Glut5 and Fructose Metabolism Inhibition in a Simulated Small Intestine Environment

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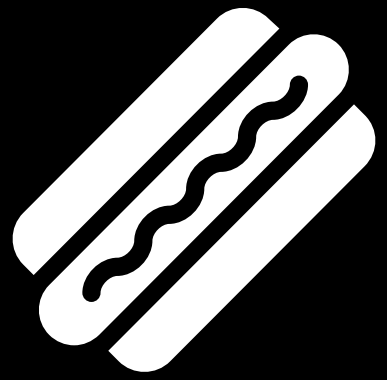
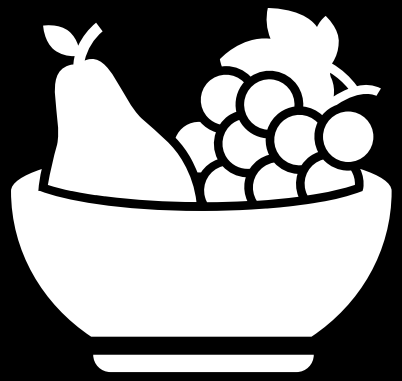
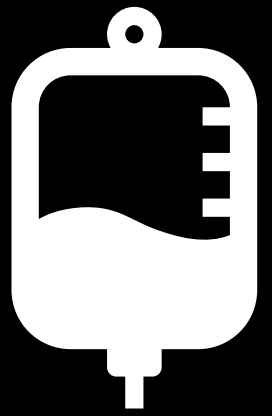
Hypothesis and Goal

N-[4-(methylsulfonyl)-2-nitrophenyl]-1,3-benzodioxol-5-amine (MSNBA) inhibits Glut5 function and fructose metabolism in a simulated small intestine environment.

The goal of this project was to obtain data on ways to treat or circumvent the effects of Hereditary Fructose Intolerance.

Background



Fructose

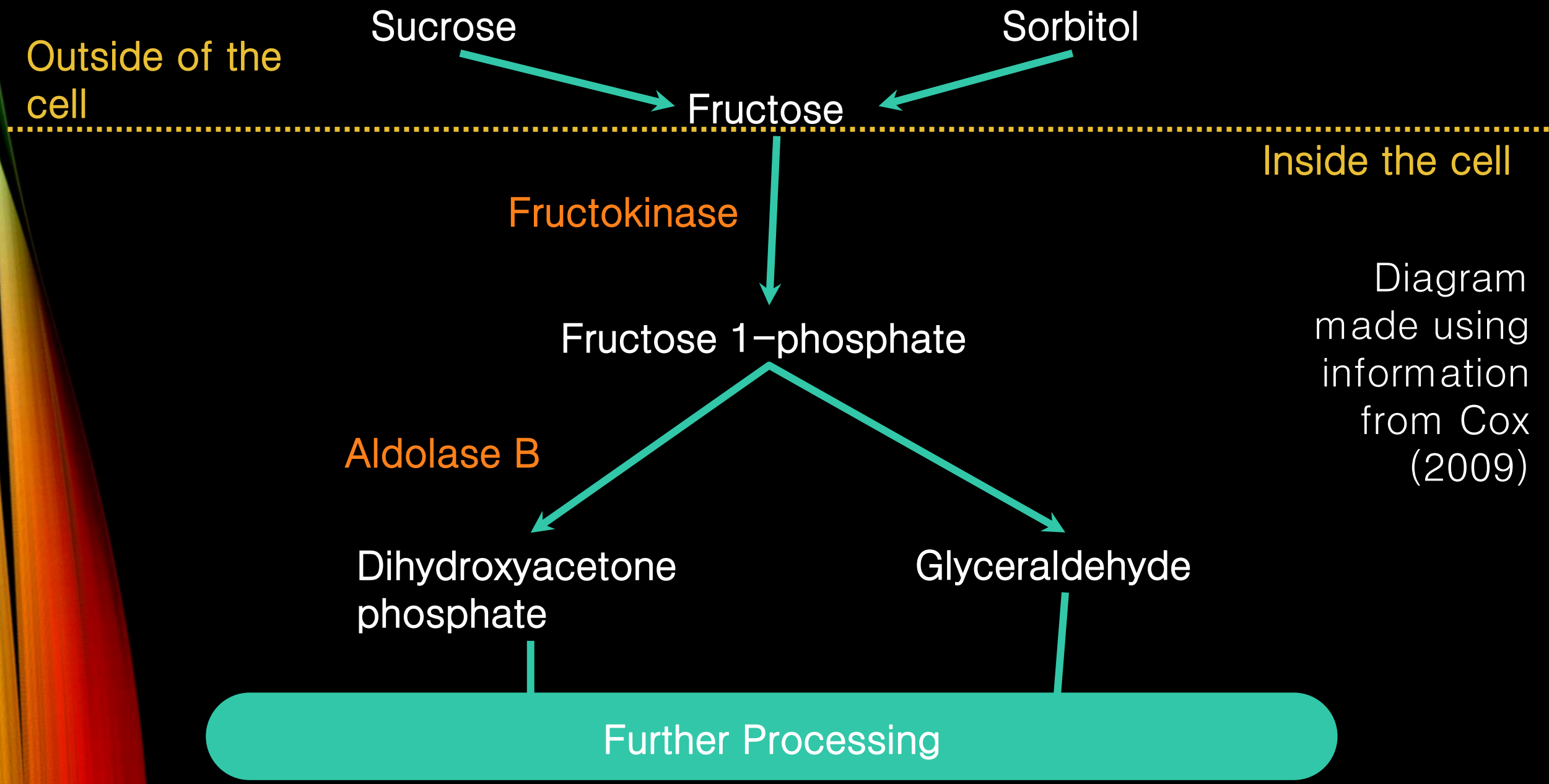
- Found in several foods
- In intravenous medications (Baker et al., 2015)

Background

Hereditary Fructose Intolerance (HFI)

(Baker et al., 2015)

- Occurs in 1 in 20,000 babies (Oppelt et al., 2015)
- Genetic disorder
- Defective Aldolase B
- Cannot process fructose, sucrose, and sorbitol
- No symptoms occur except when the above substances enter the body
- Has no cure


Sucrose
Sorbitol
Outside of the cell
Fructose
Inside the cell
Fructokinase
Fructose 1–phosphate
Diagram made using information from Cox (2009)
Aldolase B
Dihydroxyacetone phosphate
Glyceraldehyde
Further Processing

Background

The small intestine is essential for fructose metabolism (Jang et al., 2018).

Glut5 is a receptor on the outside of the cells (Ebert et al., 2017).

Glut5 moves fructose into the cells (Barone et al., 2009).

Attempts to inhibit Glut5 have been made.

Background

Plant Phytochemicals

- Nobiletin, epicatechin gallate, and tangeretin
- Inhibited fructose metabolism in Caco-2 cells (cells resembling small intestine)

(Satsu et al., 2018)

Plant Products

- Astragalin-6-glucoside inhibited Glut5
- Study focused on Glut1 and Glut5

(George Thompson et al., 2015)

MSNBA

- Picked out from a list
- Hinders only Glut5 function
- Hinders fructose metabolism in MCF7 cells (breast cancer)

(George Thompson et al., 2016)

Methods: Treatment

- FHs 74 Int cells (small intestine cells) from the American Type Culture Collection (ATCC)
- MSNBA concentrations of 50 µM, 100 µM, and 200 µM
- No fructose and 10 mM fructose solution
- 30 minutes, 2 hours, and 24 hours
- Two sets of plates for one experiment
- The entire experiment would be repeated two more times

Methods: Data Acquisition

Fructose Assay

- Fructose concentration test
- Use a kit from Sigma–Aldrich (Sigma–Aldrich Co. LLC, 2014)
- Find amount of product (Eilertsen & Schnell, 2018)
- Use fluorescence (Thermo Fisher Scientific Inc., n.d.)

Western Blotting

- Bands show how much Glut5 is there (Mahmood & Yang, 2012)
- MSNBA inhibits Glut5 competitively (George Thompson et al., 2016)
- Competitive inhibition (Bhagavan & Ha, 2015)

Project Progress

- Media was being made
- Purchased cells
- Purchasing materials for the media
- Planned to continue in the summer
- Project halted over COVID-19

Alterations – Fructose Dosing

- Designed with one concentration
- Different fructose doses
- No universal diet

Alterations – Mouse Model

Cell Lines

- Designed with cell line
  - Genetically modified (Kaur & Dufour, 2012)
- Glut5–deficient mice like in the study by Barone et al. (2009)

Small Intestine pH

- Designed for one pH range
- Mimic small intestine more closely
- pH range: 5.9–7.8 (Koziolek et al., 2015)

Microbes

- Sterile media
- Gut contains bacteria
- *Lactobacillus*, *Bifidobacterium*, and *Faecalibacterium* (Payne et al., 2012)

Conclusion

- Wanted to find ways to mitigate HFI caused by ingested fructose
- Designed an experiment involving the effects of MSNBA in the small intestine
- Started the experiment
- Could not complete the project due to COVID-19
- Possible alterations
  - Using different fructose doses
  - Using a mouse model

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