Modulation of Vitamin D Status by Gut Microbiota: Impact on Depression and Anxiety-related Behavior in Adult C57BL/6J Mice

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Introduction

Depression and anxiety disorders are among the most common mental health disorders that affect U.S. adults today (1). As a result of the COVID-19 pandemic, symptoms of both disorders were exacerbated globally (2). In addition to the classic role of vitamin D (VD) in bone health, suboptimal levels of VD has shown to increase risk for inflammation, immune function, and cognitive function. Individuals diagnosed with seasonal affective disorder, a type of depression, frequently have suboptimal levels of VD due to limited sun exposure related to seasonal changes and/or low dietary intake. Vitamin D plays a vital role in physiological functions like mitigating inflammatory status and restores calcium and neurotransmitter imbalance (3). Along with VD, growing evidence suggests gut microbiota likely play a role in neuropsychiatric disorders (4, 5) as supplementation with pre- and probiotic has shown to alleviate mental health disorders (6). To date, the mechanisms by which VD alleviate depression and anxiety-related symptoms and whether the protective effect is dependent on gut microbiota remains unclear.

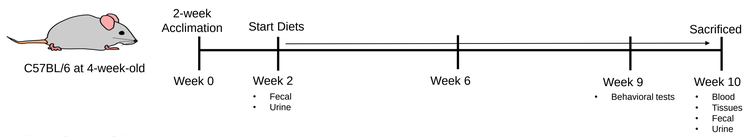
Hypothesis

1.Depletion of gut microbiota (via antibiotics) would disrupt host VD status and promote behaviors related to depression and anxiety in adult mice

2.Shifting of microbial composition via prebiotic supplementation would "rescue" or restore VD loss and ameliorate symptoms of depression and anxiety

Methods

Study Design



Experimental Groups

CTR (n = 10/sex)	AB (n = 10/sex)	VD (n = 10/sex)	VD + AB (n = 10/sex)	VD + FOS (n = 10/sex)	VD + FOS + AB (n = 10/sex)
Standard Chow	Standard Chow	Standard Chow	Standard Chow	Standard Chow	Standard Chow
	Antibiotic Cocktail*	5000 IU Vitamin D3	5000 IU Vitamin D3	5000 IU Vitamin D3	5000 IU Vitamin D3
			Antibiotic Cocktail*	5% w/w FOS	5% w/w FOS
					Antibiotic Cocktail*

CTR, Control; FOS, Fructooligosaccharide; VD, vitamin D3; AB, antibiotics

*0.5 g/L vancomycin, 1g/L neomycin sulfate, 1g/L metronidazole, 1g/L ampicillin

Behavioral testing

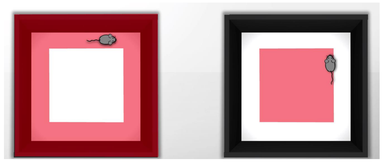
Assessment of depression-related behavior

- Forced Swim Test (FST) and Tail Suspension Test (TST)

Immobility time as measurement of depression-like behaviors

Assessment of anxiety-related behavior

- Open Field Test (OFT)



Outer edge (more anxious)

Center (less anxious)

Images adapted from Conduct Science

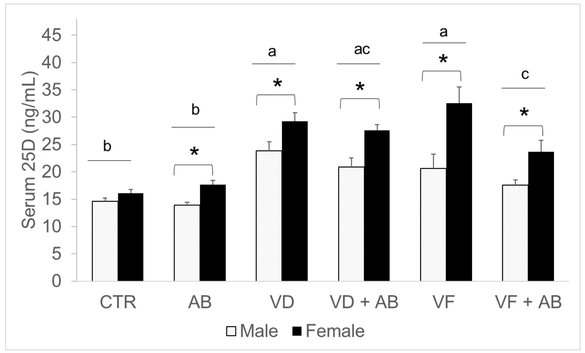
Vitamin D Status and Serotonin Levels

Vitamin D status, as indicated by serum concentrations of 25-hydroxycholecalciferol (25D) was measured by a commercially available ELISA kit (Crystal Chem, Inc). Serum serotonin was quantified using an ELISA kit (Enzo Life Sciences).

Colonic mRNA expressions of VDR and TPH1

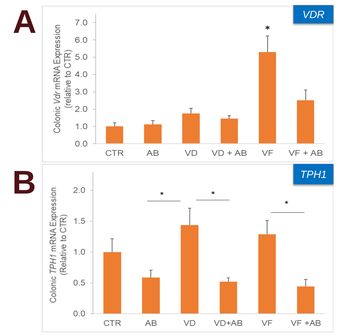
Colonic mucosa was harvested from mice at the time of euthanization, and RNA was extracted with Trizol®. The mRNA expressions of colonic vitamin D receptor (VDR) and tryptophan hydroxylase 1 (TPH1), an enzyme critical for biosynthesis of serotonin in the gut, was evaluated via real-time PCR.

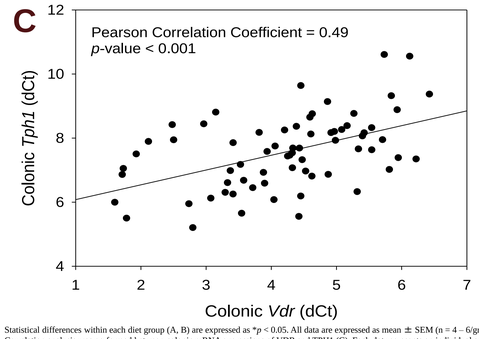
Supplementation of vitamin D improved circulating levels of 25D and that it was not affected by depletion of gut microbiota in adult mice



Circulating 25-hydroxycholecalciferol (25D) in adult mice. Different letters indicate statistical differences between diets at $p < 0.05$. Statistical differences between sex within each diet group are expressed as $*p < 0.05$. Data are expressed as mean ± SEM (n = 6 – 7/group/sex).

Combination of vitamin D and FOS (VF) upregulated colonic mRNA expression of *VDR*. Positive correlation was observed between colonic *VDR* and colonic *TPH1*, a rate-limiting enzyme responsible for serotonin synthesis in the gut





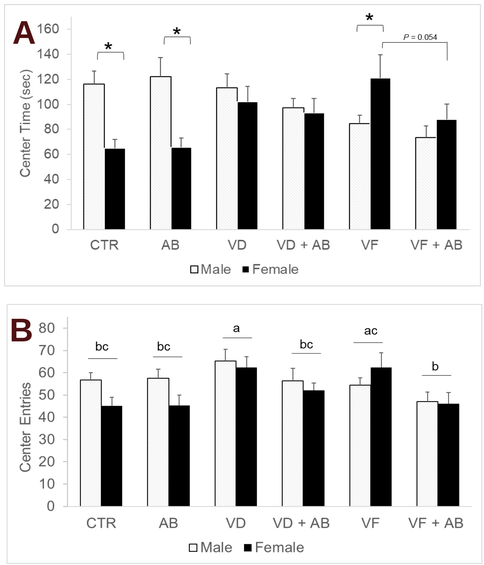
Statistical differences within each diet group (A, B) are expressed as $*p < 0.05$. All data are expressed as mean ± SEM (n = 4 – 6/group/sex). Correlation analysis was performed between colonic mRNA expressions of VDR and TPH1 (C). Each dot represents an individual sample.

Depression-related behaviors, as indicated by FST and TST, were not affected by dietary interventions

Diet	FST (sec)	TST (sec)	Total Immobility (sec)
CTR	154.63 ± 7.31	105.42 ± 12.07	260.05 ± 15.22
AB	179.84 ± 7.48	96.10 ± 9.24	276.47 ± 12.23
VD	150.26 ± 12.43	101.42 ± 10.33	251.68 ± 16.53
VD + AB	172.06 ± 10.04	101.94 ± 8.73	274.00 ± 12.23
VF	178.61 ± 11.45	99.33 ± 10.91	277.29 ± 13.39
VF + AB	164.89 ± 8.95	88.21 ± 9.17	251.44 ± 14.23

Time (seconds) spent immobile during the forced swim test (FST) and tail suspension test (TST) in adult C57BL/6J mice. Data presented are combined data of male and female mice. Data are expressed as the mean ± SEM (n = 8 – 10/group/sex) for the last 4 minutes of the observation period.

Combination of vitamin D and FOS (VF) tended to decrease anxiety-related behavior in adult female mice compared to male mice



Time (seconds) spent in the center of the open field test (A) and number of entries into the center (B) during the OFT in adult male and female C57BL/6J mice. Different letters indicate statistical significance between diets at $p < 0.05$. Statistical differences between sex within each diet group are expressed as $*p < 0.05$. Data are expressed as mean ± SEM (n = 8 – 10/group/sex) observed over a 10-minute period.

Rodents that exhibit less anxious-like behavior will spend more time in the center of the field, whereas rodents perceived as more anxious will spend more time alongside the outer edge of the field.

Serum serotonin were higher in female mice on a combination diet of vitamin D and FOS (VF) compared to female mice on a control (CTR) or vitamin D alone (VD) diets

Statistical differences between sex within each diet group (A) are expressed as $*p < 0.05$. Treatment effects within each sex was investigated (B and C). Significance between groups within each sex is expressed as $\#p < 0.05$. All data are expressed as mean ± SEM (n = 5 – 7/group/sex).

Conclusions

- **FOS enhances colonic VDR expression**, to a greater extent than VD alone, though vitamin D status did not alter among groups.
- **Modulation of gut microbiota via FOS ameliorates anxiety-related behavior**, but not depression-related behavior. This observation is in parallel with serum serotonin and that the effect is much significant in female than male mice.
- **Colonic mRNA expression of *TPH1* may be dependent on the presence of gut microbiota and that it was strongly correlated with colonic *VDR* expression**. This may suggest a role of VDR in regulating TPH1 and hence, serotonin synthesis in the gut.

Future Directions

- Profile the changes of gut microbiota and its relevance with anxiety-related behavior
- Role of FOS in the gut-brain axis, specifically on the relationship between colonic vitamin D signaling and neurotransmitter synthesis

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