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Association between vitamin D and bisphenol A levels in an elderly Italian population: results from the InCHIANTI study

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Abstract

Objective: This study aimed to evaluate the association between the endocrine-disrupting chemical, bisphenol A (BPA) on circulating levels of 25-hydroxy vitamin D (25(OH)D) and other vitamin D metabolites in an elderly population in Italy.

Methods: This was a retrospective analysis of the InCHIANTI Biobank in Italy. The association between vitamin D metabolites namely 1,25(OH)D, 25(OH)D, parathyroid hormone (PTH) and BPA levels were evaluated. Multiple regression models were used to examine the association between predictor variables with 1,25(OH)D or 25(OH)D levels.

Results: Samples from 299 individuals aged 72.8 ± 15.7 years were examined. Mean levels of BPA, 1,25(OH)D and 25(OH)D were 351.2 ± 511.6 ng/dL, 43.7 ± 16.9 pg/mL and 20.2 ± 12.1 ng/mL, respectively. One hundred eighty individuals (60.2%) were deficient (<20 ng/mL) in 25(OH)D and this population also presented higher BPA levels (527.9 ± 1289.5 ng/dL vs 86.9 ± 116.8 ng/dL, $P < 0.0001$). Univariate analysis revealed that BPA levels were negatively correlated with both 1,25(OH)D ($r = -0.67$, $P < 0.0001$) and 25(OH)D ($r = -0.69$, $P < 0.0001$). Multivariate regression revealed that PTH ($\beta: -0.23$, 95% CI: $-0.34, -0.13$, $P < 0.0001$) and BPA ($\beta: -0.25$, 95% CI: $-0.3, -0.19$, $P < 0.0001$) remained significantly associated with 25(OH)D levels while BPA was also associated with 1,25(OH)D levels ($\beta: -0.19$, 95% CI: $-0.22, -0.15$, $P < 0.0001$). Receiver operating characteristic curve analysis showed that a BPA concentration of >113 ng/dL was the best cut-off to predict individuals deficient in 25(OH)D (area under the curve: 0.87, 95% CI: 0.82–0.90, $P < 0.0001$).

Conclusion: The strong negative association between BPA and vitamin D in this elderly population warrants further investigation, particularly since this population is already at greatest risk of hypovitaminosis and fracture.

Key Words

- ▶ bisphenol A
- ▶ vitamin D
- ▶ 25(OH)D
- ▶ parathyroid hormone
- ▶ elderly
- ▶ vertebral fractures

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Introduction

Vitamin D is essential for the homeostatic regulation of calcium (1), and reduced vitamin D intake or inadequate levels can impact upon bone metabolism leading to increased parathyroid hormone (PTH) secretion and increased bone resorption (2).

Vitamin D deficiency (measured as the plasma level of the transport form of vitamin D, 25(OH)D), is a common condition worldwide, particularly in elderly and osteoporotic subjects (3, 4). Vitamin D insufficiency (<30 ng/mL) and/or deficiency (<20 ng/mL) in the elderly worsen not only due to poor exposure to sunlight but also due to reduced intestinal absorption and skin synthesis capacity (5, 6).

Besides its well-known role in bone metabolism and calcium homeostasis, vitamin D status has also been linked to muscle strength in older people (7) and the incidence of falls (8). There is also evidence of a beneficial effect of vitamin D on bone mineral density (BMD) (1) and a range of extraskeletal effects including benefits in patients hospitalized with COVID-19 (9).

Bisphenol A, (BPA), is an organic compound and is a fundamental molecule in the synthesis of some plastics and some additives (10). Suspected of being harmful to humans since the 1930s, doubts on the use of BPA made headlines in 2008, when many governments carried out studies on its safety and some sellers took products containing this compound off the market (11).

To date, BPA has been linked to numerous male sexual developmental diseases in the fetus, and a decline in fertility in men, according to a 2010 report by the Food and Drug Administration (FDA) (12). However, an assessment published by the FDA later in March 2013 dismissed the alarm stating that BPA is safe at very low levels found in some foods (13). However, recent studies have shown a high risk in the induction of metabolic processes that develop an increased risk of cancer (14).

Furthermore, it has recently been demonstrated that exposure to BPA and other compounds such as phthalates, called 'endocrine disruptor chemicals' (EDCs), could be associated with a reduction in vitamin D levels (15, 16).

In a study by Johns *et al.*, involving 4667 adults who participated in the National Health and Nutrition Examination Survey (NHANES), it was observed that individuals exposed to higher concentrations of phthalates had lower levels of vitamin D than individuals exposed to low concentrations of EDCs (15). Recent data from NHANES also revealed that higher levels of BPA exposure is associated with an increased risk of long-term (all-cause) mortality (17).

In this study, we explored the potential strength and direction of the association between vitamin D metabolites and BPA exposure in an elderly population in Italy, a population at greatest risk of hypovitaminosis and fracture. To understand whether BPA could impact bone metabolism, we also examined the association between BPA levels and vitamin D metabolites; 1,25(OH)D, 25(OH)D, parathyroid hormone (PTH) and vitamin D-binding protein (VDBP).

Methods

Study design

The InCHIANTI (Invecchiare in Chianti, aging in the Chianti area) study was originally designed in 1998 to evaluate factors contributing to the decline of mobility in late life designed by the Laboratory of Clinical Epidemiology of the Italian National Research Council on Aging, (INRCA, Florence, Italy) in collaboration with local administrators and primary care physicians of Greve in Chianti and Bagno a Ripoli in the Tuscany region (18).

This was a non-interventional monocentric cross-sectional follow-up analysis of biological samples collected from June 2013 to July 2014 and conducted in full compliance with Italian legislation regarding non-interventional studies and the principles of the Declaration of Helsinki. All participants provided written informed consent for the anonymous use of personal data which were obtained from every patient in compliance with European Legislative Decree 2016/679. This consent included permission to consult administrative and medical databases as well as to conduct future analysis. The Regional Ethics Committee for Clinical Trials of the Tuscany Region (Section, Area Vasta Centro; Careggi, Firenze) approved this study (protocol number: 13929_bio) on July 8, 2019, which was conducted according to the regulations for observational studies (19).

Study population and sample collection

Blood and urine samples were obtained from elderly subjects during the fourth follow-up (from June 2013 to July 2014) analysis (including 687 subjects aged between 35 and 100 years) who underwent medical examinations, questionnaires and related functional tests that participated in the InCHIANTI study (18).

The present retrospective analysis of samples stored in the InCHIANTI Biobank evaluated the association

between the vitamin D metabolites 1,25(OH)D, 25(OH)D, PTH, VDBP, with urinary BPA levels. Phenotypic clinical data (age, gender and BMI) were also collected.

The majority of subjects gave venous blood sample (fasting 8 h prior to sampling) that was delivered within 2 h to the laboratory in preparation for the biological setup for the Biobank. Stored samples included 0.3 mL aliquots of plasma and serum stored at -80°C in the InCHIANTI Biobank, at the Piero Palagi Hospital. For BPA assays, urine samples were collected and stored at -80°C until further use.

Laboratory assays

Assays for all variables were performed at our central laboratory, Azienda Ospedaliero-Universitaria Careggi (AOUC), Florence, Italy with the exception of BPA and VDBP, which were assayed at the Fondazione Italiana Ricerca sulle Malattie dell'Osso (FirmoLab), Florence, Italy.

Serum PTH was analyzed using a sandwich electrochemiluminescence immunoassay (ECLIA) by Elecsys 2010 Modular Analytics E170 COBAS 602 (Roche Diagnostics GmbH). Serum 1,25(OH)D and 25(OH)D levels were measured by RIA (Liason, DiaSorin, Saluggia, Italy). Serum creatinine was measured by an enzymatic method with calibration traceable to a reference procedure (isotope dilution mass spectrometry) and automatized on a Cobas 8000. Serum VDBP was measured by enzyme-linked immuno assay method (R&D Systems). BPA analysis was performed on urine samples using the HPLC/mass spectrometry by solid-phase extraction coupled with high-performance isotope dilution tandem liquid chromatography-mass spectrometry with focus peak. A comprehensive quality control system was implemented to prevent samples from being contaminated during handling, storage and analysis. For BPA concentrations below the detection level (116/1455 (8%)), a value of 0.3 ng/mL (30 ng/dL) was assigned in the NHANES survey and this value was used in the present analysis (20).

Statistical analysis

Data are presented as mean \pm s.d. for quantitative variables or number and percentages for categorical variables. Data were evaluated for normal distribution using the Kolmogorov-Smirnov test. Comparisons between continuous variables with normal distribution were assessed using the Student's *t* test, while the χ^2 test or Fisher's exact method was used to compare proportions for categorical variables. Urinary and serum laboratory

variables were log-transformed (as they were not distributed normally) prior to univariate and multivariate analyses. Both univariate regression (assessed using the Pearson's correlation coefficient, *r*) and multiple linear regression models were used to assess the strength of the association between vitamin D levels and clinical and laboratory variables. In multiple regression models, independent variables (covariates) initially included in models were age, BMI, gender, PTH, VDBP, creatinine and BPA, and the dependent variable was 1,25(OH)D or 25(OH)D levels. Receiver operating characteristic curve (ROC) analysis (expressed as area under the curve; AUC, odds ratio (OR) and respective 95% CIs) was used to assess the predictive power of BPA levels to identify individuals having deficient levels of 25(OH)D (i.e. <20 ng/mL) (21). A *P*-value of <0.05 was considered statistically significant. Statistical analysis was performed using InStat Software (GraphPad) or MedCalc Software (Broekstraat, Mariakerke, Belgium).

Results

Clinical characteristics

In this retrospective analysis of the InCHIANTI Study Biobank, samples from 299 individuals living in the Chianti area of Tuscany, Italy, were analyzed. A summary of general demographic and laboratory variables is presented in Table 1. This analysis was conducted in an elderly population (mean age, 72.8 ± 15.7 years) and 55.2% were female. Although age and BMI were similar in males and females, a higher proportion of males were previous smokers and a lower proportion were never smokers. Levels of the biologically active form of vitamin D, 1,25(OH)D, were higher in females vs males (46.9 ± 19.1 pg/mL vs 39.8 ± 13 pg/mL, $P=0.0035$), as well as the stable form 25(OH)D (21.3 ± 12.7 ng/mL vs 18.9 ± 11.1 ng/mL) in addition to VDBP levels (736.3 ± 223.3 vs 647.8 ± 150.4 mg/L, $P < 0.0001$), whereas PTH and BPA levels were similar in male and female subjects. In this elderly population, mean levels of 25(OH)D in the total population were borderline deficient (20.23 ± 12.1 ng/mL).

Characteristics of individuals deficient in 25(OH)D

Stratifying the entire population by 25(OH)D levels, we can observe that 180 (60.2%) individuals had levels that were considered deficient, that is having 25(OH)D levels <20 ng/mL according to the Endocrine Society (21). The characteristics of this population presenting with deficient

Table 1 Clinical and laboratory characteristics. Data are presented as number and % or mean and s.d.

Characteristic	Total population (n = 299)	Female (n = 165)	Male (n = 134)	P
General				
Age, years (range)	72.8 ± 15.7	72.8 ± 16.3	72.9 ± 14.9	0.95
BMI (kg/m ²)	26.8 ± 4.4	26.4 ± 4.9	27.3 ± 3.8	0.08
Smoking status, n (%)				
Never	159 (53.2)	116 (70.3)	43 (32.1)	<0.0001
Previous	116 (38.8)	39 (23.6)	77 (57.5)	<0.0001
Current	24 (8)	10 (6.1)	14 (10.4)	0.2
Laboratory				
1,25(OH)D (pg/mL)	43.7 ± 16.98	46.9 ± 19.1	39.8 ± 13	0.0035
25(OH)D (ng/mL)	20.23 ± 12.1	21.3 ± 12.7	18.9 ± 11.1	0.09
PTH (pmol/L)	5.6 ± 3.1	5.5 ± 2.8	5.6 ± 3.4	0.83
VDBP (mg/L)	698.2 ± 200.9	736.3 ± 223.3	647.8 ± 150.4	0.0001
Creatinine (mg/dL)	0.89 ± 0.53	0.75 ± 0.49	1.06 ± 0.53	<0.0001
BPA (ng/dL)	351.2 ± 511.6	337.5 ± 507.7	368.2 ± 517.8	0.61

BPA, bisphenol A; PTH, parathyroid hormone; VDBP, vitamin D-binding protein. Bold indicates statistical significance.

levels vs levels ≥ 20 ng/mL are summarized in Table 2. Features such as advanced age and higher BMI were present in the population to a greater extent in vitamin D-deficient individuals, whereas gender, smoking status, VDBP and creatinine levels were largely unchanged between the two groups. As expected, lower vitamin D metabolites (1,25(OH)D and 25(OH)D) and higher PTH levels were observed, but a pronounced and highly significant increase in urinary BPA levels was noted in the vitamin D-deficient group (527.9 ± 1289.5 ng/dL vs 86.9 ± 116.8 ng/dL, $P < 0.0001$).

Table 2 Characteristics of patients who were deficient (<20 ng/mL) and not deficient (≥ 20 ng/mL) in serum 25(OH)D. Data are presented as number and % or mean and s.d.

Characteristic	25(OH)D		P
	<20 ng/mL	≥ 20 ng/mL	
n (%)	180 (60.2)	119 (39.8)	<0.0001
General			
Female gender, n (%)	94 (52.2)	71 (59.7)	NS
Age (years)	75.6 ± 14.8	68.6 ± 16.2	<0.0001
BMI (kg/m ²)	27.3 ± 4.5	26 ± 4.2	0.0098
Smoking status, n (%)			
Never	97 (53.9)	62 (52.1)	NS
Previous	71 (39.4)	45 (37.8)	NS
Current	12 (6.7)	12 (10.1)	NS
Laboratory			
1,25(OH)D (pg/mL)	38.5 ± 13.8	51.5 ± 18.3	<0.0001
25(OH)D (ng/mL)	12.95 ± 3.9	31.2 ± 11.9	<0.0001
PTH (pmol/L)	6.32 ± 3.5	4.4 ± 1.8	<0.0001
VDBP (mg/L)	688.2 ± 219.5	709.7 ± 161.7	0.36
Creatinine (mg/dL)	0.87 ± 0.5	0.9 ± 0.56	0.48
BPA (ng/dL)	527.9 ± 1289.5	86.9 ± 116.8	<0.0001

BPA, bisphenol A; PTH, parathyroid hormone; VDBP, vitamin D-binding protein. Bold indicates statistical significance.

Association between vitamin D metabolites and BPA by univariate analysis

Having identified a range of variables potentially associated with vitamin D levels (Table 2), we next used univariate regression to evaluate the association among both laboratory and clinical variables with vitamin D metabolites. BPA levels were inversely correlated with both 1,25(OH)D ($r = -0.67$, $P < 0.0001$) and 25(OH)D levels ($r = -0.69$, $P < 0.0001$) (Fig. 1). Examining a range of variables associated with 1,25(OH)D levels (Fig. 2), as expected, 25(OH)D and VDBP were positively correlated and PTH was negatively correlated with 1,25(OH)D, whereas weaker correlations were observed for creatinine and BMI. Age was negatively correlated with 1,25(OH)D. Levels of 25(OH)D showed a similar pattern to 1,25(OH)D although the negative association with PTH levels was stronger ($r = -0.44$, $P < 0.0001$) (Fig. 3). Examining variables associated with BPA levels (Fig. 4), PTH levels ($r = 0.39$, $P < 0.0001$) and age ($r = 0.33$, $P < 0.0001$) were positively correlated with BPA, whereas other variables such as VDBP, creatinine and BMI were weakly associated with BPA.

Predictor variables associated with 25(OH)D levels by multivariate analysis

Multiple linear regression analysis was applied in order to determine the direction and strength of the association between predictor variables influencing 1,25(OH)D and 25(OH)D levels. In a first model, inserting 1,25(OH)D as the dependent variable, from a range of predictor variables, BPA ($\beta: -0.19$, 95% CI: -0.22 , -0.15 , $P < 0.0001$) emerged as the strongest predictor associated with 1,25(OH)D levels

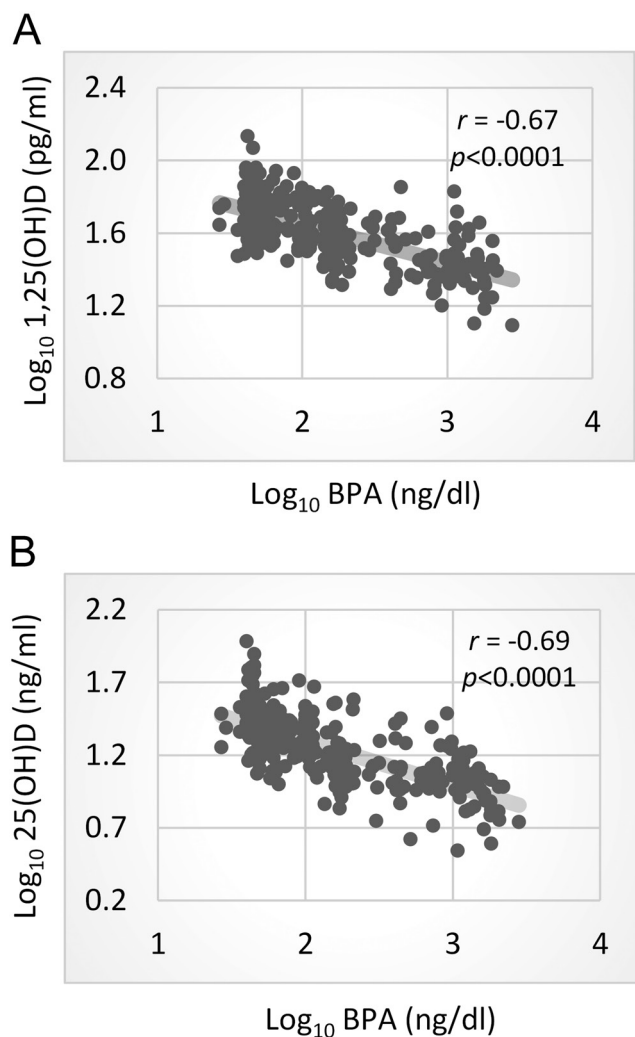


Figure 1
The association between urinary BPA levels and serum vitamin D levels. (A) The association between 1,25(OH)D and BPA. (B) The association between 25(OH)D and BPA. Regression coefficients and P-values are shown. BPA, bisphenol A.

(Table 3). Other variables associated with 1,25(OH)D levels to a lesser extent were age, gender and VDBP. In a second model, 25(OH)D was considered as the dependent variable and both BPA ($\beta: -0.25$, 95% CI: $-0.3, -0.19$, $P < 0.0001$) and PTH ($\beta: -0.23$, 95% CI: $-0.34, -0.13$, $P < 0.0001$) emerged as the only variables associated with 25(OH)D levels (Table 4). The coefficient (β) can be interpreted as the % increase in the dependent variable (25OHD or 1,25OHD) for every 1% increase in the independent variable (BPA). Therefore, for x% increase, we calculate $1.x$ to the power of the coefficient, subtracting 1 and multiplying by 100 (i.e. for every 20% increase in BPA, the dependent variable 25(OH)D changes by: $(1.20^{-0.25} - 1) \times 100 = (0.95 - 1) \times 100 = -5\%$ (22). Further models were also performed on males and females

separately and the strength of the association between BPA and 1,25(OH)D and 25(OH)D levels was maintained to a similar extent in males and females (Supplementary Tables 1 and 2, see section on supplementary materials given at the end of this article).

ROC analysis

We next wanted to determine if exposure to a specific concentration of BPA could help predict individuals deficient in serum 25(OH)D. To explore this further, all 25(OH)D values were categorized as either 'deficient', that is having 25(OH)D levels < 20 ng/mL (21) or not deficient (≥ 20 ng/mL). ROC analysis revealed a significant predictive power of BPA levels to detect individuals deficient in 25(OH)D compared to those with levels ≥ 20 ng/mL (0.87, 95% CI: 0.82–0.90, $P < 0.0001$). A BPA concentration of > 113 ng/dL was the best cut-off value to predict individuals deficient in 25(OH)D, with a sensitivity of 73.6% (95% CI: 66.5–79.9%) and specificity of 89.08% (95% CI: 82.0–94.1%) (Fig. 5).

Discussion

In this retrospective analysis of a large Biobank in the Tuscany region in Italy, we reveal a strong inverse relationship between urinary BPA concentrations and circulating serum vitamin D levels in an elderly sample of the general population. The negative correlation observed between BPA levels and circulating 25(OH)D levels persisted even after multivariate analysis, considering a range of confounding variables such as age, gender and creatine concentration. BPA was also strongly associated with 1,25(OH)D levels after multivariate analysis.

In healthy individuals, vitamin D is synthesized (or introduced into the body with food), hydroxylated first in the liver and then transformed into 25-hydroxy vitamin D₃ (25(OH)D₃) or calcidiol. Calcidiol is further hydroxylated to its active form, calcitriol; 1,25-dihydroxy vitamin D (1,25(OH)₂D₃), this second hydroxylation occurs in the kidney (21). The activation process is regulated by the circulation of serum levels of PTH, calcium and phosphorus. In turn 1,25(OH)₂D₃ controls PTH secretion through a negative feedback mechanism.

Our findings revealed a highly significant inverse relationship between PTH levels and both vitamin D metabolites, confirming our understanding of the physiology of the calcium–PTH–vitamin D axis (1). However, 60% of this elderly population (mean age of 72 years) were deficient in vitamin D; that is < 20 ng/mL

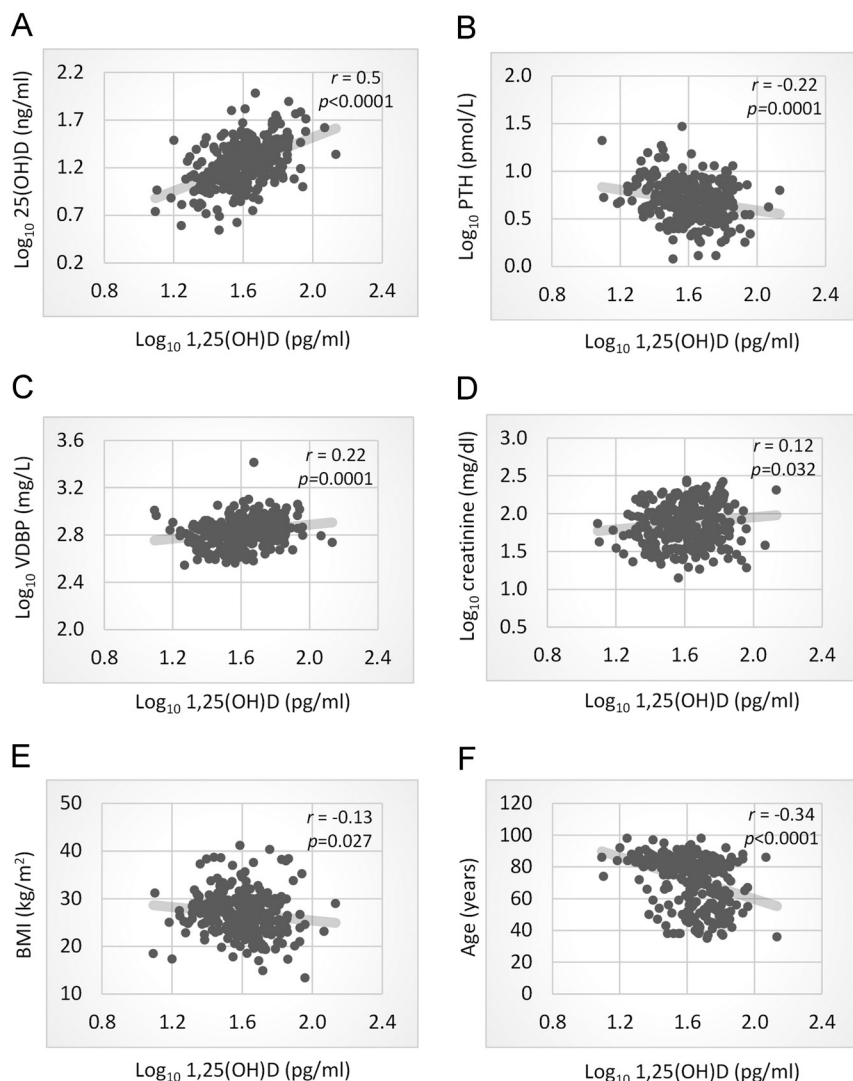


Figure 2

The association between serum 1,25(OH)D levels and vitamin D metabolites and clinical variables. (A) The association between 25(OH)D and 1,25(OH)D. (B) The association between PTH and 1,25(OH)D. (C) The association between VDBP and 1,25(OH)D. (D) The association between creatinine and 1,25(OH)D. (E) The association between BMI and 1,25(OH)D. (F) The association between age and 1,25(OH)D. Regression coefficients and *P*-values are shown. BMI, body mass index; BPA, bisphenol A; PTH, parathyroid hormone; VDBP, vitamin D-binding protein.

25(OH)D. The low levels of 1,25(OH)D in these individuals were likely due to vitamin D deficiency since 1,25(OH)D synthesis can be seen to be substrate-dependent as inferred from the positive correlation between serum of the two metabolites ($r = 0.5$, $P < 0.0001$). A similar positive correlation ($r = 0.49$, $P < 0.001$) was also observed by Lips and colleagues in patients with a femoral neck fracture, lower 1,25(OH)D levels seen in patients with impaired renal function and this correlation was absent in healthy controls (23). It is recognized that during mild vitamin D deficiency, no relationship between 25(OH)D and 1,25(OH)2D would be expected (24). However, in cases of severe vitamin D deficiency (e.g. renal failure), the production of 1,25(OH)2D would be limited, by a lack of substrate (25, 26).

We observed a positive correlation between both vitamin D metabolites, but PTH was also negatively correlated with both forms. Whether the advanced

age of our population or the direct or indirect effect of BPA exposure acting as an EDC on the enzyme kinetic equilibrium involved in the synthesis of these metabolites has been altered or disturbed, we can only speculate. Although the dynamic relationship between 1,25(OH)D, 25(OH)D and PTH is well established (27), including their temporal alterations (24), little information is currently available in the elderly population.

Indeed, in NHANES, higher urinary BPA concentrations were found to be inversely correlated with 25(OH)D levels in women but not in men (15). In women, an IQR increase (i.e. 25%) in urinary BPA was associated with a 3.7% decrease in total 25(OH)D with a similar effect seen in our population (5% decrease for every 20% increase in BPA in the entire cohort). In a subsequent study by the same authors, maternal–prenatal urinary BPA levels were found to be negatively associated with serum 25(OH)D levels and a higher risk of vitamin D deficiency (16). In a

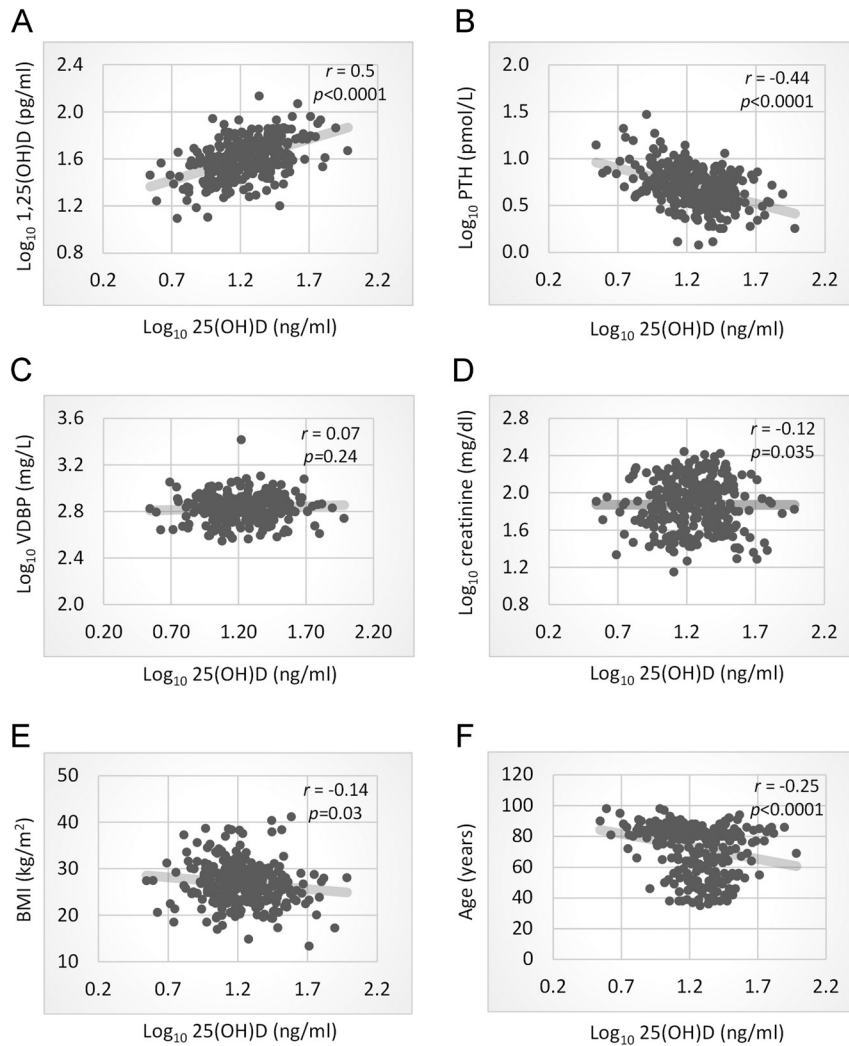


Figure 3

The association between serum 25(OH)D levels and vitamin D metabolites and clinical variables. (A) The association between 1,25(OH)D and 25(OH)D. (B) The association between PTH and 25(OH)D. (C) The association between VDBP and 25(OH)D. (D) The association between creatinine and 25(OH)D. (E) The association between BMI and 25(OH)D. (F) The association between age and 25(OH)D. Regression coefficients and *P*-values are shown. BMI, body mass index; BPA, bisphenol A; PTH, parathyroid hormone; VDBP, vitamin D-binding protein.

separate study by Erden and colleagues, it was observed that in patients with chronic obstructive apnea, serum BPA concentrations were found to be negatively correlated with serum 25(OH)D levels (28).

Our findings confirm this negative association between BPA exposure and 25(OH)D levels in addition to 1,25(OH)D that was independent of gender. In the NHANES study, the characteristics of subjects were distinctly different from ours in that they were younger (67% were <60 years of age) and the majority (70%) were overweight (37% were also obese). Although it is recognized that vitamin D status is influenced by a range of demographic, clinical, pathophysiological and genetic factors (29), the long-term impact of BPA exposure is poorly understood.

Vitamin D metabolites were also negatively correlated with BMI, confirming previous studies. Indeed, in a meta-analysis study by Zimmerman and colleagues (30), the importance of body weight for the dose-response

relationship with circulating 25(OH)D has shown that 34.5% of variation in circulating 25(OH)D was explained by body weight, followed by the type of supplement (D₂ or D₃) (9.8%), age (3.7%), calcium intake (2.4%) and basal 25(OH)D concentrations (1.9%), leaving about 50% of the variations to unknown factors.

We did not see an association between BPA levels and VDBP although a recent pilot study observed that BBP levels were negatively correlated with VDBP in women with polycystic ovary syndrome, an observation that may point toward potential liver dysfunction (31).

While the relationship between vitamin D and BPA remains poorly understood, there is pre-clinical data showing that BPA can increase urinary excretion of vitamin D₃ leading to a decrease in blood concentrations suggesting that perturbation of vitamin D₃ metabolism may be linked with BPA-related neurodevelopmental disorders (32). Another study performed in mice

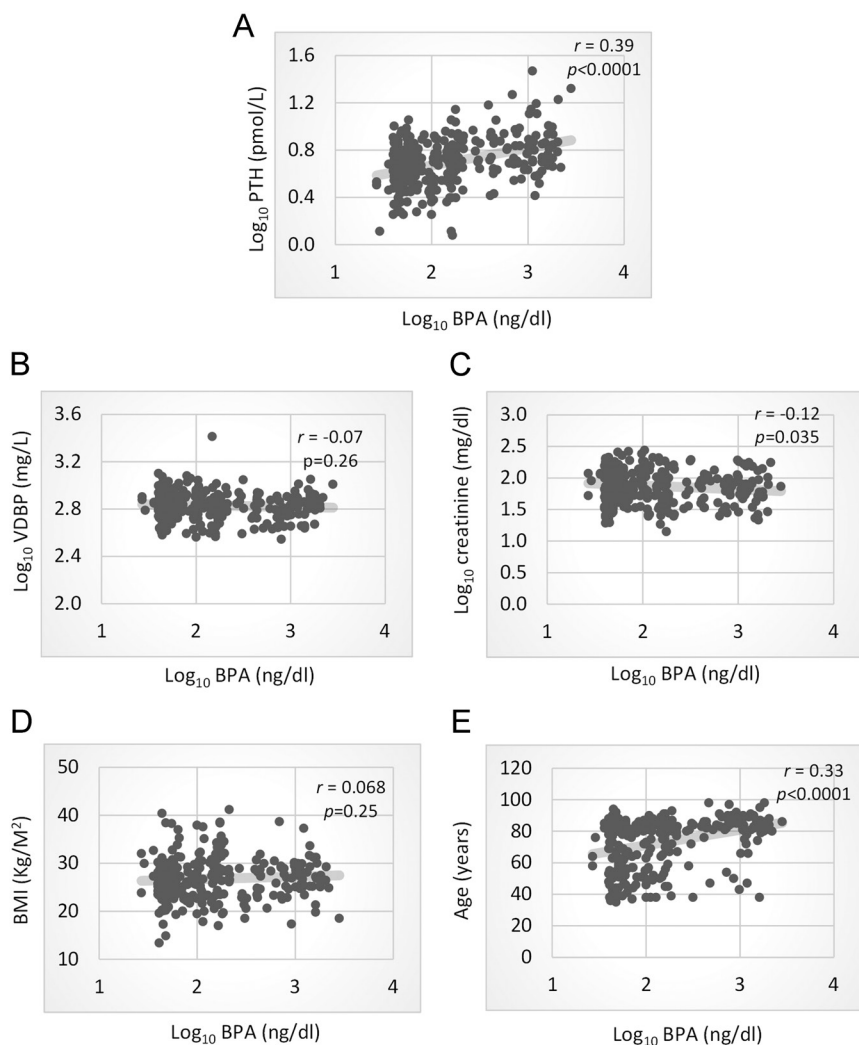


Figure 4

The association between urinary BPA levels and vitamin D metabolites and clinical variables. (A) The association between PTH and BPA. (B) The association between VDBP and BPA. (C) The association between creatinine and BPA. (D) The association between BMI and BPA. (E) The association between age and BPA. Regression coefficients and *P*-values are shown. BMI, body mass index; BPA, bisphenol A; PTH, parathyroid hormone; VDBP, vitamin D-binding protein.

revealed a repressive effect of maternal vitamin D3 supplementation on Th17 cell proliferation in offspring mice induced by perinatal BPA exposure. Authors suggest that this effect is in part due to its positive effect on the downregulation of ROR γ t mRNA expression, interleukin

(IL)-6 and IL-23, mediated via vitamin D receptor activation (33).

Age in the present study was negatively correlated with vitamin D metabolites and positively correlated with BPA levels. With increasing age exposure to BPA proportionally

Table 3 Multivariate linear logistic regression analysis of variables associated with serum 1,25(OH)D levels.

Characteristic	β (95% CI)	s.e.	t-Statistic	P
General				
Age (years)	-0.001 (-0.002, 0.000)	0.001	-2.29	0.023
BMI (kg/m ²)	-0.002 (-0.005, 0.001)	0.002	-1.22	0.23
Gender (male)	-0.046 (-0.08, 0.015)	0.016	-2.95	0.003
Laboratory^a				
25(OH)D (ng/mL)	0.045 (-0.39, 0.13)	0.06	1.06	0.29
PTH (pmol/L)	0.04 (-0.035, 0.12)	0.04	1.08	0.28
VDBP (mg/L)	0.16 (0.03, 0.29)	0.07	2.48	0.01
Creatinine (mg/dL)	0.03 (-0.04, 0.095)	0.033	0.91	0.37
BPA (ng/dL)	-0.19 (-0.22, -0.15)	0.018	-10.13	<0.0001

^aAll laboratory variables were log-transformed prior to entering in logistic regression models.

BPA, bisphenol A; PTH, parathyroid hormone; s.e., standard error; VDBP, vitamin D-binding protein.

Bold indicates statistical significance.

Table 4 Multivariate linear logistic regression analysis of variables associated with serum 25(OH)D levels.

Characteristic	β (95% CI)	s.e.	t-Statistic	P
General				
Age, years	0.000 (−0.002, 0.001)	0.001	−0.35	0.72
BMI (kg/m ²)	−0.003 (−0.007, 0.002)	0.002	−1.22	0.23
Gender (male)	−0.011 (−0.06, 0.033)	0.023	−0.51	0.61
Laboratory^a				
1,25(OH)D (pg/mL)	0.09 (−0.08, 0.26)	0.09	1.06	0.29
PTH (pmol/L)	−0.23 (−0.34, −0.13)	0.054	−4.32	<0.0001
VDBP (mg/L)	0.009 (−0.17, 0.19)	0.093	0.099	0.92
Creatinine (mg/dL)	−0.05 (−0.15, 0.04)	0.05	−1.13	0.26
BPA (ng/dL)	−0.25 (−0.3, −0.19)	0.027	−9.16	<0.0001

^aAll laboratory variables were log-transformed prior to entering in logistic regression models. BPA, bisphenol A; PTH, parathyroid hormone; s.e., standard error; VDBP, vitamin D-binding protein. Bold indicates statistical significance.

increases, low levels were seen in younger individuals (<50 ng/dL) rising to extremely high levels of BPA (1500 ng/dL) in individuals >90 years of age.

Given that this elderly population are representative of the general population for this age range and are predominantly deficient in vitamin D (60% had 25(OH)D levels <20 ng/mL), they are at increased risk of bone-related diseases such as fracture (1). If BPA exposure is truly acting as a key culprit in blunting vitamin D availability and function on a national scale, strategies to limit exposure to

BPA and counteract this effect through augmenting levels in vitamin D deficient individuals need to be implemented.

In recent months, the role of vitamin D in elderly individuals hospitalized with overt COVID-19 has been documented. In particular, patients who are deficient in or have low 25(OH)D levels experience worse outcome in terms of hospital stay, transfer to ICU or death compared to patients who are not deficient in vitamin D (34). There is also accumulating evidence reporting the potential benefit afforded in these elderly fragile patients when administered high doses of native vitamin D supplementation such as cholecalciferol (9, 35). Since the range of pathologies that are associated with BPA exposure also overlap in terms of risk factors leading to worse outcome in patients hospitalized with COVID-19, it is tempting to speculate on the indirect involvement of BPA on outcome in COVID-19 patients (36). Clearly, factors that interfere with vitamin D availability in this high-risk population such as BPA exposure warrant further evaluation.

Study limitations

The main limitation of this study was its observational design. The present analysis is strictly hypothesis-generating in that the observed associations were identified in a cross-sectional sample. We cannot claim causality since the potential impact of BPA exposure on circulating vitamin D was not evaluated by biochemical and/or mechanistic studies.

The primary aim of this analysis was to explore the association between vitamin D metabolites, namely 1,25 (OH)D and 25(OH)D on urinary BPA levels. The elderly population included in this analysis are representative of the general population, in that no strict inclusion–exclusion criteria were employed. Although the sample size (*n*=299) permitted sub-analysis and multivariate

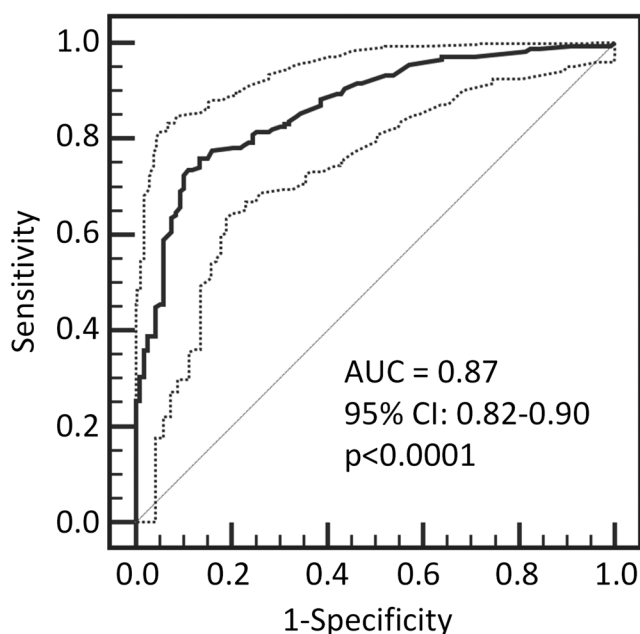


Figure 5 ROC curve to evaluate the predictive power of BPA levels to detect individuals deficient in 25(OH)D (<20 ng/mL) compared to those with levels ≥20 ng/mL. AUC, area under the curve; BPA, bisphenol A; ROC, receiver operating characteristic. ROC curve is represented by heavy line and 95% CIs are represented by dotted lines.

analysis to identify variables associated with vitamin D levels in this elderly population, data are cross-sectional and lack a follow-up period to identify factors linking BPA via vitamin D depletion such as hard outcome measures (BMD, fracture, falls, etc.).

It is a limitation of this paper that information on comorbid diseases was not collected from this database for this study so as to rule out any potential residual confounding from elderly patients with pathologies such as kidney disease. Information on comorbid diseases (as well as concomitant medication) would undoubtedly aid our understanding of the role of BPA in the involvement of other pathologies beyond those linked to vitamin D deficiency.

Conclusion

This was a cross-sectional study where biological samples were collected over a period of 1 year. We observed a strong inverse relationship between urinary BPA concentrations and circulating serum vitamin D levels in an elderly population. Further observational studies with long follow-up periods in addition to pre-clinical mechanistic studies will be necessary to elucidate the exact mechanisms through which compounds such as BPA interact with the vitamin D endocrine system. Hard endpoints such as BMD, fracture and mortality will also aid our understanding of the long-term impact of BPA exposure.

Supplementary materials

This is linked to the online version of the paper at <https://doi.org/10.1530/EC-21-0571>.

Declaration of interest

M L B has received honoraria/grants or speaker fees from Abiogen Pharma, Alexion, Amgen, Bruno Farmaceutici, Eli Lilly, Kyowa Kirin, MSD, NPS, Servier or Shire. All other authors declare no conflict of interest. F N, R G and S B are employees of Abiogen Pharma Spa, Pisa, Italy.

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Author contribution statement

M L B, S B and L F were involved in the conception and design of the study; T I and F G analyzed patient samples and data acquisition; M L B, S B, L F, C G E, F N, S B and R G were involved in the analysis and interpretation of data; all authors contributed to drafting the paper/revising it critically

for intellectual content. All authors have read and agreed to the published version of the manuscript.

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