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Original Article

Effects of cholecalciferol supplementation on depressive symptoms, C-peptide, serotonin, and neurotrophin-3 in type 2 diabetes mellitus: A double-blind, randomized, placebo-controlled trial

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Abstract

The coexistence of depression and type 2 diabetes mellitus (T2DM) can significantly worsen disease prognosis and lower quality of life. Emerging evidence suggests that vitamin D deficiency contributes to the progression of T2DM and is closely associated with the development of depression. The aim of this study was to investigate the effects of cholecalciferol on depression in patients with T2DM, exploring its mechanisms by analyzing its impact on C-peptide, serotonin, and neurotrophin-3 levels. A double-blind, randomized, placebo-controlled clinical trial was conducted at Cipto Mangunkusumo General Hospital, Jakarta, Indonesia, from April 2021 to September 2022. Patients with T2DM and depressive symptoms were randomly assigned to two groups: received 4000 IU of cholecalciferol daily and received a placebo for 12 weeks. Depression was assessed using the Beck Depression Inventory-II (BDI-II) before and 12 weeks after the intervention. The levels of C-peptide, serotonin, and neurotrophin-3 were measured at the end of the fourth week of intervention using the enzyme-linked immunosorbent assay (ELISA) method. Between-group comparisons were made using independent Student t-tests and Mann-Whitney U tests. Paired Student t-tests or Wilcoxon tests were applied for within-group comparisons between pre- and post-intervention. A total of 70 T2DM patients with depression were included in this study, comprising 38 patients in the cholecalciferol group and 32 in the placebo group. C-peptide levels increased significantly in the cholecalciferol group compared to the placebo group (p=0.006). No significant differences were observed in serotonin and NT-3 levels between the cholecalciferol group compared to the placebo group. The cholecalciferol group had a significantly greater reduction in BDI-II scores compared to the placebo group (p<0.001). This trial highlights that taking cholecalciferol might help ease mild to moderate depression symptoms in patients with T2DM by enhancing c-peptide levels, though its effects on serotonin and neurotrophin-3 are still unclear.

Keywords: Type 2 diabetes mellitus, depression, serotonin, vitamin D, neurotrophin-3

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Introduction

Depression and type 2 diabetes mellitus (T2DM) represent significant global public health challenges, with their coexistence exacerbating the impact of both conditions. Comorbid depression in individuals with T2DM complicates clinical management, leading to poorer glycemic control, increased complications, non-compliance with treatment, and a decline in quality of life [1,2]. The financial burden of care for those with T2DM and concurrent depression is notably higher compared to individuals with T2DM alone [3,4]. This bidirectional relationship not only positions T2DM as a risk factor for depression but also reveals that individuals with T2DM face an elevated risk of experiencing depressive symptoms [5,6]. The underlying causes of depression are multifactorial, involving genetic predisposition, dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, deficiencies in monoamines, and alterations in various brain regions [7]. The incidence of depression among individuals with T2DM is reported to be twice that of the general population, with one in four individuals with T2DM experiencing depression [8]. The global prevalence of depression in T2DM population ranges from 18% to 25% [9].

Recent studies have shown that vitamin D insufficiency has been linked to reduced neuroplasticity, potentially hindering recovery in those suffering from depression [10,11]. This vitamin is crucial for mood regulation, influencing serotonin synthesis, a neurotransmitter that significantly impacts emotional well-being. A previous study has demonstrated a clear connection between low vitamin D levels and increased vulnerability to depressive symptoms, underscoring the importance of addressing vitamin D deficiency in this population [12]. Additionally, vitamin D plays a vital role in metabolic processes, including insulin production and glucose metabolism, with low levels being associated with an increased risk of metabolic diseases such as diabetes and obesity [13]. Improving vitamin D status may thus yield benefits for both mental and metabolic health, thereby reducing the risk of related disorders [14].

In recent years, alternative treatments for depression beyond conventional antidepressants have garnered attention, with vitamin D emerging as a potential therapeutic option. The pathophysiology of depression has been associated with vitamin D receptors located in various brain regions, including the cingulate cortex and hippocampus [15]. Notably, reduced levels of neurotrophin-3 (NT-3)—a protein critical for neuron growth and survival—have been correlated with low vitamin D levels [10]. NT-3 is integral to neurobiological processes underlying mood and anxiety disorders, particularly in neurogenesis [16]. Post-mortem studies have shown decreased NT-3 levels in the parietal cortex of depressed patients, with peripheral NT-3 levels also diminished in those with depression [17,18]. In addition, serotonin (5-hydroxytryptamine, 5-HT) is another key player in mood regulation, influencing various physiological functions, including mood, sleep, appetite, and energy expenditure [19]. In peripheral tissues, serotonin enhances insulin secretion; thus, decreases in extracellular serotonin can lead to fluctuations in insulin production. A study has demonstrated that serotonin metabolism is impaired in individuals with depression and in those who succumb to depression-related causes [20]. Additionally, C-peptide serves as a marker for pancreatic islet cell function, providing insights into endogenous insulin deficiency [21]. Elevated C-peptide levels two hours postprandial correlate with improved outcomes in T2DM, while lower levels are linked to increased depressive symptoms [22]. Fluctuations in blood glucose, resulting from low C-peptide levels, can provoke anxiety and exacerbate depressive symptoms [23].

Given its accessibility and low cost, vitamin D supplementation presents a promising avenue for alleviating depressive symptoms in individuals with T2DM. While current studies remain inconclusive, there is potential for vitamin D to positively influence depression in the T2DM population. The aim of this study was to investigate the effects of cholecalciferol (vitamin D) on depression in patients with T2DM, exploring its mechanisms by analyzing its impact on C-peptide, serotonin, and NT-3 levels.

Methods

Study design and setting

A double-blind, randomized, placebo-controlled clinical trial was conducted at Cipto Mangunkusumo General Hospital, Jakarta, Indonesia, from April 2021 to September 2022. The trial was registered on ClinicalTrials.gov (NCT04917458). The report was prepared following the CONSORT checklist guidelines [24]. The study was conducted on T2DM patients with depressive symptoms recruited using consecutive sampling techniques. The study aimed to evaluate the effects of cholecalciferol administration compared to placebo on depressive symptoms assessed using the Beck Depression Inventory-II (BDI-II) scores as the primary outcome and on NT-3, serotonin, and C-peptide as secondary outcomes.

Patients

This study included patients with T2DM and depression. The diagnosis of T2DM was based on the American Diabetes Association's guidelines [25], with patients having previously been diagnosed by an endocrinologist through fasting blood glucose (FBG) levels exceeding 126 mg/dL. Depression severity, ranging from mild to moderate, was assessed using the BDI-II scale [26]. Additional inclusion criteria required that patients had not used vitamin D supplements for at least three months before and throughout the trial period. Patients with pre-existing conditions that could affect calcium or vitamin D metabolism (such as liver, kidney, skin, or nervous system diseases) were excluded, as well as those on high doses of steroids or immunomodulators. Pregnant or breastfeeding women and patients receiving antidepressant medications were also excluded from the study. Loss to follow-up patients were those who were unable to complete the study due to unwillingness or side effects of the intervention.

Written informed consent was obtained from all patients who agreed to participate in the clinical trial. Patients continued receiving standard treatment during the study. Weekly check-ins were conducted to ensure adherence to the treatment protocol and address any concerns or complaints.

Sample size and randomization

A formula to calculate the minimum sample size for a randomized control trial [27] was followed. Considering a 20% dropout rate, the minimum sample size was determined to be 76 patients across the two groups (cholecalciferol and placebo). Randomization was conducted digitally using a block-permuted randomization technique with block sizes of 4 and 6. To maintain the integrity of the treatment sequence, the randomization intervention code was concealed in a sealed envelope. The Department of Pharmacy of Cipto Mangunkusumo General Hospital, Jakarta, Indonesia, held the code for the type of therapy (either cholecalciferol or placebo) and was responsible for preparing both the cholecalciferol and placebo drugs used in this study, thereby ensuring a double-blind design. Consequently, neither the investigators, the attending physicians, nor the patients were aware of the type of therapy administered.

Baseline assessment

To ensure that patients with T2DM and depression did not differ significantly between the cholecalciferol and placebo groups, baseline characteristics were assessed. Sociodemographic data and anthropometric measurements were collected. Information regarding sex, age, and marital status was primarily obtained from the National Identity Card. Anthropometric measurements (body weight, height, and body mass index) were performed using standardized procedures and calibrated equipment. To ensure accuracy, duplicate measurements were taken, and in cases of discrepancies, additional measurements were conducted until a precise value was obtained. Patients self-reported their highest educational attainment, the type of anti-diabetic regimen, and the duration since their T2DM diagnosis. Blood pressure (BP), including diastolic and systolic values (DBP and SBP, respectively), was measured using a standard sphygmomanometer while the patients were seated.

Additionally, triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels were assessed using an autoanalyzer. Vitamin D intake, vitamin D status, and the conversion of daily sun exposure to vitamin D were calculated based on a subjective

questionnaire. Ultraviolet (UV) dose and vitamin D production were estimated using Holick's rule, which states that sun exposure of one-quarter of the minimum erythemal dose (MED) on one-quarter of the body area is equivalent to 1000 IU of oral vitamin D3 [28,29]. To measure this, data were collected on the duration and time of sun exposure, specifically asking patients about their average time spent outdoors. The percentage of body area exposed to sunlight was determined based on the Lund Browder criteria (face: 9%, hands: 6%, forearms: 6%, feet: 3%, and face, hands, forearms, and feet: 24%) [30,31]. The standard erythemal dose (SED) was calculated using the formula 0.9×UV index, with Indonesia's UV index considered to be 6 [32-34]. Thus, MED was calculated as 2× SED [35]. Vitamin D dosage was derived from Holick's rule: ¼×MED×body area exposed, which translates to ¼×1000 [36,37].

A food frequency questionnaire was utilized to measure daily vitamin D intake, asking patients to report how often they consumed specific foods divided into four categories (never, once/month, twice/month, three times/month, and more than four times/month). Foods high in vitamin D, such as dairy products and fish, as well as fortified foods like juices and cereals, received special attention [38,39]. Finally, BDI-II scores and categorical statuses were assessed by a certified internist specializing in psychosomatic and palliative medicine.

Intervention

Two groups of patients were assigned: one group received 4000 IU of cholecalciferol daily, while the other group was given a placebo, with the placebo capsules composed of a lactose-based shell. The size, color, and shape of the cholecalciferol and placebo capsules were identical to maintain blinding. The intervention was conducted over 12 weeks. Throughout the trial, patients continued their regular anti-diabetic medications and were advised to adhere to their usual diets and exercise routines.

Outcomes and measurements

The primary outcome, depression, was assessed using the BDI-II scale after the 12-week intervention. The BDI-II score was derived from responses to 21 items, each rated on a four-point Likert scale (0–3). Depression levels were categorized as mild (scores 14–19), moderate (scores 20–28), and severe (scores 29–63) [26].

Blood samples were collected from the median cubital vein before the intervention started and at the end of the fourth week of intervention to evaluate the secondary outcomes (NT-3, serotonin, and C-peptide). The Samples were drawn into tubes containing potassium ethylenediaminetetraacetic acid (EDTA) and analyzed using ELISA kits (all from Elabscience, Wuhan, China). For the C-peptide analysis, 100 µL of blood was used (Human CP(C-Peptide) ELISA Kit, E-EL-H6020, Elabscience), while serotonin required 50 µL (ST/5-HT(Serotonin/5-Hydroxytryptamine) ELISA Kit, E-EL-0033, Elabscience), and NT-3 required 100 µL (Human NT-3(Neurotrophin 3) ELISA Kit, E-EL-H1896, Elabscience).

Serum 25-hydroxyvitamin D (25-OH vitamin D) levels were measured using an ELISA kit (25-OH Vitamin D Total ELISA Kit, DS167701, Diasino, Zhengzhou, China). Vitamin D status was categorized into three groups: sufficient (≥30 ng/mL), insufficient (20–29 ng/mL), and deficient (<20 ng/mL) [40].

Statistical analyses

The normality of the parameters was evaluated using the Kolmogorov-Smirnov test. Demographic characteristics were summarized through descriptive statistics, with mean and standard deviation (SD) reported for continuous variables, and frequencies and percentages for categorical variables. Anthropometric, clinical, and biochemical factors between the two groups were compared using the Student independent t-test or the Mann-Whitney test for the continuous variables and the Chi-squared test for categorical variables. Additionally, paired student t-test or Wilcoxon test were used, as appropriate, to compare values before and after the intervention. Statistical significance was considered at a *p*-value of less than 0.05. All analyses were performed using SPSS software (IBM SPSS Corp., Chicago, USA) version 29.0.2 for MacOS.

Results

Characteristics of the patients

Out of the 118 patients who provided consent, 41 of them were excluded due to various factors such as severe depression, prior vitamin D supplementation, kidney or liver issues, or neurological disorders. Consequently, 77 eligible patients were included in the study. These patients were divided into two groups, including 40 who received cholecalciferol, while 37 were given a placebo. Seven patients did not complete the study and were excluded from the final analysis. One patient from the placebo group complained of nausea, leading to the discontinuation of treatment. The other six patients, four patients from placebo groups and two patients from the cholecalciferol group, were unwilling to continue the trial due to personal or family reasons. The detailed flow diagram of each step from recruitment to analysis is presented in **Figure 1**.

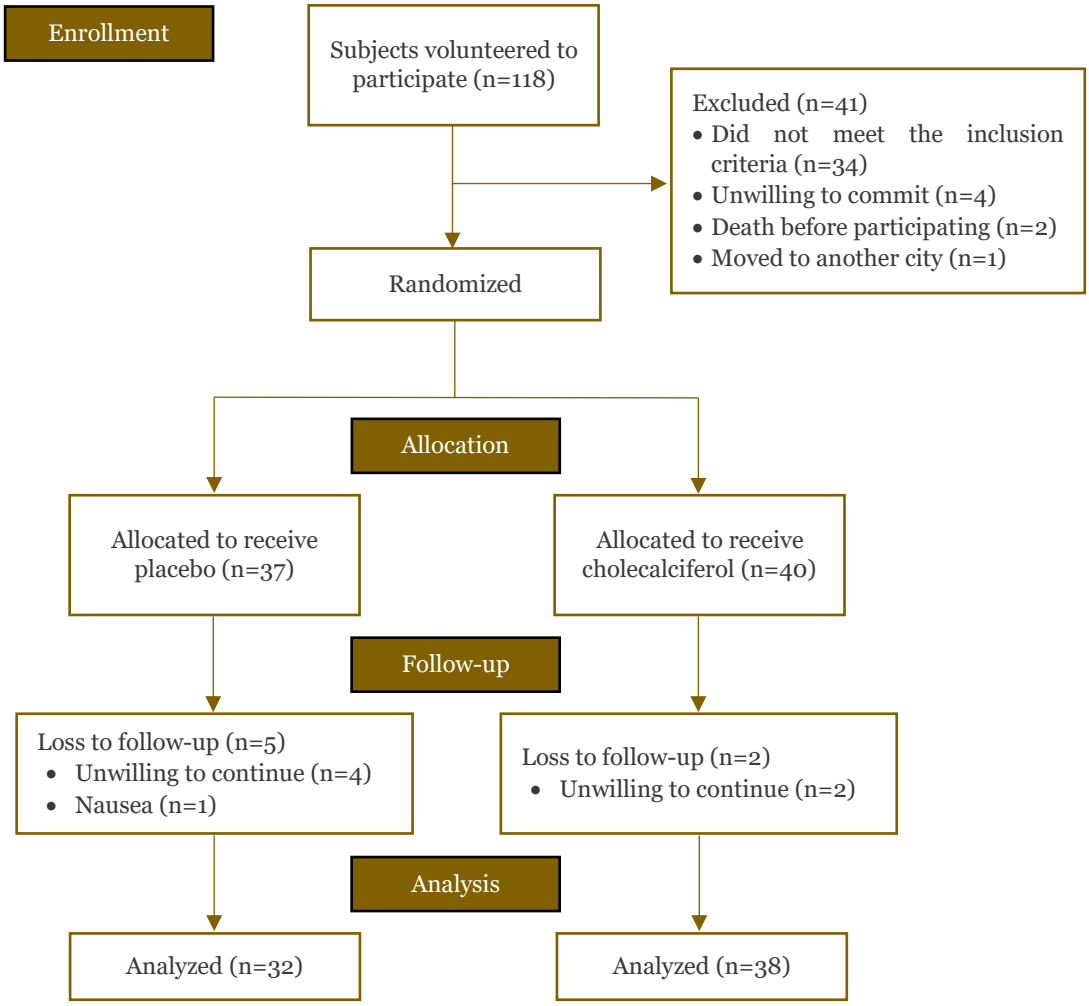

Figure 1. Consort flow diagram of the clinical trial.

A total of 70 T2DM patients with depression were included in this study, as presented in **Table 1**. Most patients were female, with an average age of 54 years in both groups. The mean BMI for both groups was 26 kg/m^2. More than half of the patients in each group were on a combination regimen of insulin injections and oral antidiabetic medications. Overall, most patients had T2DM for more than five years. According to the BDI-II status, the majority fell into the mild depression category, with 78.9% in the cholecalciferol group and 68.8% in the placebo group, resulting in average scores of 16.79±2.56 and 17.84±3.27, respectively. Regarding vitamin D status, most patients were classified as insufficient, with 63.2% in the cholecalciferol group and 65.6% in the placebo group, reporting average vitamin intakes of 62.07±61.68 IU and 56.03±56.48 IU, respectively. Based on the conversion of sun exposure to vitamin D, the

cholecalciferol group had a mean level of 2114.53±705.59 IU, while the placebo group had 2057.06±673.95 IU. There were no significant differences in patient characteristics between the cholecalciferol and placebo groups (**Table 1**). Additionally, there were also no significant differences in clinical and laboratory parameters across the groups (**Table 2**).

Table 1. Characteristics of type 2 diabetes mellitus patients with depression included in the study (n=70)

Characteristics	Frequency (%)		*p*-value
	Cholecalciferol (n=38)	Placebo (n=32)	
Sex			
Male	8 (21.1)	6 (18.75)	0.810[a]
Female	30 (78.9)	26 (81.25)	
Age (years), mean±SD	54.47±8.70	54.03±8.91	0.835[b]
Educational level			
Not educated	1 (2.6)	0 (0.0)	0.491[a]
Elementary school	2 (5.3)	4 (12.5)	
Primary school	3 (7.9)	1 (3.1)	
Senior high school	18 (47.4)	12 (37.5)	
University	14 (36.8)	15 (46.9)	
Marital status			
Not married	4 (10.5)	5 (15.6)	0.291[a]
Married	27 (71.1)	25 (78.1)	
Divorce	7 (18.4)	2 (6.3)	
Body weight (kg), mean±SD	64.08 (9.64)	65.80 (13.42)	0.548[b]
Body height (cm), mean±SD	155.11 (8.20)	157.09 (7.12)	0.287[b]
Body mass index (kg/m^2), mean±SD	26.655 (3.58)	26.652 (5.10)	0.998[b]
Anti-diabetic regimen			
Oral only	16 (42.1)	12 (37.5)	0.279[a]
Insulin oral	1 (2.6)	4 (12.5)	
Combination	21 (55.3)	16 (50.0)	
Duration of T2DM (years)			
<5	15 (39.5)	10 (31.3)	0.571[a]
5–10	12 (31.6)	14 (43.8)	
>10	11 (28.9)	8 (25.0)	
BDI-II scores, mean±SD	16.79±2.56	17.84±3.27	0.208[c]
BDI-II status			
14–19 (mild)	30 (78.9)	22 (68.8)	0.311[a]
20–28 (moderate)	8 (21.1)	10 (32.0)	
Vitamin D intake (IU), mean±SD	62.07±61.68	56.03±56.48	0.663[c]
Vitamin D status			
Normal	0 (0.0)	0 (0.0)	0.830[a]
Insufficiency	24 (63.2)	21 (65.6)	
Deficiency	14 (36.8)	11 (34.4)	
Conversion of sun exposure to vitamin D (IU), mean±SD	2114.53±705.59	2057.06±673.95	0.682[c]

BDI-II: Beck Depression Inventory-II
[a] Analyzed with Chi-squared test
[b] Analyzed with independent Student t-test
[c] Analyzed with Mann-Whitney test

Table 2. Baseline clinical and laboratory levels of type 2 diabetes mellitus patients with depression included in the study (n=70)

Parameters	Mean±SD		*p*-value
	Cholecalciferol (n=38)	Placebo (n=32)	
Clinical parameters			
Systolic blood pressure (mmHg)	128.03±15.42	130.63±18.33	0.399[a]
Diastolic blood pressure (mmHg)	73.29±9.69	75.09±13.07	0.510[b]
Laboratory			
Fasting blood glucose (mg/dL)	153.55±60.56	150.78±58.23	0.907[b]
HbA1c (%)	8.295±2.24	8.244±1.97	0.921[b]
Triglyceride (mg/dL)	170.158±107.47	150.406±67.72	0.686[b]
High-density lipoprotein (mg/dL)	48.684±11.19	47.188±8.76	0.541[b]
Low-density lipoprotein (mg/dL)	111.053±35.75	114.906±26.50	0.616[b]
Total cholesterol (mg/dL)	193.79±41.73	193.34±34.39	0.962[b]
25(OH)D level serum (ng/mL)	12.585±9.16	14.251±8.11	0.427[b]

[a] Analyzed with Mann-Whitney test
[b] Analyzed with independent Student t-test

Comparison of C-peptide, serotonin, and neurotrophin-3 levels

There was no significant difference in the mean levels of C-peptide, serotonin, and NT-3 between the two groups prior to the intervention, indicating comparable baseline levels (**Table 3**). However, significant changes were observed before and after the intervention (**Table 3**). In the cholecalciferol group, the mean concentration of C-peptide increased significantly (123.14±66.66 vs 184.76±119.63 pg/mL; p=0.006). Additionally, notable changes in NT-3 levels were recorded in both groups, with the cholecalciferol and placebo groups exhibiting a significant increase (p<0.001 and p=0.047, respectively). Although the mean change in NT-3 (delta NT-3) for the cholecalciferol group was greater than that of the placebo group, the differences in changes between the cholecalciferol and control groups were not statistically significant (p=0.111) (**Table 3**).

Table 3. Comparison of C-peptide, serotonin, and neurotrophin-3 concentrations before and after intervention among groups

Variables	Mean±SD		p-value
	Cholecalciferol (n=38)	Placebo (n=32)	
C-peptide (pg/mL)			
Baseline	123.14±66.66	136.85±98.74	0.930[a]
After 4 weeks	184.76±119.63	129.67±97.08	0.028[b]
Delta	61.61±129.71	-7.18±96.92	0.006[a]
p-value	0.006[c]	0.695[d]	
Serotonin (ng/mL)			
Baseline	414.21±407.21	513.25±575.24	0.525[b]
After 4 weeks	312.19±250.83	488.68±518.70	0.211[b]
Delta	-102.02±428.58	-24.57±501.27	0.540[a]
p-value	0.133[d]	0.588[d]	
Neurotrophin-3 (pg/mL)			
Baseline	434.14±247.65	415.77±246.73	0.724[a]
After 4 weeks	722.22±531.72	540.31±262.17	0.259[b]
Delta	288.12±470.82	88.54±242.47	0.111[a]
p-value	<0.001[d]	0.047[d]	

[a] Analyzed with Mann-Whitney test
[b] Analyzed with Student independent t-test
[c] Analyzed with Student paired t-test
[d] Analyzed with Wilcoxon test

Comparison of depression level before and after intervention

The mean BDI-II scores in the cholecalciferol and placebo groups did not differ significantly before the intervention (p=0.208). However, after 12 weeks of intervention, a significant difference in BDI-II scores was observed between the two groups (p<0.001) (**Table 4**). The mean BDI-II scores were reduced after the intervention within each group, with p<0.001 for both groups. The mean decrease (delta) in the BDI-II score for the cholecalciferol group was -10.053, while the placebo group experienced a decrease of -5.656 (**Table 4**). The reduction of BDI-II scores in the cholecalciferol group was statistically significantly greater than that in the placebo group (p<0.001) (**Table 4**). Changes in the BDI-II scores of the patients between pre- and post-12-week intervention are presented in **Figure 2**.

Table 4. Comparison of depression levels based on Beck Depression Inventory-II (BDI-II) scores before and after intervention between groups

Variables	Mean±SD		p-value
	Cholecalciferol (n=38)	Placebo (n=32)	
BDI-II scores			
Baseline	16.79±2.56	17.84±3.27	0.208[a]
After 12 weeks	6.74±4.63	12.19±5.01	<0.001[b]
Delta	-10.05±4.98	-5.66±5.03	<0.001[b]
p-value	<0.001[c]	<0.001[d]	

[a] Analyzed with Mann-Whitney test
[b] Analyzed with Student independent t-test
[c] Analyzed with Wilcoxon test
[d] Analyzed with Student paired t-test

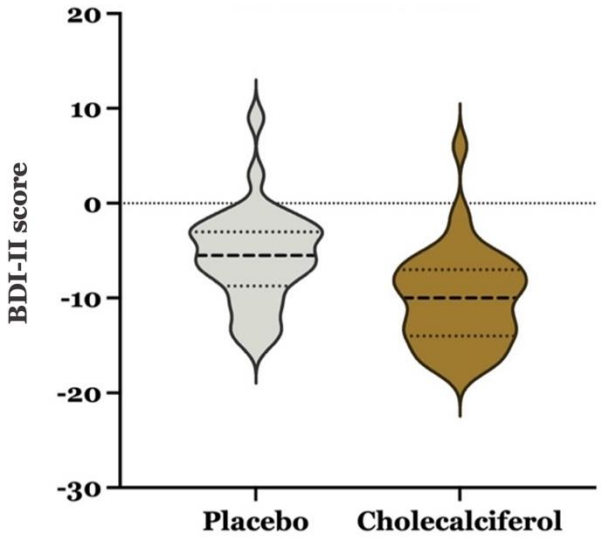

Figure 2. Violin plot showing the delta score of Beck Depression Inventory-II (BDI-II) of the placebo and cholecalciferol group.

Discussion

Depression in patients with T2DM warrants special attention due to its potential to diminish adherence to therapy. This decline in adherence can lead to poorer blood glucose control, an increased risk of complications, heightened disability, reduced productivity, elevated therapy costs, and a decreased quality of life [41-43]. Furthermore, previous studies have demonstrated that the coexistence of depression and T2DM elevates the risk of morbidity and mortality, adversely affecting not only patients but also their families and communities [44-47]. This study findings revealed that cholecalciferol significantly reduced BDI-II scores after 12 weeks of treatment and increased C-peptide and NT-3 levels after 4 weeks.

This study revealed that cholecalciferol was able to significantly increase the mean C-peptide level from 123.14 pg/mL at admission to 184.76 pg/mL after 4 weeks of intervention. Our findings align with a previous study in overweight African American patients [48]. However, they contradict earlier studies involving pre-diabetic patients [49-51]. As a vitamin D supplement, cholecalciferol has been shown to enhance insulin sensitivity and promote pancreatic beta cell health [52,53], resulting in improved insulin secretion and a corresponding increase in c-peptide levels [38,54,55]. Furthermore, cholecalciferol's anti-inflammatory properties contribute to the reduction of chronic inflammation associated with insulin resistance, thereby further enhancing metabolic function and insulin secretion [56].

On the other hand, the cholecalciferol intervention over four weeks did not significantly affect the mean serotonin level, which changed from 414.21 ng/mL to 312.19 ng/mL. In this study, the impact of vitamin D on serotonin levels was not significantly different. A study using real-time quantitative polymerase chain reaction (PCR), showed that TPH-1 and TPH-2 mRNA were induced 28- to 33-fold by treatment with 10 nM vitamin D 1.25 in cultured rat serotonergic nerve cells (RN46A-B14), and the increase in TPH-2 mRNA was dependent on the degree of neuron-like characteristics of the cells [57]. However, studies in humans, both in the general population and in those with irritable bowel syndrome, did not show significant differences, similar to our study [58,59]. To the best of our knowledge, this study is the first to examine the effect of cholecalciferol on plasma serotonin in a population with T2DM.

The four-week intervention of either cholecalciferol or placebo in this study demonstrated a significant difference in the mean NT-3 levels (p<0.001 and p=0.043). This finding may be attributed to both groups receiving anti-diabetic drug therapy, which is known to enhance the expression of neurotrophic factors, including NT-3, through the activation of AMP-activated Protein Kinase (AMPK) and subsequent signaling pathways that contribute to neuroprotection and neuronal health [60,61]. Although the comparison of the mean delta NT-3 between the two groups was not statistically significant (p=0.111), the observed mean delta values indicated that

cholecalciferol caused a greater increase in NT-3 levels compared to the placebo group. Cholecalciferol is converted into calcitriol, which binds to the vitamin D receptor (VDR) in various tissues, including the brain [62,63]. Cholecalciferol is converted into calcitriol and binds to the vitamin D receptor (VDR) in many sites, including the brain [64]. The VDR-calcitriol complex then translocates to the nucleus, where it influences neuronal signaling and promotes NT-3 synthesis. Overall, cholecalciferol plays a crucial role in maintaining neural health by supporting neuronal survival and neuroprotection [65,66].

Depression frequently coexists with T2DM, leading to a high prevalence of antidepressant use among these patients [67,68]. One of the significant side effects of antidepressants is weight gain, which can contribute to insulin resistance [69,70]. A cohort study conducted in Japan involving 90,530 subjects, found that antidepressant use was associated with an increased risk of developing T2Dm [69]. While antidepressants may alleviate depressive symptoms, they can also raise blood glucose levels and body weight [71-74]. Therefore, there is a need for adjuvant therapy for patients with both T2DM and depression. Additionally, a cross-sectional study reported a relationship between vitamin D deficiency and depression [75]. Previous studies have indicated that vitamin D may have antidepressant effects [76-79]. Several other studies also suggest that vitamin D supplementation can improve both physical and mental symptoms in T2DM patients [74,80-85].

In this study, both the cholecalciferol and placebo groups demonstrated a significant decrease ($p<0.001$ for both). This outcome may be attributed to the anti-diabetic drug therapy received by both groups, considering the bidirectional correlation between depression and T2DM. Anti-diabetic medications can improve depression through several interconnected pathways. For instance, enhanced insulin sensitivity may mitigate the effects of insulin resistance, which is known to cause inflammation and depressive symptoms [86-88]. Additionally, the glucagon-like peptide-1 (GLP-1) agonist drug class promotes neurogenesis and synaptic plasticity, thereby supporting brain health and cognitive function, which may alleviate depressive symptoms [89-95]. Insulin therapy also helps maintain stable blood glucose levels, preventing mood swings and irritability associated with glucose fluctuations [90,96,97]. Furthermore, anti-diabetic medications that promote weight loss, such as GLP-1 receptor agonists, can enhance self-esteem and reduce body image-related depression [98-100]. Lastly, some anti-diabetic drugs influence neurotransmitter systems, particularly dopamine, which plays a crucial role in mood regulation [101-103].

The comparison of mean delta BDI-II scores between the two groups revealed that the BDI-II scores in the cholecalciferol group decreased significantly more than in the placebo group ($p<0.001$). This finding aligns with a previous study that demonstrated a significant improvement in depression scores following three months of vitamin D supplementation intervention [14]. Another study reported improvements in depression and anxiety symptoms among women with T2DM and vitamin D deficiency after receiving injections of 50,000 IU of vitamin D3 every two weeks for sixteen weeks [104]. Several studies have also indicated that vitamin D can serve as an effective adjuvant therapy for treating depression in patients with major depressive disorder [76,78,79,105,106]. The use of cholecalciferol in this study, along with previous studies, yielded positive results [81,107]; in contrast, another study utilized alfacalcidol [108]. Cholecalciferol is widely recognized for its efficacy in treating various conditions associated with primary or secondary vitamin D deficiency. Moreover, it is cost-effective, has a favorable safety profile, and poses fewer risks compared to its analogs and metabolites [109].

The mechanisms underlying vitamin D's effectiveness in improving depression remain incompletely understood. However, it is well-established that vitamin D receptors are present in various regions of the brain and that vitamin D can cross the blood-brain barrier. Furthermore, vitamin D plays a crucial role in regulating serotonin production, a key neurotransmitter implicated in depression [110]. Studies have also demonstrated that NT-3 can modulate serotonin levels and activity in the brain; simultaneously targeting both pathways may enhance treatment outcomes in managing depression [111,112].

During this clinical trial, one participant in the placebo group complained of nausea and vomiting then decided to stop taking medication. However, it does not call for further attention subsequently. Numerous studies demonstrate that hypercalciuria is a common adverse

consequence of high-dose vitamin D treatment [113-116]. The 4000 IU dose employed in this study was found to be quite safe in several other investigations [117].

This study has several limitations. First, vitamin D intake and sun exposure were assessed using subjective questionnaires, which means the data obtained were only estimates. Second, post-intervention vitamin D levels were not evaluated, preventing an assessment of whether they fell within normal limits. Third, in exploring the etiology of depression, not all relevant parameters could be studied, including inflammation, genetics, neuroplasticity, gut microbiota patterns, the HPA axis, tryptophan hydroxylase 1 (TPH1) and TPH2, as well as habits and psychosocial stressors. Fourth, the ongoing use of anti-diabetic medication may have had mixed effects on some parameters.

Conclusion

In summary, this clinical trial concluded that cholecalciferol supplementation is beneficial for alleviating mild to moderate depression symptoms in patients with T2DM. After 12 weeks of treatment, significant reductions in BDI-II scores were observed, along with notable improvements in C-peptide and NT-3 levels after 4 weeks. However, the impact on serotonin levels was not significant. Further exploration is needed to elucidate the mechanisms or pathways through which cholecalciferol intervention may improve depression.

Ethics approval

Written informed consent was signed by each patient. Following clearance from the Universitas Indonesia Ethics Committee (KET-464/UN2.F1/ETIK/PPM.0002/2020).

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Competing interests

The authors declare that there is no conflict of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

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References

1. Bogner HR, Mcclintock HFDV. Costs of coexisting depression and diabetes. J Gen Intern Med 2016;31:594-595.
2. Nanayakkara N, Pease A, Ranasinha S, *et al.* Depression and diabetes distress in adults with type 2 diabetes: Results from the Australian National Diabetes Audit (ANDA) 2016. Sci Rep 2018;8(1):7846.
3. Brüne M, Linnenkamp U, Andrich S, *et al.* Health care use and costs in individuals with diabetes with and without comorbid depression in Germany: Results of the cross-sectional DiaDec study. Diabetes Care 2021;44(2):407-415.

4. Bogner HR, McClintock HF de V. Costs of coexisting depression and diabetes. J Gen Intern Med 2016;31(6):594.
5. Abuhegazy H, Mujairi A, Banah F, *et al.* Depression and associated risk factors among type 2 diabetic patients: A cross sectional study on a convenience sample from the diabetic center, Khamis Mushait; Saudi Arabia. Neuropsychiatr Dis Treat 2022;18:1975-1984.
6. Dziedzic B, Sienkiewicz Z, Leńczuk-Gruba A, *et al.* Prevalence of depressive symptoms in the elderly population diagnosed with type 2 diabetes mellitus. Int J Environ Res Public Health 2020;17(10):3553.
7. Dean J, Keshavan M. The neurobiology of depression: An integrated view. Asian J Psychiatr 2017;27:101-111.
8. Strothers HS. Depression in the primary care setting. N Engl J Med 2019;380(6):559-568.
9. Darwish L, Beroncal E, Sison MV, *et al.* Depression in people with type 2 diabetes: Current perspectives. Diabetes Metab Syndr Obes 2018;11:333-343.
10. Sheldrick A, Camara S, Ilieva M, *et al.* Brain-derived neurotrophic factor (BDNF) and neurotrophin 3 (NT3) levels in post-mortem brain tissue from patients with depression compared to healthy individuals – a proof of concept study. Eur Psychiatry 2017;46:65-71.
11. Jiang C, Stephen R. Salton. The role of neurotrophins in major depressive disorder. Transl Neurosci 2013;4(1):46-58.
12. Berridge MJ. Vitamin D and depression: Cellular and regulatory mechanisms. Pharmacol Rev 2017;69(2):80-92.
13. Wimalawansa SJ. Associations of vitamin D with insulin resistance, obesity, type 2 diabetes, and metabolic syndrome. J Steroid Biochem Mol Biol 2018;175:177-189.
14. Omidian M, Mahmoudi M, Abshirini M, *et al.* Effects of vitamin D supplementation on depressive symptoms in type 2 diabetes mellitus patients: Randomized placebo-controlled double-blind clinical trial. Diabetes Metab Syndr 2019;13(4):2375-2380.
15. Alghamdi S, Alsulami N, Khoja S, *et al.* Vitamin D supplementation ameliorates severity of major depressive disorder. J Mol Neurosci 2020;70(2):230-235.
16. Shimazu K, Zhao M, Sakata K, *et al.* NT-3 facilitates hippocampal plasticity and learning and memory by regulating neurogenesis. Learn Mem 2006;13(3):307-315.
17. Sheldrick A, Camara S, Ilieva M, *et al.* Brain-derived neurotrophic factor (BDNF) and neurotrophin 3 (NT3) levels in post-mortem brain tissue from patients with depression compared to healthy individuals - a proof of concept study. Eur Psychiatry 2017;46:65-71.
18. Ogłodek EA, Just MJ, Szromek AR, *et al.* Melatonin and neurotrophins NT-3, BDNF, NGF in patients with varying levels of depression severity. Pharmacol Rep 2016;68(5):945-951.
19. Choi W, Moon JH, Kim H. Serotonergic regulation of energy metabolism in peripheral tissues. J Endocrinol 2020;245(1):R1-R10.
20. Esparza RP. Pharmacological treatment of depression: Current and future directions. Rev Fac Med 2017;60(5):7-16.
21. Jones AG, Hattersley AT. The clinical utility of C-peptide measurement in the care of patients with diabetes. Diabet Med 2013;30(7):803-817.
22. Sang YM, Wang LJ, Mao HX, *et al.* Correlation of lower 2 h C-peptide and elevated evening cortisol with high levels of depression in type 2 diabetes mellitus. BMC Psychiatry 2020;20(1):490.
23. Takekawa D, Kudo T, Saito J, *et al.* Higher plasma leptin and lower C-peptide levels are associated with depression: A cross-sectional study. J Affect Disord 2019;243:70-74.
24. Schultz KF, Altman DG, Moher D. CONSORT 2010 Statement: Updated guidelines for reporting parallel group randomised trials. BMC Med 2010;8(18):1-9.
25. Committee ADAPP, ElSayed NA, Aleppo G, *et al.* 2. Diagnosis and classification of diabetes: Standards of care in diabetes—2024. Diabetes Care 2024;47 Suppl 1:S20-S42.
26. Ginting H, Näring G, Van Der Veld WM, *et al.* Validating the beck depression inventory-II in Indonesia's general population and coronary heart disease patients. Int J Clin Health Psychol 2013;13(3):235-242.
27. Zhong B. How to calculate sample size in randomized controlled trial? J Thorac Dis 2009;1(1):51.
28. Dowdy JC, Sayre RM, Holick MF. Holick's rule and vitamin D from sunlight. J Steroid Biochem Mol Biol 2010;121(1-2):328-330.
29. Holick MF, Jenkins M. The UV advantage. California: iBooks; 2005.
30. Murari A, Singh KN. Lund and Browder chart-modified versus original: A comparative study. Acute Crit Care 2019;34(4):276-281.
31. Lund CC, Browder NC. The estimation of areas of burn. Surg Gynecol Obstet 1944:79;352.

32. Schmalwieser AW, Lohr MA, Daly SM, *et al.* Modeling acute and cumulative erythemal sun exposure on vulnerable body sites during beach vacations utilizing behavior-encoded 3D body models. Photochem Photobiol Sci 2023;22(1):1-20.
33. Buxton LS, Reeder AI, Marsh L, *et al.* Erythemal ultraviolet radiation exposure of high school rowers in Aotearoa/New Zealand. J Photochem Photobiol B 2021;222:112254.
34. Stalgis-Bilinski KL, Boyages J, Salisbury EL, *et al.* Burning daylight: Balancing vitamin D requirements with sensible sun exposure. Med J Aust 2011;194(7):345-348.
35. Setiati S. Vitamin D status among Indonesian elderly women living in institutionalized care units. Acta Med Indones 2008;40(2):78-83.
36. Dowdy JC, Sayre RM, Holick MF. Holick's rule and vitamin D from sunlight. J Steroid Biochem Mol Biol 2010;121(1-2):328-330.
37. Bogen S, Legenbauer T, Gest S, *et al.* Lighting the mood of depressed youth: Feasibility and efficacy of a 2 week-placebo controlled bright light treatment for juvenile inpatients. J Affect Disord 2016;190:450-456.
38. Watkins S, Freeborn E, Mushtaq S. A validated FFQ to determine dietary intake of vitamin D. Public Health Nutr 2021;24(13):4001-4006.
39. Aji AS, Erwinda E, Yusrawati Y, *et al.* Vitamin D deficiency status and its related risk factors during early pregnancy: A cross-sectional study of pregnant Minangkabau women, Indonesia. BMC Pregnancy Childbirth 2019;19(1):183.
40. Pludowski P, Holick MF, Grant WB, *et al.* Vitamin D supplementation guidelines. J Steroid Biochem Mol Biol 2018;175:125-135.
41. Gonzalez JS, Peyrot M, McCarl LA, *et al.* Depression and diabetes treatment nonadherence: A meta-analysis. Diabetes Care 2008;31(12):2398-2403.
42. Molosankwe I, Patel A, José Gagliardino J, *et al.* Economic aspects of the association between diabetes and depression: A systematic review. J Affect Disord 2012;142 Suppl:S42-S55.
43. Egede LE, Hernández-Tejada MA. Effect of comorbid depression on quality of life in adults with Type 2 diabetes. Expert Rev Pharmacoecon Outcomes Res 2013;13(1):83-91.
44. Egede LE, Ellis C. Diabetes and depression: Global perspectives. Diabetes Res Clin Pract 2010;87(3):302-312.
45. Salinero-Fort MA, Gómez-Campelo P, Cárdenas-Valladolid J, *et al.* Effect of depression on mortality in type 2 diabetes mellitus after 8 years of follow-up. The DIADEMA study. Diabetes Res Clin Pract 2021;176:108863.
46. Jeong JH, Um YH, Ko SH, *et al.* Depression and mortality in people with type 2 diabetes mellitus, 2003 to 2013: A nationwide population-based cohort study. Diabetes Metab J 2017;41(4):296.
47. Khubchandani J, Banerjee S, Gonzales-Lagos R, *et al.* Depression increases the risk of mortality among people living with diabetes: Results from national health and nutrition examination survey, USA. Diabetes Metab Syndr 2023;17(11):102892.
48. Chandler PD, Giovannucci EL, Scott JB, *et al.* Effects of vitamin D supplementation on c-peptide and 25-hydroxyvitamin D concentrations at 3 and 6 months. Sci Rep 2015;5:10411.
49. Rasouli N, Pittas AG. Effects of vitamin D supplementation on insulin sensitivity and secretion in prediabetes. J Clin Endocrinol Metab 2022;107(7):230-240.
50. Gagnon C, Daly RM, Carpentier A, *et al.* Effects of combined calcium and vitamin D supplementation on insulin secretion, insulin sensitivity and β-cell function in multi-ethnic vitamin D-deficient adults at risk for type 2 diabetes: A pilot randomized, placebo-controlled trial. PLoS One 2014;9(10):e109607.
51. Varshney S, Khadgawat R, Gahlot M, *et al.* Effect of high-dose vitamin D supplementation on beta cell function in obese Asian-Indian children and adolescents: A randomized, double blind, active controlled study. Indian J Endocrinol Metab 2019;23(5):545-551.
52. Leung PS. The potential protective action of vitamin D in hepatic insulin resistance and pancreatic islet dysfunction in type 2 diabetes mellitus. Nutrients 2016;8(3):147.
53. Mitri J, Dawson-Hughes B, Hu FB, *et al.* Effects of vitamin D and calcium supplementation on pancreatic β cell function, insulin sensitivity, and glycemia in adults at high risk of diabetes: The calcium and vitamin D for diabetes mellitus (CaDDM) randomized controlled trial. Am J Clin Nutr 2011;94(2):486-494.
54. Rasouli N, Younes N, Ghosh A, *et al.* Longitudinal effects of glucose-lowering medications on β-cell responses and insulin sensitivity in type 2 diabetes: The GRADE randomized clinical trial. Diabetes Care 2024;47(4):580-588.
55. Bornstedt ME, Gjerlaugsen N, Pepaj M, *et al.* Vitamin D increases glucose stimulated insulin secretion from insulin producing beta cells (INS1E). Int J Endocrinol Metab 2019;17(1):15-19.

56. Argano C, Mirarchi L, Amodeo S, *et al.* The role of vitamin D and its molecular bases in insulin resistance, diabetes, metabolic syndrome, and cardiovascular disease: State of the art. Int J Mol Sci 2023;24(20):15485.
57. Sabir MS, Haussler MR, Mallick S, *et al.* Optimal vitamin D spurs serotonin: 1 , 25-dihydroxyvitamin D represses serotonin reuptake transport (SERT) and degradation (MAO-A) gene expression in cultured rat serotonergic neuronal cell lines. Genes Nutr 2018;13:1-11.
58. Kaviani M, Nikooyeh B, Zand H, *et al.* Effects of vitamin D supplementation on depression and some involved neurotransmitters. J Affect Disord 2020;269:28-35.
59. Sikaroudi MK, Mokhtare M, Shidfar F, *et al.* Effects of vitamin D3 supplementation on clinical symptoms, quality of life, serum serotonin (5-hydroxytryptamine), 5-hydroxy-indole acetic acid, and ratio of 5-HIAA/5-HT in patients with diarrhea-predominant irritable bowel syndrome: A randomized clinical trial. EXCLI J 2020;19:652-667.
60. Eyileten C, Kaplon-Cieslicka A, Mirowska-Guzel D, *et al.* Antidiabetic Effect of Brain-Derived Neurotrophic Factor and Its Association with Inflammation in Type 2 Diabetes Mellitus. J Diabetes Res 2017;2017:2823671.
61. Zou MH, Kirkpatrick SS, Davis BJ, *et al.* Activation of the AMP-activated protein kinase by the anti-diabetic drug metformin in vivo: Role of mitochondrial reactive nitrogen species. J Biol Chem 2004;279(42):43940-43951.
62. de Miranda AS, de Barros JLVM, Teixeira AL. Is neurotrophin-3 (NT-3): A potential therapeutic target for depression and anxiety? Expert Opin Ther Targets 2020;24(12):1225-1238.
63. Shatri H, Pranandi M, Ardani Y, *et al.* The role of Neurotrophin 3 (NT-3) on neural plasticity in depression: A literature review. Intisari Sains Med 2023;14(3):1103-1108.
64. Noh K, Chow ECY, Quach HP, *et al.* Significance of the vitamin D receptor on crosstalk with nuclear receptors and regulation of enzymes and transporters. AAPS J 2022;24(4):71.
65. Orme RP, Middleditch C, Waite L, *et al.* The role of vitamin D3 in the development and neuroprotection of midbrain dopamine neurons. Vitam Horm 2016;100:273-297.
66. AlJohri R, AlOkail M, Haq SH. Neuroprotective role of vitamin D in primary neuronal cortical culture. ENeurologicalSci 2019;14:43-48.
67. Roopan S, Larsen ER. Use of antidepressants in patients with depression and comorbid diabetes mellitus: A systematic review. Acta Neuropsychiatr 2017;29(3):127-139.
68. Deuschle M. Effects of antidepressants on glucose metabolism and diabetes mellitus type 2 in adults. Curr Opin Psychiatry 2013;26(1):60-65.
69. Miidera H, Enomoto M, Kitamura S, *et al.* Association between the use of antidepressants and the risk of type 2 diabetes: A large, population-based cohort study in Japan. Diabetes Care 2020;43(4):885-893.
70. Puzhko S, Schuster T, Barnett TA, *et al.* Evaluating prevalence and patterns of prescribing medications for depression for patients with obesity using large primary care data (Canadian primary care sentinel surveillance network). Front Nutr 2020;7:24.
71. Gafoor R, Booth HP, Gulliford MC. Antidepressant utilisation and incidence of weight gain during 10 years' follow-up: Population based cohort study. BMJ 2018;361:k1951.
72. Kukucka T, Ferencova N, Visnovcova Z, *et al.* Mechanisms involved in the link between depression, antidepressant treatment, and associated weight change. Int J Mol Sci 2024;25(8):4511
73. Gill H, Gill B, El-Halabi S, *et al.* Antidepressant medications and weight change: A narrative review. Obesity 2020;28(11):2064-2072.
74. Miidera H, Enomoto M, Kitamura S, *et al.* Association between the use of antidepressants and the risk of type 2 diabetes: A large, population-based cohort study in Japan. Diabetes Care 2020;43(4):885-893.
75. Christakos S, Dhawan P, Verstuyf A, *et al.* Vitamin D: Metabolism, molecular mechanism of action, and pleiotropic effects. Physiol Rev 2015;96(1):365-408.
76. Moreira RT, Rodrigues LARL, de Farias LM, *et al.* Effect of vitamin D supplementation on depression treatment. Rev Assoc Med Bras 2021;67(8):1192-1197.
77. Gammoh O, Ibrahim A, Qnais E, *et al.* Vitamins C and D exhibit similar antidepressant effects to escitalopram mediated by NOx and FKBPL in a stress-induced mice model. Nutrients 2023;15(12):2692.
78. Mikola T, Marx W, Lane MM, *et al.* The effect of vitamin D supplementation on depressive symptoms in adults: A systematic review and meta-analysis of randomized controlled trials. Crit Rev Food Sci Nutr 2023;63(33):11784-11801.
79. Shi H, Wang B, Xu X. Antidepressant effect of vitamin D: A literature review. Neuropsychiatry 2018;7(4):337-341.
80. Bojar I, Raczkiewicz D, Sarecka-Hujar B. Depression, Metabolic Syndrome, Serum TSH and Vitamin D Concentrations in Rural and Urban Postmenopausal Women. Medicina 2020;56(10):511.

81. Penckofer S, Byrn M, Adams W, *et al.* Vitamin D supplementation improves mood in women with type 2 diabetes. J Diabetes Res 2017;2017:8232863.
82. Cojic M, Kocic R, Klisic A, *et al.* The effects of vitamin D supplementation on metabolic and oxidative stress markers in patients with type 2 diabetes: A 6-month follow up randomized controlled study. Front Endocrinol (Lausanne) 2021;12:610893.
83. Davoudi M, Allame Z, Niya RT, *et al.* The synergistic effect of vitamin D supplement and mindfulness training on pain severity, pain-related disability and neuropathy-specific quality of life dimensions in painful diabetic neuropathy: A randomized clinical trial with placebo-controlled. J Diabetes Metab Disord 2021;20(1):49-58.
84. Omidian M, Mahmoudi M, Abshirini M, *et al.* Effects of vitamin D supplementation on depressive symptoms in type 2 diabetes mellitus patients: Randomized placebo-controlled double-blind clinical trial. Diabetes Metab Syndr 2019;13(4):2375-2380.
85. Fazelian S, Amani R, Paknahad Z, *et al.* Effect of vitamin D supplement on mood status and inflammation in vitamin D deficient type 2 diabetic women with anxiety: A randomized clinical trial. Int J Prev Med 2019;10(1):17.
86. Wiernsperger NF, Bailey CJ. The antihyperglycaemic effect of metformin therapeutic and cellular mechanisms. Drugs 1999;58 Suppl 1:31-39.
87. Kemp DE, Ismail-Beigi F, Ganocy SJ, *et al.* Use of insulin sensitizers for the treatment of major depressive disorder: A pilot study of pioglitazone for major depression accompanied by abdominal obesity. J Affect Disord 2012;136(3):1164-1173.
88. Zou XH, Sun LH, Yang W, *et al.* Potential role of insulin on the pathogenesis of depression. Cell Prolif 2020;53(5):e12806.
89. Salcedo I, Tweedie D, Li Y, *et al.* Neuroprotective and neurotrophic actions of glucagon-like peptide-1: An emerging opportunity to treat neurodegenerative and cerebrovascular disorders. Br J Pharmacol 2012;166(5):1586.
90. Diz-Chaves Y, Herrera-Pérez S, González-Matías LC, *et al.* Effects of Glucagon-like peptide 1 (GLP-1) analogs in the hippocampus. Vitam Horm 2022;118:457-478.
91. Bae CS, Song J. The role of Glucagon-like peptide 1 (GLP1) in type 3 diabetes: GLP-1 controls insulin resistance, neuroinflammation and neurogenesis in the Brain. Int J Mol Sci 2017;18(11):2493.
92. Gunturu S. The potential role of GLP-1 agonists in psychiatric disorders: A paradigm shift in mental health treatment. Indian J Psychol Med 2024;46(3):193-195.
93. Luan S, Cheng W, Wang C, *et al.* Impact of glucagon-like peptide 1 analogs on cognitive function among patients with type 2 diabetes mellitus: A systematic review and meta–analysis. Front Endocrinol (Lausanne) 2022;13:1047883.
94. Kim YK, Kim OY, Song J. Alleviation of depression by glucagon-like peptide 1 through the regulation of neuroinflammation, neurotransmitters, neurogenesis, and synaptic function. Front Pharmacol 2020;11:1270.
95. Arillotta D, Floresta G, Guirguis A, *et al.* GLP-1 Receptor agonists and related mental health issues; Insights from a range of social media platforms using a mixed-methods approach. Brain Sci 2023;13(11):1503.
96. Hlscher C. Potential role of glucagon-like peptide-1 (GLP-1) in neuroprotection. CNS Drugs 2012;26(10):871-882.
97. Lennox R, Porter DW, Flatt PR, *et al.* Comparison of the independent and combined effects of sub-chronic therapy with metformin and a stable GLP-1 receptor agonist on cognitive function, hippocampal synaptic plasticity and metabolic control in high-fat fed mice. Neuropharmacology 2014;86:22-30.
98. Ahmed NSJI, Alsayed N. The weight loss effects of popular anti-diabetic medications: A literature review. In: Kacprzyk, editor. Studies in systems, decision and control. Cham: Springer; 2024.
99. Haddad F, Dokmak G, Bader M, *et al.* A comprehensive review on weight loss associated with anti-diabetic medications. Life 2023;13(4):1012.
100. Lazzaroni E, Ben Nasr M, Loretelli C, *et al.* Anti-diabetic drugs and weight loss in patients with type 2 diabetes. Pharmacol Res 2021;171:105782.
101. Hu L, Wang W, Chen X, *et al.* Prospects of antidiabetic drugs in the treatment of neurodegenerative disease. Brain-X 2024;2(1):e52.
102. Wang SY, Wu SL, Chen TC, *et al.* Antidiabetic agents for treatment of parkinson's disease: A meta-analysis. Int J Environ Res Public Health 2020;17(13):1-11.
103. Koshatwar M, Acharya S, Prasad R, *et al.* Exploring the potential of antidiabetic agents as therapeutic approaches for alzheimer's and parkinson's diseases: A comprehensive review. Cureus 2023;15(9):e44763.
104. Fazelian S, Amani R, Paknahad Z. Effect of vitamin D supplement on mood status and inflammation in vitamin D deficient type 2 diabetic women with anxiety: A randomized clinical trial. Int J Prev Med 2019;10:17.

105.Albuloshi T, Dimala CA, Kuhnle GGC, *et al.* The effectiveness of vitamin D supplementation in reducing depressive symptoms: A systematic review and meta-analysis of Randomized Controlled Trials (RCTs). Nutr Healthy Aging 2021;6(4):301-318.

106.Penckofer S, Byrn M, Adams W, *et al.* Vitamin D supplementation improves mood in women with type 2 diabetes. J Diabetes Res 2017;2017:8232863.

107.Raygan F, Ostadmohammadi V, Bahmani F, *et al.* The effects of vitamin D and probiotic co-supplementation on mental health parameters and metabolic status in type 2 diabetic patients with coronary heart disease: A randomized, double-blind, placebo-controlled trial. Prog Neuropsychopharmacol Biol Psychiatry 2018;84(Pt A):50-55.

108.Achiron A, Givon U, Magalashvili D, *et al.* Effect of alfacalcidol on multiple sclerosis-related fatigue : A randomized , double-blind placebo-controlled study. Mult Scler 2015;21(6):767-775.

109.Voynikov Y. Cholecalciferol (Vitamin D3) – pharmacological properties, therapeutic utility and potential new fields of clinical application. Pharmacia 2017;63(1):29-66.

110.Patrick RP, Ames BN. Vitamin D and the omega-3 fatty acids control serotonin synthesis and action, part 2: Relevance for ADHD, bipolar disorder, schizophrenia, and impulsive behavior. FASEB J 2015;29(6):2207-2222.

111.Ogłodek EA, Just MJ, Szromek AR, *et al.* Melatonin and neurotrophins NT-3, BDNF, NGF in patients with varying levels of depression severity. Pharmacol Rep 2016;68(5):945-951.

112.Hock C, Heese K, Müller-Spahn F, *et al.* Increased cerebrospinal fluid levels of neurotrophin 3 (NT-3) in elderly patients with major depression. Mol Psychiatry 2000;5(5):510-513.

113.Malihi Z, Lawes CMM, Wu Z, *et al.* Monthly high-dose vitamin D supplementation does not increase kidney stone risk or serum calcium: results from a randomized controlled trial. Am J Clin Nutr 2019;109(6):1578-1587.

114.Gallagher JC, Smith LM, Yalamanchili V. Incidence of hypercalciuria and hypercalcemia during vitamin D and calcium supplementation in older women. Menopause 2014;21(11):1173-1180.

115.Lee M, Lee HI, Song K, *et al.* Association of hypercalciuria with vitamin D supplementation in patients undergoing ketogenic dietary therapy. Front Nutr 2022;9:970467.

116.Malihi Z, Wu Z, Lawes CMM, *et al.* Adverse events from large dose vitamin D supplementation taken for one year or longer. J Steroid Biochem Mol Biol 2019;188:29-37.

117.Billington EO, Burt LA, Rose MS, *et al.* Safety of high-dose Vitamin D supplementation: Secondary analysis of a randomized controlled trial. J Clin Endocrinol Metab 2020;105(4):1261-1273.