Vitamin D Antagonizes Effects of High-fat Diet on Brain Transcriptome

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Introduction

Low vitamin D levels correlate with type 2 diabetes. However, the mechanisms by which vitamin D might regulate glucose metabolism are not well studied. We previously published that vitamin D acts in the paraventricular nucleus of the hypothalamus to improve glucose tolerance. Additionally, we showed that the vitamin D receptor (VDR) is required for normal glucose tolerance when on a high-fat diet in mice. However, the molecular mechanisms by which vitamin D or VDR action might alter glucose homeostasis is unknown. Since RNA-sequencing (RNA-seq) is a powerful tool to determine gene expression, we utilized this technique to determine the RNA expression patterns of the hypothalamus after vitamin D treatment in rats.

Hypothesis

We hypothesized that vitamin D alters genes important in the regulation of glucose.

Methods

- Male Long-Evans rats were divided into 2 different diets: High fat diet (45% fat) or standard chow for 20 weeks.
- Brain cannulations performed at least 1 week prior to studies.
- 0.1 mcg 1,25-hydroxyvitamin D_3 (1,25D3) was given into the third ventricle (i3vt) 120 minutes prior to sacrifice. The vehicle was hydroxypropyl-β-cyclodextrin (THPB-EC; CTD, Inc). We used 4 groups: 1) HFD-fed + 1,25D3, 2) HFD-fed + vehicle, 3) chow-fed + 1,25D3, and 4) chow-fed + vehicle.
- After sacrifice, the hypothalamus was dissected, RNA extracted, cDNA libraries created and then polyA tails added via Illumina TruSeq RNA prep Kit.
- RNA sequencing was performed on Genomic and RNA Profiling Core at Baylor College of Medicine on Illumina HiSeq 2500 Sequencing System.
- Data were mapped with TopHat2 onto the mouse genome build UCSCmm10. Expression was computed with Cufflinks2.
- Genes with >1.25 fold increase were analyzed by Gene Set Enrichment Analysis.

Results

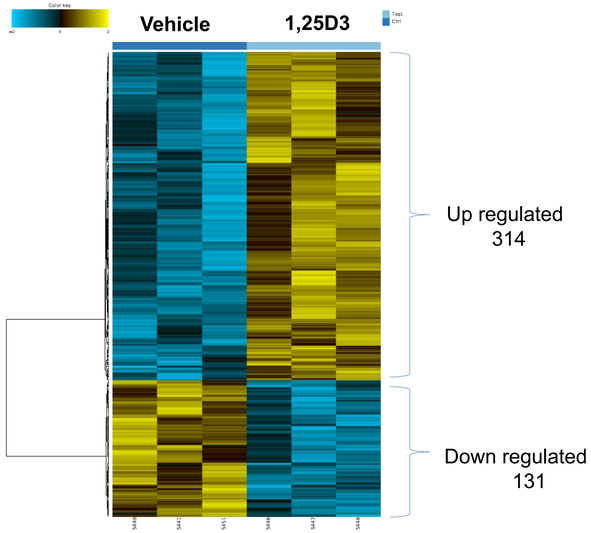

Fig. 1. Vitamin D treatment differentially regulates genes. Correlation-based clustered Heat-Map depicting relationships across 6 high-fat fed samples (3 vehicle treated controls and 3 1,25D3 treated rats) when normalized gene expression data is used for 445 genes identified to have significant differential expression between vitamin D treatment and control.

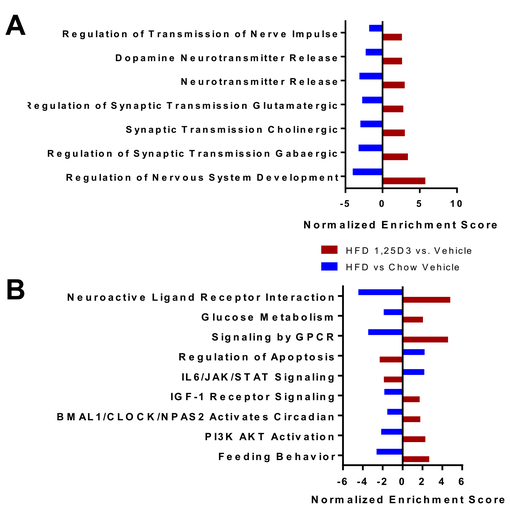

Fig. 2.GSEA Pathway analysis. A) Vitamin D treatment upregulated multiple pathways important in nerve function, which are downregulated by high-fat diet alone.
B) Vitamin D treatment significantly altered genes important for multiple pathways involved in metabolism, in an opposite fashion to the effects of high-fat diet alone.

Gene Symbol	Fold Change (linear	Q-value	Name of Gene
TNFSF15	3.1591	0.0098	TNF Superfamily member 15
CRYAA	2.8835	0.0347	Crystallin Alpha A
SELV	2.6676	0.0157	Selenoprotein V
LOC100911595	2.5787	0.0301	Unknown
KCNH7	2.5447	0.0297	Potassium Voltage-Gated Channel Subfamily H Member 7
AOC3	2.3876	0.0438	Amine Oxidase, Copper Containing 3
ZFP704	2.2821	0.0201	Unknown
IL6	2.2563	0.0319	Interleukin 6
KCNH5	2.2483	0.0264	Potassium Voltage-Gated Channel Subfamily H Member 5
ERBB4	2.2208	0.0378	Erb-B2 Receptor Tyrosine Kinase 4
TNNT2	2.1904	0.0360	Troponin T2, Cardiac Type
ADRA1A	2.1534	0.0127	Adrenoceptor Alpha 1A
BEND4	2.1472	0.0320	BEN Domain Containing 4
GPRC5A	2.0806	0.0117	G Protein-Coupled Receptor Class C Group 5 Member A
ADAMTS8	2.0635	0.0285	ADAM Metallopeptidase With Thrombospondin Type 1 Motif 8
AABR06076899.1	2.0216	0.0222	Unknown
RN50_8_0646.1	2.0078	0.0350	Unknown
AABR06025587.1	1.9995	0.0314	Unknown
RGD1309903	1.9905	0.0307	Unknown
AABR06030841.1	1.9899	0.0282	Unknown
RN50_13_0822.1	1.9888	0.0228	Unknown
KCNA2	1.9194	0.0218	Potassium Voltage-Gated Channel Subfamily A Member 2
GABRB1	1.8747	0.0443	Gamma-Aminobutyric Acid Type A Receptor Beta1 Subunit
AFF2	1.8597	0.0239	AF4/FMR2 Family Member 2
BCHE	1.8540	0.0120	Butyrylcholinesterase
PPARA	1.8528	0.0001	Peroxisome Proliferator Activated Receptor Alpha
NOS1	1.8438	0.0392	Nitric Oxide Synthase 1
RGD1562276	1.8382	0.0058	Unknown
CDKL5	1.8250	0.0236	Cyclin Dependent Kinase Like 5
FAM122B	1.8194	0.0215	Family With Sequence Similarity 122B
HTR4	1.8050	0.0342	5-Hydroxytryptamine Receptor 4
KCNQ3	1.7749	0.0394	Potassium Voltage-Gated Channel Subfamily Q Member 3
FOSL1	1.7713	0.0211	FOS Like 1, AP-1 Transcription Factor Subunit
PSD3	1.7710	0.0286	Pleckstrin And Sec7 Domain Containing 3
THSD7A	1.7584	0.0277	Thrombospondin Type 1 Domain Containing 7A
SLC2A4	1.7320	0.0011	Solute Carrier Family 2 Member 4 (GLUT4)

Table 1. Top 35 genes upregulated in high-fat fed rat hypothalamic after treatment with 1,25D3.

Conclusions

- **Vitamin D differentially regulates multiple genes involved in nerve transmission, neurotransmitter release, and multiple ion channels.**
- **Transcriptional effects of vitamin D are often in an opposite direction to the effects of high-fat diet on the brain.**

Acknowledgements

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