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ASSOCIATION BETWEEN VITAMIN D STATUS AND DEPRESSION IN MALE TWINS

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Abstract Cover Page

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By

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Doctor of Medicine

Karaganda State Medical Academy

1997

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Abstract

ASSOCIATION BETWEEN VITAMIN D STATUS AND DEPRESSION IN MALE TWINS

By
Andrey V. Gavrilyuk

Background: Depression and vitamin D deficiency are two major global health problems. To date it is still not clear if vitamin D deficiency plays a role in the rising incidence of major depression. Observational studies and randomized clinical trials have provided no definitive answer due to conflicting results.

Objective: To examine the relationship between vitamin D status and a clinical diagnosis of major depression, adjusting for potential confounding factors.

Design: We studied 509 middle-aged male twins, including 153 pairs and 5 unpaired monozygotic (MZ) and 97 pairs and 4 unpaired dizygotic (DZ) twins from the Vietnam War Era Twin Registry, who underwent a clinical interview for the assessment of major depression (lifetime history) and measurement of serum vitamin D[25(OH)]. Vitamin D levels were classified according to accepted clinical categories: deficient (<30 ng/mL), insufficient (30-49 ng/mL), and sufficient/optimal (50-80 ng/mL). We used generalized estimating equations (GEE) to test the association between vitamin D level as a continuous variable, as well as vitamin D clinical categories, with major depression.

Results: When vitamin D was treated as continuous variable, the odds of depression were 26% lower per incremental ng/mL of vitamin D, but the association was not significant (odds ratio [OR]=0.74, 95% confidence interval [CI]:0.51-1.10). After adjusting for age, physical activity, alcohol use and BMI, the estimate remained similar (OR=0.78, 95% CI: 0.53-1.16). When clinical categories of vitamin D were compared, using the sufficient/optimal category as reference, the odds of depression in the deficient category were significantly higher (OR=1.72, 95% CI: 1.01- 2.95), while the odds in the insufficient category were similar (OR=1.18, 95% CI=0.69-2.02) compared with the sufficient category. The association was slightly attenuated after adjustment for age, BMI, physical activity, and alcohol consumption (OR=1.65, 95% CI: 0.95-2.84, comparing vitamin D deficiency to sufficiency). There was no significant association within either MZ or DZ twin pairs, and the interaction with zygosity was not significant.

Conclusions: Although there is a cross-sectional association between clinical categories of vitamin D and depression, such association is in part explained by other lifestyle factors and possibly by familial factors shared by the twins.

COVER PAGE

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INDRODUCTION

Depression is the second leading cause of disability among people all over the world (1). It is estimated that depression affects more than 350 million people around the globe. In the United States depression affects 18.8 million adults in any given year and more than 200 million workdays are lost each year due to depression, which cost employers from $17 to $44 billion (1, 2). Vitamin D deficiency is another global health problem that affects people in many countries and is more common than originally thought, especially in middle-aged and elderly people (3-8). Besides its main function on calcium homeostasis, recent studies uncovered other physiological functions of vitamin D in non-skeletal tissues (5, 9) . The major breakthrough was the discovery of vitamin D receptors in many important cells of the body, including brain cells (10-14). It has been hypothesized that activation of these receptors affects the level of important neurotransmitters in the brain tissue, potentially predisposing individuals to developing clinical signs and symptoms of depression and other mental disorders (12, 13, 15-19). Other research suggests that vitamin D deficiency is a potential source of inflammation which may contribute to the signs and symptoms of depression (20, 21).

The aging population is particular prone to have both depression and vitamin D deficiency, not only due to lifestyle factors such as limited outdoor activities and changes in diet, but most importantly due to atrophic skin changes with low amount of a vitamin D precursor, 7- dehydrocholesterol (7-DHC), that lead to lower vitamin D production when exposed to solar UV-B radiation (3, 5, 22-25) . It remains to be established whether vitamin

D deficiency leads to signs and symptoms of depression or depression leads to vitamin D deficiency due to health behaviors such as loss of interest in outdoor activities (26, 27).

Numerous cross-sectional studies in different countries reached diverging conclusions. Studies by Almeida et al. in Western Australia (2015) and Toffanello et al. in Italy (2014) concluded that vitamin D deficiency had no effect on the onset of late-life depressive symptoms (28, 29). Contrary to those studies, the research done by Kerr et al. among female undergraduates in the Pacific Northwest, by Mizoue et al. in Japan (2015), by Black et al. in Australia , by Chung et al. (2014) in Korea, by Milaneschi et al. (2014) in Netherlands, by Polak et al. in New Zealand (2014), by Lapid et al. in the upper Midwest, and by Verhoeven et al. in Belgium (2012), came to the conclusion that low serum 25-hydoxyyvitamin D [25(OH)D] concentration was associated with signs and symptoms of depression (6, 30-34).

There is concern about comparability of various cross-sectional studies of vitamin D and mental health as researchers used different assays to determine circulating serum 25(OH)D levels. Those methods included competitive protein binding assay (CPBA), high performance liquid chromatography (HPLC), liquid chromatography-tandem mass spectrometry (LC_MS/MS), and radioimmunoassay (RIA), and enzyme immunoassay (35, 36). Moreover, establishing association in cross-sectional studies is not in fact establishing causation. Tolppanen et al. (2012) conducted a longitudinal study in South West England and determined that the association between serum 25(OH)D level and depression emerged in childhood. The observed association in this study was independent of potential confounding factors and proved to be stronger with more time elapsing between the assessment of vitamin D level and the symptoms of depression (37) .

Findings from randomized clinical trials (RCTs) examining the effect of vitamin D supplementation and development of signs and symptoms of depression versus placebo are controversial as well. Sanders et al. (2011) in a randomized clinical trial with annual high-dose vitamin D administration found no significant protective effect of vitamin D on development of depression (38). On the other hand, a clinical trial conducted by Mozaffari-Khosravi et al. (2013) in Iran with a single administration of various doses of vitamin D to patients diagnosed with depression and who had low serum 25(OH)D levels, yielded improvement of depression symptoms in the highest single-dose group compared with the untreated group (39). The differences in trial results could be attributed to different duration of trials, different dosages used, methods of measurement for depressive symptoms, whether a placebo group was used, as well as baseline 25(OH)D concentrations.

A recent systematic review with meta-analysis conducted by Li et al. (2014) of six RCTs, two in Norway, two in the United States, one in Australia, and one in Iran led to the conclusion that there is insufficient evidence to support the efficacy of vitamin D supplementation to ameliorate depression symptoms (40). On the other hand, a review by Shaffer et al. (2014) suggested a moderate statistically significant effect in those who had clinically significant depressive symptoms, and non-significant effect for those without clinically significant depression (41). Thus, the conclusion was that vitamin D supplementation may be effective in reducing depressive symptoms in patients with clinically significant depression (41). Another review with meta-analysis conducted by Spedding (2014) pointed out a confounding effect of variations in quality of RCTs. He suggested that studies without biological flaws were more likely to show an improvement

in depression scores with vitamin D supplementation, while studies with limitations were more likely to report null findings (42, 43).

As of today, it is still not clear if low serum 25(OH)D level is indeed a risk factor for depression. More rigorous studies are needed to support a possible association while controlling for potential confounders, as the true link between vitamin D status and depression has yet to be established. The demonstration of such an association would support Vitamin D supplementation as a simple and inexpensive solution for ameliorating depression in those who are affected by this disabling condition (43-45).

Therefore, we conducted an analysis of the cross-sectional longitudinal twin study with the purpose: first, to examine a relationship between different clinical vitamin D categories and a lifetime occurrence of depression among monozygotic (MZ) and dizygotic (DZ) middle-aged (47-64 years old) male twins; second, to assess if the severity of depression has a direct associated with the severity of vitamin D deficiency.

SUBJECTS AND METHODS

Design

Our analytical sample was derived from the Emory Twin Study (ETS), which provided the opportunity for a rigorous, quasi-experimental design of twins discordant for depression. The details on the ETS recruitment have been previously described in detail (46). The present sample comprised 510 participants who were selected to participate in ETS from the Vietnam Era Twin Registry from those who were born from 1946 to 1956 based on whether they were discordant for major depression or for posttraumatic stress disorder (PTSD). A random sample of non-affected twin pairs was also included. The original Vietnam Era Twin Registry included 4,774 male-male twin pairs with the year of birth ranging from 1939 to 1957. The inclusion criteria in this registry was service in the United States military during the Vietnam War for both twin brothers. The registry was developed for the purpose of studying the long-term health consequences of service in Vietnam. The registry utilized three main sources to collect initial data: military service records, health survey questionnaires, and computer databases from the Department of Veteran Affairs (47).

The Emory University General Clinical Research Center provided support to examine all twins in pairs under controlled conditions during an approximately 24-hour admission. Approval for the study came from the Emory Institutional Review Board and informed consent was obtained from all subjects who participated in the study.

Measurements

Twins in each pair were tested during admission at Emory Clinical Research Center, when blood samples were drawn in the morning after overnight fast, medical histories obtained, and physical examinations conducted under controlled conditions. The body mass index (BMI) was calculated based on measured weight and height as weight in kilograms divided by the height in square meters. Blood pressure was measured in the right arm using a mercury sphygmomanometer after 10 minute of rest with subjects sitting. Two measurements for blood pressure were obtained within 5 minutes, and the average was used in the analysis. We defined hypertension as a blood pressure greater than 140 mm Hg for systolic pressure, or a blood pressure greater than 90 mm Hg for diastolic pressure. Diabetes was defined as a fasting blood glucose greater than 126 mg/dL or current treatment with either insulin or other oral hypoglycemic medications. The modified version of the Baecke Questionnaire of Habitual Physical Activity was utilized to assess physical activity, with values ranging from 3.4 (minimal activity) to 13.0 (intense activity) (48). Lifetime history of major depression was defined based on the Structured Clinical Interview for DSM-III-R (49). Severity of depressive symptoms was assessed by using the Beck Depression Inventory (BDI-II), a 21-question self-report instrument, with score range from 0-63. Scores of 0-13 indicate minimal depression, scores of 14-19 indicated mild depression, scores of 20-28 indicated moderate depression, and scores of 29-63 indicated severe depression (50). Somatic and cognitive depressive symptoms scores from the BDI were computed (51). Alcohol consumption was measured as a total number of alcoholic beverages in a typical week, and ranged from 0-90. Questions were also asked about smoking status; current smoking was used in the analysis.

To determine serum vitamin D [25(OH)] level, an enzyme-linked immunosorbent assay was used (IDS Inc, Fountain Hills, AZ). Blood samples from both twins were processed in the same analytical run. Three major clinical categories of vitamin D status were constructed based on vitamin D Council and testing laboratories guidelines, and included vitamin D deficiency (vitamin D [25(OH)] less than 30 ng/mL), vitamin D insufficiency/suboptimal (vitamin D [25(OH)] between 30 ng/mL and 49 ng/mL), and vitamin D sufficiency/optimal (vitamin D [25(OH)] between 50-80 ng/mL) (52-54). Vitamin D [25(OH)] levels above 80 ng/mL, but less than 100 ng/mL are considered to be high normal, but vitamin D [25(OH)] levels between 100 -150 ng/mL are thought to be excessive. We only had 17 subjects with vitamin D [25(OH)] greater than 80 ng/mL but less than 100 ng/mL level, and we had 14 subjects with vitamin D [25(OH)] level between 100-150 ng/mL. There is no clear evidence to show any benefit or harm of vitamin D above 80 ng/mL level. We found only one study where vitamin D level greater that 100 ng/mL was associated with increased risk of atrial fibrillation (55). Thus, we included all subjects with vitamin D[25(OH)] level between 50-150 ng/mL into sufficient/optimal category. Since there is a lack of evidence if vitamin D levels greater than 150 ng/mL are safe, we excluded 1 subject above this level from our analysis (56).

Data analysis

In descriptive analyses, we compared mean values and proportions of co-variables according to the three vitamin D[25(OH)] level categories according to the clinical classification. To assess associations among clinical vitamin D [25(OH)] level categories and other covariates, we used the PROC GENMOD procedure in SAS, version 9.2 (SAS Institute, Cary, North Carolina). Initially, we treated vitamin D as a continuous variable

(vitamin D level in ng/mL) to estimate the association with major depression for each unit difference in vitamin D level. Vitamin D level was not normally distributed, therefore, it was log transformed. Multiple demographic variables were also included in our models as covariates, including age, physical activity, alcohol consumption, and BMI. The choice of covariates (a priori) was driven by our literature review of factors that could affect both vitamin D levels and occurrence of depression, thus, potentially confounding our findings. In our twin study we compared twins using within- and between pair effects. We calculated the between pair effect as the average of the two twins' values, whereas the within pair effect was calculated as each twin's deviation from the pair average. Demographic, shared familial, and early environmental factors are shared by the twins and thus are controlled by estimating the within-pair effect. Any difference in major depression seen within MZ twin pairs can be ascribed to environmental factors since monozygotic pairs share 100 % of their genetic material in addition to their common early environment. On the other hand, differences in major depression within DZ twin pairs can only partially explained by the environment, since dizygotic twin pairs on average share only 50% of genetic material. Thus, comparing effect sizes among MZ and DZ twins gives us clues if familial or shared environment confounding exists (57).

We repeated the analysis with three clinical categories of vitamin D (our main risk factor of interest), including a "sufficient/optimal" category for vitamin D levels between 50 -150 ng/mL, an "insufficient/suboptimal" category with vitamin D levels between 30-49 ng/mL, and, finally, a "deficient" category with vitamin D levels less than 30 ng/mL. We compared categories against one another in unadjusted and adjusted models to determine the clinical category of vitamin D where major depression was more common.

We also tested the interaction between vitamin D level and zygosity to assess the impact of genetic and environmental factors on the associations of interest.

Our main analyses used lifetime history of major depression and vitamin D clinical categories as categorical variables, but in secondary analyses we also examined the BDI-II total score as continuous variables. To estimate the parameters of our models we utilized a generalized estimating equation (GEE). GEE is considered to be a class of semiparametric regression techniques used to handle many types of unmeasured dependence between outcomes. We used GEE modeling (PROC GENMOD in SAS) to fit logistic regression with major depression as the outcome variable and vitamin D categories as independent variables (unadjusted), then with vitamin D, age, physical activity, alcohol use and BMI (adjusted) as independent variables. The REPEATED statement invoked the GEE method, specified the correlation structure. To specify the final working correlation display we used the CORRW option (58). All statistical tests were 2-sided with significance level inferred at calculated probability value less than 0.05.

RESULTS

Demographics

Of 566 twins (283 pairs) in the study, 510 had available 25-hydroxivitamin D [25(OH)] data. We restricted our analysis only to observations with a value of 25-hydroxivitamin D [25(OH)] less or equal to 150 ng/mL (excluded N=1). Thus, 509 observations were included in the analysis, consisting of 250 complete pairs and 9 unpaired twins. Three hundred and eleven twins (61.1%), including 153 pairs and 5 unpaired twins, were MZ, and 198 twins (38.9%), including 97 pairs and 4 unpaired twins, were DZ. Ethnic background in our sample was represented by mostly white males (N=490, 96.3%), while other ethnicities (Hispanics, African-Americans, and Asians) contributed only a small number (N=19, 3.7%).

Two hundred twenty-one subjects (43.4%) were classified as ***deficient*** based on their vitamin D [25(OH)] status, and 182 (35.8%) subjects were classified as vitamin D [25(OH)] ***insufficient/suboptimal***. Only 106 (20.8%) twins were in the ***sufficient/optimal*** category for vitamin D [25(OH)]. As shown in Table 1, vitamin D [25(OH)] deficiency was significantly associated with higher BMI, diabetes, and lower physical activity score. Subjects in the lower vitamin D level categories were also more likely to smoke, to have a history of hypertension, coronary heart disease, and PTSD, and to be taking cardiovascular medications (statins, aspirin and beta-blockers), although these differences did not reach statistical significance. Notably, subjects who were deficient in vitamin D[25(OH)], when compared to the insufficient/suboptimal and sufficient/optimal categories, were more likely to meet criteria for lifetime diagnosis of major depression (p=0.03) and tended to

have higher total, somatic and cognitive BDI mean scores (p=0.011, p=0.09 and p=0.21, respectively).

Association between 25-hydroxyvitamin D [25(OH)] status and depression

We compared lifetime history of major depression status based on 25-hydroxivitamin D [25(OH)D] clinical classification. There was a progressively higher frequency of depression going from the sufficient category to the insufficient/suboptimal and the deficient category for vitamin D level (**Figure 1**), showing a clear dose-response relationship between vitamin D level and frequency of depression. We also used box plots to compare severity for depression based on total BDI score as continuous variable according to vitamin D groups (**Figure 2**). This comparison showed slightly higher mean and median BDI scores in the deficient group compared to the sufficient group, but mean and median BDI scores were almost the same in the insufficient group compared to the sufficient group, and there was no clear dose-response relationship.

We used GENMOD models to assess the association between vitamin D [25(OH)] level as a continuous variable and depression status (**Table 2).** In unadjusted analysis, considering twins as separate individuals, we found that the odds of depression were 26% lower per each higher ng/mL of vitamin D, but the association was not statistically significant (OR=0.74, 95% CI: 0.51-1.10, p-value=0.1). When we adjusted for possible confounders in our model such as age, physical activity, alcohol use and BMI, our results still showed a non-significant 22 % lower odds of depression per unit change of vitamin D (OR=0.78, 95% CI: 0.53-1.16, p-value=0.2).

We also utilized GENMOD models with twin pair as repeated measure to compare twins within pairs, stratified by zygosity. The results of within-pair analyses were non-significant for both DZ pairs (within pairs: OR=0.97, 95% CI: 0.29-3.21, p-value=0.09) and MZ pairs (within pairs: OR=1.65, 95% CI: 0.67-4.07, p-value=0.3).

When we used logistic regression and compared different clinical categories for vitamin D level (**Table 3**), we observed that the odds of depression in the deficient clinical category were 1.72 times higher the odds of depression in the sufficient/optimal clinical category (OR=1.72, 95% CI:1.01-2.95, p-value=0.047). In contrast, the odds of depression in the insufficient category were not significantly higher than the sufficient category. The combined deficient and insufficient categories were also not significantly different from the sufficient category. However, the combined insufficient and sufficient categories were statistically significant compared with the deficient category (data not shown): the odds of depression in the deficient category were 1.59 times higher the odds of depression in both other categories combined (OR=1.59, 95% CI:1.06- 2.39, p-value=0.026). When we made adjustments for age, BMI, physical activity score, and alcohol consumption, we still observed that the odds of depression were 1.65 times higher in the deficient category compared to those in the sufficient/optimal clinical category, but these results did not reach statistical significance (OR=1.65, 95% CI:0.95-2.84, p-value=0.075) (**Table 4**). However, the combined insufficient and sufficient categories remained statistically significant in comparison to the deficient category, OR=1.54, 95% CI:1.01-2.34, p-value=0.0455 (data not shown). We have also explored the effect modification of zygosity when comparing different clinical categories for vitamin D, but we did not find significant associations neither among MZ twins nor DZ twins.

DISCUSSION

In our cross-sectional study of twins, we found that that major depression was more prevalent in twins who were classified as vitamin D deficient compared to those in the vitamin D sufficient (optimal) clinical group, although the association was partially explained by other lifestyle factors. Although not statistically significant, results were overall consistent when vitamin D was considered as a continuous variable.

Our results agree with recent cross-sectional studies conducted in the United States and other countries and among different populations. Studies conducted by Verhoeven et al. in Belgium (2012) and Lapid et al. in the upper Midwest (2013) were focusing on geriatric populations (22,29). However, older people often have numerous comorbidities, which can confound the association since they can affect mental function and also vitamin D levels. Yet, the study by Milaneschi et al. (2014) in a cohort of young men and women with a mean age 40.9-43.4 years old in the Netherlands showed an inverse association for vitamin D level and depressive symptoms. Another study by Kerr et al. among female undergraduates in the Pacific Northwest (2015) demonstrated similar results. Furthermore, the studies by Mizoue et al. in Japan (2015) among Japanese workers with mean age 41.8-47.0, as well as by Polak et al. in New Zealand (2014) in young student volunteers aged 17-25, came to the same conclusion (37,38,44,45). Another study, conducted by Toffanello et al. in Italy (2014) found an inverse association between vitamin D and depression cross-sectionally (among women only), but not prospectively, and suggested that other factors (i.e. physical activity), could play a significant role in the association (28). The study conducted by Almeida et al. in Perth, Western Australia (2015) among elderly men, concluded that even though there was association between current level of vitamin D and

current depression, there was no such an association for past of future depression (29). Several randomized trials of vitamin D supplementation were also negative, although results are mixed (42, 43). Poor RCT design may be an important reason for failure to find improvement in depression scores. In his meta-analysis comparing studies with and without biological flaws, Spedding concluded that poorly designed trials were more likely to report a null association, but studies without biological flaws showed improvement of depression with vitamin D supplementation (42, 43). Measurement error can also explain some of the variation in results (59).

A major strength of our study is the use of a twin study design which enables us to consider the effects of genetic factors and common and unshared environments on the association (60). Our analysis demonstrated that there was no significant difference in major depression within either MZ or DZ twin pairs. This suggests that the difference found in the overall analysis, where twins were considered as separate individuals, could be explained by genetics or shared familial environmental factors. Thus, there may be unmeasured confounding familial factors which may influence the occurrence of major depression as well as the risk of vitamin D deficiency in the study population.

Limitations to our study include the cross-sectional design, which may show associations, but does not establish causations between 25-hydroxyvitamin D [25(OH)D] categories and depression. Second, our study subjects came from a twin registry of Vietnam War veterans who were mostly of middle age, white males. Therefore, we ought to be careful not to apply these results to females, individuals who are younger, or any other racial of ethnic groups. Third, we are unable to rule out bias due to residual confounding or unmeasured factors.

In summary, our findings suggest that there may be a link between vitamin D clinical category level and signs and symptoms of depression. Our study design is cross-sectional, so our results should be confirmed by randomized clinical trials to account for unmeasured potential confounders and establish an adequate vitamin D dose schedule. If confirmed, vitamin D supplementation may represent a cost-effective measure to improve depression

FUTURE DIRECTIONS

If the rate of depression continues to rise worldwide, especially in the developed countries, it will soon become one of the leading causes of global disability (61). It has been shown that current treatments for depression failed to provide the desired results (62). Moreover, side effects with antidepressant treatment are very common, which contributes to noncompliance by patients. As a result, the relapses are very common (63). A cytokine-induced inflammation was recently looked at as a precursor for development of major depression (64). Vitamin D treatment costs a lot less and does not have such severe side effects as currently used antidepressant medications. However, the molecular mechanism of vitamin D action in neural tissue as well as its effects on the immune system is poorly understood. It is worth mentioning that brain matter contains important immune mediators, such as microglia and astrocytes (64). Although vitamin D receptors (VDR) were shown to be present throughout the brain tissue, the research directed to study the interaction between VDR and glia is very limited (10-14, 64). A major need in this area is a well-designed randomized trial. This trial should test the hypothesis that high-dose vitamin D supplementation of patients with clinically diagnosed major depression is safe and better than low-dose supplementation (or placebo) with respect to its therapeutic effects. Preferably, the trial should be multicenter, double-blinded, with standardized method to measure vitamin D in patients' serum, and of sufficient size to ensure adequate power.

Vitamin D deficiency and major depression are two important conditions observed all over the world (1, 3-8). We need to acknowledge that gaps in knowledge continue to exist. It is important to pursue new knowledge to confirm the efficacy of vitamin D in treating or preventing depression, and possible underlying mechanisms, such as the role of

inflammation for the glio-pathogenesis of depression and the impact of vitamin D on potentially mitigating this process.

TABLES AND FIGURES.

Table 1. Demographic characteristics among twins by 25-hydroxivitamin D [25(OH)D] (vitamin D) status based on vitamin D Council and testing laboratories guidelines. (N=509)

	25 (OH)D status			
	Deficient <30 ng/mL (N=221)	**Insufficient/suboptimal 30-49 ng/mL (N=182)**	**Sufficient/optimal 50-150 ng/mL (N=106)**	**(MH) p-value**
Demographic characteristics				
Age (years)	55.3±2.7[1]	54.7±3.0	55.5±2.7	0.6
White race [n (%)]	208 (94.1%)[2]	179 (98.4%)	103(97.2%)	0.08
Zygosity of twins				
Monozygotic [n(%)]	144 (65.2%)	107(58.8 %)	60(56.6%)	0.1
Dizygotic [n(%)]	77 (34.8%)	75 (41.2%)	46 (43.4%)	0.1
Medical history [n (%)]				
Coronary heart disease	21(9.5%)	10(5.5%)	9(8.5%)	0.5
Hypertension	79(35.9%)	67(36.8%)	38(36.2%)	0.93
Diabetes	35(15.8%)	17(9.3%)	8(7.6%)	0.02
PTSD	31(14.0%)	24(13.2%)	15(14.2%)	0.98
Cardiovascular risk factors				
Physical activity score	7.1±2.0	7.3±1.7	7.5±1.6	0.058
Smoking [n(%)]	180(81.5%)	151(83.0%)	84(79.3%)	0.74
Alcohol consumption[3]	5.4±10.2	4.5±9.2	5.6±9.5	0.94
BMI (kg/m^2)	30.0±5.4	29.5±4.9	28.6±3.7	0.02

Current Medications [n (%)]				
Statins	61(27.6%)	49(26.9%)	22(20.8%)	0.2
Aspirin	63(28.5)	44(24.2%)	29(27.4%)	0.7
β-blockers	16 (7.2%)	13(7.1%)	10(9.4%)	0.5
Outcome				
Major depression (life time history)	66(29.9%)	41(22.5%)	21(19.8%)	0.03
BDI total score	6.7±8.1	5.6±7.6	5.4±6.8	0.11
BDI score (somatic)	4.6±5.1	3.9±4.7	3.7±4.4	0.09
BDI score (cognitive)	2.1±3.5	1.7±3.3	1.6±3.0	0.21

[1]Indicates Mean value and SD. [2] Due to missing values rows may not add up to 509. [3]Alcohol average number drinks per week.

Table 2. Logistic models for the association between major depression with vitamin D as a continuous variable.

Model	OR (per ng/mL)	95% CI[1] Lower	Upper	p-value
Unadjusted (crude estimate)	**0.74**	**0.51**	**1.10**	**0.1**
Adjusted (adjusted for age, physical activity, etoh[2], bmi[3])	**0.78**	**0.53**	**1.15**	**0.2**
Unadjusted (crude estimate) stratified by the within and between pair by zygosity				
MZ [4] BETWEEN	**0.67**	**0.36**	**1.23**	**0.2**
WITHIN	**1.65**	**0.67**	**4.07**	**0.3**
DZ[5] BETWEEN	**0.55**	**0.27**	**1.11**	**0.96**
WITHIN	**0.97**	**0.29**	**3.21**	**0.09**

[1]Confidence Interval.[2]Alcohol consumption. [3]Body Mass Index. [4]Monozygotic twin pairs. [5]Dizygotic twin pairs.

Table 3. Unadjusted logistic regression models for the association between major depression and vitamin D clinical categories.

Categories compared	OR (per ng/mL)	95% CI[1] Lower	Upper	p-value
Sufficient	**1.00**			**(ref.)**
Insufficient	**1.18**	**0.69**	**2.02**	**0.556**
Deficient	**1.72**	**1.01**	**2.95**	**0.047**
Deficient + Insufficient	**1.42**	**0.87**	**2.33**	**0.159**

Confidence Interval. Our reference group included subject with "Sufficient/optimal" level for vitamin D.

Table 4. Adjusted logistic regression models for the association between major depression and vitamin D clinical categories (adjusted for age, alcohol consumption, body mass index, and physical activity score).

Categories compared	OR (per ng/mL)	95% CI[1] Lower	Upper	p-value
Sufficient	**1.0**			**(ref.)**
Insufficient	**1.15**	**0.66**	**1.99**	**0.622**
Deficient	**1.65**	**0.95**	**2.84**	**0.075**
Deficient + Insufficient	**1.37**	**0.83**	**2.27**	**0.213**

Confidence Interval. Our reference group included subject with "Sufficient/optimal" level for vitamin D.

Figure 1. Histogram comparing lifetime depression frequency according to clinical classification of 25-hydroxivitamin D [25(OH)D] levels.

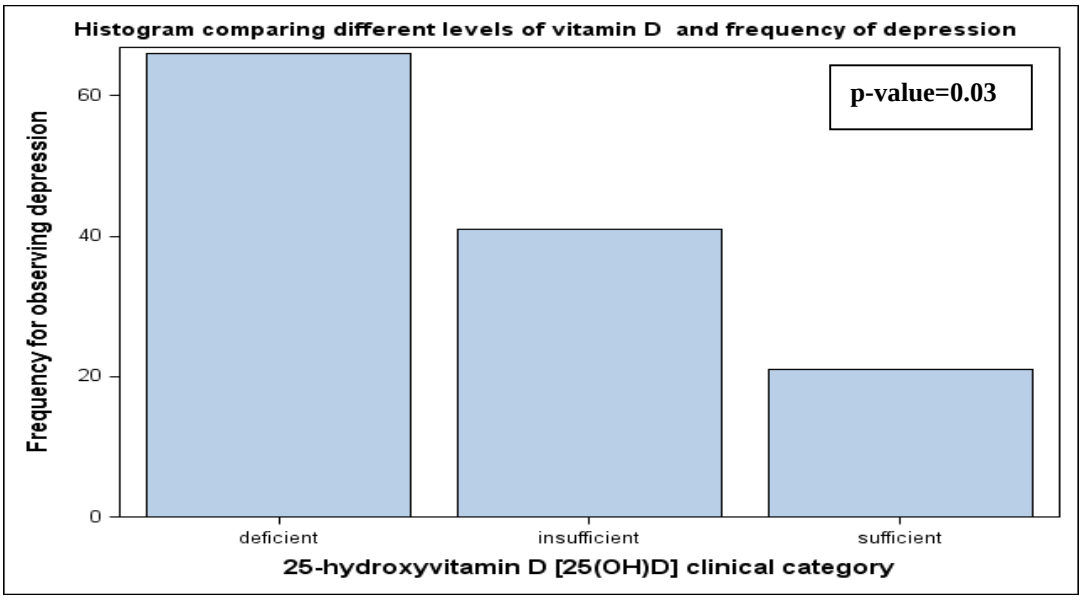
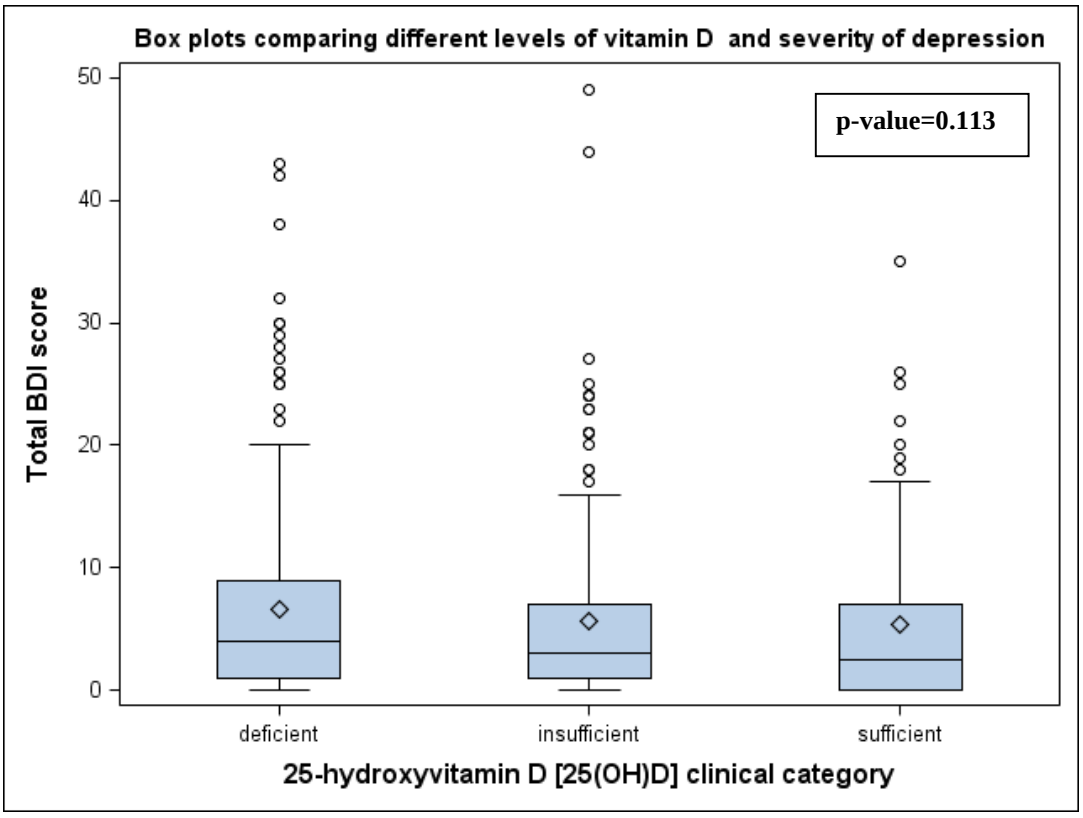


Figure 2. Box plot comparing severity of depression (total BDI score) according to clinical classification of 25-hydroxivitamin D [25(OH)D] levels.



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