] elevated fasting glucose,[43] early-onset type 2 diabetes,[44] and the prevalence of the MS.[45,46]

In conclusion, using an integrated approach, we showed in a general population that an increased ratio of PTH/25-OH-D_3 is associated with increased abdominal obesity, impaired glucose tolerance, an increased prevalence of the MS and its components, and increased CIMT. However, CIMT was not associated with MS. These findings are novel and in keeping with observations in obese children, in whom impaired glucose tolerance was a better predictor of CIMT than different definitions of the MS. Dysregulation of calcium homeostasis might therefore be a common mechanism leading to both the MS and arterial calcification. Prospective and intervention studies should address the causality of these relationships. When addressing multiple risk factors in patients affected by the MS, attention should also go to appropriate dietary calcium intake and sufficient outdoor activity with responsible solar exposure. Future studies are needed to clarify the long-term effects of dietary calcium intake and vitamin D obtained from sunlight or external sources in the pathogenesis of cardiometabolic disease and the prevention of the MS.

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