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. We added via Illumina TruSeq RNA-Seq (Illumina, CA) 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.

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 D₃ (1,25D₃) was given into the third ventricle (3Vt) 120 minutes prior to sacrifice. The vehicle was hydroxypropl-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.
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• 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 among 8 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.

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.

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