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## Glut5 and Fructose Metabolism Inhibition in a Simulated Small Intestine Environment

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**GLUT5 and Fructose Metabolism Inhibition in a Simulated Small Intestine Environment**

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HNRS 4990: Honors College Thesis

Dr. Huffman

May 25, 2020

### **Abstract**

This thesis describes an experiment that was designed in order to obtain information that could be useful for treating individuals with HFI. The author hypothesized that MSNBA would inhibit GLUT5 and fructose metabolism in the small intestine. The final experimental design involves exposing a small intestine cell line to fructose and MSNBA. The cell line would be exposed to MSNBA over the course of three different periods, and data was intended to be obtained using western blotting and a fructose assay kit. Although the final experimental design project was not fully carried out due to circumstances surrounding COVID-19, a plausible experimental design was produced. The experimental design has the potential to be modified in ways that could be used to test the effects of MSNBA with more informative results. The modifications could be used to plan a study that could obtain more useful data than the final experimental design described in this work.

*Keywords:* hereditary fructose intolerance, MSNBA, GLUT5, western blotting, fructose metabolism, fructose malabsorption, FHS 74 Int

## **GLUT5 and Fructose Metabolism Inhibition in a Simulated Small Intestine Environment**

This project was designed to obtain data regarding the inhibition of fructose metabolism in the small intestine, and the objective of this experiment was to study potential treatments for disorders like hereditary fructose intolerance (HFI).

### **Fructose Metabolism Disorders**

Unfortunately, not everyone can digest fructose. In fact, Tran (2017) has found that there are three types of disorders in fructose metabolism. These disorders are called essential fructosuria, fructose-1,6-bisphosphatase (FBPase) deficiency, and hereditary fructose intolerance. Essential fructosuria has no symptoms and is not usually treated. FBPase deficiency and HFI are potentially life-threatening disorders caused by exposure to fructose, sucrose, and/or sorbitol (Baker et al., 2015; Tran, 2017). FBPase and essential fructosuria will not be discussed further in this work.

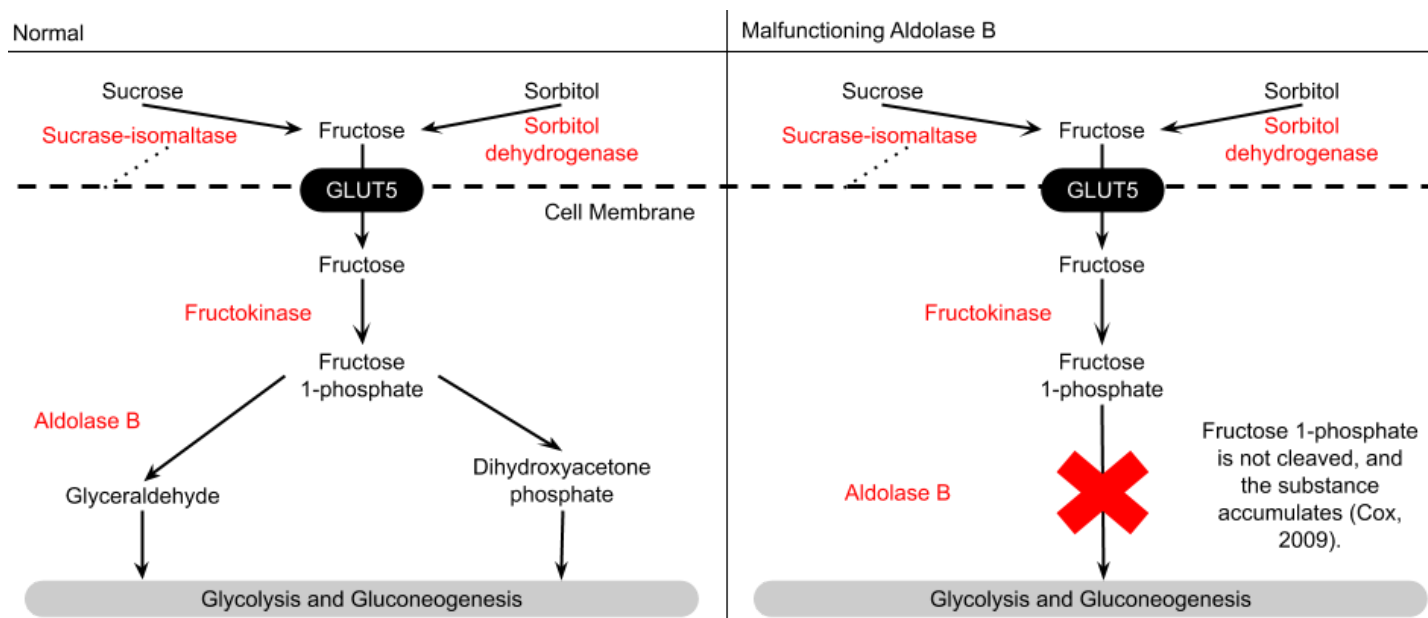
Potentially deadly disorders like HFI are concerning because fructose is so common. In fact, Baker et al. (2015) states that nuts, wax beans, green beans, potatoes, onions, spinach, cabbage, lettuce, asparagus, celery, and cauliflower are the few vegetables that people with HFI are recommended to eat because all other vegetables contain fructose. Additionally, fruits and foods with added sugars are considered prohibited in HFI dietary guidelines. Limitations regarding medication use are also present due to the presence of fructose in intravenous medications. Needless to say, fructose is quite prevalent in daily existence.

In addition to the hardships that can come with such dietary restrictions, HFI is a worrisome disorder because there is no cure for it (Baker et al., 2015). The fact that a disorder with a potentially heavy impact on people's lives has no cure interested the author, and further steps were taken to learn more about fructose metabolism.

### ***Hereditary Fructose Intolerance***

HFI is a genetic disorder that causes the production of a malfunctioning version of an enzyme called Aldolase B (Baker et al., 2015). According to Cox (2009), Aldolase B is essential for fructose metabolism, and it can be found in the kidneys, liver, upper small intestine, and the duodenum of the small intestine. Normally, ingested fructose is absorbed using facilitated diffusion by cells in the mucous membrane of the small intestine through GLUT5 and converted into fructose 1-phosphate by fructokinase. Next, Aldolase B cleaves fructose 1-phosphate into glyceraldehyde and dihydroxyacetone phosphate for further processing in glycolysis and gluconeogenesis. This mechanism, as illustrated in Figure 1 on the next page, primarily takes place in the small intestine, and excess fructose that cannot be chemically altered by the organ is transported to the liver through the hepatic portal vein (Jang et al., 2018).

Figure 1

**Fructose Metabolism Comparison**

*Note.* The information used to make this figure is from Cox (2009) and Gericke et al. (2017). The figure illustrates fructose metabolism in the small intestine when Aldolase B does and does not function properly. Sorbitol is converted into fructose by sorbitol dehydrogenase in the lumen (Cox, 2009). Sucrase-isomaltase—an enzyme usually embedded in the cell membrane of small intestine cells—hydrolyzes the  $\alpha$ -1,2-glucosidic bonds of sucrose to produce fructose (Gericke et al., 2017).

Cox (2009) states that fructose 1-phosphate accumulates in the body when Aldolase B is unable to cleave it. Aldolase A and phosphorylase A are competitively inhibited by fructose 1-phosphate, and this halts gluconeogenesis and glycolysis, respectively. A variety of detrimental effects also occur (Cox, 2009). One of these effects is a harsh drop in the amount of glucose in the blood (Tran, 2017). Baker et al. (2015) explain that HFI initially manifests with symptoms of nausea, abdominal distress, vomiting, and inability to grow. These symptoms tend to be experienced by infants starting to eat food containing fructose or sucrose; they can experience more serious clinical manifestations—seizures, lethargy, and/or progressive

coma—after eating a large amount of fructose. People who are continuously exposed to fructose, sucrose, and/or sorbitol can suffer from kidney damage and liver damage extensive enough to cause kidney failure and/or liver failure. The only way to avoid the symptoms of HFI is to abstain from foods and medications containing fructose, sucrose, and/or sorbitol. Due to dietary restrictions, individuals with HFI consume fewer varieties of vegetables and fruits; therefore, to prevent nutrient deficiencies, multivitamins must be used. For instance, supplements for vitamin C have been recommended (Guery et al., 2007). Those individuals affected by HFI also would need to abstain from foods containing polysorbate and sucralose (Baker et al., 2015).

### **GLUT5**

GLUT5 is a type of transporter belonging to a family of 14 glucose transporters (GLUT) (Douard & Ferraris, 2008), and isoform 1 of GLUT5 is 501 amino acids long (National Center for Biotechnology Information & U.S. National Library of Medicine, n.d.-a). GLUT5 is 54,973.98 Da (Swiss Institute of Bioinformatics, n.d.), which is around 54.97 kDa. This molecular weight was found by taking the amino acid sequence from the National Center for Biotechnology Information and the U.S. National Library of Medicine (n.d.-a) and inputting it into the ProtParam tool provided by the Swiss Institute of Bioinformatics (n.d.). Isoforms 2, 3, and 4 are 244, 457, and 354 amino acids long, respectively (National Center for Biotechnology Information & U.S. National Library of Medicine, n.d.-b, n.d.-c, n.d.-d). Isoforms 2, 3, and 4 are likely to be fragments because GLUT5 is 501 amino acids long in general (InterPro, n.d.).

GLUT5 (synonymous with Slc2a5) lines the small intestine to bring fructose into small intestine cells (Ebert et al., 2017). It is also found in smaller quantities in the skeletal muscle, brain, kidney, testes, and adipose tissue (Nomura et al., 2015). Despite the name of the family it

belongs to, GLUT5 is unable to transport glucose (Douard & Ferraris, 2008). GLUT5 has been found to be essential for fructose metabolism in mice because mice without the GLUT5 transporter experienced an inability to digest fructose (Barone et al., 2009).

GLUT5 has also been found to be important in cancer research. For instance, multiple types of cancer cells express a high amount of GLUT5 due to their need for a high amount of sugar (Nomura et al., 2015). In fact, George Thompson et al. (2016) writes that breast cancer cell lines MCF7 and MDA-MB-231 take in fructose and contain mRNA that encodes for GLUT5. In contrast, cells that make up breast tissue do not usually have GLUT5. Cancerous cells in the uterus, testis, colon, lung, liver, and brain have been shown to express high levels of GLUT5 as well (Douard & Ferraris, 2008).

### **GLUT5 Inhibition**

A few experiments studied chemicals that could hamper GLUT5. For instance, Caco-2 cells, cells resembling human intestinal epithelia, were exposed to three phytochemicals called nobiletin, epicatechin gallate (ECg), and tangeretin, and the treated cells absorbed fructose at a rate that was 60% of the untreated cells (Satsu et al., 2018). Another study found that astragalin-6-glucoside, a plant product, inhibited GLUT5 function without affecting GLUT1 (George Thompson et al., 2015). A third experiment found that N-[4-(methylsulfonyl)-2-nitrophenyl]-1,3-benzodioxol-5-amine (MSNBA) inhibited GLUT5 exclusively in MCF7 cells (George Thompson et al., 2016).

The author of this paper decided to work on an experimental design around MSNBA because the study from George Thompson et al. (2016) showed that MSNBA specifically affected GLUT5. In contrast, the study by George Thompson et al. (2015) focused on the effects that plant products had on GLUT1 and GLUT5 without investigating GLUT2, 3, and 4 to the



extent that the George Thompson (2016) study did. The Satsu et al. (2018) study, which concentrated on the effects of plant phytochemicals on nutrient uptake, also did not investigate GLUT2, 3 and 4 to the same degree as the George Thompson (2016) study.

### **Other Design Choices**

At first, the experimental design was concentrated on supplementing the small intestine with functional Aldolase B; however, this plan proved to not be feasible. Plans were then made for a project studying methods of halting fructose absorption.

### ***Two Designs***

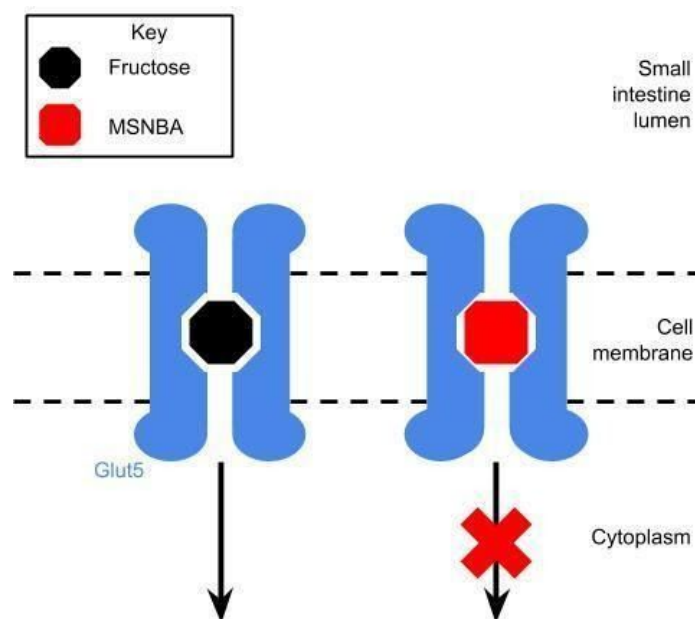
The hypothesis of the first experimental design was that supplementing the small intestine lumen with Aldolase B would increase the rate of fructose digestion in the cells. This design could not be carried out for multiple reasons. The main reason is that Aldolase B cleaves fructose 1-phosphate instead of fructose (Cox, 2009), so adding Aldolase B in a small intestine environment would not affect fructose digestion. Sibley and Lehninger's (1949) journal was the source for the assay described in the first design. However, their work investigated the concentration of the products made by the breakdown of fructose 1,6-bisphosphate in tissues, which are glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. Although Aldolase B reacts to fructose 1,6-bisphosphate and fructose 1-phosphate in equal rates (Cox, 2009), glyceraldehyde 3-phosphate and glyceraldehyde are not the same compounds, so the procedure written by Sibley and Lehninger (1949) would not have provided any data that could support or falsify the original hypothesis. More details about this initial design can be found in Appendix 1: Aldolase B in the Small Intestine.

After further literature research, the author altered the experiment's approach. The new procedure involved impairing the function of GLUT5. Because MSNBA was shown to

specifically prevent GLUT5 from importing fructose in breast cancer cells (George Thompson et al., 2016), the project's efforts went towards investigating MSNBA's effects on GLUT5 in small intestine cells. After all, the small intestine has conditions that differ from those of breast cancer cells. When the author created the second experimental design, no studies had been published containing information on MSNBA's effects on the small intestine, so plans for the project were formulated as soon as that information was known. It was hypothesized that MSNBA would halt fructose metabolism in small intestine cells by inhibiting the function of the GLUT5 transporter as demonstrated in Figure 2.

**Figure 2**

***MSNBA on GLUT5***



*Note.* This figure was created from information and illustrations in Nomura et al. (2015) and George Thompson et al. (2016). Because George Thompson et al. (2016) found that MSNBA inhibits GLUT5 competitively, the author of this project created the above figure to show how MSNBA has the potential to prevent facilitated diffusion of fructose via GLUT5 in the small intestine.

### ***Protein Analysis***

It was decided that the amount of GLUT5 proteins would be obtained via a process called western blotting instead of through any tests on mRNA translation. The study from George Thompson et al. (2016) showed that MSNBA competitively inhibits GLUT5. Specifically, the study showed that MSNBA directly affects the GLUT5 protein; however, it does not prevent the production of the transporter. According to Mahmood and Yang (2012), western blotting uses gel electrophoresis to separate proteins. The gel is stacked onto the membrane, which allows the proteins to move successfully onto the membrane. At this point, the proteins are gathered in the form of bands. Next, the membrane is first exposed to a primary antibody that binds to the protein of interest and is then exposed to a secondary antibody. Once the secondary antibody binds to the primary antibody, the band bound to the primary antibody is detectable (Thermo Fisher Scientific Inc., n.d.-b). After the band is detectable, one can ascertain how much protein is found in the sample based on how thick the band is (Mahmood & Yang, 2012).

### ***Fructose Kit***

The author planned to use a High Sensitivity Fructose Assay Kit from Sigma-Aldrich® to detect the amount of fructose in a sample of cells; using a kit would be less difficult than attempting to create a new method for investigating whether fructose was imported inside of the small intestine cells. The author planned to use a coupled enzyme assay kit (Sigma-Aldrich Co. LLC, 2014). As Eilertsen and Schnell (2018) explain, a coupled enzyme assay consists of two reactions; the first reaction uses an enzyme to convert the substrate into the first product. Another enzyme is then used to convert the first product into a second product. In the case of

this assay, the second product is fluorescent (Sigma-Aldrich Co. LLC, 2014), so it gives off light at a particular wavelength when it is exposed to light (Thermo Fisher Scientific Inc., n.d.-a).

### **Experimental Design**

This experiment could not be attempted due to circumstances described in the section, Experimental Attempt. Consequently, despite the author's efforts to provide exact details on how that experiment could be conducted, some methods of that experiment likely need to be modified. Without performing the experiment, there is no certain way of knowing what parts of the design, if any, should be altered and what the alterations would look like.

### ***Final Design***

**Cell Culture.** In the second experimental design, the author planned to use a simulated small intestine environment made by using Fhs 74 Int cells, a small intestine cell line from ATCC® and by following instructions posted at American Type Culture Collection (2019). According to manufacturer's instructions, FHs 74 Int cells would be grown and maintained in 100 mm cell culture plates in ATCC® Hybri-Care Medium supplemented with 30 ng/mL human epidermal growth factor (HEGF) and fetal bovine serum. The HEGF would be supplied from Gibco™. Based on a discussion with Gabriel Almeida Alves, a current PhD candidate in Biology at Western Michigan University, the author agreed that Gibco™ 100X Antibiotic-Antimicotic would also be supplemented in the growth media. 100 mm cell culture plates are estimated to contain  $5.5 \times 10^6$  cells (American Type Culture Collection, 2014), and this plate size would allow enough cells to grow to provide a sample for the fructose assay kit.

**Treatment.** Individual cell culture plates would be grouped according to the treatment they would receive. Because digestion is not instantaneous and occurs over time, periods of treatment would occur for 0 minutes, 30 minutes, 2 hours, or 24 hours. To investigate the effects

of MSNBA on fructose metabolism, FHs 74 Int cells in plates would be divided into groups based on exposure to either 0  $\mu\text{M}$ , 50  $\mu\text{M}$ , 100  $\mu\text{M}$ , or 200  $\mu\text{M}$  concentrations of MSNBA dissolved in dimethyl sulfoxide (DMSO) and to exposure to a fructose solution or a solution lacking fructose. The MSNBA would be supplied by Enamine, and the fructose solution would consist of 10 mM fructose dissolved in ATCC<sup>®</sup> Hybri-Care Medium without any additional supplements. The number of plates per group would have been decided based on the amount of supplies available. The treatments would have been administered to plates in the manner described in Table 1.

**Table 1**

***Volume of Treatment***

Solution	Treatment Group							
	TG 1	TG 2	TG 3	TG 4	TG 5	TG 6	TG 7	TG 8
50 $\mu\text{M}$ MSNBA	100	0	0	0	100	0	0	0
100 $\mu\text{M}$ MSNBA	0	100	0	0	0	100	0	0
200 $\mu\text{M}$ MSNBA	0	0	100	0	0	0	100	0
10 mM Fructose	200	200	200	200	0	0	0	0
Media without the Supplements	200	200	200	200	400	400	400	500

*Note.* Table 1 provides the volume of the treatment solution that would be added to each plate in an assigned treatment group of cell culture plates. The total volume for these treatment solutions would be 500  $\mu\text{L}$  each. All volumes in this table are in microliters. TG stands for “Treatment Group.” TG 4 was the control for the small intestine cells exposed to a fructose solution, and TG 8 was the control for all of the TGs.

The cycle of treatments would be repeated to obtain enough protein for the western blotting assay and enough of a supernatant for the fructose assay.

**Western Blotting.** The lysate would have been prepared according to instructions found in a tutorial created by NovusBiologicals (2012). As stated in the tutorial by NovusBiologicals (2012), the cells would be washed in ice-cold PBS twice, covered with lysis buffer, and scraped into a centrifuge tube in an ice bucket. The cells would be pipetted up and down, and the centrifuge tube would be left to rest in the ice bucket for 30 minutes. Once that incubation period is complete, the centrifuge tube would be centrifuged to produce a cell pellet and a supernatant, and the supernatant would be collected while the pellet would be discarded. The only modification to the steps of the tutorial would be that a CHAPS lysis buffer would be prepared using instructions from “CHAPS Lysis Buffer” (2014) instead of using a RIPA buffer mentioned in the tutorial provided by NovusBiologicals (2012). CHAPS would be used because it can separate membrane proteins from the membrane (Lorch & Batchelor, 2011).

10% mini polyacrylamide gels would be used because they can separate proteins weighing between 15-100 kDa (Abcam, n.d.). The mini polyacrylamide gels would be made using a protocol from Life Technologies Corporation (2014). The samples would be placed in the wells of mini polyvinylidene difluoride (PVDF) membranes with 0.45  $\mu\text{m}$  pores, which is the appropriate pore size for proteins larger than 20 kDa (Li-COR, 2020). Based on a discussion with Jaafar Hachem, a current PhD candidate in Chemistry at Western Michigan University, a 5% fat-free bovine serum albumin (BSA) solution would be used as the blocking buffer for the immunoblotting process, and a tris-buffered saline-Tween (TBST) solution would be used to wash the membrane.

A rabbit anti-human GLUT5 polyclonal antibody from Thermo Fisher Scientific Inc. (Catalog # PA5-82190) would be used for the primary antibody, and a goat anti-rabbit IgG (H+L) highly cross-adsorbed secondary antibody (Alexa Fluor Plus 488) (Catalog # A32731) from Thermo Fisher Scientific Inc. would also be used. The membrane would be incubated in a chemiluminescent reagent.

**Fructose Assay.** A High Sensitivity Fructose Assay Kit from Sigma-Aldrich® would be used to determine the extent to which fructose metabolism would have occurred. The kit would be used according to the protocol written by Sigma-Aldrich Co. LLC (2014). That protocol states that the end product of the coupled enzyme assay should give off light at 587 nm when exposed to light at a wavelength of 535 nm. The kit is capable of detecting as little as 20-100 pmole/well of D-fructose. Each sample would consist of 1-50  $\mu$ L of the supernatant from  $5 \times 10^6$  cells centrifuged at  $13,000 \times g$  and mixed in ice-cold phosphate-buffered saline at a pH of around 8, and the samples would be administered in wells of a 96 well flat-bottom plate. The concentration of the fructose would be calculated using equations listed in the Sigma-Aldrich Co. LLC (2014) protocol.

## **Experiment**

Once the final plans for the experiment were drafted, the author attempted to make a growth environment suitable for the small intestine cells. During this period, materials were being gathered and prepared for the cells. However, safety concerns related to the 2020 COVID-19 pandemic resulted in the cessation of further progress as the laboratory where the experiment was being conducted was eventually closed to students. Because the experiment was never completed, this section details what was accomplished. After this stage, supplies were being gathered, and manufacturers were being called to ask for help with some

last-minute questions. The ATCC<sup>®</sup> FHs 74 Int cells, ATCC<sup>®</sup> Hybri-Care Medium, Gibco<sup>™</sup> 100X Antibiotic-Antimicotic, and fetal bovine serum were acquired. The ATCC<sup>®</sup> Hybri-Care Medium was also reconstituted and filtered with a 0.22 µm filter.

Additionally, because the Gibco<sup>™</sup> HEGF was required, the author was about to purchase it, but could not successfully login to the vendor's website at the time. Consequently, a price quote for the item and an eventual purchase could not be made. Shortly after those events took place, the project was stopped because Western Michigan University ceased all in-person lectures and class laboratory activities. Due to the pandemic, it became clear that the likelihood of the author continuing to work on the project would be extremely low.

## **Discussion**

If this project is attempted in the future, modifications could be made to provide a more realistic model of the human body. The first modification would be to use different doses and concentrations of sugars. Additionally, an animal model could be used to achieve more comprehensive results than what could be achieved using a cell line.

### ***Modification 1***

This project was designed to use a 10 mM fructose solution. However, because individuals eat varying amounts of fructose, only so much information can be inferred from this project. Additionally, future attempts with MSNBA testing could be done with a mixture of fructose, sucrose, and/or sorbitol. A caveat to using different combinations and doses of sugar is that individuals eat different quantities of sugar worldwide, so figuring out how much sugar can or should be used to test the effectiveness of MSNBA could be difficult.

Rao et al. (2007) found that the uppermost limit for a single dose of fructose could be 50 g because healthy participants in their study who consumed that much fructose in one serving



exhaled more hydrogen than the baseline value. This result is a sign of fructose malabsorption. Ozaki et al. (2018) found that fructose that is not taken into the intestine is left to be fermented by bacteria into gases like hydrogen and short-chain fatty acids, and this hydrogen can enter the bloodstream and be exhaled out in measurable quantities. Fructose malabsorption can lead to fructose intolerance, which Ozaki et al. (2018) defines as a series of abdominal issues such as vomiting, gas, diarrhea, abdominal pain, abdominal distension, and nausea. Rao et al. (2007) found that among participants who drank a 10% (w/v) solution with 50 g of fructose, 69% experienced an increase in the exhalation of hydrogen, and portions of this group stated that they had such symptoms as gas (30%), diarrhea (15%), and abdominal pain (15%). The authors also found that a portion of healthy individuals experienced some of the symptoms that Ozaki et al. (2018) has noted to be indicative of fructose intolerance. Therefore, 50 g could be used as an upper benchmark for a single dose of fructose.

As for the lowest single dose of fructose, two things could be considered. First, data from Rao et al. (2007) indicated that healthy individuals who consumed 15 g of fructose did not experience any symptoms of fructose malabsorption or an increase in hydrogen exhalation. Additionally, a regular soft drink can contain approximately 20 g of fructose (Douard & Ferraris, 2013). Considering that Rao et al. (2007) found that a healthy individual does not experience malabsorption when consuming 15 g of fructose, 15 g could be categorized as a low benchmark for fructose dosing because that quantity can be surpassed with 1 soft drink.

### **Modification 2**

One of the major modifications to the project would be to use a mouse model instead of a cell line model. If *in vitro* tests show promising results, then *in vivo* studies could be conducted using, for example, GLUT5 knockout mice similar to what Barone et al. (2009) used in their

GLUT5 research. To have a more detailed idea of the extent to which MSNBA inhibits GLUT5 metabolism, comparisons could be done between 1) GLUT5 knockout mice with MSNBA administered to them, 2) GLUT5 knockout mice with no MSNBA administered to them, 3) wild-type mice with MSNBA administered to them, and 4) wild-type mice with no MSNBA administered to them. Further data could be acquired with Aldolase-B knockout mice, which have been found to experience effects similar to humans affected by HFI (Oppelt et al., 2015).

The use of a mouse model would incorporate certain variables that the use of a cell model would not account for. For instance, the pH of the small intestine has been found to vary from a range of 5.9—6.3, in the beginning of the organ, to a range of 7.4—7.8, towards the end of the organ (Koziolek et al., 2015).

The current cell model design does not completely replicate the conditions of the human small intestine, which is why a mouse model could provide more pertinent data for a project of this nature. Compared to the media used to grow the cells in the current design, the human small intestine, as Koziolek et al. (2015) note, has a fairly wide pH range throughout the organ. When ATCC® Hybri-Care Medium is prepared as per the instructions of American Type Culture Collection (2018), the pH range should be 7.0-7.4. Because the pH range of the growth media matches that of only a portion of the small intestine, a mouse model would likely provide more information about how MSNBA would affect the small intestine.

A mouse model could also provide a microbiome that would produce a more accurate representation of the interstitial fluid to which the MSNBA and small intestine epithelium would be subjected. A microbiome is crucial because MSNBA has the potential to cause fructose malabsorption. Due to MSNBA's properties as a competitive inhibitor (George Thompson et al., 2016), inhibiting the function of GLUT5 could cause fructose to stay in the lumen of the small

intestine to ferment. After all, the gut is far from sterile, and Payne et al. (2012) notes that the gut contains *Lactobacillus*, *Bifidobacterium*, and *Faecalibacterium*, which are all bacteria capable of processing fructose. Since the fermentation of fructose can lead to symptoms of fructose malabsorption and intolerance (Ozaki et al., 2018), some considerations might need to be made if future evidence supports the idea that the substance could be used as a drug for those with HFI. Specifically, the patient's level of comfort should be accounted for in case symptoms of fructose malabsorption and intolerance manifest.

### **Conclusion**

This experiment was designed in order to obtain information that could be useful for treating individuals with HFI. It was hypothesized that MSNBA would inhibit GLUT5 and fructose metabolism in the small intestine. Although this project was not fully carried out, a plausible experimental design was produced. Had this experiment been attempted, the design could have been modified to test the effects of MSNBA, which could have provided more informative results. These alterations could have been used to plan a study that could obtain more useful data than the final experimental design described in this work.

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## **Appendix 1: Aldolase B in the Small Intestine**

This portion of the work details the initial design for this project, and it was not implemented because it proved to not be feasible. The exact details for the design such as the names of the products that would have been purchased are not provided because the author realized that the design was not feasible during the process of organizing its implementation.

### **Cell Culture**

The cell culture methods for Aldolase B in the Small Intestine would be the same as the methods used in GLUT5 and Fructose Metabolism Inhibition in a Simulated Small Intestine Environment.

### **Treatment**

Individual cell culture plates would be exposed to Aldolase B and fructose. One group of plates would be exposed to fructose without any Aldolase B. Another group of plates would be exposed to Aldolase B without being exposed to fructose. A final group of plates would be exposed to Aldolase B and fructose. The details for this section were not completed because the author learned while attempting to make plans for this design that this method would not answer the first hypothesis.

### **Assay**

The data would be gathered using an assay that would require the use of methods written by Sibley and Lehninger (1949), which would require the use of a photoelectric colorimeter. As per the procedure written by Sibley and Lehninger (1949), the sample would be mixed with 0.25 mL of fructose 1,6-bisphosphate and 0.25 mL of a hydrazine solution in a test-tube. The test-tube would also contain 1 mL of 0.1 M tris(hydroxymethyl)aminomethane buffer and would be filled with enough deionized water to bring the total test tube volume to 2.5

mL of solution. The test-tube would be placed in a water bath set at 38°C for 30 minutes. To stop the chemical reaction of the sample, 2 mL of 10% trichloroacetic acid would be used. A blank test tube would be prepared for each sample by repeating the preparations described above without adding fructose 1,6-bisphosphate until after the reaction.

Continuing to pattern the methods developed by Sibley and Lehninger (1949) all test tubes would be centrifuged, and 1 mL of the supernatant for each tube would be placed in colorimeter tubes in order to obtain a reading. The colorimetric tubes would be placed in a 38°C water bath for 10 minutes after 1 mL of 2,4-dinitrophenylhydrazine solution is added to it. The colorimeter would be set at 100% for its transmission when the experimental sample is measured, and a standard curve would be generated to show how the percent transmission is affected by the amount of fructose 1,6-bisphosphate that is split. The color released has a maximum absorption of 540 nm. The enzyme concentration should be calculated based on the fact that the concentration is proportional to the intensity of the color.