Bile acids and their role in microbial control of phenotypic programming *in utero*

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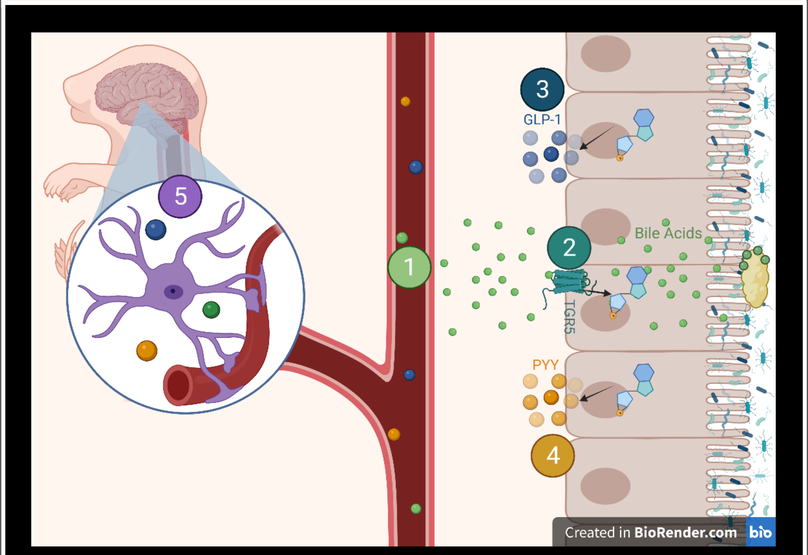
Background

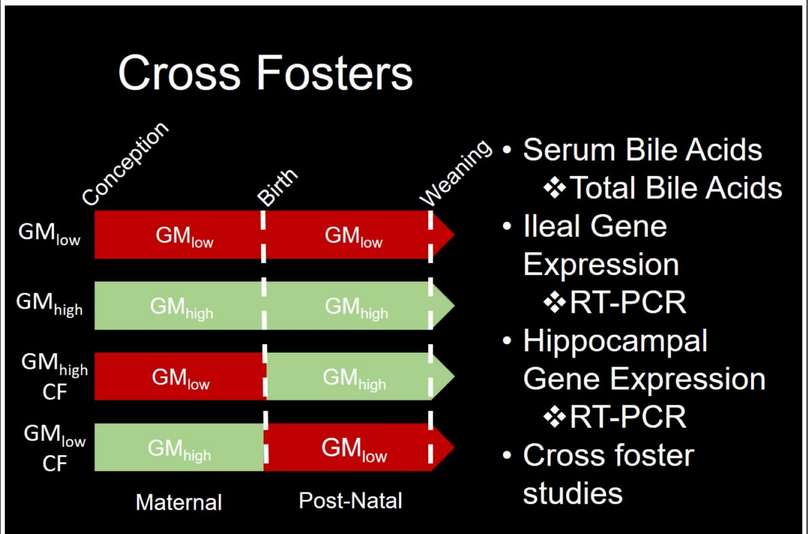
- Differences in phenotype linked to gut microbiome (GM).
- Cross foster (CF) studies show that GM-mediated effects are programmed *in utero.*
- Secondary bile acids are microbial metabolites under investigation for their effects.
- Luminal and intra-arterial administration of bile acids increases TGR5-dependent secretion of GLP-1 and PYY in the colon.

Hypotheses



1. Serum bile acids will be increased in GM_{low} vs. GM_{high}
2. Bile acid receptor *Tgr5* will be increased in GM_{low}
3. *Glp-1* expression will be increased in GM_{low}
4. *Pyy* expression will be increased in GM_{low}
5. Neural GLP-1 and PYY receptors will be greater in GM_{low}
6. Mice will mimic their surrogate dam in ileal gene expression and their birth dam in hippocampal gene expression

Methods

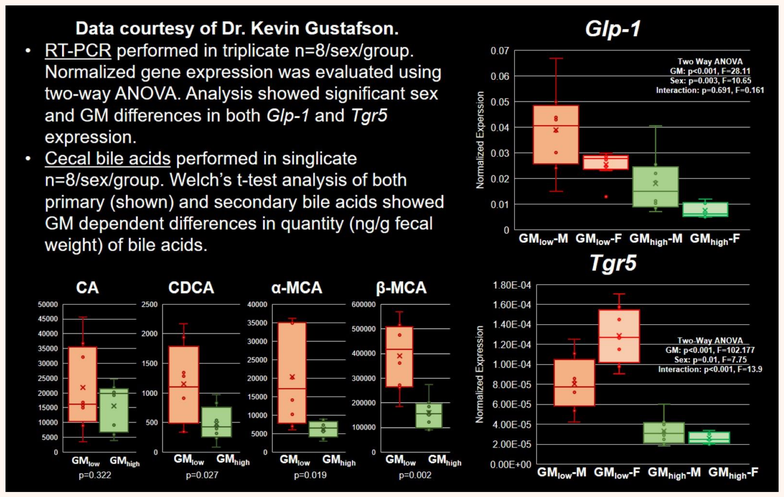


- Serum Bile Acids
  - ❖Total Bile Acids
- Ileal Gene Expression
  - ❖RT-PCR
- Hippocampal Gene Expression
  - ❖RT-PCR
- Cross foster studies

Previous data shows colonic *Tgr5* and *Glp-1* expression and cecal bile acids are significantly greater in GM_{low} mice

Data courtesy of Dr. Kevin Gustafson.

- RT-PCR performed in triplicate n=8/sex/group. Normalized gene expression was evaluated using two-way ANOVA. Analysis showed significant sex and GM differences in both *Glp-1* and *Tgr5* expression.
- Cecal bile acids performed in singlicate n=8/sex/group. Welch's t-test analysis of both primary (shown) and secondary bile acids showed GM dependent differences in quantity (ng/g fecal weight) of bile acids.



1 Due to high variability, there was no statistical difference detected in serum bile acids

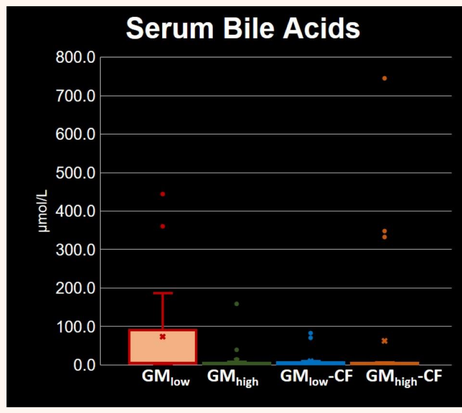


FIGURE 1: Data obtained by total bile acids assay n=12/sex/group. Due to non-normal distribution, serum levels of bile acids were evaluated with Kruskal–Wallis one-way ANOVA on ranks p=0.071. Analysis did not detect a significant difference in bile acids between groups; however, averages were higher for GM_{low} and GM_{high} cross-fostered mice due to a higher number of elevated samples from those two groups.

2 Ileal expression of *Tgr5* higher in GM_{high} mice

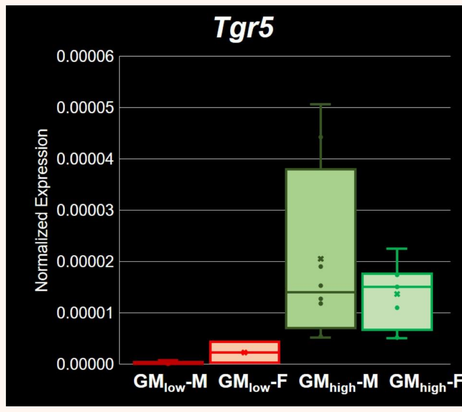


FIGURE 2: Data obtained from RT-PCR performed in triplicate n=2-8/sex/group. Normalized gene expression was evaluated using two-way ANOVA
GM: p=0.005, F=9.88,
Sex: p=0.628, F=0.242,
GM x Sex: p=0.384, F=0.791
Analysis shows significant difference between GMs but did not detect a significant effect of sex or an interaction.

3 Ileal expression of *Glp-1* higher in GM_{high} mice

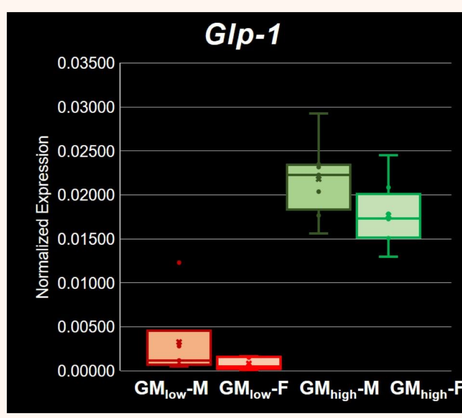


FIGURE 3: Data obtained from RT-PCR performed in triplicate n=5-8/sex/group. Normalized gene expression was evaluated using two-way ANOVA
GM: p<0.001, F=285.9
Sex: p=0.157, F=2.14
GM x Sex: p=0.043, F=4.58
Analysis shows significant difference between GMs and a possible GM x sex interaction.

4 *Pyy* shows significant sex-dependent difference in the ileum

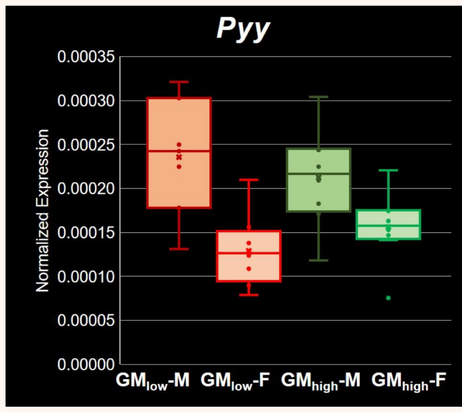


FIGURE 4: Data obtained from RT-PCR performed in triplicate n=8/sex/group. Normalized gene expression was evaluated using two-way ANOVA
GM: p=0.918, F=0.011
Sex: p<0.001, F=19.14
GM x Sex: p=0.186, F=1.84
Analysis shows significant differences between sexes but did not detect a significant effect of GM or an interaction.

5 Hippocampal *Npy2r* shows higher expression in GM_{low}

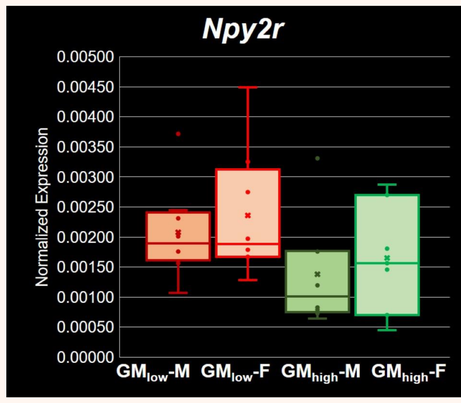


FIGURE 5: Data obtained from RT-PCR performed in triplicate n=8/sex/group. Normalized gene expression was evaluated using two-way ANOVA
GM: p=0.042, F=4.543
Sex: p=413, F=0.692
GM x Sex: p=0.992, F<0.001
Analysis shows a significant difference in hippocampal expression of PYY receptor, NPY2R, between GMs.

6 Expect PYY receptor to show patterns of fetal programing in hippocampal tissues

- Ongoing studies will determine if *Tgr5*, *Glp-1, Pyy* and/or *Npy2r* show patterns of fetal programming.
- I hypothesize that *Tgr5, Glp-1* and *Pyy* expression will be guided by postnatal microbial composition while downstream receptor, *Npy2r*, will show evidence of fetal programming.

Conclusions

- Frequency of intake may be one possible explanation for the high variability seen in serum bile acids.
- Data indicate that *Tgr5* and *Glp-1* expression is higher in the ileum of GM_{high} mice relative to GM_{low} mice.
- *Pyy* does not show a significant difference in expression between GMs in the ileum.
- There are different patterns of bile acid signaling molecule expression between the colon and ileum.
- Hippocampal expression of *Npy2r* is significantly higher in GM_{low} mice.
- No expression of *Glp-1r* was found in the hippocampus.

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