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Testing Lansoprazole on Human Gut Microbiota using a Gastroesophageal Reflux Model *In Vitro*

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ABSTRACT

One in five Americans experience Gastroesophageal Reflux Disease, but the disease has multiple treatments, such as Proton Pump Inhibitors (PPI). However, the effects of PPIs may induce more harmful effects on the gut microbiome, and less treatable symptoms such as inflammatory bowel disease, diabetes, obesity, and even cancer may occur. PPIs reduce acid secretion within the stomach and reduce stomach acid in the esophagus. The side effects of PPIs on the gut microbiome have yet to be researched extensively. This study suggests that Lansoprazole, a PPI, may cause significant changes in gut bacteria growth. An adapted method of *in vitro* digestion modeled the digestive systems of a human. Solutions replicating the environment of the mouth, stomach, and intestines were combined with a human dosage of Lansoprazole, and the pH was adjusted at different segments to model the low pH environments of the stomach and the neutral pH of the intestines. The respective solutions were then exposed to gut bacteria, *E. coli*, and *S. epidermidis*. These gut bacteria represent specific bacteria species within the intestinal tract. Data indicated that *E. coli* grew approximately 50% as much as the control at 20-minutes and less than 25% as much as the control at 40-minutes. *S. epidermidis* grew significantly less than the control, with approximately 60% as much at 20-minutes and approximately 15% as much at 40-minutes. In conclusion, studies indicate Lansoprazole treats GERD, but side effects from consuming the drug may cause gut dysbiosis, which may lead to the side effects previously mentioned.

Introduction

Gastroesophageal Reflux (GERD) is a chronic disease resulting in stomach acid (hydrochloric acid) flowing into the esophagus. It is very common to experience acid reflux as much as a few times a month, but chronic acid reflux (more than once a week) indicates the onset of GERD (Thrift et al., 2013). Acid reflux is caused when the lower esophageal sphincter does not relax after eating, allowing acid to flow back into the esophagus (Figure 1). A sphincter is a ring of muscles that serves as a barrier between two body parts. Sphincters stay contracted or closed unless relaxed, such as when a person eats. The lower esophageal sphincter is the barrier between the esophagus and the stomach that blocks stomach acid from flowing into the esophagus. However, the esophageal side of the sphincter has similar properties to the esophagus, and the gastric side of the sphincter can be exposed to the stomach's acidity (Rosen & Winters, 2022). Stomach acid in the esophagus irritates the mucus lining and leads to symptoms such as heartburn and chest pain because the esophagus is not protected by acid exposure (Clarrett & Hachem., 2018). Currently, there are over-the-counter and prescription medications that can treat GERD, such as Proton Pump Inhibitors. This study looks at how one of those treatments, Lansoprazole, affects the human gut microbiota and understands whether or not the medication will result in stable or dysfunctional gut bacterial growth.

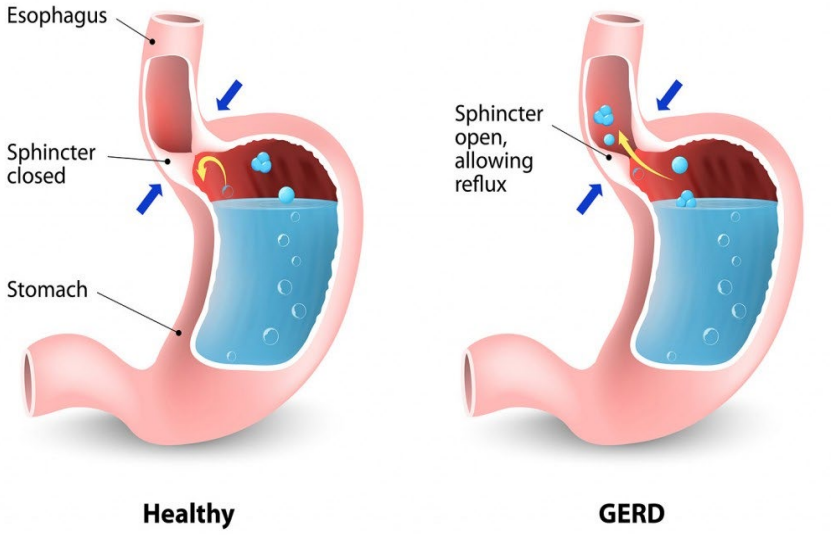

***Figure 1*:** The distinction between a healthy stomach and esophagus versus a stomach and esophagus in a patient with GERD. The open sphincter results in acid flowing up the esophagus and damaging the esophageal mucus lining. *Image from* Gastrohealth.com in an article titled "Gastroesophageal Reflux Disease – A Clinical Discussion on the Pathophysiology, Symptoms, Diagnosis and Treatment"

Proton pumps are a gastric enzyme called Hydrogen Potassium ATPase (H+/K+-ATPase) and are one of the final enzymes that function to secrete acid into the stomach (Shin et al., 2008). The acid secretion into the stomach lowers the pH and creates a stable gastric environment for other enzymes like pepsin that break down foods. Proton Pump Inhibitors (PPI) are a class of medication that reduces acid secretion. Proton Pumps Inhibitors block the gastric Hydrogen Potassium ATPase proton pump, resulting in less acid secretion into the stomach. Antacids, H2RAs, and PPIs are all used to help reduce acid secretion, but currently, PPIs are more effective because they maintain stable and predictable pH control. PPIs are also recommended over other options due to their results in treating GERD and repairing peptic ulcers (Clarrett & Hachem., 2018). Peptic ulcers are bumps in the esophageal lining due to gastric acid damage. Lansoprazole is a common Proton Pump Inhibitor that can help with prolonged acid reflux, a symptom of GERD. However, Lansoprazole is being used as opposed to other PPIs because it has 26% negative reviews and a list of side effects (Sinha, 2023). Additionally, studies show that other PPIs may be more efficient at treating GERD than Lansoprazole (Javed et al., 2020). This means that there may be a factor to Lansoprazole that makes it have negative reviews, and the effect on the human gut microbiota may be causing those side effects.

Human gut microbiota are microorganisms that exist within the human digestive tract. They are a necessary part of humans as it provides immune response and metabolic traits (Afzaal et al., 2022). The growth of certain gut microbiota can result in a healthy organism. Some gut bacteria are necessary for non-digestible foods such as fibers (Valdes, 2018). These bacteria produce enzymes that break down the carbohydrates into simple sugars, then turn the sugars into fatty acids that human cells can absorb (Inman, 2011). Other gut bacteria are necessary for immune response because the bacteria produce antiviral proteins. These are proteins that kill off viruses, thus providing a non-infected organism (Maldonado-Contreras, 2021). An unnatural gut bacterial growth is known as gut dysbiosis, which leads to harmful gastrointestinal disorders (Wei et al., 2021). *Escherichia coli* and *Staphylococcus epidermidis* were used in this study because they are very common bacteria in the digestive tract and provide an effect on two widely different bacteria. *E. coli* is located in the large intestine of humans (Katouli, 2010) and is used to break down food within the digestive tract (Ben-Joseph, 2022). On the other hand, *S. epidermidis* is used to fight off pathogens that enter the system but is also part of the Gastrointestinal tract (Akinkunmi et al., 2014). The two bacteria represent a

broad spectrum of gut microbiota. *E. coli* is a gram-negative bacterium, which means that it has a thin cell wall (Lim et al., 2010), and *S. epidermidis* is a gram-positive bacterium, meaning it has a thick cell wall (Namvar et al., 2014). A thicker cell wall allows bacteria to retain water; however, a thin cell wall in gram-negative bacteria allows the bacteria to grow and be hosted easier in the digestive tract (Mitchell, 2020). This may cause *S. epidermidis*, the gram-positive bacteria, to have more variance in growth due to not being hosted as well within the digestive tract.

In vitro digestion is a method that replicates the digestive tract using laboratory materials rather than a model organism. Using digestive procedures when studying the gut microbiome allows for consumables to be exposed to the environment of the digestive tract before being exposed to the gut microbiome, similar to what occurs in a human. *In vitro* is experimentation done outside any living organisms. A static *in vitro* digestion model uses a variety of solutions that model the environment of the mouth, esophagus, stomach, and intestines. A static digestion model indicates that each portion of digestion is simulated individually. There is no engineering adaptation to replicate the flow of enzyme secretion and gasses into the system (Lee et al., 2018); however, other methods are used in order to model enzyme interaction and pH change. Static digestion can be better than other dynamic digestion methods because it involves a simpler procedure that can be adapted to meet experimental needs. To model the esophagus of patients with GERD, the *in vitro* model was initially set to a pH of 4.0. Solutions that model the chemical and enzyme environment of the digestive system were combined with Lansoprazole in order to replicate a human consuming medication. *In vitro* digestion can be better than using the digestive system inside an animal model because there is reduced access to the gut of an animal model unless invasive methods are used (Biagini et al., 2018).

Methods

Bacteria Culturing

E. coli and *S. epidermidis* are both cultured in a nutrient broth. The nutrient broth is a culture media that includes beef extract and peptones, which provide vital compounds to microorganisms, such as amino acids and carbon (Liofilchem, 2017). To combine the bacteria with the nutrient broth, sterile inoculation loops swiped the bacterial slant 4-6 times, then were mixed in the nutrient broth flask (*Figure 2)*. Once inoculated, the broth is placed in a water bath shaker (New Brunswick Co.) and set to 135 rotations per minute at 37℃ for 24 hours. 37℃ is the ideal temperature for *E. coli* growth (Ferrer et al., 2003) because it represents the internal temperatures of humans and, thus, the environmental temperature of the gut microbiome. The water bath shaker is used in order to mix the bacteria with the broth evenly in order to give the bacteria nutrients to feed off of and allow them to grow. During experimentation, bacteria were used within two days of the first inoculation in the broth in order to maintain a stable level of nutrients for the bacteria.

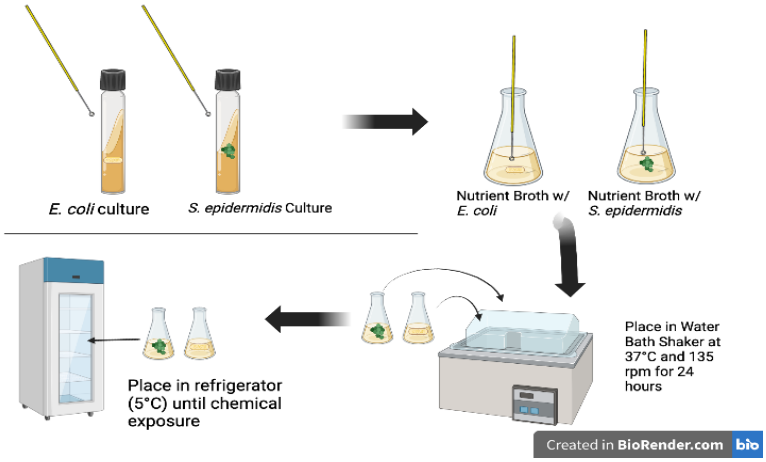

Figure 2: Inoculation procedure representation, which prepares gut bacteria for Lansoprazole exposure. *Image made on* BioRender.com

In Vitro Digestion (Adapted from Brodkorb et al., 2019)

In vitro digestion is done by simulating different parts of the human digestion system using a series of solutions and enzymes and completing numerous pH changes during the process to represent the environment of the digestive system *(Figure 3)*.

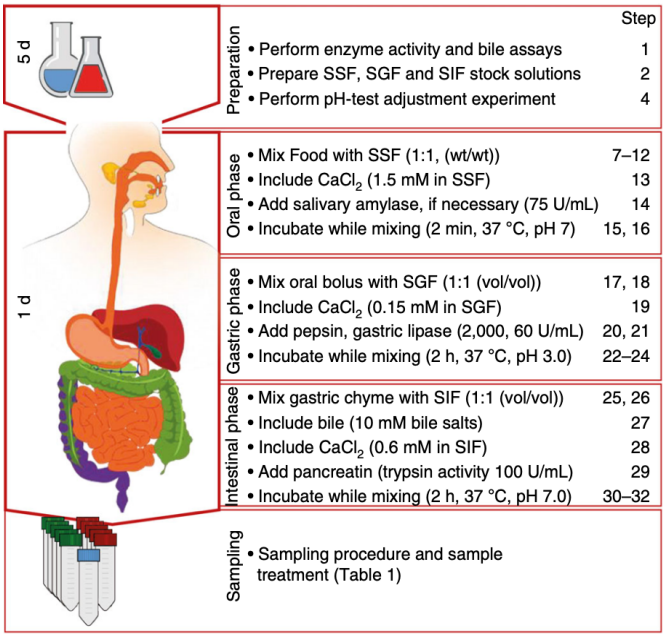

Figure 3: The breakdown of the different stages and preparations of the static *in vitro* digestion method. Image from *Brodkorb et al., 2019.*

Human digestion consists of 3 stages, esophageal stage, stomach stage, and intestinal stage. To start human digestion, nutrients are consumed and broken down by the teeth and salivary enzymes. Then, the ground food passes down the esophagus. The esophagus consists of a muscle system that contracts and pushes food down. Then the food enters the stomach, where stomach acid further breaks down any food into smaller and smaller pieces. Stomach enzymes such as pepsin flow into the stomach and aid in breaking down harder-digested consumables such as proteins. Pepsin turns these proteins into simpler amino acids so that the small intestines absorb the nutrients later in the digestive tract (Heda et al., 2022). Following the stomach, food passes into the small intestine, where pancreatic and liver juices break down other types of difficult-digested foods. The pancreatic enzymes, such as lipase, break down fats. Protease is another enzyme that is from the pancreas that breaks down additional proteins, and then amylase breaks down starches into simpler sugars (Johns Hopkins Medicine). Liver juices such as bile aid in these processes as well. As nutrients pass through the small intestines, fats, carbohydrates, vitamins, protein, and water are absorbed by the small intestines. Those absorbed nutrients then go on to give energy and nutrients to the body and cells (National Cancer Institute). Any food that remains after absorption in the small intestine flows into the large intestine. Within the large intestine, more nutrients and water is absorbed, and any substances left are formed into bowel movement and released through the anus (Gibson et al., 1996).

Digestion can be modeled *in vitro* by replicating different structures of the digestive tract and different electrolyte fluids of the digestive tract. There are three stages to the *in vitro* procedure: the esophageal phase, the gastric phase (also known as the stomach phase), and the intestinal phase.

Table 3 | Volumes of electrolyte stock solutions of digestion fluids for a volume of 400 mL diluted with water (1.25× concentrations)

			SSF (pH 7)		SGF (pH 3)		SIF (pH 7)	
Salt solution added	Stock concentrations		Milliliters of stock added to prepare 0.4 L (1.25×)	Final salt concentration in SSF	Milliliters of stock added to prepare 0.4 L (1.25×)	Final salt concentration in SGF	Milliliters of stock added to prepare 0.4 L (1.25×)	Final salt concentration in SIF
	(g/L)	(M)	(mL)	(mM)	(mL)	(mM)	(mL)	(mM)
KCl	37.3	0.5	15.1	15.1	6.9	6.9	6.8	6.8
KH_2PO_4	68	0.5	3.7	3.7	0.9	0.9	0.8	0.8
$NaHCO_3$[a]	84	1	6.8	13.6	12.5	25	42.5	85
NaCl	117	2	-	-	11.8	47.2	9.6	38.4
$MgCl_2(H_2O)_6$	30.5	0.15	0.5	0.15	0.4	0.12	1.1	0.33
$(NH_4)_2CO_3$*	48	0.5	0.06	0.06	0.5	0.5	-	-
HCl		6	0.09	1.1	1.3	15.6	0.7	8.4
$CaCl_2(H_2O)_2$[b]	44.1	0.3	0.025	1.5	0.005	0.15	0.04	0.6

[a]The use of carbonate salts in the electrolyte solutions requires the use of sealed containers with limited headspace, see also CRITICAL STEP in Step 24. [b]$CaCl_2(H_2O)_2$ should be added immediately before use. Volumes in Table 2 are indicated for a typical experiment of 5 mL of SSF.

Figure 4: The different simulated digestion fluids that are made by combining multiple electrolyte stock solutions. *Image from* Brodkorb et al., 2019.

During the esophageal phase, a simulated salivary fluid (Figure 4) is combined with the chemical Lansoprazole and 5mL of DI water or with 5 mL of DI water and no chemical, depending on whether it was an experimental group or control group, respectively. The solutions were then set to a pH of 4 by adding small quantities of hydrochloric acid in order to model the relative pH of the esophagus in a person with gastroesophageal reflux (Tutuian & Castell, 2006). The resulting mixture is then put in a water bath shaker at 37°C for 2 minutes. This shaker models the movement in the digestive system due to muscular contraction and external physical movements in humans (e.x. Walking, jumping, etc). The temperature used in the water bath shaker is the same as the internal temperature of humans.

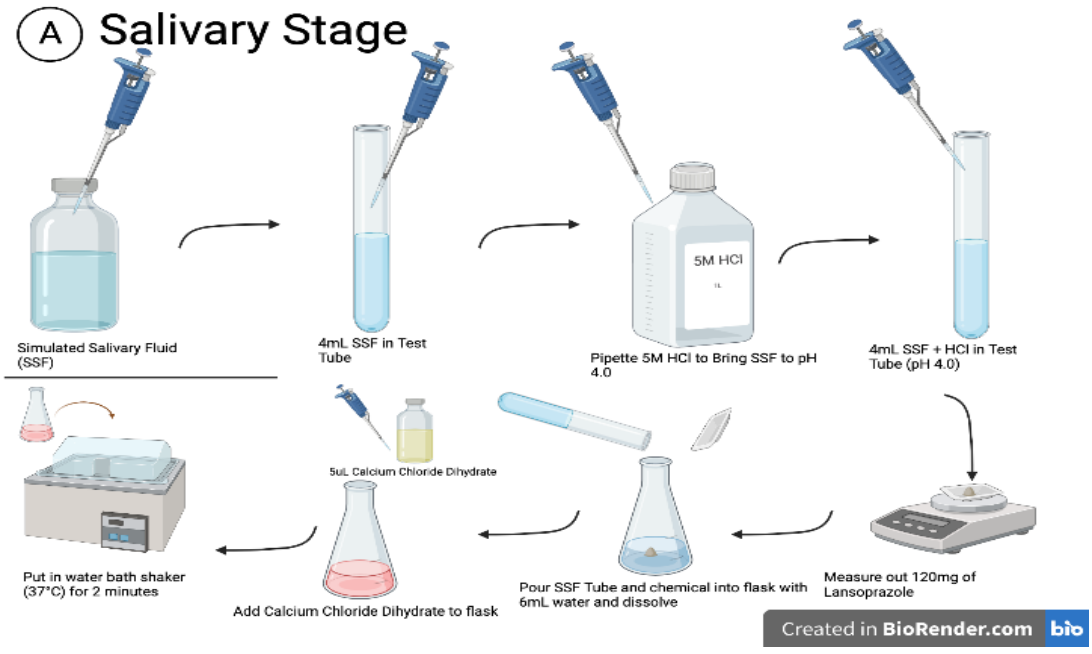

Figure 5: The steps taken to complete the salivary stage of *in vitro* digestion and the materials used during the procedure. *Image made from* BioRender.com

After, the mixture is removed from the water bath shaker and combined with the simulated gastric fluid. The gastric stage uses a similar set of electrolyte solutions in order to model the environment of the stomach *(Figure 4)*. In 200 microliter increments, Hydrochloric acid is added to bring the solution to a pH of 3. Pepsin is then added to the solution. Pepsin is an enzyme in the stomach that digests protein which will be used to keep the procedure controlled without determining if the enzyme has any effect on the gut microbiome. The enzyme used in this study was derived from a porcine mucosa which means it was taken from the stomach lining of a pig. After the enzyme is added, the solution is placed in a water bath shaker set to 37℃ and shaken at 135 rotations per minute for 2 hours to replicate gastric movement.

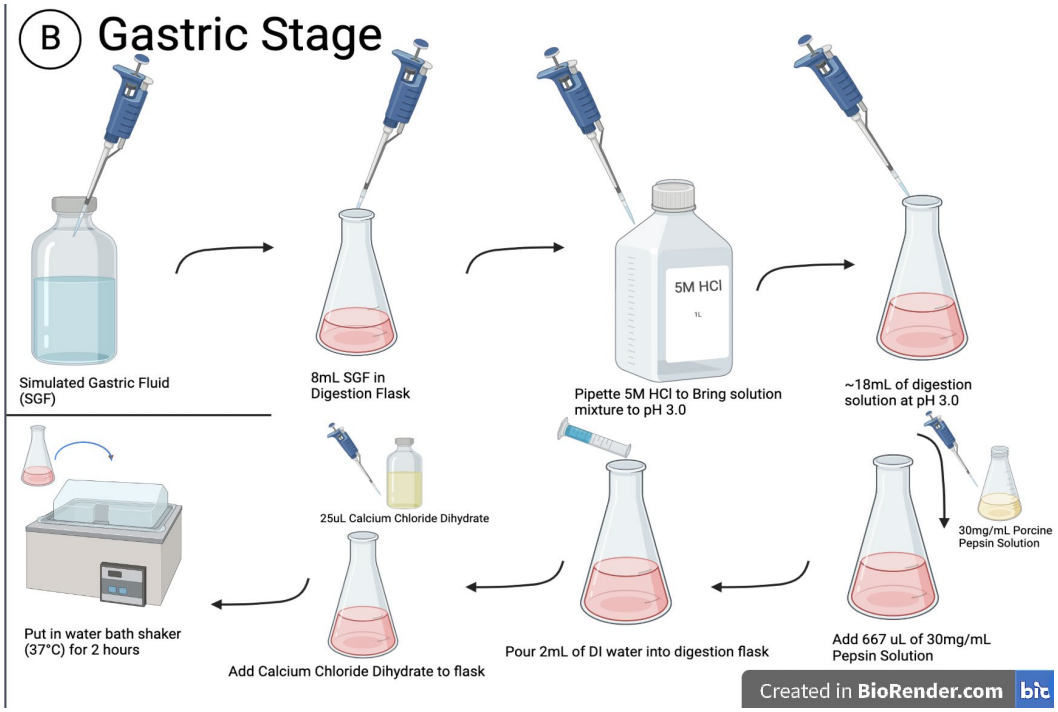

Figure 6: The steps taken to complete the gastric stage of *in vitro* digestion. *Image made from* BioRender.com

Following the two hours in the water bath shaker, the intestinal phase commences by combining the gastric chyme (result of the gastric stage) with the simulated intestinal fluid (*Figure 4)*. Then, pancreatin is added, which is an enzyme in the small intestine that helps with additional protein breakdown. There was no protein in this study, however similar to pepsin, pancreatin is added in order to keep enzymatic activity controlled. Pancreatin used in the procedure was derived from the pancreas of a pig, but it modeled a similar structure to the enzyme within humans. Once added, the solution is placed in the water bath shaker at 37 degrees Celsius for 2 hours. The resulting solution can now be combined with bacteria in order to expose the proton pump inhibitor to the gut bacteria.

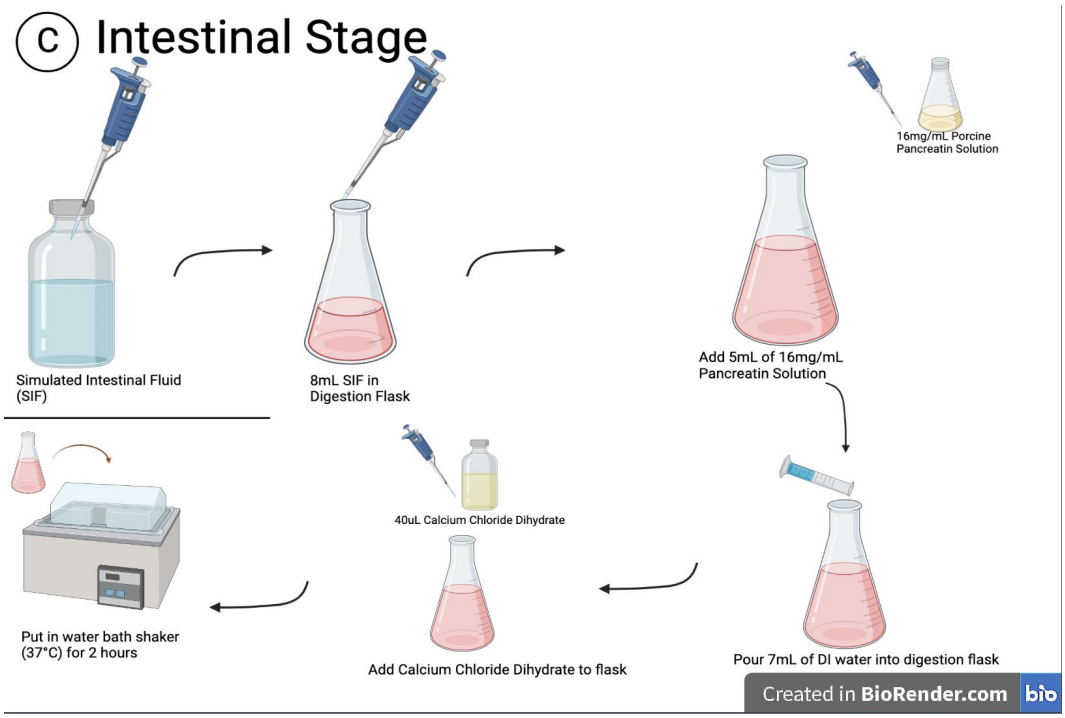

Figure 7: The steps taken to complete the gastric stage of *in vitro* digestion. *Image made from* BioRender.**com**

Lansoprazole Concentration

The dosage of Lansoprazole will replicate a human dosage. The human dosage of Lansoprazole is 30mg/day (Cunha, 2021), and in this experiment, relation to humans in as many ways possible is the goal. The human dosage is being used in order to be in parallel to the human digestive *in vitro* procedure. Being able to use a human dosage allows a conclusion based on a realistic dosage rather than a serial dilution to the weight of another organism. The result of the digestion with the chemical will be exposed at an amount of solution that would contain 30mg of Lansoprazole.

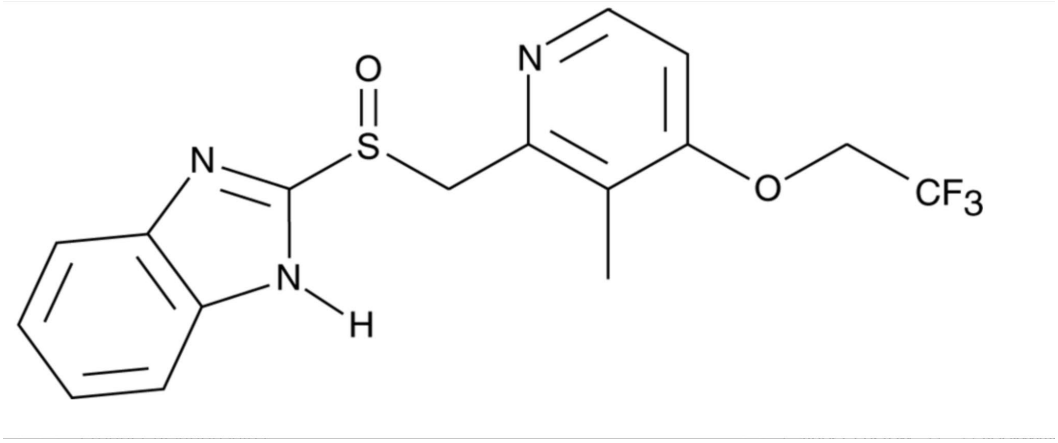

Figure 8: The chemical structure of Lansoprazole. *Image from* Cayman Chemical.

Spectrophotometry

A spectrophotometer determines how much light is absorbed by a solution, and that determines the liquid's optical density or concentration of bacteria. Different colors of light are represented at different wavelengths. In a spectrophotometer, a specific wavelength of light is shot through the system, then comes into contact with the solution. The amount of light absorbed by the solution determines the optical density. In this study, the optical density was calibrated around nutrient broth because that was the base solution. When bacteria were added to the nutrient broth (Figure 9), higher optical densities appeared because the bacteria in the broth made a cloudier solution which caused more light to be absorbed. Optical density was tested at a wavelength of 399 nm because when comparing the optical density from wavelengths of 300 nm to 800 nm, the peak of the optical density was always between 398.5 nm and 399.3 nm. Additionally, when comparing experimental and control groups, the most variance in optical density was around 399 nm which allowed for more significance and clarity on the growth of bacteria. The optical densities were recorded in triplicate samples, meaning for each flask of bacteria, three samples were recorded for optical density, and then the average was calculated. The bacteria and broth were always reshaken manually (by swirling a flask), with the water bath shaker, or with a vortex mixer before recording optical density in order to homogenize the microorganisms within all the broth. The growth of bacteria was found by comparing the control group, with water, to the experimental group with the proton pump inhibitor. The results were represented by considering the control group as healthy growth. The control group's growth was set to 100% because it was 100% of what was considered healthy. Any variance in healthy growth was set to a higher or lower percentage representing how much difference there was in the percent growth between groups. For example, if an experimental group grew 50% less than the control group, the control group was represented as 100%, and the experimental group was represented as 50%. Three-time intervals were taken: the initial absorbance, 20-minute absorbance, and 40 min absorbance. The data will then be compared with a graph showing the percent change from the control.

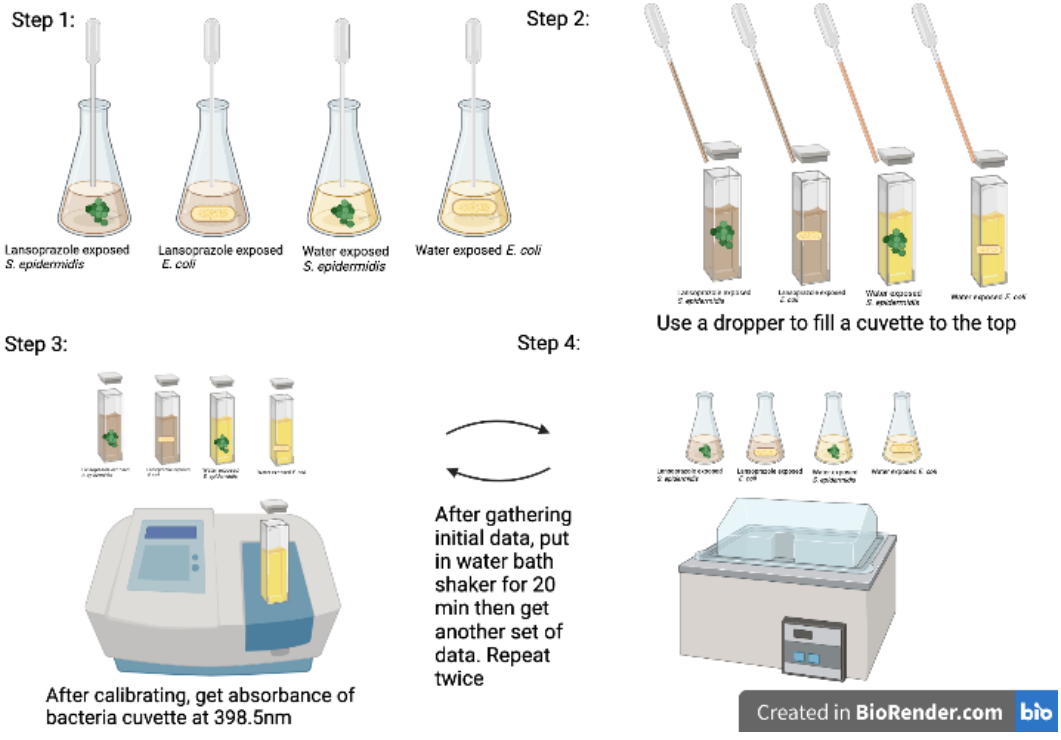

Figure 9: The steps taken for data collection by calculating the absorbance (optical density) using a spectrophotometer. *Image from* BioRender.com

Statistical Analysis

A statistical analysis was conducted after completing data collection. In this study specifically, an Anova test built by Navendu Vasavada (astatsa.com) was conducted, then further a Tukey test was calculated to compare experimental groups.

An ANOVA test compares the means within each group and then determines whether a relationship exists. The test analyzes the variance between the data points, the means, and the means of the other experimental groups.

Then, a Tukey test quantifies how significant the relationship between each group is. It determines a p-value that is based on how likely the relationship between groups is due to chance rather than due to a significant difference. A p-value of less than 0.05, or 5%, states that more than 95% of the time the results presented are not due to chance. Any p-value less than 0.05 is considered statistically significant, and additionally p-values less than 0.01 are considered even more statistically significant because it states that less than 1% of the time your results are due to chance.

Results

To represent the results accurately, the Lansoprazole exposed groups were presented as a percentage of the control. The control was the water-exposed bacteria which represents the natural growth of gut bacteria. The control group replicated no external effect on the bacteria other than the effect of water. For clarity, the control group was represented as 100% growth, and any other groups that differed from the control would be represented with a larger or smaller value based on how much more or less growth it had compared to the control.

Additionally, in each graph, error bars were represented by plus and minus double the standard error of the mean (± 2SEM). This was held constant throughout every trial and group in order to represent the significance and overlap between the control and the experimental groups better. When using double the standard error of the mean, the significance would usually correlate with whether or not the error bars overlap. However, in order to confirm statistical significance, a one-way ANOVA statistical test was used, then additionally, a Tukey test was conducted. As explained in the methods, these tests determine if a relationship exists between two groups based on the variance within the standard error of the mean and whether the relationship is significant or entirely due to chance.

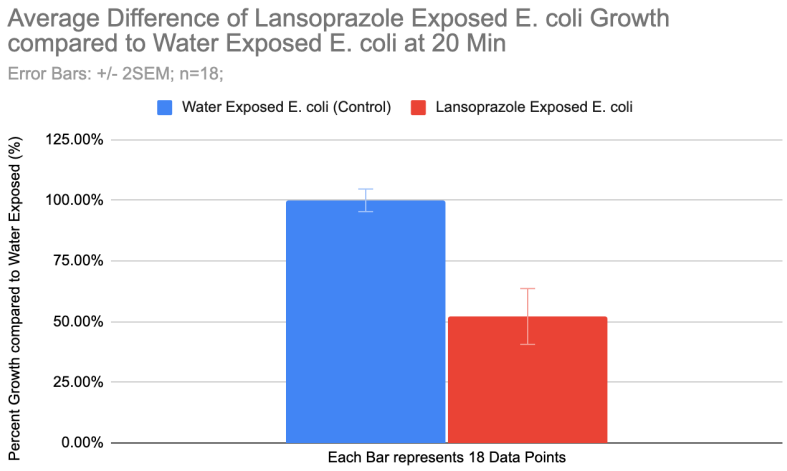

Figure 10: Graphical representations of the difference in the growth of *E. coli* exposed to Lansoprazole, and the growth of *E. coli* exposed to just water (control) at the 20-minute time interval. *Graph made from* Google Sheets.

Figure 10 represents the growth between *E. coli* exposed to Lansoprazole and the control at the 20-minute increment. At this increment, the growth of the PPI exposed *E. coli* was 52.14% as much as the control which means it grew 47.86% less than what was determined to be normal growth. The error bar on the control group remained small as data didn't vary as much throughout each data point. This suggests that the growth stayed constant throughout the entire bacteria culture. Conversely, the Lansoprazole exposed *E. coli* had an error bar value ranging ± 11% meaning there was less consistency between each data point and each culture. The error bars still did not overlap and an additional Anova test was conducted to determine how likely the results were due to chance, which is explained after all graphs.

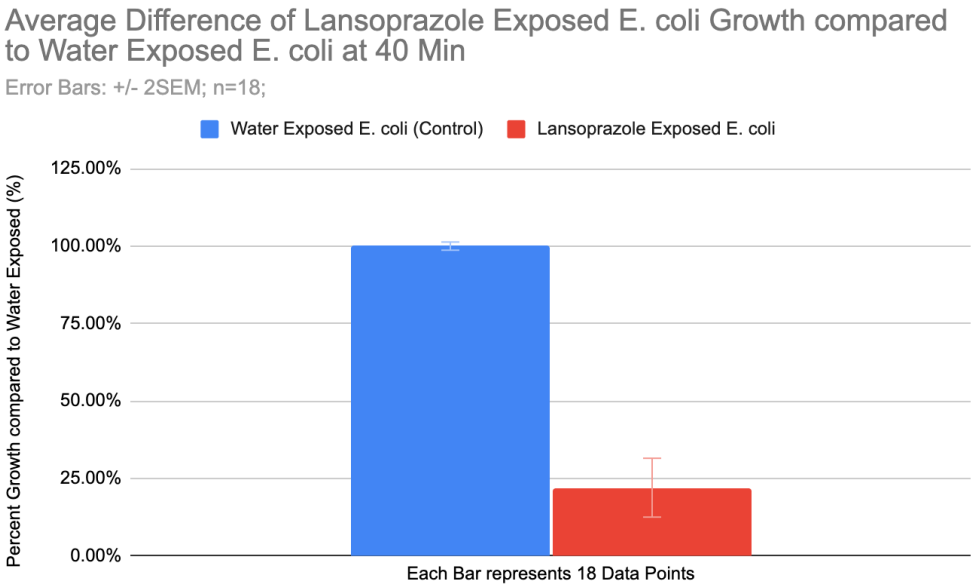

Figure 11: Graphical representations of the difference in the growth of *E. coli* exposed to Lansoprazole, and the growth of *E. coli* exposed to just water (control) at the 40-minute time interval. *Graph made from* Google Sheets.

In the second graph, *Figure 11*, the data represents the growth of *E. coli* exposed to Lansoprazole after 40-minutes. The growth of the Lansoprazole exposed *E. coli* was now 22.00% as much as the control growth over 40-minutes. Similarly, error bars were larger on the Lansoprazole exposed *E. coli,* however the top of the error bar was still less than the natural growth of *E. coli,* and would be further supported by the statistical analysis test.

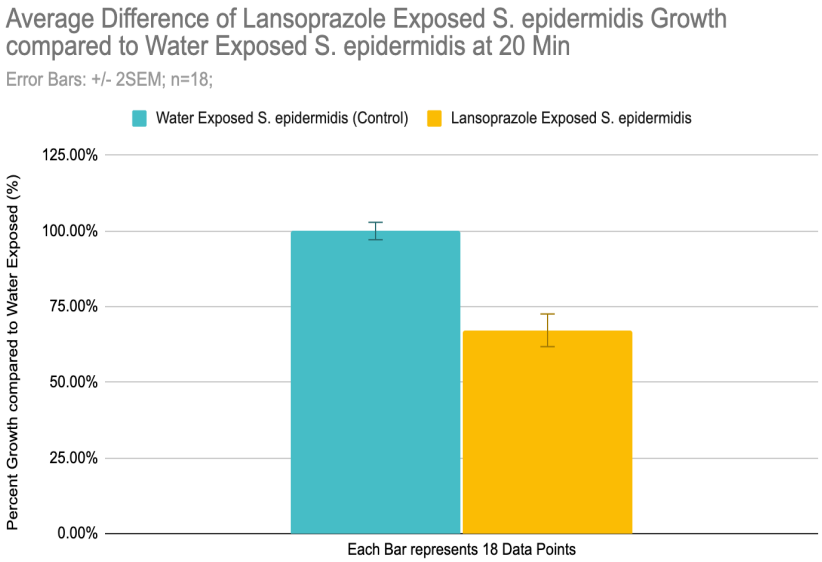

Figure 12: Graphical representations of the difference in the growth of *S. epidermidis* exposed to Lansoprazole, and the growth of *S. epidermidis* exposed to just water (control) at the 20-minute time interval. *Graph made from* Google Sheets.

Similar to *E. coli, S. epidermidis,* also a bacteria within the intestinal tract, resulted in less growth when exposed to Lansoprazole. In *figure 12,* the comparison between the water exposed *S. epidermidis* and the Lansoprazole exposed *S. epidermidis* is shown. After 20-minutes, the *S. epidermidis* grew only 67.19% as much as the control. Error bars did not overlap in this graph which determined there was likely a significant difference between the two groups, but an Anova and Tukey test was conducted to confirm the significant difference. Error bars on the Lansoprazole exposed group were larger than the control and this is likely due to small differences in the dosage of Lansoprazole the specific bacteria culture measure was exposed too, and will be further analyzed in the limitations section.

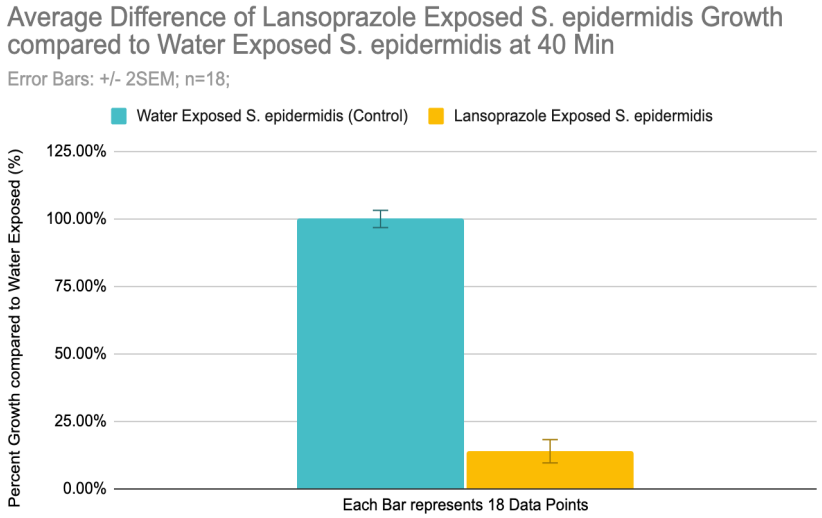

Figure 13: Graphical representations of the difference in the growth of *S. epidermidis* exposed to Lansoprazole, and the growth of *S. epidermidis* exposed to just water (control) at the 40-minute time interval. *Graph made from* Google Sheets

Finally, this graph *(Figure 13)* represents the comparison of water exposed *S. epidermidis* and Lansoprazole exposed *S. epidermidis* at 40-minutes. This final interval resulted in the largest difference between the control group and the Lansoprazole group. The *S. epidermidis* that were exposed to the chemical grew 13.94% as much as the control group, with error bars being the smallest in comparison to the other graphs. The reason for the smaller error bars here may be due to less variance in the spectrophotometry results at the given time. The spectrophotometer as stated in the methods was calibrated every three runs due to variance, so in this trial, the variance in between every run may have been smaller than normal which resulted in smaller error bars.

Statistical Analysis

For data collections done at 20-minutes, both bacteria expressed p-values of less than 0.05 on the Tukey (t-test), whilst at the 40-minute data collections, the test expressed p-values of less than 0.01. The statistical significance test further amplifies the results presented in relation to the reduced growth of *E. coli* and *S. epidermidis* when exposed to Lansoprazole. The significance of the statistical analysis test allows for a stronger conclusion based on the results.

Tukey Statistical Analysis Test

Water *E. coli* vs Lansoprazole *E. coli* at **20-minutes** (**Refer to *Figure 10***)	p<0.05 - Significant
Water *E. coli* vs Lansoprazole *E. coli* at **40-minutes** (**Refer to *Figure 11***)	p<0.01 - Significant
Water *S. epidermidis* vs Lansoprazole *S. epidermidis* at **20-minutes** (**Refer to *Figure 12***)	p<0.05 - Significant
Water *S. epidermidis* vs Lansoprazole *S. epidermidis* at **40-minutes** (**Refer to *Figure 13***)	p<0.01 - Significant

The table is a representation of the Tukey test statistical analysis, depicting the significant difference between each of the experimental groups to the respective control group.

Discussion

The difference between the growth of the control bacteria versus the bacteria growth with Lansoprazole resulted in a significantly reduced growth of both *E. coli* and *S. epidermidis*. There are a number of factors that may have resulted in the reduced growth; however likely the effect is due to the change in stomach acidity when exposed to Lansoprazole. Lansoprazole reduces the amount of acid secretion into the stomach (Metz et al., 2019), which changes the pH of the stomach. In the experiment presented, the in vitro digestion procedure added a controlled amount of hydrochloric acid to bring the pH down in the experimental control group. However, within the experimental group, the PPI reduces the secretion of stomach acid and raises the pH of the solution, which likely resulted in a higher pH than the control group. This may then affect the gut microbiome, as explained in the relationship between gastric pH and gut bacterial growth (Nardone & Compare, 2015).

Additionally, over time the growth of the bacteria exposed to Lansoprazole was reduced. Within the *E. coli* data, at 20-minutes, the experimental group grew 52% as much as the control group at 20-minutes. However, at 40-minutes, the experimental group only grew 22% as much as the control group at 20-minutes. This may suggest that

without necessary treatments, the growth of gut bacteria when consuming Lansoprazole will result in a continually worsening effect on the gut microbiome; however, future studies would confirm the claim.

Conclusion

The data presented achieves the objective of determining if there are any harmful effects of consuming Lansoprazole in regards to the gut microbiome, along with determining what those harmful effects may be and what they can lead to. It was determined that Lansoprazole reduces the growth of *E. coli* and *S. epidermidis* to a significant extent. The reduction in the growth of *E. coli* and *S. epidermidis* can lead to Enteric Infections, Inflammatory Bowel Disorders (Zhang et al., 2014). Enteric infections are an illness within the intestinal tract that is caused by bacterial infections. Diarrhea is one of the main symptoms of the infection, and it affects many young people in developing countries (Petri et al., 2008). Inflammatory Bowel Disease is an inflammation in the intestinal tract that is caused by bacterial interactions (Fakhoury et al., 2014). Finally, the most severe side effect directly linked to the reduction in *S. epidermidis* is a worsened immune system. *S. epidermidis* is vital to fighting off pathogens and viruses that enter the intestinal tract, so the reduction in bacterial growth may lead to viruses (Vitale et al., 2021)

Another conclusion that can be suggested due to the significance of this study is that Lansoprazole may not be a good recommendation for those with underlying gut disorders. Approximately 2 million people in the United States experience Inflammatory bowel disease, a disease that's started with gut dysbiosis (DeGruttola et al., 2016). With future research, the combination of Lansoprazole with subjects that have underlying gut dysbiosis may experience worsening effects on their health.

Limitations

The data collected indicates possible harmful effects of Lansoprazole: however, uncontrollable factors contribute to the limitations of the project.

Firstly, research was conducted in a High School Laboratory, and despite using some advanced equipment, the laboratory does not replicate a highly controlled laboratory. In this lab, multiple people conducted widely different research that changed environmental conditions. For example, the water bath shaker was always within 2 degrees Celsius of 37 degrees; however, due to other researchers needing equipment, it could only sometimes be set to exactly 37 degrees. This means the temperature was not precisely the internal temperature of a human the whole time.

Furthermore, the experiment presented used a static *in vitro* method which modeled digestion with electrolyte solutions and pH adjustments. Due to the lack of advanced equipment, the muscular construction of a human and the secretion of acids similar to a human could not be replicated accurately. The digestive system could be modeled simplistically; however, complex processes within humans may cause shifts in the results. Future studies would confirm any changes.

Finally, Lansoprazole was suspended in the digestion solutions and did not dissolve. Between certain parts of digestion, some chemicals would fall toward the bottom. Despite constant mixing and vortexing within flasks, some chemical may have stuck to the sides or fallen out of the suspension. This would cause the exact dosage to be slightly different.

Future Studies

In order to support the results presented in this study, future research and data collection needs to be gathered. A critical study that needs to be done is comparing how further the growth of the gut microbiome is with patients who have GERD. GERD may cause dysbiosis in the human gut microbiota (Meng et al. 2018), so comparing growth in a healthy organism and growth in a GERD patient may clarify the claim. Additionally, the data gathered from that future

study may support the effect of PPIs on the human gut microbiota. Suppose the dysbiosis within patients who consume Lansoprazole is worse than within patients who have GERD and do not consume Lansoprazole. That will corroborate the claim that Lansoprazole may cause harmful effects on the gut microbiome.

Another critical study that needs to be done before any action can be done is testing Lansoprazole in more developed organisms. Specifically, a study suggests that mice can be induced with Gastroesophageal Reflux disease, which may allow testing treatments and effects of different PPIs within a non-human model (Nu-ri et al., 2021). The mouse is a model that should be used for testing because it has a gut microbiome that can be in some ways similar to humans (Wang et al., 2019). If initial testing in mice results in the same gut dysbiosis exhibited in my study, it would allow for a more decisive conclusion on some of the harmful effects of Lansoprazole and allow for necessary precautions to be taken before testing in humans.

Along with testing Lansoprazole, other PPIs need to be tested to understand which other PPIs cause similar harmful effects as shown in this study and which PPIs allow a patient to keep a stable gut microbiome. As explained previously, Omeprazole is one of the most well-known PPIs on the market, and a study shows that it is more effective at reducing gastric acidity than Lansoprazole (Javed et al., 2020). Studying if this PPI also has a similar effect on the gut microbiome will allow researchers to infer whether a chemical part of Lansoprazole is the issue or if PPIs generally contain a compound harmful to the gut microbiome.

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