CLINICAL STUDY

Vitamin D, parathyroid hormone and the metabolic syndrome in middle-aged and older European men

David M Lee, Martin K Rutter¹, Terence W O'Neill, Steven Boonen², Dirk Vanderschueren³, Roger Bouillon⁴, Gyorgy Bartfai⁵, Felipe F Casanueva^{6,7}, Joseph D Finn, Gianni Forti⁸, Aleksander Giwercman⁹, Thang S Han¹⁰, Ilpo T Huhtaniemi¹¹, Krzysztof Kula¹², Michael E J Lean¹³, Neil Pendleton¹⁴, Margus Punab¹⁵, Alan J Silman, Frederick C W Wu¹⁶ and the European Male Ageing Study Group

ARC Epidemiology Unit, The University of Manchester, Manchester M13 9PT, UK, ¹Manchester Diabetes Centre, The University of Manchester, Manchester, UK, ²Division of Gerontology and Geriatrics and Centre for Musculoskeletal Research, Department of Experimental Medicine, ³Department of Andrology and Endocrinology and ⁴Department of Experimental Medicine, Katholieke Universiteit Leuven, Leuven, Belgium, ⁵Department of Obstetrics, Gynaecology and Andrology, Albert Szent-Gyorgyi Medical University, Szeged, Hungary, ⁶Department of Medicine, Santiago de Compostela University, Complejo Hospitalario Universitario de Santiago (CHUS), Santiago de Compostela, Spain, ⁷CIBER de Fisiopatología Obesidad y Nutrición (CB06/03), Instituto Salud Carlos III, Santiago de Compostela, Spain, ⁸Andrology Unit, Department of Clinical Physiopathology, University of Florence, Florence, Italy, ⁹Reproductive Medicine Centre, Malmö University Hospital, University of Lund, Malmö, Sweden, ¹⁰Department of Endocrinology, Royal Free and University College Hospital Medical School, Royal Free Hospital, Hampstead, London, UK, ¹¹Department of Reproductive Biology, Imperial College London, Hammersmith Campus, London, UK, ¹²Department of Andrology and Reproductive Endocrinology, Medical University of Lodz, Lodz, Poland, ¹³Department of Human Nutrition, University of Glasgow, Glasgow, UK, ¹⁴Clinical Gerontology, The University of Manchester, Hope Hospital, Salford, UK, ¹⁵Andrology Unit, United Laboratories of Tartu University Clinics, Tartu, Estonia and ¹⁶Department of Endocrinology, Manchester Royal Infirmary, The University of Manchester, Manchester, UK

(Correspondence should be addressed to D M Lee; Email: david.m.lee@manchester.ac.uk)

Abstract

Objectives: Low serum 25-hydroxyvitamin D (25(OH)D) and elevated parathyroid hormone (PTH) levels have been linked to insulin resistance, the metabolic syndrome (MetS) and its components. Data in healthy, community-dwelling Europeans are lacking, and previous studies have not excluded subjects receiving drug treatments that may distort the relationship between 25(OH)D/PTH and MetS. The aim of our analysis was to examine the association of 25(OH)D and PTH with Adult Treatment Panel III-defined MetS in middle-aged and older European men.

Design: This was a population-based, cross-sectional study of 3369 men aged 40–79 years enrolled in the European Male Ageing Study.

Results: After exclusion of subjects with missing data, 3069 men with a mean ($\pm$ s.d.) age of 60 ± 11 years were included in the analysis. Age-adjusted 25(OH)D levels were inversely associated with waist circumference, systolic blood pressure (BP), triglycerides, and glucose (all $P < 0.01$). Age-adjusted PTH levels were only associated with waist and diastolic BP (both $P < 0.05$). After adjusting for age, centre, season and lifestyle factors the odds for MetS decreased across increasing 25(OH)D quintiles (odds ratios 0.48 (95% confidence intervals 0.36–0.64) highest versus lowest quintile; $P_{\text{trend}} < 0.001$). This relationship was unchanged after adjustment for PTH, but was attenuated after additional adjustment for homeostasis model assessment of insulin resistance (0.60 (0.47–0.78); $P_{\text{trend}} < 0.001$). There was no association between PTH and MetS.

Conclusions: Our results demonstrate an inverse relationship between 25(OH)D levels and MetS, which is independent of several confounders and PTH. The relationship is partly explained by insulin resistance. The clinical significance of these observations warrants further study.

European Journal of Endocrinology 161 947–954

Introduction

25-Hydroxyvitamin D (25(OH)D) and parathyroid hormone (PTH) are important physiological regulators of extracellular calcium homeostasis. A number of recent population-based, cross-sectional studies suggest additional metabolic roles for these hormones (1–5). Data from the California-based Rancho Bernardo study, in which subjects have high sunlight exposure and high vitamin D levels, indicated that 25(OH)D was not

related to prevalent metabolic syndrome (MetS) (4), whereas US population-based data from NHANES, in which vitamin D levels were lower, indicated a marked inverse relationship between these variables (5). Both studies also suggested that PTH was related to prevalent MetS in older men. There is some biological plausibility for these relationships because low vitamin D levels, and to a lesser extent elevated PTH levels, have been associated with glucose intolerance and insulin resistance (6–10).

Although the studies by Reis (4, 5) and others (3, 11) were population based and some were adjusted for several confounders including PTH and 25(OH)D levels, subjects taking anti-hypertensive and lipid-lowering medications were not excluded from these analyses. We are not aware of any previous studies that have specifically explored the effects of these groups of drugs on the relationship between the vitamin D/PTH axis and MetS, though it is possible that any associations may potentially be distorted by medication use targeting component parts of the MetS.

The purpose of our study was to examine the cross-sectional associations of 25(OH)D and PTH levels with MetS in a large population-based cohort of European men and to contrast the strength of any associations after excluding those taking medications that might influence these relationships.

Methods

Subjects

The European Male Ageing Study (EMAS) is a prospective, non-interventional cohort study of male ageing in Europe. Details regarding recruitment, response rates and assessments have been previously described (12). Briefly, non-institutionalised men aged 40–79 years were recruited from municipal or population registers in eight centres: Florence (Italy); Leuven (Belgium); Lodz (Poland); Malmö (Sweden); Manchester (UK); Santiago de Compostela (Spain); Szeged (Hungary); Tartu (Estonia). For the baseline survey, stratified random sampling was used with the aim of recruiting equal numbers of men into each of four age bands (40–49, 50–59, 60–69 and 70–79 years). Subjects were invited by letter to complete a short postal questionnaire and to attend for screening at a local clinic. The mean adjusted response rate across the eight centres was 43% (range 24–60%). The study was funded by the European Union, and ethical approval for the study was obtained in accordance with local institutional requirements in each centre.

Assessments

The postal questionnaire included items concerning demographic, health and lifestyle information. Subjects were asked about tobacco use (response set=current/past/non-smoker) and typical alcohol consumption during the preceding month (response set=every day/5–6 days/week/3–4 days/week/1–2 days/week/< once/week/not at all). Those who agreed to participate subsequently attended a research clinic to complete an interviewer-assisted questionnaire (IAQ) and physiological assessments. The IAQ included the physical activity scale for the elderly (PASE) (13) to assess physical activity levels. Blood pressure measurements were performed after a 5-min rest period to the nearest 1 mmHg with subjects seated using an Omron

500I automated sphygmomanometer (Omron Healthcare Ltd, Milton Keynes, UK). Height was measured barefoot to the nearest 1 mm using a stadiometer (Leicester Height Measure, SECA UK Ltd, Birmingham, UK), and weight to the nearest 0.1 kg using an electronic scale (SECA, model no. 8801321009, SECA UK Ltd) with subjects wearing light clothing. Each centre's electronic scales and stadiometers were calibrated on a monthly basis. Waist circumference was measured thrice to the nearest 1 mm using anthropometric tape, mid-way between the lowest rib and the iliac crest with the subject standing, and the median used to score. Current prescription and non-prescription drug use was recorded with participants bringing in all medications for confirmation.

Biochemistry

Phlebotomy was performed prior to 1000 h to obtain a fasting blood sample from all subjects. Processed serum was stored and protected from light at -80°C prior to analysis and shipped on dry ice to central laboratories for measurement of 25(OH)D (Katholieke Universiteit Leuven) and PTH (University of Santiago de Compostela). Serum 25(OH)D levels were determined using RIA (RIA kit: DiaSorin, Stillwater, MN, USA). Intra- and inter-assay coefficients of variation (CV) for 25(OH)D were 11 and 8% respectively. The detection limit of the RIA kit was 5.0 nmol/l 25(OH)D. Serum was assayed for PTH using a chemiluminescence immunoassay (Nichols Advantage Bio-Intact PTH assay, Quest Diagnostics, Madison, NJ, USA). Intra- and inter-assay CV for PTH were 6 and 2.8% respectively. The detection limit of the chemiluminescence immunoassay was 0.16 pmol/l.

Analyses for cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were performed locally in all centres using commercially available enzymatic methods. Fasting glucose was measured using standard hexokinase enzymatic assays. Insulin was assayed using quimioluminescence (University of Santiago de Compostela). Insulin resistance was calculated using the homoeostasis model assessment of insulin resistance (HOMA-IR) (14). All clinical pathology laboratories were accredited by the relevant national authorities and adhered to current guidelines on Good Laboratory Practice as specified by EU Directive 2004/9/EC (15).

Metabolic syndrome

To assess the prevalence of MetS among the EMAS cohort, we used the current ATP III guidelines (16). The ATP III definition of MetS was met if subjects had at least three of the following: a waist circumference >102 cm, a triglyceride level ≥ 1.7 mmol/l, a HDL cholesterol level <1.03 mmol/l, systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg, and fasting glucose ≥ 5.6 mmol/l.

Analysis

Statistical analyses were performed using Intercooled Stata version 9.2 (StataCorp., College Station, TX, USA). Subjects with missing 25(OH)D and PTH measurements ($n=151$), or incomplete MetS data ($n=149$) were excluded, leaving 3069 men in the main analysis. 25(OH)D and PTH were examined as continuous variables or classified into quintiles. Age, the PASE score and individual components of the MetS were treated as continuous variables in linear regression models. Season of attendance at the clinic was defined as winter (January–March), spring (April–June), summer (July–September) and autumn (October–December). The associations of 25(OH)D and PTH with covariates were examined using Pearson correlation and one-way ANOVA.

We initially explored the association of both 25(OH)D and PTH levels with MetS components using linear regression (adjusting for age), with results expressed as β coefficients and 95% confidence intervals (CI). Mean levels ($\pm$ S.E.M.) of each MetS component by quintile of either 25(OH)D or PTH were then estimated using multiple linear regression models adjusting for potential confounders (age, physical activity and season). In addition, to allow for the likelihood that observations are independent across centres, but not necessarily within centres, robust standard errors were requested using Stata's *cluster* subcommand with centre as the clustering variable. Linear trends were assessed by using the quintiles of 25(OH)D or PTH as ordinal terms in the regression model. Finally, multivariable logistic regression models assessed the individual and combined associations of 25(OH)D and PTH with MetS after serial adjustments for potential confounders, including HOMA-IR, with results expressed as odds ratios (ORs) and 95% CI. Effect modification by age was also assessed by inclusion of interaction terms between 25(OH)D or PTH concentration and age (by decade) in the logistic regression models.

Results

Subjects

The clinical characteristics of the 3069 men included in the analysis sample are shown in Table 1.

25(OH)D and PTH

25(OH)D was inversely related to PTH ($r=-0.18$, $P<0.001$) and varied markedly by season of measurement (mean $\pm$ S.D.): summer, 85 ± 33 versus winter, 50 ± 26 nmol/l ($P<0.001$). 25(OH)D was inversely related to body mass index (BMI) ($r=-0.10$, $P<0.001$), and positively related to PASE score ($r=0.10$, $P<0.001$). There were significant between-centre differences in mean levels of 25(OH)D, ranging from 77 nmol/l in Malmö to 47 nmol/l in Tartu (adjusted for season, $P<0.001$).

Table 1 Baseline characteristics ($n=3069$).

Variable	Mean (S.D.)
Age (years)	60.0 (11.0)
Waist circumference (cm)	98.4 (10.9)
Systolic blood pressure (mmHg)	146 (21)
Diastolic blood pressure (mmHg)	87 (12)
HDL cholesterol (mmol/l)	1.4 (0.4)
Triglycerides (mmol/l)	1.6 (1.1)
Glucose (mmol/l)	5.6 (1.4)
Body mass index (kg/m ²)	27.6 (4.1)
25(OH)D (nmol/l)	62.3 (31.3)
Parathyroid hormone (pmol/l)	3.1 (1.5)
Physical activity scale for the elderly	195 (92)
	%
ATPIII metabolic syndrome	31.7
Waist circumference >102 cm	33.6
Blood pressure $\geq 130/85$ mmHg ^a	84.9
HDL cholesterol <1.03 mmol/l	12.9
Triglycerides ≥ 1.7 mmol/l	30.3
Glucose ≥ 5.6 mmol/l ^b	36.0
Diabetes ^c	7.6
Current smoker	21.1
Alcohol consumption (≥ 1 day/week)	56.2
Lipid-lowering medication ^d	13.0

^aMeasured blood pressure and/or using anti-hypertensive drugs.

^bMeasured blood glucose and/or using anti-diabetic drugs.

^cSelf-report and/or using anti-diabetic drugs.

^dIncludes fibrate derivatives, bile acid sequestrants, niacin and HMG-CoA reductase inhibitors.

Higher levels of PTH were observed in the winter (3.2 ± 1.7 pmol/l) as opposed to the summer months (2.9 ± 1.5 pmol/l), and these seasonal differences were statistically significant ($P=0.03$). PTH levels increased with age ($r=0.13$, $P<0.001$), BMI ($r=0.08$, $P<0.001$) and were inversely related to PASE score ($r=-0.13$, $P<0.001$). Current smokers had lower levels of both 25(OH)D and PTH than non-smokers ($P<0.01$). Subjects reporting drinking one or more alcoholic drinks per week had higher 25(OH)D and lower PTH levels than those drinking less frequently (both $P<0.01$). As with 25(OH)D, mean levels of PTH varied between centres ranging from 2.7 pmol/l in Szeged to 3.4 pmol/l in Leuven (adjusted for season, $P<0.001$). Using linear regression, we did not find a significant association between the latitude of each centre, which ranged from 42.88°N (Santiago) to 58.38°N (Tartu), and either 25(OH) ($\beta=-0.10$, $P=0.3$) or PTH ($\beta=-0.01$, $P=0.1$) after adjusting for season of attendance.

Metabolic syndrome

The MetS was present in just under one-third of subjects (Table 1). Among those not using anti-hypertensive, diabetes and/or lipid-lowering medications, this fell to one quarter (24.7%). The prevalence of individual MetS components according to the current ATPIII guidelines ranged from 13% of subjects with low HDL cholesterol to 85% of subjects with hypertension. Age-adjusted associations between 25(OH)D and PTH

Table 2 Age-adjusted associations between 25-hydroxyvitamin D (25(OH)D) or parathyroid hormone (PTH) and components of the metabolic syndrome: linear regressions.

Dependent variables	Independent variables	
	25(OH)D – (per 10 nmol/l)	PTH – (per pmol/l)
	β Coefficients (95% CI) ^a	
Waist circumference (cm)	–0.413 (–0.536, –0.291)*	0.596 (0.344, 0.847)*
Systolic blood pressure (mmHg)	–0.548 (–0.773, –0.323)*	0.406 (–0.058, 0.870)*
Diastolic blood pressure (mmHg)	–0.214 (–0.354, –0.075)*	0.525 (0.238, 0.811)*
HDL cholesterol (mmol/l)	0.006 (0.002, 0.010)*	–0.009 (–0.017, –0.001)*
Triglycerides (mmol/l)	–0.037 (–0.050, –0.025)*	–0.008 (–0.033, 0.018)*
Glucose (mmol/l)	–0.032 (–0.048, –0.016)*	–0.019 (–0.052, 0.013)*

* $P < 0.05$. Note: although the distribution of triglycerides was positively skewed, using log-transformed triglycerides in the above regressions did not change the significance of the associations.

^aAdjusted for age.

and components of the MetS are presented in Table 2. Increasing 25(OH)D levels were associated with lower values for waist circumference, systolic blood pressure, diastolic blood pressure, triglycerides and glucose, and higher values for HDL cholesterol. However, increasing levels of PTH were only significantly associated with higher values for waist circumference and diastolic blood pressure, and lower values for HDL cholesterol. When the regressions of 25(OH)D and PTH versus blood pressure, lipids and glucose were additionally adjusted for waist circumference, the associations between 25(OH)D and both diastolic blood pressure and HDL cholesterol, and PTH and HDL cholesterol was no longer significant (all $P > 0.07$; data not shown).

Adjusted means for individual components of MetS across 25(OH)D or PTH quintiles are shown in

Table 3. Waist circumference, systolic blood pressure, triglycerides and glucose levels were inversely associated with increasing 25(OH)D quintiles (all $P_{\text{trend}} \leq 0.05$), and these relationships were essentially unchanged after additional adjustment for smoking, alcohol consumption and other MetS components (all $P_{\text{trend}} < 0.05$, data not shown). Increasing PTH quintiles were positively related to waist circumference, systolic blood pressure and diastolic blood pressure (all $P_{\text{trend}} \leq 0.02$), and these trends remained significant for waist circumference and diastolic blood pressure (both $P_{\text{trend}} < 0.02$) after additional adjustment for smoking, alcohol consumption and other MetS components, but not for systolic blood pressure ($P_{\text{trend}} = 0.6$, data not shown).

The associations of 25(OH)D or PTH with MetS are summarised in Table 4. The adjusted odds for MetS

Table 3 Adjusted means (S.E.M.) for components of the metabolic syndrome versus quintiles of 25(OH)D and PTH.

Dependent variable	Quintiles of 25(OH)D (range nmol/l)					P_{trend}
	<i>n</i> =617	<i>n</i> =620	<i>n</i> =615	<i>n</i> =610	<i>n</i> =607	
	I (<35.7)	II (35.7–49.4)	III (49.5–65.1)	IV (65.2–85.9)	V (>85.9)	
Waist circumference (cm)	99.2 (0.7)	99.3 (0.7)	99.0 (0.7)	98.2 (1.0)	96.0 (1.1)	0.05
Systolic blood pressure (mmHg)	148.1 (2.2)	146.4 (2.4)	145.6 (1.9)	145.7 (1.5)	142.8 (1.4)	0.05
Diastolic blood pressure (mmHg)	87.9 (1.5)	87.5 (1.6)	87.4 (1.6)	86.9 (1.1)	86.1 (0.7)	0.3
HDL cholesterol (mmol/l)	1.40 (0.04)	1.38 (0.05)	1.40 (0.03)	1.39 (0.05)	1.46 (0.06)	0.4
Triglycerides (mmol/l)	1.73 (0.08)	1.65 (0.13)	1.58 (0.09)	1.54 (0.10)	1.30 (0.06)	0.001
Glucose (mmol/l)	5.84 (0.12)	5.70 (0.11)	5.61 (0.13)	5.63 (0.10)	5.48 (0.10)	0.002

Dependent variable	Quintiles of PTH (range pmol/l)					P_{trend}
	<i>n</i> =614	<i>n</i> =614	<i>n</i> =614	<i>n</i> =614	<i>n</i> =613	
	I (<2.01)	II (2.01–2.55)	III (2.56–3.10)	IV (3.11–3.86)	V (>3.86)	
Waist circumference (cm)	97.6 (0.7)	98.0 (0.9)	97.9 (0.9)	98.9 (0.7)	99.5 (0.8)	0.02
Systolic blood pressure (mmHg)	145.3 (2.1)	144.7 (2.0)	145.6 (1.6)	145.1 (2.0)	148.0 (2.0)	0.05
Diastolic blood pressure (mmHg)	86.3 (1.4)	86.5 (1.3)	86.5 (1.0)	87.6 (1.2)	88.8 (1.5)	0.004
HDL cholesterol (mmol/l)	1.41 (0.05)	1.41 (0.05)	1.43 (0.05)	1.40 (0.04)	1.38 (0.04)	0.3
Triglycerides (mmol/l)	1.66 (0.14)	1.61 (0.10)	1.49 (0.09)	1.53 (0.07)	1.53 (0.05)	0.2
Glucose (mmol/l)	5.84 (0.13)	5.68 (0.17)	5.61 (0.11)	5.51 (0.07)	5.63 (0.06)	0.1

Values shown are means (S.E.M.). Adjusted for age, physical activity, season and centre. 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone. Note: although the distribution of triglycerides was positively skewed, using log-transformed triglycerides in the above regressions, P_{trend} remained unchanged.

Table 4 Adjusted odds ratios (95% confidence intervals) for the association of 25-hydroxyvitamin D (25(OH)D) and parathyroid hormone (PTH) with the metabolic syndrome.

	Quintiles of 25(OH)D (range nmol/l)					<i>P</i> _{trend}
	I (<35.7)	II (35.7–49.4)	III (49.5–65.1)	IV (65.2–85.9)	V (>85.9)	
Mean 25(OH)D (nmol/l)	26.2	42.7	57.2	74.5	111.9	
Model 1	1.00 (referent)	0.97 (0.65–1.43)	0.75 (0.51–1.10)	0.63 (0.39–1.01)	0.48 (0.36–0.64)	<0.001
Model 2	1.00 (referent)	0.96 (0.65–1.41)	0.75 (0.50–1.11)	0.62 (0.40–0.97)	0.47 (0.37–0.62)	<0.001
Model 3	1.00 (referent)	0.94 (0.62–1.43)	0.78 (0.56–1.08)	0.61 (0.36–1.04)	0.60 (0.47–0.78)	<0.001

	Quintiles of PTH (range pmol/l)					<i>P</i> _{trend}
	I (<2.01)	II (2.01–2.55)	III (2.56–3.10)	IV (3.11–3.86)	V (>3.86)	
Mean PTH (pmol/l)	1.59	2.29	2.81	3.46	5.22	
Model 1	1.00 (referent)	0.92 (0.66–1.26)	0.77 (0.50–1.19)	0.88 (0.59–1.31)	0.95 (0.59–1.55)	0.8
Model 2	1.00 (referent)	0.88 (0.65–1.20)	0.72 (0.47–1.11)	0.80 (0.55–1.18)	0.82 (0.51–1.33)	0.4
Model 3	1.00 (referent)	0.97 (0.72–1.32)	0.86 (0.55–1.35)	1.03 (0.84–1.26)	0.98 (0.71–1.36)	0.9

Model 1: adjusted for age, smoking, alcohol consumption, physical activity, season and centre. Model 2: adjusted for all variables in Model 1 plus PTH in 25(OH)D analysis and 25(OH)D in PTH analysis. Model 3: adjusted for all variables in Model 2 plus HOMA-IR. Note: there was no evidence that age modified the relationship between either 25(OH)D or PTH and the metabolic syndrome in any of the models ($P_{\text{interaction}} > 0.1$ in all cases).

decreased by more than 50% across increasing quintiles of 25(OH)D (Model 1), and this relationship was unchanged after adjustment for PTH (Model 2), but was attenuated (~20%) after additional adjustment for HOMA-IR (Model 3). There was a significant linear trend across 25(OH)D quintiles for each model (Table 4), primarily driven by the significant OR in the highest quintile. There was no evidence that the association of 25(OH)D with MetS differed by age decade ($P_{\text{interaction}} > 0.1$). No association between PTH and MetS was observed in any of the models, and there was no evidence that the PTH–MetS association was modified by age ($P_{\text{interaction}} > 0.1$).

When the above logistic regression models were repeated following exclusion of subjects receiving diabetes, anti-hypertensive and/or lipid-lowering medications ($n=1960$), the results were broadly unchanged. In Model 1, the adjusted odds for MetS decreased ~40% across increasing quintiles of 25(OH)D (OR 0.56 (95% CI 0.33, 0.94) for highest versus lowest quintile; $P_{\text{trend}}=0.01$). The association was unchanged following adjustment for PTH (Model 2) (0.57 (0.35, 0.93) for highest versus lowest quintile; $P_{\text{trend}}=0.01$), but additional adjustment for HOMA-IR (Model 3) again attenuated the relationship (0.70 (0.47, 1.04) for highest versus lowest quintile; $P_{\text{trend}}=0.04$). As with the analysis using the entire sample, we observed no relationship between PTH and MetS in models excluding men using specific medications (all $P_{\text{trend}} > 0.3$, data not shown).

Discussion

Main findings

In this population-based study of middle-aged and older European men, we showed that age-adjusted 25(OH)D levels were significantly associated with all

MetS components. Waist circumference, systolic blood pressure, triglycerides and glucose levels were inversely associated with 25(OH)D after additional adjustment for physical activity, season and centre. The odds of MetS decreased ~50% across increasing 25(OH)D quintiles, and this relationship was in part explained by insulin resistance. Waist circumference, diastolic blood pressure and systolic blood pressure were associated with PTH levels after multivariable adjustment, but there was no evidence of an association between PTH levels and MetS.

Previous studies of 25(OH)D and MetS

Several factors might explain why population-based studies exploring the association of 25(OH)D with MetS, and its components have yielded conflicting results. Ford and co-workers showed in NHANES subjects that the adjusted risk for MetS was inversely associated with 25(OH)D levels, but this study was limited by the failure to adjust for PTH (1). This is potentially important because low calcium and vitamin D levels are physiological stimuli for PTH synthesis, and there is evidence that elevated PTH may influence the risk for MetS (17–24). In a subsequent analysis that adjusted for PTH levels, Reis and co-workers found no association between MetS with 25(OH)D levels in men or women (4). These Rancho Bernardo study participants resided in southern California where high levels of exposure to u.v. radiation probably contributed to the relatively high 25(OH)D levels (mean ~105 nmol/l). In a separate study in NHANES subjects, Reis and co-workers reported a strong inverse relationship between 25(OH)D levels and prevalent MetS that was independent of PTH levels and other important confounders (5). The mean 25(OH)D level among this representative sample of the non-institutionalised US population was

~62 nmol/l. These findings were in stark contrast to those of the Rancho Bernardo study and suggested that a threshold may exist whereby vitamin D deficiency may influence incident MetS (5), whereas higher levels may not (4). Our results and mean 25(OH)D levels (62 nmol/l) are very similar to Reis' recent NHANES study (5) and, therefore, lend some support to the threshold hypothesis. The different 25(OH)D levels reported in the Rancho Bernardo study (4) and the NHANES study (5) should be interpreted cautiously, however, as different assays were used to measure 25(OH)D. Whether the relationships observed are explained by variation in sun exposure is uncertain and our results did not change following additional adjustment for latitude – a surrogate measure of sun exposure (data not shown).

The mechanism(s) by which low vitamin D could be associated with MetS remain speculative (5). Data in humans suggest that low 25(OH)D levels are associated with glucose intolerance and insulin resistance (6, 9, 10). In support of this, we showed that the relationship between MetS and 25(OH)D was in part explained by insulin resistance. The cross-sectional nature of our data does not exclude the possibility that obesity and hypertension, and associated co-morbid conditions, could reduce levels of outdoor physical activity and sun exposure. This explanation may be less likely because even short duration sun exposure from late spring to early autumn will stimulate vitamin D synthesis and when we additionally excluded many of our less healthy subjects who were receiving therapy for diabetes, hypertension and/or dyslipidaemia, our results were largely unchanged. Residual confounding from low 25(OH)D levels acting as a marker for a less healthy lifestyle and diet not identified by the measured covariates might also explain our findings.

Previous studies of PTH and MetS

In the Rancho Bernardo study and in NHANES subjects, Reis and co-workers showed that prevalent MetS was positively related to PTH concentration among older men but not women (4, 5). In contrast, we found no relationship between MetS and PTH levels in men and no evidence of an age interaction even in minimally adjusted models ($P_{\text{interaction}} > 0.1$). It is possible that when we excluded men who were using medications targeting component parts of the MetS, our data reflect the true physiology more closely. However, we found no evidence of an association between PTH and MetS even when subjects receiving diabetes, anti-hypertensive and/or lipid-lowering medications were excluded from the analysis.

Our mean PTH levels (3 pmol/l $\approx$ 28 pg/ml) were lower than compared with Reis' studies; 51 pg/ml (4) and 42 pg/ml (5) respectively, and it is possible,

therefore, that a threshold also exists for PTH whereby elevated PTH may influence the development of MetS whereas lower levels do not. In either case, our data do not support an independent association between the MetS and PTH in men.

Strengths and limitations

We have studied a large, population-based sample of European men. We adjusted for physical activity, 25(OH)D and PTH status which were assessed by standardised methods (12) and the age-stratified enrolment facilitated robust exploration of age interactions. To allow for possible distortion of the relationship between the vitamin D/PTH axis and MetS by medication use targeting diabetes, hypertension or dyslipidaemia, we repeated our logistic regression models excluding subjects who were currently treated for pre-existing components of the MetS. Although our rationale to exclude these men was theoretical, it was based on the premise that the association between 25(OH)D/PTH and MetS may be falsely inflated if subjects with currently treated components of the MetS were included in the analysis. Evidence of a stronger relationship between 25(OH)D and the MetS in analyses using the entire EMAS sample supports this notion, although the overall pattern of associations was unchanged.

Our study has a number of limitations. The cross-sectional design limits conclusions about causal relationships. We enrolled non-institutionalised, primarily Caucasian men with a study response rate of 43%, which could limit the generalisability to other groups. 25(OH)D and PTH were assayed from a single measurement and, consequently, the strength of the observed associations are likely to be conservative. However, given that the prevalence of MetS in our sample was relatively common and we used a logistic regression model to determine ORs from cross-sectional data, it is possible that we may have overestimated the magnitude of the observed associations (25). Finally, we did not adjust for calcium intake, although adjustment for PTH in the 25(OH)D analysis should partly compensate for this.

Clinical implications

Studies in animals (26–28) and some (29, 30) but not all (31) data in humans suggest that vitamin D therapy could improve glucose intolerance and insulin resistance (29). These studies and the data presented here could inform therapeutic trials of vitamin D on incident MetS and ultimately on incident diabetes and/or cardiovascular disease in subjects with low 25(OH)D levels. However, it is premature to suggest vitamin D therapy to prevent MetS, diabetes or cardiovascular

disease particularly since the relationship between 25(OH)D and cardiovascular risk may be non-linear and possibly U-shaped (32).

Conclusion

We have shown that in a population-based study of European men, low 25(OH)D levels were linked to prevalent MetS and that this relationship was partially mediated by insulin resistance. In contrast to previous studies, PTH levels were not associated with MetS. Further prospective studies are needed to ascertain the relationship between vitamin D and MetS.

Declaration of interest

The authors have no financial arrangements or conflict of interest to disclose concerning this manuscript.

Funding

The European Male Ageing Study is funded by the Commission of the European Communities Fifth Framework Program 'Quality of Life and Management of Living Resources' Grant QLK6-CT-2001-00258. Additional support was also provided by the Arthritis Research Campaign (UK).

Acknowledgements

The EMAS study group: Florence (Gianni Forti, Luisa Petrone, Antonio Cilotti); Leuven (Dirk Vanderschueren, Steven Boonen, Herman Borghs); Lodz (Krzysztof Kula, Jolanta Slowikowska-Hilczar, Renata Walczak-Jedrzejowska); London (Ilpo Huhtaniemi); Malmö (Alexander Giwercman); Manchester (Frederick Wu, Alan Silman, Neil Pendleton, Terence O'Neill, Joseph Finn, Philip Steer, Abdelouahid Tajar, David Lee, Stephen Pye); Santiago (Felipe Casanueva, Mary Lage); Szeged (Gyorgy Bartfai, Imre Földesi, Imre Fejes); Tartu (Margus Punab, Paul Korrovitz); Turku (Min Jiang). The authors wish to thank the men who participated in the eight countries, the research/nursing staff in the eight centres: C Pott, Manchester; E Wouters, Leuven; M Nilsson, Malmö; M del Mar Fernandez, Santiago de Compostela; M Jedrzejowska, Lodz; H-M Tabo, Tartu; A Heredi, Szeged for their data collection, C Moseley, Manchester for data entry and project co-ordination, and E Van Herck, Leuven for performing the 25(OH)D assays.

References

- 1 Ford ES, Ajani UA, McGuire LC & Liu S. Concentrations of serum vitamin D and the metabolic syndrome among US adults. *Diabetes Care* 2005 **28** 1228–1230.
- 2 Gannage-Yared MH, Chedid R, Khalife S, Azzi E, Zoghbi F & Halaby G. Vitamin D in relation to metabolic risk factors, insulin sensitivity and adiponectin in a young Middle-Eastern population. *European Journal of Endocrinology* 2009 **160** 965–971.
- 3 Lu L, Yu Z, Pan A, Hu FB, Franco OH, Li H, Li X, Yang X, Chen Y & Lin X. Plasma 25-hydroxyvitamin D concentration and metabolic syndrome among middle-aged and elderly Chinese individuals. *Diabetes Care* 2009 **32** 1278–1283.
- 4 Reis JP, von Muhlen D, Kritiz-Silverstein D, Wingard DL & Barrett-Connor E. Vitamin D, parathyroid hormone levels, and the prevalence of metabolic syndrome in community-dwelling older adults. *Diabetes Care* 2007 **30** 1549–1555.
- 5 Reis JP, von Muhlen D & Miller ER III. Relation of 25-hydroxyvitamin D and parathyroid hormone levels with metabolic syndrome among US adults. *European Journal of Endocrinology* 2008 **159** 41–48.
- 6 Chiu KC, Chu A, Go VL & Saad MF. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. *American Journal of Clinical Nutrition* 2004 **79** 820–825.
- 7 Chiu KC, Chuang LM, Lee NP, Ryu JM, McGullam JL, Tsai GP & Saad MF. Insulin sensitivity is inversely correlated with plasma intact parathyroid hormone level. *Metabolism* 2000 **49** 1501–1505.
- 8 Norman AW, Frankel JB, Heldt AM & Grodsky GM. Vitamin D deficiency inhibits pancreatic secretion of insulin. *Science* 1980 **209** 823–825.
- 9 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. *Journal of Nutrition* 2009 **139** 329–334.
- 10 Forouhi NG, Luan J, Cooper A, Boucher BJ & Wareham NJ. Baseline serum 25-hydroxy vitamin D is predictive of future glycemic status and insulin resistance: the Medical Research Council Ely Prospective Study 1990–2000. *Diabetes* 2008 **57** 2619–2625.
- 11 Hyponen E, Boucher BJ, Berry DJ & Power C. 25-Hydroxyvitamin D, IGF-1, and metabolic syndrome at 45 years of age: a cross-sectional study in the 1958 British Birth Cohort. *Diabetes* 2008 **57** 298–305.
- 12 Lee DM, O'Neill TW, Pye SR, Silman AJ, Finn JD, Pendleton N, Tajar A, Bartfai G, Casanueva F, Forti G, Giwercman A, Huhtaniemi IT, Kula K, Punab M, Boonen S, Vanderschueren D & Wu FC. The European Male Ageing Study (EMAS): design, methods and recruitment. *International Journal of Andrology* 2009 **32** 11–24.
- 13 Washburn RA, Smith KW, Jette AM & Janney CA. The physical activity scale for the elderly (PASE): development and evaluation. *Journal of Clinical Epidemiology* 1993 **46** 153–162.
- 14 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF & Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985 **28** 412–419.
- 15 Directive 2004/9/EC of the European Parliament and of the Council on the inspection and verification of good laboratory practice (GLP) 2004.
- 16 Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA & Costa E. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005 **112** 2735–2752.
- 17 Bell NH, Epstein S, Greene A, Shary J, Oexmann MJ & Shaw S. Evidence for alteration of the vitamin D-endocrine system in obese subjects. *Journal of Clinical Investigation* 1985 **76** 370–373.
- 18 Bolland MJ, Grey AB, Gamble GD & Reid IR. Association between primary hyperparathyroidism and increased body weight: a meta-analysis. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 1525–1530.
- 19 Cheung PS, Thompson NW, Brothers TE & Vinik AI. Effect of hyperparathyroidism on the control of diabetes mellitus. *Surgery* 1986 **100** 1039–1047.
- 20 Jorde R, Sundsfjord J, Haug E & Bonaa KH. Relation between low calcium intake, parathyroid hormone, and blood pressure. *Hypertension* 2000 **35** 1154–1159.
- 21 Jorde R, Svartberg J & Sundsfjord J. Serum parathyroid hormone as a predictor of increase in systolic blood pressure in men. *Journal of Hypertension* 2005 **23** 1639–1644.
- 22 Kamycheva E, Sundsfjord J & Jorde R. Serum parathyroid hormone level is associated with body mass index. The 5th Tromso study. *European Journal of Endocrinology* 2004 **151** 167–172.
- 23 Snijder MB, van Dam RM, Visser M, Deeg DJ, Dekker JM, Bouter LM, Seidell JC & Lips P. Adiposity in relation to vitamin D status and parathyroid hormone levels: a population-based study in older men and women. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 4119–4123.

- 24 Taylor WH. The prevalence of diabetes mellitus in patients with primary hyperparathyroidism and among their relatives. *Diabetic Medicine* 1991 **8** 683–687.
- 25 Lee J. Odds ratio or relative risk for cross-sectional data? *International Journal of Epidemiology* 1994 **23** 201–203.
- 26 Ayesha I, Bala TS, Reddy CV & Raghuramulu N. Vitamin D deficiency reduces insulin secretion and turnover in rats. *Diabetes, Nutrition and Metabolism* 2001 **14** 78–84.
- 27 Bourlon PM, Billaudel B & Faure-Dussert A. Influence of vitamin D3 deficiency and 1,25 dihydroxyvitamin D3 on *de novo* insulin biosynthesis in the islets of the rat endocrine pancreas. *Journal of Endocrinology* 1999 **160** 87–95.
- 28 de Souza Santos R & Vianna LM. Effect of cholecalciferol supplementation on blood glucose in an experimental model of type 2 diabetes mellitus in spontaneously hypertensive rats and Wistar rats. *Clinica Chimica Acta* 2005 **358** 146–150.
- 29 Borissova AM, Tankova T, Kirilov G, Dakovska L & Kovacheva R. The effect of vitamin D3 on insulin secretion and peripheral insulin sensitivity in type 2 diabetic patients. *International Journal of Clinical Practice* 2003 **57** 258–261.
- 30 Chiu KC, Chuang LM & Yoon C. The vitamin D receptor polymorphism in the translation initiation codon is a risk factor for insulin resistance in glucose tolerant Caucasians. *BMC Medical Genetics* 2001 **2** 2.
- 31 Tai K, Need AG, Horowitz M & Chapman IM. Glucose tolerance and vitamin D: effects of treating vitamin D deficiency. *Nutrition* 2008 **24** 950–956.
- 32 Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, Benjamin EJ, D'Agostino RB, Wolf M & Vasan RS. Vitamin D deficiency and risk of cardiovascular disease. *Circulation* 2008 **117** 503–511.

Received 22 July 2009

Accepted 9 August 2009