mating. During pregnancy/lactation, obese dams continued on the HFD (HFD/HFD), or transitioned to CD (HFD/CD). Lean dams remained on CD (CD/CD). Offspring were weaned at 3 weeks and placed on a CD. M and F pups underwent neurobehavioral testing at 4 weeks (juvenile) and 11 weeks (adult). 27-44 M and 28-41 F offspring/sex/diet group were tested. Hippocampal learning was evaluated with fear conditioning (FC) at 4 weeks and Morris Water Maze (MWM) at 11 weeks. Kruskal-Wallis and Mann Whitney testing determined significant differences between groups.

RESULTS: On day two of FC, HFD/HFD M and F juvenile offspring had significantly lower percent freezing vs CD/CD ($p < 0.0001$), indicating hippocampal learning/memory deficits (Fig 1). HFD/CD pups had significantly higher percent freezing vs CD/CD ($p<0.0001$), suggesting maladaptive anxiety. In the MWM, only MATOB-exposed Ms had significant hippocampal learning deficits (increased total latency to platform and indirect search strategies, Fig 2A/B). MATOB-exposed Ms also showed increased anxiety-like behaviors on MWM (excessive thigmotaxis, $p = 0.004$).

CONCLUSION: MATOB and pregnancy diet significantly affect offspring memory and anxiety. MATOB is associated with hippocampal learning deficits in juveniles; abnormalities persisted into adulthood in Ms only. Maternal switch to CD in pregnancy led to increased anxiety in juveniles, again persisting into adulthood in Ms only. Together with our prior findings that MATOB has a greater effect on the M embryonic brain, these data suggest that MATOB has a more profound and persistent effect on M brain development and cognition from fetal life to adulthood.

Figure 1: MATOB Associated with Juvenile Hippocampal Learning Deficits (HFD/HFD) and Increased Anxiety (HFD/CD) on Fear Conditioning

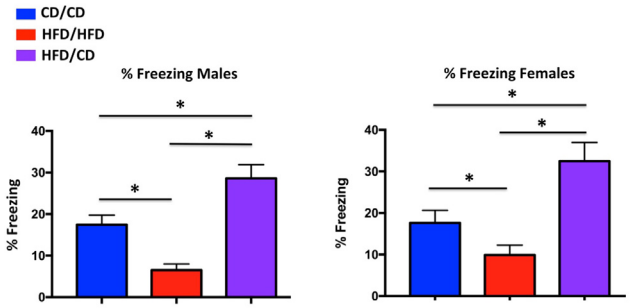
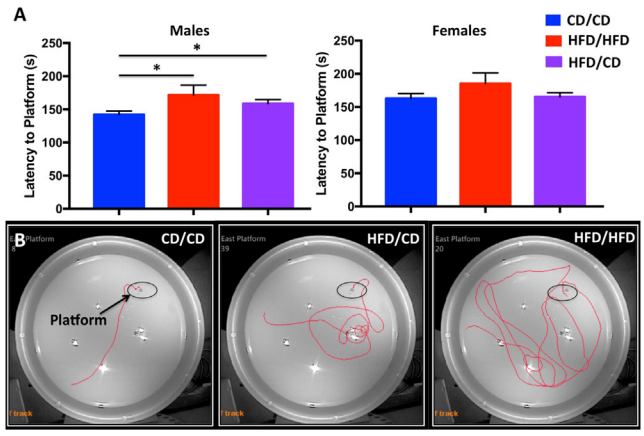


Figure 2: MATOB Associated With Adult Hippocampal Learning Deficits Morris Water Maze Total Latency to Platform (A) and Search Strategies (B)



22 The hepatic expressed circadian gene, npas2, influences the developing got microbiome with restricted feeding

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OBJECTIVE: We have recently shown in both non-human primates and in rodents that fetal and neonatal expression of the circadian transcription factor, *Npas2*, plays a critical role in establishing life-long metabolic homeostasis. Similarly, we and others have also established the importance of establishment of the early gut microbiome on later in life metabolism and notably with malnutrion. We hypothesized that altered circadian gene expression would result in gut microbiome dysbiosis, especially with diet-induced metabolic stress (*i.e.,* restricted feeding). Using our novel murine model of conditional knock-out (cKO) of *Npas2* in the neonatal liver, we aimed to determine the role of the circadian machinery in gut dysbiosis with restricted feeding.

STUDY DESIGN: We collected fecal samples from cKO and control (WT) mice before (day 0) and after the restricted feeding study (day 17). Extracted DNA was sequenced using the MiSeq Illumina platform using primers specific for either the V1-V3 or V4 regions of the 16S rDNA gene. The resulting sequences were quality filtered, aligned, and assigned taxonomy. Principal coordinate analysis was performed on unweighted and weighted UniFrac distances between samples as shown in **Figure 1**, with Permutation ANOVA to assess clustering significance between groups. Microbial taxa that significantly differ between groups of interest was determined using Linear Discriminate Analysis Effect Size (LEfSe) and RandomForest.

RESULTS: Principal coordinate analysis performed on weighted UniFrac distances between male cKO and WT samples revealed that the gut microbiome of the mice did not differ by genotype (cKO vs. WT) at the start of the restricted feeding study, but did differ by virtue of genotype at the end of the study (cKO vs. WT, $p=0.001$).

CONCLUSION: Here we have provided an initial key insight into the interplay of the establishment of the peripheral circadian clock in the liver and the gut microbiome, and presumptive cooperativity to establish, adapt and regulate metabolic homeostasis. As *Npas2* expression in the liver is a target of maternal metabolic perturbations during fetal development, these findings have potential implications in the long term metabolic health of their offspring.

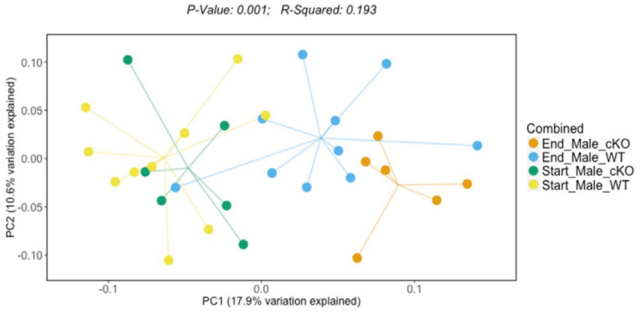

Figure 1: The gut microbiome is differentially altered in male *Npas2* cKO mice after restricted feeding. Principal coordinate analysis was performed on weighted UniFrac distances between samples. The gut microbiome of the mice did not differ by genotype (cKO vs. WT, yellow vs. green) at the start of the restricted feeding study, but did differ by virtue of genotype at the end of the study (cKO vs. WT, blue vs. orange). Significance was determined by PERMANOVA (p=0.001). These findings suggest that neonatal loss of *Npas2* is associated with a poorly adapted response to restricted feeding in adult life.