In Vitro Investigation of the Mechanisms by Which Intestinal Microbiota Regulate Hepatic Gluconeogenesis

Results

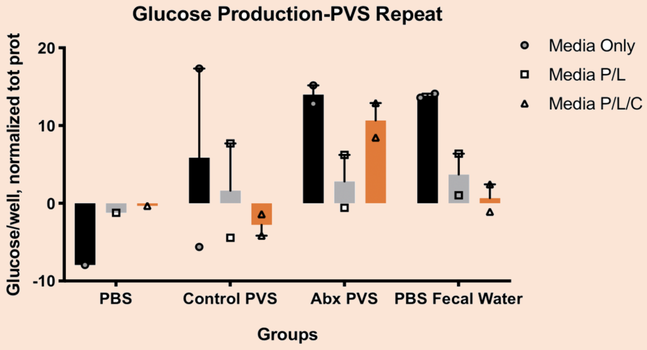

Figure 1. Glucose production as a function of portal vein serum treatment. Initial experiments for optimization of the glucose production protocol revealed unexpected responses of the primary hepatocytes to the three types of media (Figure 1).

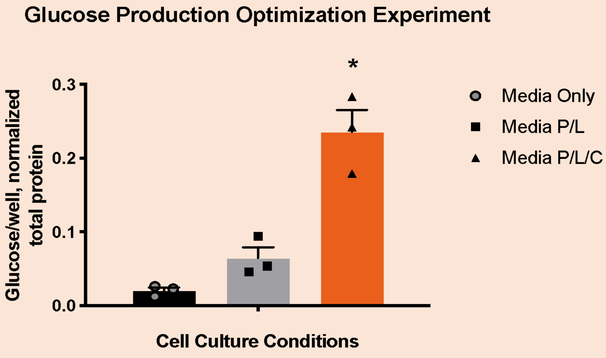

Figure 2. Optimization of the Glucose Assay. The next experiments were to optimize the glucose production assay.

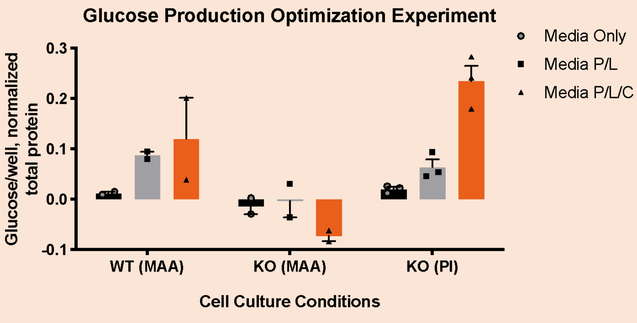

Figure 3. Expected glucose production response was not seen in the same primary hepatocytes plated by a different investigator 4 hours earlier. When a different investigator had plated the same types of cells four hours earlier, there were unexpected results.

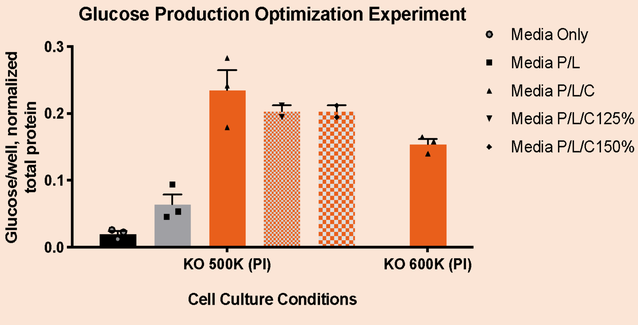

Figure 4. Permutations: Increase in cell density or cAMP. In the same set of cells, permutations were added. Increased cell density and cAMP were used to see if these permutations affect glucose production.

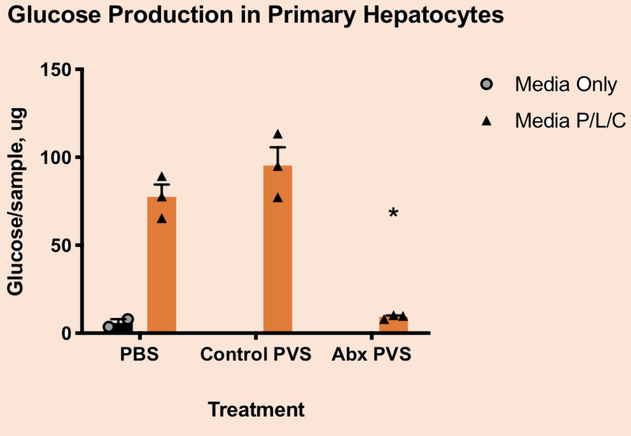

Figure 5. Preliminary Data. In a preliminary experiment, different serums were used. PBS, Control portal vein serum, and Antibiotic portal vein serum were all tested.

Review of Literature

- The impact of cell culture innovation has been enormous on human society. For example, advancement in biology in recent years has been heavily dependent on technology for cell culture (Yao, 2017).
- In the human body, the liver is an important organ. The liver plays an important part in the metabolism of the body and has a variety of functions including the processing of insulin, protein synthesis and drug detoxification (Lumen, 2019).
- Glucose is a type of sugar that is obtained from foods, and it is used by your body for energy. It's called blood glucose or blood sugar as it moves through your bloodstream into your cells (Dansinger, 2016).
- The gut microbiome is the accumulation of microorganisms, including bacteria, viruses, and fungi, as well as their shared genetic material, that are found in the gastrointestinal tract (GIT), and includes both commensal and pathogenic microorganisms (Lederberg, 2019).
- The intestinal microbiome plays an important role in the absorption of nutrients and minerals, the synthesis of enzymes, vitamins and amino acids, and the production of short-chain fatty acids (SCFAs) (Lederberg, 2019).

Hypothesis

- The gut microbiome regulates host mammalian blood glucose homeostasis by delivering metabolites, pyruvate, lactate and cAMP through the portal vein.

Methodology

Cell Culture- Primary hepatocytes were prepared

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Glucose Production Assay- Cell densities (500K, 600K)

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Measurement of Glucose

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Quantification of Protein

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Statistical Analysis- Microsoft Excel: Student's T-test to compare groups
Prism 8.0: Data was graphed and figures were exported for publication ($p < 0.05$ considered significant)

Discussion

- Mice lacking a gut microbiome have a clear and consistent decrease in blood sugar in both fasting and fed states, it might be due to alterations in amino acid metabolism that affect hepatic gluconeogenesis.
- Initial experiments produced results that were both unexpected and quite variable.
- Those that tested various densities of cell seeding as well as concentrations of cyclic AMP, suggested that these conditions were not the primary culprit.
- It appeared that duration of cell growth on the culture plates prior to fasting might play a key role.
- My final experiments demonstrated a significantly reduced glucose production in primary hepatocytes treated with the portal vein serum from antibiotic (commensal depleted) mice.

Future Research

- Repetition in experiments to confirm the validity of the findings.
- If the portal vein serum of antibiotic mice demonstrates a consistent difference from that of conventional mice with regards to glucose production in hepatocytes, this would justify further investigations into the precise metabolites or microbial-derived molecules.
- Applying these findings to human health and disease would be an important goal to improve metabolic diseases such as diabetes and obesity.

Conclusion

- The human microbiome has progressed into an extremely diverse, sophisticated and environmentally friendly ecosystem.
- An experiment in which many tests and different permutations were used, tried to help the scientific community further their knowledge on the microbiome.
- More experiments should be done to conclude and verify these findings, there is without a doubt that this is important research and it has great implications for the field even despite the limitations that the research came across.

References

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