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Clinical Research Article

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Sex-Dependent Association of Vitamin D With Insulin Resistance in Humans

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Abbreviations: CRP, C-reactive protein; iPTH, intact parathyroid hormone; WBC count, white blood cell count; 25(OH)D, 25-hydroxyvitamin D.

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Abstract

Background: Animal studies suggested that vitamin D might decrease insulin resistance. Estrogen increased insulin sensitivity and glucose tolerance in rodents. However, sex-specific association of vitamin D with insulin resistance in humans remains unclear.
Objectives: To investigate the sex-dependency of the association of insulin resistance and 25-hydroxyvitamin D [25(OH)D] in a large Caucasian population.
Methods: Cross-sectional study from out-patients' blood samples with measurements of 25(OH)D and homeostatic model assessment of insulin resistance (HOMA-IR) drawn at exactly the same day (n = 1887). This cohort was divided into 3 groups: (1) group with vitamin D deficiency (n = 1190), (2) group with vitamin D sufficiency (n = 686), and (3) vitamin D excess groups (n = 11); the vitamin D excess group was excluded from further analysis due to the small size.
Results: Analysis of the entire study population showed that serum 25(OH)D was inversely associated with HOMA-IR [Spearman correlation coefficient (r_s) = –0.19, $P < 0.0001$]. When considering the vitamin D status, this association was only seen in the vitamin D deficiency group but not in the vitamin D sufficient group. The correlation was sex-dependent: HOMA-IR was inversely correlated with vitamin D in women with vitamin D deficiency (r_s = –0.26, $P < 0.0001$) but not in men with vitamin D deficiency (r_s = 0.01,

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$P = 0.714$). After multivariate linear regression analysis considering confounding factors, this relationship was again only seen in women.

Conclusion: Vitamin D was inversely and independently associated with insulin resistance only in women with vitamin D deficiency. Based on our data, we suggest that in particular vitamin D deficient women might benefit from vitamin D substitution by improving insulin resistance. This, however, needs to be proven in adequately designed double-blind placebo-controlled clinical studies.

Key Words: 25-hydroxyvitamin D, HOMA-IR, sex dependent

Insulin resistance—besides pancreatic beta cell failure—plays a key role in the pathophysiology of type 2 diabetes, a chronic metabolic disorder that is becoming increasingly common as a global public health threat (1). The International Diabetes Federation reported that in 2015 about 415 million people worldwide had type 2 diabetes, and this number is expected to rise to 642 million by 2040 (2). It is therefore necessary to find effective therapeutic approaches to reduce insulin resistance—beside approaches to avoid/reduce pancreatic beta cell failure. Insulin resistance is caused by multiple defects in the insulin signaling pathway in insulin-sensitive tissues.

Some studies show that vitamin D deficiency is involved in abnormal glucose metabolism, and vitamin D supplementation can optimize glucose homeostasis and reduce insulin resistance. This was seen not only in type 2 diabetes patients but also in subjects without diabetes (3-6). Cutaneous synthesis through UVB radiation of 7-dehydrocholesterol to previtamin D3 is the major route to obtain vitamin D of human (7). Vitamin D3 which originates from previtamin D3 by temperature-dependent rearrangements of 3 double bonds need further enzymatic conversion to its active forms, converts to 25-hydroxyvitamin D3 in the liver first and then to 1,25-dihydroxyvitamin D3 in the kidney (8). Vitamin D plays an important role in the pathogenesis of many diseases, in particular disorder involved in bone metabolism. But there is also evidence that 1,25-dihydroxyvitamin D is involved in glucose metabolism for example in gestational diabetes. Some studies showed that vitamin D supplementation improves glucose control in women with gestational diabetes mellitus (9,10). Other clinical studies have shown that serum 25-hydroxyvitamin D [25(OH)D] concentration is negatively correlated with insulin resistance, particularly in patients with obesity, diabetes, and polycystic ovary syndrome (11,12). A recent cross-sectional study analyzing a Han China population described an inverse association between 25(OH)D and homeostatic model assessment of insulin resistance (HOMA-IR) in overweight men but not women (13); it was thought that the link between vitamin D and insulin resistance might be sex-specific. We therefore investigated whether the suspected association between vitamin D and insulin resistance is sex-dependent in a large Caucasian population.

Materials and Methods

Study population

This was a cross-sectional study in 1887 patients out of the 56 0578 patients having measurements of serum 25(OH)D concentration and HOMA-IR from blood samples drawn at exactly the same day between December 2012 and April 2020 at the Institute for Medical Diagnostics Berlin, Germany (IMD Berlin; https://www.imd-berlin.de/en/laboratory.html) (14). From the same sample available, we also included data on measurements of cystatin C, intact parathyroid hormone (iPTH), calcium, phosphate, serum creatinine, total cholesterol (TC), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), C-reactive protein (CRP), white blood cell (WBC) count, and hemoglobin into the final data set for further analysis.

The 1887 patients having HOMA-IR and 25(OH)D measurements were divided into 3 groups: (1) vitamin D deficiency group (n = 1190), (2) vitamin D sufficiency group (n = 686), and (3) vitamin D excess group (n = 11). Grouping was based on international guidelines for vitamin D (15,16). Vitamin D deficiency is defined as serum 25(OH)D concentrations below 30 ng/mL; vitamin D sufficiency is defined as serum 25(OH)D concentrations between 30 and 100 ng/mL; and vitamin D excess is defined as serum 25(OH)D concentrations above 100 ng/mL (14). The vitamin D excess group was excluded from further analysis due to low numbers (n = 11). This study was conducted according to the ethical standards of the Institute for Medical Diagnostics Berlin (IMD Berlin), which waived the requirement for informed consent and conducted it in accordance with its ethical standards and the Declaration of Helsinki Principles for data collection.

Clinical and laboratory parameters

The following parameters were collected: age, sex, and date of blood taking as well as laboratory parameters such as 25(OH)D, cystatin C, iPTH, calcium, phosphate, fasting plasma glucose, fasting insulin, serum creatinine, TC, LDL, HDL, CRP, WBC count, and hemoglobin. These parameters were measured using standard tests of the Institute for Medical Diagnostics Berlin (IMD Berlin, Germany; see https://www.imd-berlin.de/nc/en/tests-a-z.html). HOMA-IR was calculated using the equation HOMA-IR = fasting insulin (µU/mL) × fasting plasma glucose (mmol/L)/22.5 (17). 25(OH)D was measured by Abbott Architect i2000 (Abbott Laboratories, Wiesbaden, Germany) using the automated chemiluminescent microparticle immunoassay 25(OH)D by Abbott Architect (Abbott Laboratories, Wiesbaden, Germany). This kit has been standardized on standard reference materials of the National Institute of Standards and Technology (NIST SRM 2972). All clinical and laboratory data undergo subject to rigorous data quality assurance and quality control testing.

Statistical analysis

The data were downloaded from the server of the IMD Berlin and entered into a SPSS database (14). The data were analyzed using SPSS version 23.0 (IBM corporation, New York, USA). All parameters were presented as median (interquartile range). To compare the characteristics between 2 groups, the Mann-Whitney U-test for continuous variables was used, and the Chi-square test was used to analyzed categorical variables. The Spearman correlation analysis was performed to assess correlation. Parameters that show a significant correlation with HOMA-IR were subsequently included into a multivariate linear regression model. For parameters representing the same pathophysiological pathway, just 1 of these parameters with more data was selected, such as cystatin C (n = 50) and serum creatinine (n = 957), which represent kidney function, and CRP (n = 863) and WBC count (n = 999), which respond to inflammation. The multivariate linear regression model was therefore adjusted for age, 25(OH)D, phosphate, serum creatinine, HDL, WBC count, and hemoglobin. A P value of less than 0.05 was considered significant.

Results

Characteristics of study population

All continuous variables were found to be nonnormally distributed by Kolmogorov-Smirnov test ($P < 0.05$). Parameters such as cystatin C, blood lipids (TC, HDL), iPTH, calcium, phosphate, CRP, and WBC count were overall like the normal reference values of the IMD for these parameters (Table 1). Biochemical parameters such as calcium, serum creatinine, TC, and LDL were lower in the vitamin D deficient group than in the vitamin D sufficient group, while HOMA-IR, iPTH, CRP, and WBC count were higher (Table 1). When comparing all parameters between men and women, women had higher 25(OH)D, iPTH, phosphate, and blood lipid (TC, HDL) concentrations, while HOMA-IR, serum creatinine, cystatin C, hemoglobin, and age were lower than those of men (14).

Correlation of serum 25(OH)D with clinical chemistry parameters in men and women

The analysis of HOMA-IR group showed that most parameters in men and women were correlated with vitamin D, except cystatin C, phosphate, HDL, and hemoglobin (Table 2). Serum 25(OH)D was inversely correlated with HOMA-IR ($r_s = -0.19$, $P < 0.0001$) (Table 2). To determine whether these correlations are consistent at different 25(OH)D levels, a correlation analysis was performed in both vitamin D deficient and vitamin D sufficient groups. Since there were only 11 subjects in the vitamin D excess group, no correlation analysis was performed in this group.

Results from the vitamin D deficient group showed that serum 25(OH)D was inversely correlated with HOMA-IR in women ($r_s = -0.26$, $P < 0.0001$) but not in men ($r_s = 0.01$, $P = 0.714$) (Table 2, Fig. 1). In contrast, serum 25(OH)D was associated with iPTH ($r_s = -0.38$, $P = 0.005$), calcium ($r_s = 0.10$, $P = 0.015$), TC ($r_s = 0.13$, $P = 0.001$), and LDL ($r_s = 0.15$, $P < 0.0001$) in men but not in women (Table 2). The correlation of 25(OH)D and TC went into opposite directions in men and women (Table 2). In the vitamin D sufficient group, there was no significant correlation of serum 25(OH)D and HOMA-IR, either in women or men (Table 2).

Multivariate linear regression for HOMA-IR and 25(OH)D in men and women

Spearman's correlation analysis showed a significant correlation between 25(OH)D and HOMA-IR in women with vitamin D deficiency (Table 2). Therefore, a multivariate linear regression was performed in this group. The parameters that showed a significant correlation with HOMA-IR were included into the multivariate linear regression model, such as age, 25(OH)D, phosphate, serum creatinine, HDL, WBC count, and hemoglobin (Table 3). For parameters representing the pathophysiological pathway, just 1 of these parameters with more data was selected, such as cystatin C (N = 50) and serum creatinine (n = 957), CRP (n = 863),

Table 1. Characteristics of the study population (N = 1887)

Parameters	IMD reference range[a]	All (N = 1887)	0-30 (n = 1190)	30-100 (n = 686)	≥100 (n = 11)
Age (year)	—	51 (41-59)	50 (39-57)[b]	54 (45-62)	70 (57-78)
Sex (female)[c]	—	678 (35.90)	413 (34.70)	264 (38.50)	1 (9.10)
25(OH)D (ng/mL)	30.00-100.00	26.00 (17.00-35.00)	20.00 (13.00-25.00)[b]	39.00 (34.00-47.50)	145.00 (134.00-300.00)
HOMA-IR	—	2.77 (1.61-5.69)	3.17 (1.81-6.79)[b]	2.30 (1.39-4.04)	2.01 (1.52-3.01)
Fasting insulin (µU/m)	2.60-24.90	11.00 (6.60-20.50)	12.35 (7.30-23.40)[b]	8.85 (5.68-15.20)	8.00 (5.40-10.80)
FPG (mg/dL)	60.00-100.00	103.00 (95.00-114.00)	103.00 (95.00-114.00)	103.00 (95.25-113.00)	111.00 (102.00-114.00)
Cystatin C (mg/L)	0.70-1.55	0.93 (0.81-1.07)	0.93 (0.79-1.11)	0.93 (0.81-1.06)	0.99 (0.87-1.10)
Serum creatinine (mg/dL)	0.16-0.95	0.98 (0.84-1.11)	0.95 (0.82-1.08)[b]	1.04 (0.89-1.18)	0.96 (0.82-1.32)
iPTH (pg/mL)	15.00-65.00	38.10 (29.05-47.20)	42.70 (32.68-59.70)[b]	33.95 (27.55-42.43)	39.50 (18.35-41.35)
Calcium (mmol/L)	1.90-2.75	2.34 (2.28-2.40)	2.33 (2.28-2.39)[b]	2.35 (2.30-2.40)	2.43 (2.32-2.54)
Phosphate (mmol/L)	0.81-2.42	1.00 (0.88-1.13)	1.00 (0.88-1.14)	1.00 (0.88-1.12)	—
TC (mg/dL)	< 200.00	190.00 (164.00-217.00)	187.50 (162.00-215.00)[b]	194.00 (169.00-222.00)	163.50 (154.25-178.00)
LDL (mg/dL)	< 115.00	121.00 (96.00-147.00)	118.00 (95.00-144.00)[b]	125.00 (101.75-150.00)	105.00 (101.00-109.00)
HDL (mg/dL)	> 45.00	50.00 (41.00-61.00)	50.00 (41.00-61.00)	49.00 (41.00-61.00)	53.50 (49.00-58.00)
CRP (mg/L)	0.00-5.00	1.60 (0.50-3.80)	1.80 (0.50-4.20)[b]	1.20 (0.50-3.29)	1.05 (0.26-6.83)
WBC count (10^9/L)	3.60-16.20	6.30 (5.20-7.70)	6.40 (5.30-7.80)[b]	6.10 (5.10-7.50)	5.80 (5.05-7.15)
Hemoglobin (g/dL)	9.40-17.20	14.30 (13.40-15.20)	14.30 (13.30-15.20)	14.30 (13.60-15.10)	13.80 (13.05-15.60)

Data are given as median (25th-75th percentile) unless otherwise noted. All parameters are nonnormally distributed except for sex.
Abbreviations: 25(OH)D, 25-hydroxyvitamin D; CRP, C-reactive protein; FPG: fasting plasma glucose; iPTH, intact parathyroid hormone; WBC, white blood cell.
[a]Reference range from the Institute for Medical Diagnostics Berlin (https://www.imd-berlin.de).
[b]Used to mark *P* value < 0.05, while comparing with vitamin D sufficient group (30-100 ng/mL).
[c]Data given as n (%).

and WBC count (n = 999) (Table 3). This was done to avoid collinearity. In women, analysis of multivariate linear regression showed that 25(OH)D was significantly associated with HOMA-IR (unstandardized coefficients B: –0.24, $P = 0.024$) (Table 4). In men, however, this association did not exist ($P = 0.698$) (Table 4).

Discussion

Our study showed that serum 25(OH)D was inversely associated with HOMA-IR ($r_s = -0.19$, $P < 0.0001$) (Table 2). However, the comparison of patients with deficient and sufficient vitamin D status showed that this association was only present in the group of patients with vitamin D deficiency. In patients with sufficient vitamin D levels, the association was not detectable (Table 2). When women and men were analyzed separately in patients with vitamin D deficiency, we saw that serum 25(OH)D concentrations were inversely associated with HOMA-IR in women ($r_s = -0.26$, $P < 0.0001$) but not in men ($r_s = 0.01$, $P = 0.714$) (Table 2). A multivariate linear regression analysis confirmed that this relationship is present only in women. These results suggested that the effect of vitamin D deficiency on insulin resistance is sex dependent.

The link between vitamin D and insulin resistance has been reported in several clinical studies (18,19), but it is still controversial (20). Based on our data, we suggest that one reason for these inconsistent findings might be the fact that most of these studies did not do a subgroup analysis based on vitamin D concentration and sex simply because their data sets were too small and did not allow it. A recent placebo-controlled, double-blind clinical trial examined the effect of vitamin D replacement on the incidence of type 2 diabetes, which contradicts our findings (21). The design of the study most likely explains the contradictions. The main inclusion criterion in that study was the

Table 2. Correlation of 25(OH)D with clinical chemistry parameters in men and women in vitamin D deficient and sufficient group

Parameters	25(OH)D 0-30ng/mL				25(OH)D 30-100 ng/mL				25(OH)D ng/mL	
	Men		Women		Men		Women		All	
	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*	r_s	*P*
Age (years)	0.25	<0.0001	0.07	0.164[a]	0.08	0.095	0.10	0.091	0.24	<0.0001
HOMA-IR	0.01	0.714	−0.26	<0.0001[a]	−0.06	0.253	0.02	0.702	−0.19	<0.0001
Fasting insulin (µU/mL)	0.01	0.793	−0.24	<0.0001[a]	−0.08	0.115	0.00	0.958	−0.20	<0.0001
FPG (mg/dL)	0.07	0.071	−0.16	0.002[a]	0.07	0.153	0.11	0.085	−0.01	0.695
Cystatin C (mg/L)	0.10	0.578	−0.64	0.014[a]	0.16	0.435	−0.14	0.515	−0.02	0.833
Serum creatinine (mg/dL)	0.20	<0.0001	0.10	0.101[a]	−0.01	0.845	0.06	0.551	0.21	<0.0001
iPTH (pg/mL)	−0.38	0.005	−0.20	0.156[a]	−0.36	0.002	−0.17	0.121[a]	−0.39	<0.0001
Calcium (mmol/L)	0.10	0.015	0.06	0.434[a]	0.09	0.115	0.07	0.590	0.12	<0.0001
Phosphate (mmol/L)	−0.11	0.015	0.26	0.003	0.02	0.689	−0.05	0.758	−0.05	0.103
TC (mg/dL)	0.13	0.001	0.00	0.964[a]	−0.02	0.726	0.09	0.347	0.10	<0.0001
LDL (mg/dL)	0.15	<0.0001	0.03	0.571[a]	−0.03	0.537	0.02	0.798	0.11	<0.0001
HDL (mg/dL)	0.03	0.463	0.16	0.008[a]	−0.03	0.626	0.10	0.278	0.05	0.096
CRP (mg/L)	−0.13	0.001	−0.17	0.012	0.04	0.478	−0.13	0.117	−0.15	<0.0001
WBC count (10^9/L)	−0.17	<0.0001	−0.18	0.001	0.03	0.596	0.08	0.354	−0.13	<0.0001
Hemoglobin (g/dL)	0.04	0.268	0.07	0.225	0.01	0.864	0.04	0.667	0.03	0.330

The Spearman correlation analysis was performed to assess correlations, since all parameters were found to be not normally distributed ($P < 0.05$).
Abbreviations: 25(OH)D, 25-hydroxyvitamin D; CRP, C-reactive protein; FPG, fasting plasma glucose; iPTH, intact parathyroid hormone; r_s, Spearman correlation coefficient; WBC, white blood cell.
[a]Used to mark when there was a huge difference in *P*-values between men and women.

presence of biochemical signs of prediabetes—but not of vitamin D deficiency. This key inclusion criterion leads to an enrichment of study participants with a predisposition to type 2 diabetes for whatever reason, such as genetic causes or familial predisposition, obesity, lifestyle, dietary habits, etc. Under these conditions, vitamin D deficiency is only one of many factors predisposing to diabetes; its effect might be diluted by other reasons, increasing the likelihood of diabetes. Besides the selection bias of this study, it is even more important that this study does not examine patients with significant vitamin D deficiency. A total of 2423 participants underwent randomization (1211 to the vitamin D group and 1212 to the placebo group). By month 24, the mean serum 25(OH)D level in the vitamin D group was 54.3 ng/mL (from 27.7 ng/mL at baseline), as compared with 28.8 ng/mL in the placebo group (from 28.2 ng/mL at baseline). In other words, not only the treatment group but also the placebo group did not really suffer from major vitamin D deficiency. Statements on the relationship between vitamin D deficiency and insulin resistance can therefore only be made with great caution in that study because only 525 out of 2423 patients were vitamin D deficient in that study. We, on the other hand, investigated the relationship between insulin resistance in patients with and without vitamin D deficiency and hence came to different conclusions. However, regarding patients without vitamin D deficiency our study results are in good agreement with the previously discussed study indicating that these will not benefit from vitamin D substitution. The new finding in our study is the demonstration of a sex dependent association of vitamin D deficiency with insulin resistance.

An early event in the pathogenesis of type 2 diabetes is the development of insulin resistance. To maintain normal glucose concentrations, the pancreatic β-cells initially overcome this resistance by releasing more insulin, Once this compensation fails, type 2 diabetes develops. Vitamin D deficiency plays a pivotal role in both processes. Vitamin D acts to reduce inflammation. Inflammation, however, is one of the key players in inducing insulin resistance. Vitamin D also maintains the normal resting concentrations of Ca^{2+} and reactive oxygen species in the pancreatic β-cells that are elevated during hyperglycemia. More recent studies also indicate that vitamin D also has an important impact on the epigenetic control of gene expression. Epigenetic alterations are a hallmark of type 2 diabetes by which many diabetes-related genes are inactivated by hypermethylation. Vitamin D acts to prevent such harmful hypermethylation by increasing the expression of enzymes such as DNA demethylases that prevent hyperglycemia-induced hypermethylation of multiple gene promoter regions of many diabetes-related genes (22-26).

It is important to note that the correlation between 25(OH)D and HOMA-IR in our study was detectable only in women and not in men. This sex-dependent correlation could be due to differences in sex steroid hormones and the distribution of fat tissue between men and women. Some studies have shown that sex steroid hormones significantly regulate insulin resistance. Importantly, the effects of estrogen and androgen were different (27,28). For example, estrogen inhibited beta cell apoptosis of the pancreas by reducing oxidative stress, while androgen acted in the opposite direction (29,30). Many studies have found that estrogen stimulated insulin sensitivity and increased glucose tolerance in the skeletal muscles of adult mice (31,32). This may be due to the high expression of the estrogen receptor α in skeletal muscle (33,34). Estrogen supplementation has also been shown to reduce the incidence of diabetes in postmenopausal women by improving glucose-stimulated insulin secretion (35,36). In comparison, offspring of baboons in which estrogen was suppressed during the second half of pregnancy showed insulin resistance after puberty (37). In addition, adipose tissue as an important target tissue for insulin is also involved in the development of insulin resistance. Interestingly, numerous studies have shown sex differences in the function of fat tissue. Women had a higher proportion of body fat than men and stored it in a different pattern (38,39). This female fat distribution could protect the body from metabolic diseases such as type 2 diabetes (40,41). In other words, women can accumulate more adipose tissue without having harmful metabolic consequences. Our study also showed that blood lipid levels (TC, LDL, HDL) were higher in women than in men, while HOMA-IR was lower in women (14). In addition, adipose tissue expressed both subtypes of the estrogen receptor (ER; ERα as well as ERβ) (42-44). In women, estrogen thus might influence insulin resistance by binding to ERα or ERβ in the adipose tissue (45,46). However, since we performed a cross-sectional analysis, the opposite relationship (ie, insulin sensitivity may affect vitamin D metabolism) might also be possible.

From what we have discussed, we have learned that vitamin D reduces insulin resistance by affecting insulin-sensitive tissues, such as adipose tissue. Therefore, differences in the distribution of adipose tissue and sexual steroid hormones between women and men are likely to be the underlying factors for the sex-specific association of vitamin D with insulin resistance. An animal study indicates an interaction between insulin, sex steroids, and

Table 3. Correlation of HOMA-IR with clinical chemistry parameters in the vitamin D deficient group (0-30 ng/mL)

Parameters	HOMA-IR		
	N	r_s	*P*
Age (year)	1190	0.15	<0.0001
25(OH)D (ng/mL)	1190	−0.00	0.011
Cystatin C (mg/L)	50	0.30	0.036
Serum creatinine(mg/dL)	957	0.10	0.001
iPTH (pg/mL)	108	0.06	0.534
Calcium (mmol/L)	769	0.00	0.987
Phosphate (mmol/L)	641	−0.16	<0.0001
TC (mg/dL)	888	−0.01	0.791
LDL (mg/dL)	945	0.00	0.919
HDL (mg/dL)	935	−0.36	<0.0001
CRP (mg/L)	863	0.19	<0.0001
WBC count (10^9/L	999	0.13	<0.0001
Hemoglobin (g/dL)	997	0.12	<0.0001

The Spearman correlation analysis was performed to assess correlations, since all parameters were found to be not normally distributed ($P < 0.05$).
Abbreviations: 25(OH)D, 25-hydroxy-vitamin D; CRP, C-reactive protein; FPG, fasting plasma glucose; iPTH, intact parathyroid hormone; r_s, Spearman correlation coefficient WBC, white blood cell.

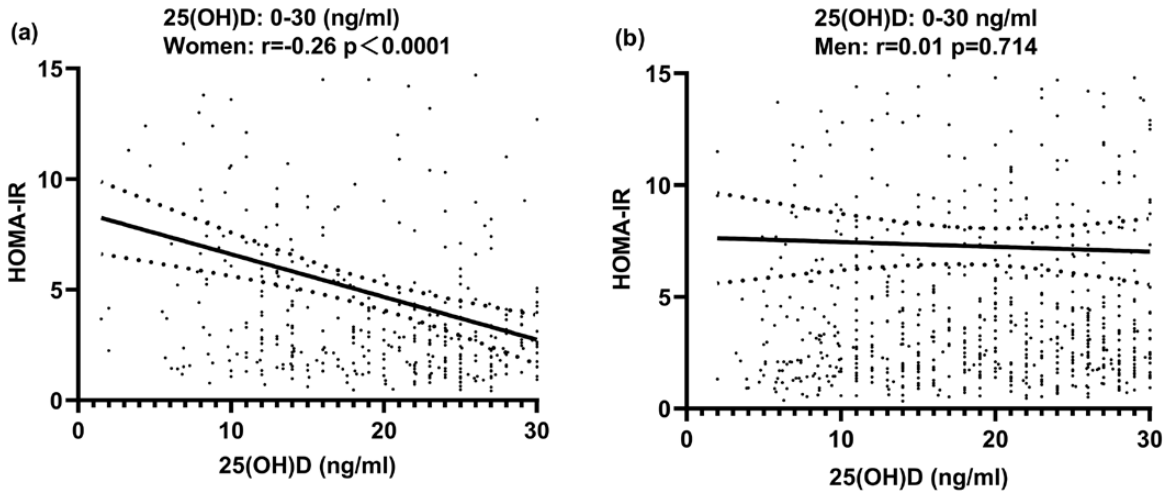

Figure 1. 25(OH)D and HOMA-IR in vitamin D deficient group. Data are given as mean ± 95% confidence intervals. In the vitamin D deficient group, 25(OH)D was inversely associated with HOMA-IR in women ($r_s = -0.26$, $P < 0.0001$) (A), but not in men ($P = 0.714$) (B).

Table 4. Multivariate linear regression for HOMA-IR in men and women with vitamin D deficiency (0-30 ng/mL)

Independent variable	Men			Women		
	P-value	Unstandardized coefficients B	95% confidence interval for B	*P*-value	Unstandardized coefficients B	95%confidence interval for B
(Constant)	0.365	7.72	−9.00~24.44	0.038	18.79	1.02~36.56
25(OH)D	0.698	0.03	−0.12~0.18	0.024	−0.24	−0.44~−0.03
Age	0.064	0.09	−0.01~0.18	0.173	0.07	−0.03~0.16
Phosphate	0.181	−3.73	−9.19~1.74	0.875	0.73	−8.43~9.88
Serum creatinine	0.811	−0.51	−4.68~3.66	0.532	−2.31	−9.62~4.99
HDL	0.000	−0.21	−0.28~−0.13	0.082	−0.06	−0.14~0.01
WBC count	0.754	−0.08	−0.59~0.42	0.071	0.58	−0.05~1.22
Hemoglobin	0.116	0.66	−0.16~1.48	0.130	−0.76	−1.76~0.23

We included parameters in the model showing a significant correlation with HOMA-IR in the univariate analysis; see Table 3.
Abbreviations: 25(OH)D, 25-hydroxyvitamin D; WBC, white blood cell.

vitamin D metabolism. Male rats had higher concentrations of vitamin D than female rats, but this sex difference disappeared after gonadectomy and were reversible after subsequent testosterone treatment. This suggests that sex steroids may be involved in the metabolism of vitamin D. This study also showed that vitamin D concentrations were reduced in both male and female diabetic rats with gonadectomy but were increased again by insulin treatment. This study also showed that androgens and estrogens have an opposite effect on vitamin D concentrations (47)

Taken together as previously outlined, there is preclinical evidence that both vitamin D and also estrogens might affect insulin resistance means reduce insulin resistance. Both molecular mechanisms may act synergistically. This might explain our finding of the relationship between insulin resistance and vitamin D status in women. This effect becomes obvious when the vitamin D concentrations in the women is low. Our study suggests a threshold for this effect, since this close interaction is only seen in women with vitamin D deficiency but not in women with adequate concentrations of vitamin D. This hypothesis to explain our findings needs, of course, be further analyzed in preclinical studies as well as in controlled clinical trials of vitamin D supplementation in women with vitamin D deficiency.

Although our study is a huge cross-sectional study, it inevitably has limitations. We used all data on vitamin D measurements done in our institute in the past years (14). We are an institute for clinical chemistry analyzing blood samples as service for over 800 primary care physicians in the Berlin-Brandenburg area of Germany. This explains the size of our database. However, we do just have patient data such as age and sex; other data are not provided by the primary care physicians. In addition, the vitamin D excess group was excluded from analysis due to low numbers (n = 11); thus, the association between vitamin D and insulin resistance in this group remains unclear.

Conclusions

Overall, our study showed that vitamin D was inversely and independently of other confounding factors associated with insulin resistance in women but not men with vitamin D deficiency. This sex-dependent association may be due to a synergistic interaction of estrogens and vitamin D. There seems to be, however, a threshold for this effect, since we observed this association only in women with vitamin D deficiency.

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