# Is Apple Cider Vinegar Effective for Reducing Heartburn Symptoms Related to Gastroesophageal Reflux Disease?

by

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ABSTRACT

Drinking vinegar is a popularly discussed remedy for relieving heartburn symptom, as can be read on many websites; however, there has been no scientific research or theory to support its efficacy. This randomized, placebo-controlled, double-blind, cross-over research study tested the efficacy of the organic apple cider vinegar, with mother, on alleviation of the heartburn symptom related to Gastro-esophageal reflux disease (GERD). A minimum of one week separated the four trial arms: chili (placebo), antacid after chili meal (positive control), vinegar added to chili, and diluted vinegar after chili meal. Twenty grams of vinegar were used in both vinegar treatments, and 10 grams of liquid antacid were used in the antacid trial. A five-point Likert scale and a 10-cm visual analogue scale (VAS) were used to assess heartburn severity during a 120 minutes testing time. Seven of 15 recruited subjects' data was usable for statistical analysis (age: 39.6 ± 12.2 y, body mass index (BMI): 29.4 ± 4.2 kg/$m^2$, waist circumference: 36.4 ± 4.1 inch). There was no statistically significant difference among the mean and incremental area-under-the-curve (iAUC) heartburn scores among different trials (Likert scale questionnaire p= .259, VAS questionnaire p= .659, iAUC Likert scale p= .184, iAUC VAS p= .326). Seven participants were further divided into antacid responder (n=4) and antacid non-responder groups (n=3). Likert scale mean heartburn score and iAUC data in antacid responder group had significant finding (p= .034 and p= .017 respectively). The significance lay between antacid and 'vinegar added to chili' trials. Effect size was also used to interpret data due to the small sample size: Likert scale: mean heartburn score= .444, iAUC= .425; VAS mean heartburn score= .232, iAUC .611. Effect size for antacid responder group was Likert scale: mean heartburn score= .967, iAUC= .936. Future research is needed to examine whether

ingesting organic vinegar benefits alleviation of heartburn symptom related to GERD for people who do not respond well to antacid.

# DEDICATION

I would like to dedicate this thesis to my family. Thank you all for your support and all the help and time during my long journey of schooling. I love you all!

My Husband - Allen Lee

My Parents - Farn-Shyong Yeh & Aa Yn Yeh-Lin

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# CHAPTER 1

# INTRODUCTION

The United States reports the highest prevalence of 17.6 - 28.8% gastroesophageal reflux disease (GERD) during the last two decades, based on the questionnaires of 379 - 1524 sample size compared to the Asia and the Europe (Ronkainen & Agreus, 2013). One major cause of the GERD is the refluxing of stomach or intestinal contents back to the esophagus. Heartburn is a chest-burning sensation which generates a discomfort at the substernal region, and it is one of the most common symptoms of GERD (Dore, Pedroni & Pes et al., 2007).

Over-the-counter medications and home remedies are popular strategies to alleviate these symptoms (Rodriguez, Miner & Robinson et al., 1998; Collings, 2002). In 1998, more than one billion dollars was spent on over-the-counter medications treating heartburn in the U.S., and since then the clinical problem of GERD has been increasing globally (Rodriguez et al., 1998; Hartono, Qua & Goh, 2011). Gastric acid has been blamed as the source of the discomfort. One of the most effective acid-suppression medications in today's market is the proton-pump inhibitor (PPI). In 2009, the total sale of PPIs was approximately $13.6 billion worldwide (Gatyas, 2009). However, there are concerns about long-term treatment with PPIs. The therapy may create a dependency in that halting treatment induces rebound acid hypersecretion and makes it difficult to discontinue use (Reimer, Sondergaard, Hilsted & Bytzer, 2009). On top of rebound acid hypersecretion, hip fracture, enteric infections, drug interaction, reduction of vitamin B12, and hypergastrinemia are also the safety issues relate to long-term PPI treatments (Hershcovici, Mashimo & Fass, 2011; Tari, 1997). The risk of esophageal adenocarcinoma increases among the people who use PPIs chronically, having

GERD with erosive endoscopic findings or diagnosed with Barrett's esophagus (Lada, Niemam & Han et al., 2013; Erichsen, Robertson & Farkas et al., 2012).

With the side effects of the acid-suppression medications in mind, the interest in studying dysfunction of the lower esophageal sphincter (LES) has increased. Pharmacology has shifted its focus on treating GERD from acid suppressant such as PPIs in the last few decades to improving the function of LES, which is the key physiological anti-reflux mechanism in the body with the crural diaphragm (Hershcovici et al., 2011).

It is thought that transient lower esophageal sphincter relaxation (TLESR) is the most mutual causal mechanism of GERD, especially for the patients who have non-erosive reflux disease (NERD) and do not respond to PPIs well (Hershcovici et al., 2011). NERD patients have normal endoscopy findings and the majority of the GERD patients fall under this category. For example, in the Asia-Pacific region up to 75% of GERD patients are in NERD group (Hartono et al., 2011). It is suggested that the purpose of the TLESR is letting the gas out of the stomach. Currently a great deal of research is focused on reducing the TLESR in order to reduce the acid reflux episodes or performing surgery to fix the deficiency of the LES (Crookes, 2006). However, bloating of the stomach might be a possible side effect of the reducing TLESR approach, if the reason behind having TLESR is letting the gas out of the stomach, and surgery is not cost effective.

In the esophagus, both chemical and physical reactions are used to clear the acidic content from the stomach due to reflux. Saliva secretion is increased when the esophageal body senses acid because saliva acts as a buffer, which neutralizes the acid through chemical reactions (Crookes, 2006; Dutta, Agrawal & Mahmoud, 2010). Collings and colleagues

successfully used calcium carbonate gum to neutralize the acid in the esophagus and relieve the heartburn symptoms after a meal without dramatically change the pH in the stomach (Collings, 2002), which is the main underlying source of many side effects of PPIs. The physical process of clearing acidic content is through esophageal emptying by peristalsis (Crookes, 2006). Research evaluated the effects of acidification on the esophageal motor activity by Corazziari et al. and Bontempo et. al., has demonstrated that, in the esophageal mucosa, there are acid sensitive receptors that can sense the change in the intraluminal pH. Acid infusion at the lower part of esophageal body could induce esophageal peristaltic contractions of the smooth muscle that are independent of swallowing, and these contractions are referred to as secondary peristalsis. Furthermore acid infusion increased the frequency of dry and wet swallowing, which triggered the primary peristalsis. The movement of this acid-induced primary peristalsis is longer in duration, bigger in amplitude (especially the upper third body), and lower in propagation velocity when compared to saline-infusion phase (Corazziari, Pozzessere, Dani, Anzini & Torsoli, 1978; Bontempo, Piretta & Corazziari et al., 1994).

In general, patients with NERD condition have a hypersensitive esophagus compared to the asymptomatic ERD patients who have a hyposensitive esophagus. NERD patients, besides acid reflux, the non-acidic content can also produce the symptoms of GERD (Hartono et al., 2011). Without the acid in the reflux content, the acid-induced contraction is not stimulated, so the emptying of the esophagus does not occur. This might be the underlying reason that PPIs don't work well with this group of patients. On the other hand, people with ERD who have a hyposensitive esophagus, even though the acid reflux has entered the lumen, the receptors in the esophageal mucosa are not responding to the

acid well, so the acidic content stays in the esophagus longer. In this situation damage to the esophageal mucosa might take place. Drinking organic vinegar is a popularly discussed remedy, which relieves heartburn symptom, on the website; however, there is no scientific research or theory to support its efficacy. Vinegar is cost effective, simple, readily available, and there is no concern of rebound acid hypersecretion and other side effects previously mentioned that relate to long term PPI treatment.

**Purpose of Study**

The purpose of this experiment was to test the efficacy of the apple cider vinegar on alleviation of the heartburn symptoms related to GERD, and provide scientific research for a popularly discussed remedy on the website.

**Hypotheses**

**H1.** Compared to the both vinegar trials, the antacid trial shows significant alleviation of the heartburn sensation.

**H2.** Compared to the placebo trial, the vinegar trials do not show significant alleviation of the heartburn sensation.

**Definition of Terms**

- Acetic Acid: A weak strength and sharp flavored acid, which is the main component of vinegar. Its chemical form is $CH_3COOH$. The average acidity of vinegar is 5%.
- Acid reflux: A condition in which the content of the stomach containing gastric acid flows back into the esophagus.

- Asymptomatic erosive reflux disease (ERD): Patients experience no discomfort of GERD symptoms, but endoscopy findings show damage of the esophageal mucosa because of acid reflux.
- Esophageal adenocarcinoma: A type of cancer derived from glandular tissue of esophageal epithelia.
- Gastroesophageal reflux disease (GERD): a chronic digestive disorder that stomach contents flow back into the esophagus through the lower esophageal sphincter (LES) causing symptoms of heartburn, chest pain, regurgitation, dry cough, nausea, sore throat, and feeling of a lump in the throat. According to patients' endoscopic findings, GERD can usually be allocated into three categories: Non-erosive reflux disease (NERD), symptomatic and asymptomatic erosive reflux disease (ERD).
- Heartburn: A chest-burning sensation which generates a discomfort at the sub sternal region.
- Hypergastrinemia: An excess of gastrin, a gastric hormone stimulates secretion of gastric acid, in the blood.
- Lower esophageal sphincter (LES): An area where the esophagus merges with the stomach and it relaxes during swallows. From manometry perspective it is the high pressure zone which consists of two kinds of musculature.
- Non-Erosive Reflux Disease (NERD): Patients who have GERD symptoms, but with normal endoscopy results, however histological test might show some changes of esophageal epithelium.

- Proton-pump inhibitor (PPI): A kind of medication that reduces gastric acid production. It is one of the most effective medicines available for treating acid-reflux on the current market.
- Rebound acid hypersecretion: An increase in gastric acid secretion, which is more than pretreatment level, within two weeks after discontinuing of antacid treatment.
- Transient lower esophageal sphincter relaxation (TLESR): A spontaneous relaxation of LES lasting 10 - 60 seconds that is independent of swallowing, but with simultaneous relaxation of the crural diaphragm. TLESR is thought to be a natural pathway to vent air in the stomach, and also the most mutual causal mechanism of GERD.

**Delimitations and Limitations**

The symptom assessment of heartburn severity in this research was mainly based on each participant's subjective sensation. Vinegar has a very distinctive smell and flavor although the effort had been made to mask its characteristic, some participants might still guess they were in the experimental trial, hence bias could arise. Lacking of quantitative data, such as intraluminal pH and pressures readings of the esophagus, and the possibility of participants noticed vinegar and knowing they were in the experimental trials might play a role during the symptom assessments. Many participants had early work shifts, and some needed to get up at 4 am. Even though all participants stated with fasting until 8 am on the study day; skipping coffee or gum was still a challenge during that 4-hour window.

This study excluded certain subcategories of GERD patients who have had the following diagnosis: asymptomatic ERD, hiatal hernia, Barrett's esophagus, and

adenocarcinoma patients. GERD patients with active antacid prescriptions were also excluded. Therefore the results of this study cannot be generalized to these populations, as well as pregnant women.

# CHAPTER 2

# REVIEW OF LITERATURE

## Gastroesophageal Reflux Disease

**Definitions and diagnoses.** There were various definitions of gastroesophageal reflux disease (GERD) among different countries because patients' clinical symptoms have wide ranges of manifestations. This situation made clinical diagnosis and research purposes regarding GERD challenging. In 1994, a classification of the esophagitis was proposed at the Los Angeles World Congress of Gastroenterology, and later this classification became known as the Los Angeles classification (Sami & Ragunath, 2013).

***The Los Angeles classification.*** In 1996, David Armstrong and colleagues validated the Los Angeles classification through 59 endoscopists from different countries. The report examined the similarity of the participants' diagnoses from endoscopic still images and video images. Among all participants, the recognized complications had a more consistent recording; however, the problem of assessing "the circumferential extent and number of mucosal breaks" existed even among experienced endoscopists. The researcher of the study thought the design of the evaluation worksheet might be at fault (Armstrong et al., 1996, pp. 89-90). In 1999, Lars Lundell and colleagues did further validation of the Los Angeles classification. At that time, the grading definition of esophagitis had changed (Table 1). Forty-six endoscopists from different countries were recruited. The mean K value of 0.4 was the result of the "circumferential extent" of esophagitis evaluation. The closer the K value is to one, the more consistent the data is. Lundell et al. pointed out that based on the Los Angeles classification, the diagnostic agreement of the esophagitis'

circumferential extent in this study had a K value of o.4, which is only at an acceptable level. However, Lundell et al. also mentioned that the outcome of an alternative method had a mean K value of 0-0.15, which is considered unacceptable (p. 172).

Table 1
*The Los Angeles classification of esophagitis*[1]

| | |
|---|---|
| Grade A | One (or more) mucosal break no longer than 5 mm, that does not extend between the tops of two mucosal folds |
| Grade B | One (or more) mucosal break more than 5 mm long that does not extend between the tops of two mucosal folds |
| Grade C | One (or more) mucosal break that is continuous between the tops of two or more mucosal folds but which involves less than 75% of the circumference |
| Grade D | One (or more) mucosal break which involves at least 75% of the oesophageal Circumference |

[1] Lundell, Dent & Bennett et al., 1999, p. 173

As the number of patients with symptoms of reflux grew in the clinical primary care setting, a need for a symptom-based definition for GERD was increasing. The majority of patients who show symptoms of reflux do not go through further diagnostic tools, such as endoscopy. They are either self-treated with over-the-counter antacid or prescribed proton-pump inhibitors (PPIs) through their primary care providers. These treatments are currently believed to be the most cost-effective or responsive for treating GERD symptoms regardless of their potential risk and side effects of long-term use (more details are in the treatment section below). These patients are only referred to an endoscopist or gastroenterology specialist when their symptoms persist or relapse after the treatment (Zschau, Andrews &

Holloway et al., 2013). The Los Angeles classification fell short of diagnosing this population of GERD patients, and defining the complication of vast symptoms associated with the disease. Thus in 2007, Dr. Nimish Vakil and his colleagues developed the Montreal definition and classification of gastroesophageal reflux disease (pp. 1900, 1903 & 1914).

***The Montreal definition.*** The final agreement of the Montreal definition was reached by a repeated anonymous voting process through a group of 44 experts from 18 countries (Vakil, Zanten & Kahrilas et al., 2007). Dr. Vakil and his group (2007) approached this definition differently from the Los Angeles classification because they wanted the Montreal definition to be "patient-centered" and "independent of endoscopic findings." This definition is patient-centered meaning each patient's well-being is considered when the symptoms become troublesome. For example, having symptoms, such as heartburn or related pain, "on two or more days a week" is considered mild, but, if the patient's quality of life is seriously affected due to the symptoms, then the frequency and duration become less concerning, and the patient's symptoms are considered worse instead of mild. They also emphasized that the definition is independent of endoscopic findings because many people might not undergo the endoscopy process or they used different diagnostic tools. Furthermore, the endoscopic finding may vary depending on the modernity of the diagnostic technology used, so the Montreal definition "is likely to endure despite changes in technology" (Vakil et al., 2007).

The Montreal Definition of GERD, according to Dr. Vakil and his group's initiative result (2007), is "…a condition which develops when reflux of stomach contents causes troublesome symptoms and/or complications" (p. 1903). Nonerosive reflux disease (NERD), which associates with the Los Angeles classification, is described as "esophageal

symptomatic syndromes" under the Montreal definition. Erosive reflux disease (ERD), which based on patient's endoscopy findings according to the Los Angeles classification, would be described as "esophageal syndromes with esophageal injury." Additionally "reflux esophagitis" is used when there is a sign of the esophageal injury, rather than erosive esophagitis. The Montreal definition is able to include many more people with various GERD symptoms, and patients who used different diagnostic tests, besides endoscopy, such as various esophageal pH-monitoring tools, reflux monitoring, esophageal acid exposure recording, and the correlation between reflux episodes and symptom occurrences.

**Triggering factors.** GERD has vast varieties of manifestations with wide ranges of frequency and duration. The variable complications may affect the stomach, esophagus, throat, teeth, lungs, and ultimately the quality of life. There is no clear-cut explanation for GERD symptoms and in current state, many underlying factors are needed further investigations or remain unclear. Due to the safety concerns and side effects of PPIs long-term use, the research trend has shifted GERD treatment focus from PPIs over the last few decades to understanding the underlying causes, hence readjusting and discovering better therapy options.

***Brain processing.*** In 2010, a group of scholars in Shanghai, China compared the brain cerebral cortex reactions upon esophageal chemical stimulations among healthy volunteers and different types of GERD patients (Xu, Zheng & Zhao et al., 2010). They concluded that when comparing how the brain receives and integrates sensations from esophageal stimulations, there are significant differences among healthy volunteers and within each GERD groups. The result supports the proposition that brain sensory center

strongly influences "esophageal visceral sensation" and thus, leads to different manifestations among GERD subgroups (Xu et al., 2010).

Siwiec et al. in their 2015 article examined functional connectivity between esophageal acid stimulation and insular responding to the sensory information in GERD patients comparing to the healthy control. The study tested the functional connectivity under 4 circumstances. There was no difference between GERD patients and healthy subjects during pre-infusion phase. Some regions between insula and thalamic had significantly higher activities in GERD patients during neutral saline, acid infusion and post-infusion phases, however, some regions only showed significant reaction during acid infusion and post-infusion phase. Interestingly, the right dorsal posterior insula and the right amygdala only showed significant connectivity during acid-infusion phase. Furthermore, the connectivity between insular and hippocampus only showed significant difference during post-infusion phase. The result of this study suggests that GERD patients might have abnormal highly active sensory afferents in the esophagus, "esophago-cortical neuraxis", and this could be an important underlying factor for NERD patients (Siwiec, Babaei, Kern, Samuel, Li & Shaker, 2015).

***Psychological factors.*** Many studies have pointed out that psychological issues might be one of the essential factors in GERD. Ronnie Fass and his colleagues did an experiment in 2008 on patients with typical GERD symptoms and healthy volunteers. They introduced auditory stress to participants via a validated method by delivering "folk music in foreign language" to one ear and "heavy metal music" to the other ear at the same time (p. 699). The result demonstrated that stress can enhance GERD patients' perception to intraesophageal acid exposure, and worsen the symptoms. The greater the emotional

response to the stressor, relates to a higher intensity of the intraesophageal acid perception. Fass et al. also points out that previous studies suggest that anxiety could also influence brain function on sensitization of GERD symptoms, such as heartburn, without the acid exposure to the esophagus (R. Fass, Naliboff & S. Fass et al., 2008). Another research reported that a highly stressful life style for over a 6-month period would most likely increase heartburn symptom severities, but not the frequency, the following four months despite the actual reflux episodes might not match the upsurge (Naliboff, Mayer & Fass et al., 2004). These results also support the proposal that stress enhances the sensory afferents of the esophagus.

***Transient lower esophageal sphincter relaxation (TLESR).*** The term is defined as the relaxations of the lower esophageal sphincter when the action of swallowing is absent. Several studies described TLESR as the major cause to GERD (Schneider, Kuper, Konigsrainer & Brucher, 2009; Wu, Mui, Cheung, Chan & Sung, 2007; Pandolfino, Zhang, Ghosh, Han, Boniquit & Kahrilas, 2006). However, the research studied frequency comparisons of TLESR among healthy individuals and GERD patients, the results were not consistent. One research shows GERD patients have significant higher episodes of TLESR compared to healthy control group (Schneider et al., 2009), while another shows there is no consistent difference between two groups (Pandolfino et al., 2006). However, Pandolfino et al. pointed out that there are more acid reflux occurrences accompanied TLESR in GERD population than healthy ones. Therefore, targeting TLESR reduction in GERD patients has been a new strategy for pharmaceutical development.

Pandolfino and his colleagues (2006) examined the process between the transient relaxation of the LES and the opening of the esophagogastric junction. They found out that the mechanics, also involves "crural diaphragm inhibition, esophageal shortening, and a

positive pressure gradient between the stomach and the esophagogastric junction lumen" (p. 1725). The increased pressure from the stomach distention explains why often troublesome GERD symptoms occur after meals.

In 2009, Schneider and his colleagues studied whether TLESR was the underlying cause of the connection between morbid obesity and GERD (p. 595). In the article, they defined several types of TLESR. Isolated TLESR takes place as single event. Post-swallow TLESR occurs when the action of swallowing induces the LES relaxation. There is also paired TLESR, which comes as double TLESR, and in this rare situation, the LES relaxes longer than other types of TLESRs. The normal duration of the TLESR is 5 seconds. The degree of the LES relaxation also classified TLESRs into complete and incomplete based on if the gastric baseline is reached. Different types of TLESRs were compared among the groups, and with an upright posture, two hour after meal observed the highest frequency of TLESRs. In the conclusion, GERD patients either with normal weight or obese GERD patients had significant higher postprandial TLESR frequency when compared to the control group (healthy individuals). Regarding the TLESRs type, obese patients had majority complete isolated TLESRs and percentage is similar to the healthy control group. Incomplete TLESRs are significantly less in obese GERD group. It is noteworthy that all the participants in the obese GERD group have had hiatal hernias. This research also shows there is no association between TLESRs and LES pressure (Schneider, Kuper, Konigsrainer & Brucher, 2009). As stated earlier, it is the positive gradient between stomach and the LES could trigger TLESR. In a 2006 article, Crookes mentioned that typical western diet played an important role in production of TLESR, and carbonated beverages are included. These beverages increase gastric distention and reduce LES pressure, thus lessen the threshold to

produce TLESR as well (Crookes, 2006). As TLESR number creases, so as the possible episodes of reflux.

***Obesity.*** The connection between obesity and GERD has been studied for decades, but the results are quite controversial. A higher rate of hiatal hernias is noted in overweight and obese populations, which might due to a higher intra-abdominal pressure, and the association of the GERD symptoms, such as heartburn and acid regurgitation (Schneider et al., 2009). Patients with hiatal hernia have compromised crural diaphragm contraction protection; hence acid reflux is more likely occurs. Crookes pointed out in a 2006 article (p. 465) that 228 symptomatic GERD patients sequentially selected in a foregut surgical practice; the mean BMI is 28 $kg/m^2$. According to the chart, patient number dramatically decreased when BMI is higher than 35 $kg/m^2$ (Crookes, 2006). However, a variable was not considered with this statement. Patients with BMI higher than 35 $kg/m^2$ might be advised to lose some weight before undergo a foregut surgery or patients in this population might have more complications with their health condition that prevent them from surgeries.

Corley and colleagues did a cross-sectional study in San Francisco and Oakland, California in 2006 to evaluate the association among GERD symptoms, abdominal obesity, ethnicity and gender. The sample size of this study is 80110 and the sample frame is the subjects who were interviewed in a cohort study, which was implemented between 1964 and 1968. Many gastroenterological risk factors have been studied through this cohort, such as gastric cancer, *Helicobacter pylori*, gastric lymphoma etc. The result shows that there is an independent association between GERD symptoms and increasing abdominal diameters in white subjects, but this association is missing in black and Asian participants. The association between GERD symptoms and BMI is also stronger in white populations than

black or Asian subjects. Although men in this study have larger abdominal diameters in average, there is no association between GERD symptoms and gender (Corley, Kubo & Zhao, 2007).

Pandolfino and colleagues analyzed gastro-esophageal pressure gradient and its relationship with obesity through high-resolution manometry in another article in 2006 (pp. 639-649). The results of higher pressure gradient between the stomach and the esophagus in obese subjects explains the likelihood of gastric juice flow into the esophagus, especially when the crural diaphragm moves down during inspiration, the stomach is compressed. The gradient of this pressure has a positive correlation with increased BMI, and even stronger, when waist circumference is brought into the consideration (Pandolfino, EL-Serga, Zhang, Shah, Ghosh & Kahrilas, 2006).

Interestingly, Dore and colleagues pointed out in a 2007 research article that atypical symptoms in GERD, such as "chest pain, sialorrhea, hoarseness, globus sensation, chronic coughing, episodic bronchospasm, hiccup, eructations, laryngitis, and pharyngitis," (p. 463) are not associated with BMI. In fact, based on the study result (Table 2, p. 466), the frequency of these atypical symptoms dropped dramatically when participants' BMI were higher than 31 $Kg/m^2$ (Dore, Pedroni & Pes et al., 2007).

***Smoking.*** Several studies have shown smoking has adverse effect to GERD symptoms. The reasons are the following: (1) Smoking reduces LES pressure, which increased the chance of gastric acid reflux to the esophagus (Stanciu & Bennett, 1972). (2) Smoking reduces the salivary gland secretion. There is a large amount of bicarbonate ion in the saliva works as buffer to neutralize the acid in the mouth and the esophagus. Reduced

saliva drastically reduces the esophagus' acid buffering mechanism through chemical reactions (Ness-Jensen, Lindam, Lagergren & Hveem, 2014). (3) While smoking, the inspiration phase of breathing brought in a large amount of nicotine, and Miller et al. pointed out that enhancing nicotinic receptors can mediate relaxations in gastroesophageal muscle, which would decrease the anti-reflux barrier between the stomach and the esophagus (Miller, vegesna, Braverman, Barbe & Ruggieri Sr., 2014). (4) The stomach pressure increased during the inspiration period, thus the gastro-esophageal pressure gradient increased. Then gastric acid is driven to flow back into the esophagus. (5) A long-term usage of nicotine would damage the epithelium and mucosa (Nakajima, Nagahara & Kurosawa et al., 2011). This condition allows more chemical passing through the esophageal wall and enter the intra-cellular space, thus enhances the stimulation and reaction of the neural system.

A prospective study in 2011 by Nakajima et al., support the proclamation that quit smoking can not only alleviate GERD symptoms but also improve the quality of life (Nakajima et al., 2011). Contradictorily, a cohort study in 2014 by Ness-Jensen et al., shows the result that quitting smoke only improves GERD disease in the individual who is not overweight or obese; takes anti-reflux medication weekly; and has severe GERD symptoms (Ness-Jensen et al., 2014).

***Diet.*** A majority of GERD symptoms, especially heartburn and acid regurgitation, happen after a meal. Heartburn, based on the Montreal definition, is "a burning sensation in the retrosternal area (behind the breastbone)" (Vakil et al., 2007). Many studies assessing heartburn severity or an item's efficacy in heartburn alleviation require a reliable method to induce consistent heartburn sensation in the participants.

In 1998, Rodriguez and colleagues tested three different meal compositions based on their calories, osmolality, pH, volume and fat contents (p. 487). They evaluated each meal's heartburn-inducing capability; on-set timing of the symptoms; the number of reflux episodes; each participant's gastric pH; and the duration of symptoms. The total recording time of the symptoms was 180 minutes since starting of the meal. All three meals induced heartburn among the participants. Wendy's Chili and red wine induced the highest incidents of heartburn among the participants during the first 45 minutes of the trial, comparing to McDonald's hamburger with chocolate shake, or McDonald's sausage biscuit with chocolate milk (p. 488). Tomatoes, caffeine, peppermint, onions, chocolate, orange juice, and capsaicin are considered as irritating ingredients that might cause heartburn symptoms in GERD (Rodriguez, Miner, Robinson, Greenwood, Maton & Pappa, 1998).

***Gastric distention.*** Besides the heartburn-causing ingredients, gastric distention can also induce heartburn when a big meal expands the stomach to a certain degree. In 1985, Holloway and colleagues examined how gastric distention affects LES pressure in healthy volunteers and GERD patients (p. 779). LES pressure drops during gastric distention, and TLESR occurs. Sometimes the incidence is also followed by the opening of the esophagogastric junction, which allows the contents of the stomach to flow back to the esophagus, thus leading to reflux. Holloway et al. inserted a balloon in each participant's stomach and inflated it to 250, 500, and 750 ml for the placebo group and 250, and 500 ml for the reflux patients. They concluded that the rate of TLESR was the major factor that induced the postprandial esophageal reflux rather than the dropping of the LES pressure because there were no significant changes in LES pressure between the placebo and the experimental groups. Even though the placebo group and GERD patients had similar rate

of TLESR, GERD patients had higher percentage of "complete relaxations." According to the chart, when the balloon was inflated to the volume of 500 ml in the experimental group, the number of TLESR increased one and half fold comparing to the volume of 250 ml (Holloway, Hongo, Berger & McCallum, 1985).

***Alcohol.*** In 1997, Teyssen et al., compared alcoholic beverages made from different processes, and they discovered that the process of the alcohol production is a key variable, which influences the capacity of the alcohol, stimulating gastric acid output and releasing hormone gastrin in the stomach. When drinking the type of alcohol, which fermentation is the only process involved in the production, the output of gastric acid and releasing of the gastrin is significantly increased. These types of alcohol are "beer, wine, champagne, martini, and sherry" (Teyssen, Lenzing, Gonzalez-Calero, Korn, Riepl & Singer, 1997, p. 49). The process of distillation significantly decreases the influence of the alcohol has on the gastric acid and gastrin. Teyssen et al. prior to this research did a study in stimulants of gastric acid in beer. The result showed that the step of adding yeast during the production was crucial for the trigger of acid secretion because the byproduct, fermented glucose, from this step was the most powerful stimulant (Teyssen et al., 1997).

This condition might be explained that one of the main functions of the stomach acid is to protect our digestive system by killing the potential harmful microbe with its strong acid. A product made from fermentation process without distillation could contain a large amount of microbe, which stimulates the gastric acid secretion. Coincidentally, vinegar produced without the process of distillation consists of many similar characters that a fermented alcohol has, except the muscle relaxing effect. Rodriguez et al. pointed out the conflict reports on alcohol consumption and its effect on resting LES pressure. Both

increasing and decreasing effects on resting LES pressure were noted in different research studies (Rodriguez et al., 1998). Alcohol's ability to relax muscle contractions is commonly known, yet the gastric acid stimulating ability might connect the role of acid and its reaction with the LES structure, thus explains the result of increasing resting LES pressure.

***Acid pocket.*** The idea that acid reflux mostly happens after a meal seems contradictory because one would think that the food ingested during the meal would buffer the acid in the stomach (Rohof, Bennink, Smout, Thomas & Boeckxstaens, 2013). Indeed, in a 2001 study, which recruited dyspeptic patients with negative endoscopy results, Fletcher et al. pointed out that median fasting gastric juice pH was 1.3 (range 1.0-1.9), and the meal-related mean peak intragastric pH was 5.2 (range, 2.7-7.2). However, the median of the minimum gastroesophageal reflux content pH was 2.5 (range, 1.1-3.4) at fasting state and 3.0 (range, 2.1-3.5) postprandial. The median gastric minimum pH one minute before the reflux episode was 3.2 (range, 1.4-5.3), which had a much wider range of pH than the reflux content in both fasting and postprandial states. Though not statistical significant, it demonstrated that the acidity of gastric juice was not the main underlying cause of gastroesophageal reflux.

Fletcher et al. revealed that the body region of the stomach has a higher pH value than the antrum region postprandial. The cardia region of the stomach which is closest to the esophagus had many unbuffered acid pockets that did not get mixed with ingested food (Fletcher, Wirz, Young, Vallance & McColl, 2001). These acid pockets might explain the slightly more acidic esophageal reflux than the stomach content postprandial (Fletcher et al., 2001). Furthermore, this condition might explain the physiological mechanics of the acid sensitive nerve cells in stomach cardia region that helps the contraction of the LES.

**Subcategories of GERD.** Based on the pathogenesis of the disease and its complications, GERD can generally be categorized into the following subgroups.

***Functional heartburns (FH).*** This group of patients previously was combined with NERD and "acid sensitive esophagus" since all three groups share similar characteristics of the symptoms, such as no evidence of esophageal mucosal breakages from endoscopy results and a hyper-sensitive esophagus. However, some significant difference among these three groups has led experts in the field to re-categorized these patients so as to better diagnose each individual accordingly. Hence a more suitable treatment can be applied to each group of patients (Zerbib, Varannes, Simon & Galmiche, 2012; Savarino, Zentilin & Tutuian et al., 2012).

Compared with negative endoscopy and biopsies findings, and a normal acid esophageal exposure, the key distinction criterion to the functional heartburn group is that their symptoms have a negative association with the acid-reflux episodes. This is the only subcategory under GERD where the patients exhibit 100% reflux-like symptoms. All other GERD subgroups with "morphological changes" have some patients who are asymptomatic (Ronkainen & Agreus, 2013, p. 326). Yet, the one distinguish factor between this group of patients and healthy population is the reflux-like symptoms that are not related to acid reflux episodes. In 2012, Edoardo Savarino and his colleagues did a study to differentiate NERD from FH by impedance-pH monitoring. The result of the pH-monitoring shows no difference between FH patients and healthy volunteers, and this finding might explain the particularly high refractory rate of PPI treatment in FH group (p. 165). There are less than 10% of patients in the gastroenterologists' offices belonging to this group. However, the

estimated percentage might be higher in the primary care clinical offices (Galmiche, Clouse & Balint et al., 2006).

The reasons underlying the symptomatic presentations in the FH group are still largely unidentified. However, many studies support the fact that psychosocial factors are especially more important in this group than other variables. Anxiety disorders, depression, sleep deprivation, somatization disorder, and poor social support are reported more frequently in this group. It is also noteworthy to point out that female patients form the majority of this group.

The treatment plan for FH should base on each patient's condition, and aggressive tests need to be prescribed cautiously. Since FH generally do not respond to PPI treatments, high dosage of acid-suppressant therapy should be avoided. After all, the heartburn and pain symptoms that these patients suffer from are not associated with acid reflux in the first place. Pain management approaches currently are the most recommended by the experts even though there are not many clinical trails exist to validate these approaches (Zerbib et al., 2012).

***Non-erosive reflux disease (NERD).*** The definition of this group is mainly based on patients' negative endoscopy results that show no signs of breakage or injury of the esophageal wall. However, biopsy results might show slightly cells type change, such as "lymphocytic infiltration, papillary elongation, basal cell hyperplasia and dilated intracellular spaces" (Ronkainen et al., 2013, p. 326) on the esophageal wall due to the abnormal esophageal acid exposure. Many epidemiology and review reports indicated that 50% up to 75% of GERD population has a negative endoscopy result (Savarino et al., 2012; Hartono,

Qua & Goh et al., 2011; Goh, 2004). Under broad considerations, these patients would be considered as NERD until more detailed symptoms are investigated. Then FH might be the diagnosis instead of NERD. Patients in this group have a little better responsive rate to PPI treatments than the ones in FH group. Though in general, this group shares the fame of refractory toward PPI treatment with FH group.

NERD patients are considered having hypersensitive esophagus. Hartono et al. in a 2010 experiment compared esophageal sensitivity of different GERD groups. Based on the patients' endoscopy results, they were divided into NERD and ERD groups. Then according to the presentation of the symptoms, ERD is further divided into asymptomatic and symptomatic group. Patients who have experienced reflux symptoms more than once per week for the preceding 3 months were recruited. The result shows that NERD patients not only feel the symptoms during the acid perfusion period, but also when saline was infused (Hartono et al., 2011). Therefore, one can expect low intraesophageal pH value is not the only issue causes GERD symptoms. Conflictingly, Fletcher et al. mentioned that during esophageal acid-perfusion tests, an average threshold for symptoms is pH 2.5. When pH is above 2.5, symptoms and "acid-induced salivation" are rarely noticed (Fletcher et al., 2001). Though in Fletcher et al. study, on-set of symptoms were only measured when intraesophageal pH was lower than 4, so the non-acidic reflux was not recorded in the study. Based on Hartono et al. study, NERD patients might be suffering from both acidic and non-acidic reflux. The esophageal wall of the NERD patients show much less damages from acid when compared with ERD groups, the fact might indicate that reflux in NERD group are more prevalent in non-acidic reflux than the acid ones. There are many acid infusion experiments studied how the esophagus reacted to the acid when it was introduced through

the tube, but currently there is no study examined the esophageal reaction when the acid is ingested as food.

***Erosive reflux disease (ERD).*** ERD is defined as positive-endoscopy result with esophageal mucosa breakage, and the diagnoses based on the Los Angeles classification can be categorized into grade A, B, C and D (Table 1). The term erosive esophagitis is sometimes used as well. In this category, based on the symptoms presentation, it is further divided into symptomatic and asymptomatic. In a 2010 study, Hartono et al. demonstrated that symptomatic ERD group responded to acid reflux more significantly than the healthy volunteers, but saline infusion did not induce GERD symptoms in this group as it did in NERD group. In asymptomatic ERD group, both saline and acid infusion did not induce symptoms indicating this group has a hyposensitive esophagus (Hartono et al., 2011).

Generally ERD is more prevalent in Western world (10-20%). Recently many countries in Asia have research related to the increasing prevalence of ERD in Japan, Taiwan and Korea. In 2012, Ou and colleagues did a prevalence study in Taiwan, according to the reports, in 1995 the prevalence of ERD was 5%, and then the rate changed from 14.5% in 2000 to 23.5% in 2007. The majority of the ERD patients in this study result were grade A (71.6%), and there were very few grade C and none in grade D. The study also identified that "male sex, smoking, obesity, and hiatus hernia" were risk factors of ERD (Ou, Tu & Hsu et al., 2012, p.63).

Schneider et al. in their 2009 article related to TLESRs and morbid obesity, an interesting result was observed. In the GERD group with normal weight participants (BMI = 27 $kg/m^2$), its TLESR frequency was a lot higher in recumbent position compared to the

other three groups (healthy control, obese GERD and diffuse esophagus spasm) in the study (Schneider et al., 2009). Ribolsi et al. did an esophageal peristalsis study (2014), and observed that in the supine position, comparing to NERD, ERD needs significant longer time to clear the bolus and has a significant higher percentage of acid exposure time. According to the result, they indicated that this finding might explain the underlying cause for the erosion development and esophageal mucosal damages in ERD patients (Ribolsi, Balestrieri, Emerenziani, Guarino & Cicala, 2013).

As one expects, a more advanced endoscopic diagnostic tool would improve the detection of the breakage on the esophageal wall. Interestingly, Amano et al., did a study in 2008 in Japan compared conventional and magnifying endoscopy, and revealed a different answer. The K value was .76 between two methods, which indicated both methods had good agreement on the diagnoses despite the technology gap (Amano, Yamashita & Koshino et al., 2008).

Surprisingly, a study Jung et al. did in Republic of Korea in 2013, revealed that non-vegetarianism diet as one of the risk factor, which associates with ERD, when compared it with Buddhist priests group ($p = .03$). This study recruited 148 participants (79 male, 69 female) for each group. In Asian Buddhist priests, many people who follow the vegetarian diet, besides meat, they do not eat onion, scallion, leek, or garlic because these plant-based food was believed to disturb the calmness of one's mind. Furthermore, many of them follow a life style of no drinking and smoking. As previously discussed in the triggering factors section, onions, sausage, alcohol and smoking are the items might induce heartburn, though the Buddhist vegetarian diet's protective effect against ERD remained unclear. Compared the male participants' biomedical data of the Buddhist priests with the general

population in the study, unexpectedly, Buddhist priests group had statistically significant higher number in metabolic syndrome, weight, BMI, waist circumference, total cholesterol, total adipose tissue area (both visceral and subcutaneous), and significantly lower HDL ($p < .05$). However, despite all the biomedical factors, the reflux esophagitis rate was still significantly lower in the Buddhist priests group (Jung, Kang & Hahn et al., 2013). Oddly, the description of the study result conflicted with the data in its Table 1, which it referred to, and stated there was no statistically significant difference between the two study-groups on the factors previously mentioned.

In 2004, El-Serag et al. did a cross-sectional study of the employees at a VA medical center in Houston. The aim of this study was observing how races played a factor between GERD symptoms and ERD. The racial distribution in this study was 43% black, 34% white, 23% others ("8% Hispanic, 11% Asian, 1% Native American, and 3% others") (p. 1694). The result revealed white population is more accessible to ERD than black (El-Serag, Petersen & Carter et al., 2004). In 2005, Japan did the first national epidemiology survey related to heartburn and ERD, and out of 3608 people 602 patients reported ERD as grade A-D (16.7%), and 210 patients out of these 602 patients were asymptomatic (34.9%) (Ohara, Kouzu, Kawano & Kusano, 2005).

ERD is thought to be a progressive disease toward Barrett's esophagus, and eventually might lead to esophageal adenocarcinoma, so long-term lasting GERD symptoms are a warning sign of a more serious disease. Erichsen et al. did a cohort study using the data collected from 1996 to 2008 in Denmark, and concluded that ERD patients' absolute risk of developing esophageal adenocarcinoma after 10 years was 0.24%. The inflammation of the esophageal wall might be the source of the origin (Erichsen, Robertson & Farkas et al.,

2012). The problem is that asymptomatic patients normally do not have a sense how serious their conditions are until endoscopy is performed. Jung et al. mentioned that esophagogastro-duodenoscopy is an annual or biannual procedure in Korea as a screening tool for gastric cancer (Jung et al., 2013).

***Barrett's esophagus(BE).*** While FH and NERD groups are mostly female patients, males are more prevalent in ERD, BE and esophageal adenocarcinoma groups. After a long period of acidic reflux damaging the esophageal mucosal wall, metaplasia of the esophageal stratified squamous epithelium occurs, and turns those acid-sensitive cells into the intestinal columnar epithelium, which has a better tolerance to acid. Therefore, BE patients are less sensitive to GERD symptoms, such as heartburn. The diagnosis of BE is made when the esophagus has some pink lining at its distal end under endoscopy and biopsy result of the pink layers shows intestinal metaplasia (Cameron, 2002; Modiano & Gerson, 2009). Cameron pointed out that reflux symptoms are 2.2 to 4.8 times higher in the population who has first degree relatives with BE. Age is one of the risk factors of BE. For people 60 years and older, who have a routine endoscopic check up, 1% of them are diagnosed with BE. Male patient's prevalence is twice that of female's (Cameron, 2002).

***Esophageal adenocarcinoma (EAC).*** Currently EAC has grabbed a great deal of attention in the research because in 25 years (from 1975 to 2001) the rate of incidence has increased six folds, and mainly in the U.S. and the Western world. EAC is also the major type of histologic esophageal cancer in the United States. The long-term survival rate of this cancer is low, but in the last 30 years the rate has been improved. Besides the advanced surgical technology and therapy care, early detection should be considered as a goal for the clinical primary care setting (Modiano et al., 2009; Hur, Miller & Kong et al., 2013).

## Upper Gastrointestinal Tract Physiology

**Stomach**. Gastric acid juice used to take the main blame for the symptoms of GERD. The sale records of over-the counter antacid medication indicates that this notion still occupies a big part of the market. The strategy of the past was to increase the pH of the total stomach acid, so that even if it got into the esophagus, there would be no discomfort or damage to the esophageal wall. The main component of antacid is Calcium Carbonate ($CaCO_3$). Similarly saliva has a large amount of Carbonate in its content as well. However, instead of neutralize the pH in the esophagus; the aim of antacids is to neutralize the acid in the whole stomach.

Ayazi et al. explored the relationship between resting gastric pH and esophageal acid exposure in 2008 with 54 healthy volunteers and 1582 GERD patients' pH records from a lab. Normal gastric pH was set by healthy volunteers, and the median was 1.5 (range 1.1-1.9) with $5^{th}$ percentile was 0.3 and $95^{th}$ percentile was 2.9. The GERD patients' records were divided into normal gastric pH group and hypochlorhydria group ("gastric pH above the $95^{th}$ percentile of normal"; pH > 2.9) (p. 1970). Normal gastric pH GERD group generally had a higher rate of gastroesophageal reflux episodes. Except during the supine positon, hypochlorhydria group had more reflux, though it is not statistic significant. It is also noteworthy that in this study, patients' gastric pH records' distribution was bimodal. The median gastric pH was 1.7 in normal gastric pH GERD group, and it was slightly higher than healthy volunteers' median pH value of 1.5. This finding indicates that too much of gastric acid is not the main cause of GERD symptoms (Ayazi, Leers & Oezcelik et al., 2009).

The rat gastric stimulating studies have shown that vagal and spinal sensory neurons respond to unpleasant gastric distention or acid stimulation significantly different. These studies suggest that even though vagal and spinal afferents are both sending the sensory information to the brain, they have distinct functions. Sakurai et al. in their 2008 article mentioned that in the stomach, vagal afferents are mostly sensory neurons that carry information from noxious chemical stimulation, and spinal afferents are in charged of the perception of harmful mechanical stimuli (Sakurai, Obata & Ozaki et al., 2008; Sugiura, Dang & Lamb et al., 2005). Previously mentioned in the triggering factors section that unbuffered acid pockets in the stomach after a meal tend to locate in cardia region of the stomach, and these acid pockets contain concentrated proton, which might influence gastric vagal afferent to generate action potentials.

**Lower esophageal sphincter (LES).** LES is a very important structure between the esophagus and the stomach. With the help of the crural diaphragm to give the pressure on the exterior lining of the LES, a barrier is created to prevent the contents of the stomach to reflux into the esophagus.

In human, and most of the other animals, LES is not visible. Therefore, when conducting a research related to measuring LES pressure or TLESR, the definition of the LES has to be stated to avoid confusion. The term the esophagogastric junction is also often used. The narrowing of the esophagus where the angle of His locates is not how LES is defined. However, the sharp angle created a flap, which also helps on the mission of preventing reflux. Most of research related to GERD would define the location of the LES by manometry. It is an area, which has a higher pressure compared to the surrounding (Miller, Vegesna & Brasseur et al., 2011).

The muscle structure of the LES is quite complicated, and it has intrinsic smooth muscle and extrinsic skeleton muscle parts. One of the intrinsic parts is the flap created by the angle of His on the lower left side of the esophagogastric junction. It is composed of stomach muscle fibers, and sometimes this part is also referred to as "upper gastric sphincter." This part of the stomach muscle increases its contractility as a respond to cholinergic stimulation. During postprandial, both cholinergic antagonism and stimulation work on the stomach. Cholinergic receptors are important to gallbladder emptying, which happens when stomach is about to emptying its contents. This part of the LES contracts not only during the period of postprandial but also when the stomach is emptying (Parkman, Trate, Knight, Brown, Maurer & Fisher, 1999; Miller et al., 2011).

The other intrinsic part of the LES is the distal part of the esophagus. This area is overlapping with the cardia part of the stomach, but it is made up of the esophageal semi-circular smooth muscle. The contraction of this structure is coordinated with the third component of the LES, the extrinsic skeleton muscle of the crural diaphragm. The hiatal hernia occurs when these two parts misaligned, and considerably decreased the LES anti-reflux ability.

**Esophagus.** Esophagus is a 20 to 22 cm tubular structure connecting the pharynx and the stomach and constructed by both striated skeletal muscle (upper esophagus) and smooth muscle (lower esophagus). Esophageal peristalsis is managed by inner layer of circular muscle and outer longitudinal muscle layer with complicated nervous system, include spinal (sympathetic) and vagal (parasympathetic) pathways. The distinguish function of different sensory afferents in the stomach carried into the esophagus. The action of swallowing stimulates the vagal motor neurons in the striated muscle with a top to bottom

sequence in a combination of inhibition and excitation mode. This movement is called primarily peristalsis, and it is controlled by central nervous system (CNS). Although the peristalsis wave continues in the smooth muscle part of the esophagus, the peripheral nervous system joins the control of the contractions. The role of CNS in this part of the esophagus might involve acetylcholine receptors. Smooth muscle in the esophagus is not necessary to contract in a sequacious manner and circular contraction can be generated without simultaneously top to bottom direction (Park & Conklin, 1999; Dodds, Christensen, Dent, Wood & Arndorfer, 1978).

Previous research has pointed out that GERD patients frequently have the conditions of delayed bolus transit and trouble to clear the reflux from the stomach, especially when they are in the supine position. With a newer technology, high-resolution manometry, Ribolsi et al. examined the length of broken wave and weak peristalsis in GERD patients in their 2013 study. If wave break was more than 5 cm in the 20 mm-Hg isobaric contour in more than 20% of swallows or 2 to 5 cm in more than 30% of swallows, then the patient had a weak peristalsis. The result found that the pathological number of large breaks (>5 cm) in the peristalsis wave was associated with a statistical significant longer time required for bolus transit and reflux clearance when the patients were in the supine position. These patients also had a longer acid exposure time in the distal esophagus (Ribolsi et al., 2013). In 2012, Tsutsui et al. also did a study in Japan examined globus sensation with a subtype of GERD - laryngopharyngeal reflux disease (LPRD). Patients with LPRD feel as if a lump in their throats and previous research has shown this sensation is associated with heartburn and regurgitation. This study found association among old age, male gender, less serious reflux-related symptoms and resistance to treatment with PPI. Also, PPI-resistant

LPRD patients have significant less percentage of peristaltic contractions and complete bolus transit when comparing to the healthy control group. It is noteworthy that the mean BMI of the participants in this study was around 20 $kg/m^2$ (Tsutsui, Manabe & Uno et al., 2012).

The interior wall of the esophagus has several layers of protection to prevent acid damage from the stomach reflux. A watery layer full of bicarbonate covered the lumen of the esophagus is the first line of defense. Stratified squamous epithelium is the 2nd line of protection. It has been suggested that the lose integrity of this layer might be the underlying reason for neutral-reflux to cause heartburn because the reflux substance could enter the connective tissue below through the gaps among epithelium cells. Metaplasia of the epithelium layer due to prolonged acid exposure has been discussed in the Barrett's esophagus section.

The layer of connective tissue below is called lamina propria. This is where esophageal cardiac glands locate. Hanada et al. conducted a study in Japan in 2014 and they suggested that having esophageal cardiac glands (ECG) was an independent protective factor for reflux esophagitis, but GERD symptoms were not inhibited by the ECG (Hanada, Adachi & Mishiro et al., 2014). ECG secretes acid-neutralize mucus, however the prevalence of this gland is only 50% in the general US population and its existence is controversial (Huang, 2011). While Huang concluded that cardiac gland in the proximal stomach to be congenital, in Hanada et al. study, however, there was only 13.4% of participants had ECG. Due to the cardiac gland in the stomach mucosa locates around esophagogastric junction where LES is, it is called ECG when it locates in the esophageal mucosa. In Hanada et al. study, they detected the presence of ECG by the endoscopy

procedure and defined the yellowish lesion in the distal part of the esophagus as ECG. They pointed out the yellowish elevated areas commonly locate at left-posterior wall of the esophagus while most of the GERD related erosion or esophagitis happens on the opposite side of the esophagus wall. Furthermore, the ECG in this study, are more prominent in patients with *H. pylori* infections, and participants who have more serious gastric mucosal atrophy (Hanada et al., 2014). It might be the *H. pylori* infection reduces gastric acid secretion, and esophagitis, which is damage of the esophageal wall by gastric acid, thus reduces as well (Koike, Ohara & Sekine et al., 2001). More research is needed to investigate the association between the ECG and its protective factor for esophagitis.

Although TLESR is believed to be one of the main causes of GERD symptoms, the occurrence of TLESR sometimes are followed by esophageal motor responses. These esophageal motor responses also noticed during the acid perfusion tests in the esophagus. Sifrim et al. did a study in 1996 to investigate the association of TLESR and esophageal motor response in healthy volunteers. The result shows TLESR does not inhibit esophageal body contraction. Furthermore, introduction of acid without expanding the lower esophagus, on the other hand, increased the esophageal muscle contractility (Sifrim, Janssens & Vantrappen, 1996).

In 1978, Dodds et al. did a study in the opossum to investigate esophageal contractions induced by vagal nerve. The result showing that the esophageal smooth muscle contraction that induced by vagal stimulation maybe have a similar function of peristalsis, which is clearing the esophagus and pushing the contents toward the stomach. Most of the stimulations were focused at two-thirds of distal part of the opossum esophagus through electric frequency, bubble-free water, stroking with a cotton swab (cervical area) and

different drugs. They concluded that vagal pathway efferent mediated three types of smooth muscle contractions in the opossum esophagus. These three types contractions are A and B waves of circular contraction and longitudinal contraction (Dodds et al., 1978; Schneider, Kuper, Konigsrainer & Brucher, 2008). Several previous articles have shown that the afferent nervous system in the esophagus and the stomach involved both vagal and spinal pathways. Vagal afferents majority carries the signal from chemonociceptor and spinal afferents carry the signals of mechanical stimuli, such as the distention or stretch of the esophagus or the stomach (Sakurai et al., 2008; Sugiura et al., 2005).

**GERD Treatment**

There are many kinds of medicine that is used to raise stomach pH to help relieve discomfort symptoms of GERD because over the past few decades gastric acid has been considered the main source of the problem. Proton-pump inhibitor has been a very popular treatment choice for GERD over the past few decades. Previous research mentioned majority of PPI was prescribe in primary care setting for patients exhibited GERD symptoms before further tests, such as endoscopy, even though patients might not respond to PPIs adequately (Zschau, Andrews & Holloway et al., 2013). Dore et al. suggested that for patients exhibited atypical symptoms of GERD, prescribing PPI before referring them to specialist was cost effective therapy (2007). PPI suppresses gastric acid secretion by blocking the $H^+/K^+$ ATPase, which is the channel on the parietal cells to bring $H^+$ into gastric gland lumen. Cimitidine is another acid suppressant used to reduce gastric acid secretion. It prevents histamine stimulates parietal cells secreting HCl by blocking its binding site - $H_2$ receptors. Over-the-counter antacid, such as the one was used for the antacid trial of this study, contains a great deal of alkaline ions, such as calcium carbonate. When it is ingested

and arrives the stomach, the release of abundant $CO_3^-$ neutralizing the gastric acid directly in the stomach lumen. Feldman pointed out that calcium carbonate although works rapidly, the effect duration was only 60 minutes (Feldman, 1996). According to the result of the current study, calcium carbonate antacid seem has improved its effectiveness since 1996 because during the antacid trial, it suppressed heartburn symptoms during the whole 120 minutes testing time in the antacid responder group.

Dramatic changing the pH in the stomach causes many side effects that were mentioned in the introduction. Therefore recent research has published alternatives that might help GERD patients relieve their discomfort without targeting the gastric acid. Collings et al. showed that calcium carbonate gum (1000 mg $CaCO_3$ or 600 mg $CaCO_3$ ), which only neutralized the pH in the esophagus, significantly relieved heartburn sensation after a meal compared to the placebo treatment in a cross-over design study. It is note worthy that traditional chewable antacid was used as a positive control, and during all four trials, the mean gastric pH was never higher than 3.0. Additionally, the lower dose gum worked as well as the high dose one. By comparing to the placebo trial, two antacid gum trials had longer lasting heartburn relive than the traditional chewing antacid (2002).

In 2013, Brown et al. did another cross-over design study investigating the effectiveness of a novel gum, GutsyGum$^{tm}$, to alleviate heartburn after a meal. The gum contains 500 mg calcium carbonate, "with a proprietary blend of licorice extract, papain, and apple cider vinegar (GiGs®)."(p.1) Result showed a significant decrease on heartburn and acid reflux when compared to the placebo. However, the symptoms of nausea and belching did not show a significant decrease compared to the placebo, while the symptom of pain had a tendency to decrease although not statistically significant (p= .081) (Brown, Sam, Green &

Wood, 2014). Rohof et al. did a study with patients had symptomatic GERD and large hiatal hernias using aliginates to target acid pockets near the esophagogastric junction after a meal. Alginates are natural polysaccharide polymers that react to gastric acid and become gel. The result showed during the 2-hour recording time, alginates formed gel successfully targeted acid pockets near the esophagogastric junction and had more than 75% reduction of acid reflux episode and an hour delayed reflux compared to the 15 minutes of the antacid group (Rohof, Bennink, Smout, Thomas & Boeckxstaens, 2013).

Fundoplication is a surgical option for GERD since 1956. The procedure sews the fundus of the stomach around the LES area. However, there are many side effects related to this surgery, such as increased bloating and flatulence because the patient could not belch easily. A modern surgical option is magnetic sphincter augmentation, which is a ring-like metal structure helps LES work as a barrier. Schwameis et al. did an assessment on the efficacy, safety and feasibility of this option, laparoscopic sphincter augmentation. They concluded that this procedure was minimally invasive, feasible, and safe. Especially when compared to the fundoplication, this procedure is reversible. After 4-weeks of implementation, the result showed patients' GERD-related life quality increased significantly; PPI need reduced; and effective alleviation of GERD related symptoms. However, the concern of the metallic ring might prevent patients from magnetic resonance image test is need to be solved (Schwameis K., Schwameis M. & Zorner et al., 2014).

**Vinegar**

There has been increasing number of research related to vinegar since 1990s, especially after year 2000 and hit its peak in 2011. Johnston et al. pointed out vinegar has

been used for variety function since c. 5000BC. For example, food preservative, managing wounds, dissolving boulders, hand washing with sulfur to prevent infection, treating varies ailment, and antiglycemic agent constitute the history of the vinegar usages (Johnston & Gaas, 2006). Mitrou et al. did a study in subjects with impaired glucose tolerance. They concluded that drinking vinegar before a mixed meal (glycemic index 52, Carbohydrates < 75g, dietary fibres 3.3g), would increase skeletal muscle (forearm) blood flow. The benefit of this result was increased glucose uptake, improved insulin sensitivity, and lowered triglycerides in the blood after a meal (Mitrou, Petsiou & Papakonstantinou et al., 2015). Brown et al. mentioned that apple cider vinegar has many anecdotal reports on its helpfulness in GERD symptoms, and the explanation might be the shock of acid would close LES and hence, prevent the reflux (Brown et al., 2014). Apple cider vinegar was one of the ingredients in the antacid chewing gum in their study.

The production of vinegar involved raw materials that have starch or sugar, and the fermentation process of transforming ethanol into acetic acid (Budak, Aykin, Seydim, Greene & Guzel-Seydim, 2014). Aykin et al. explained in their article that mother of vinegar is a think and hard layer on the surface of vinegar, and it is formed by the acetic acid bacteria and extracellular cellulose. In this study, pomegranate vinegar and apple cider vinegar and their mother were compared for their amount of bioactive substances. There were some interesting detailed data reported. The mother of the vinegar had lower pH than the vinegar itself (apple cider vinegar pH 4.4± 0.9, mother of apple vinegar pH 3.23 ± 0.09, pomegranate vinegar pH 2.98 ± 0.09, mother of pomegranate vinegar pH 2.91 ± 0.08). On the website, many posts stated that organic apple cider vinegar with mother worked superior than other kinds of vinegar on the market. Following our rationale for this study,

pomegranate vinegar with mother would be ideal for inducing movement of the esophageal body because it had a lower pH, which meant more protons available. Although the result concluded that pomegranate vinegar with mother had better antioxidant function and more phenolic substance, apple cider vinegar with mother had much higher Fe and Na elements. Additionally, apple cider vinegar was composed of both chlorogenic aicd and gallic acid, unlike pomegranate had only gallic acid (Aykin, Budak & Guzel-Seydim, 2015). A research showed chlorogenic acid was beneficial in managing hypertension (Zhao, Wang, Ballevre, Luo & Zhang, 2011).

# CHAPTER 3

# METHODOLOGY

## Participants and Study Design

The recruitment of this research started after the Arizona State University Institutional Review Board, Human Subjects Committee's approval (Appendix A). Written informed consent was signed by each subject before the trial started (Appendix B). Subjects were recruited from a campus population through School of Nutrition & Health Promotion program List Serves, newspaper ads, distribution of printed flyers, and word-of-mouth.

This was a randomized, placebo-controlled, double-blind, cross-over study. Subjects were randomly assigned different sequences of four treatments (chili - placebo, antacid after chili meal - positive control, vinegar added to chili and diluted vinegar after chili meal), with one week apart. Each subject in the study served as a control for his/her trials.

The applicable sample size (Appendix C) was calculated using the data from two previous studies that have results of similar outcome variables (Hartono et al., 2011; Collings, 2002). 15 subjects (12 females and 3 males) who were 18 years or older (mean age, 39.6 $\pm$ 12.2 years; range, 21-51 years) were recruited for this study. Subjects reported heartburn symptoms related to GERD were otherwise healthy by self-report.

All subjects were screened for the willingness to follow the study design included ingesting heartburn-inducing chili on four occasions and visiting the Arizona State University's downtown Phoenix campus five times during the trial, and four of the visits would be two hours long. They were also instructed to stop heartburn medication three

days before each testing day. Female volunteers were excluded if they were pregnant or lactating. Subjects were excluded as well if they had pulmonary, renal or heart problems; had peptic ulcers or had gastric cancer; had any abdominal surgery; had been diagnosed with Barrett's esophagus, hiatal hernia, adenocarcinoma or asymptomatic ERD; or had been prescribed PPI treatment at the time of recruiting. The exclusion criteria questionnaire was created with the Survey Monkey website.

**Heartburn-Induced Meal and Symptom Assessment**

On each testing day, subjects arrived in a fasting state, no food or beverage for the last 10 hours but water was allowed. A baseline heartburn symptom was recorded prior to consuming the heartburn-inducing meal. The meal consists of: 1) 250 grams of chili with the heartburn-inducing ingredients, such as onions, tomato and fatty ground meat etc. All of the chili was cooked at once and each serving was divided into zip lock containers and stored in the freezer through out the whole study. The chili recipe is included in the appendices (Appendix D). 2) A carton of eight fl. ounce of chocolate milk (Rodriguez et al., 1998; Holloway et al., 1985). Twenty grams of apple cider vinegar (Bragg® Organic Raw Apple Cider Vinegar, Unfiltered, with the 'Mother') was used for both vinegar trials. During vinegar added to the chili trial, vinegar was added after the chili was heated in the microwave. The other three trials were offered with regular chili. Participants set off their timers right before they started eating, and they were asked to finish the meal in 15 minutes. At 15-minute mark, the after meal drink was consumed. Water was used to dilute 10 grams of liquid antacid (each 5 ml teaspoon contains: calcium 400 mg and magnesium 85 mg; Rolaids® Ultra Strength Liquid, cherry flavor), and 20 grams of vinegar to 60 grams of after

meal drink. Water with food color dye was used for the placebo trial. Gift cards were given at the end of each treatment visit at value of $10, $10, $15, $15 respectively.

The postprandial heartburn symptom assessment began at the first sign of heartburn symptom. Then 15 minutes after the start of the meal, with 5-minute intervals between 15 to 30 minutes, and then 15-minute intervals afterward until the total time span since the start of the meal was 120 minutes. Referring to previous studies, two methods were used to assess heartburn symptoms in this research: 1) 0 to 10-cm visual analogue scale (VAS) ranging from 'no pain' to 'unbearable pain', and 2) 1 to 5 five-point Likert scale ranging from 'none' to 'severe' (Appendix D) (Bytzer, 2004; Collings, 2002). During the two-hour testing period, subjects could walk around or sit upright, but were not allowed to rest at a supine position or recline.

**Statistical Analyses**

The Statistical Package for Social Sciences (SPSS) software version 22 was used for the statistical analyses of the research data. Shapiro-Wilk was used to check normality. Sphericity Assumptions was used to check the repeated measure had equal variances. If the Sphericity Assumptions was violated (Epsilon < 0.75), then Greenhouse-Geisser adjustment was applied to obtain corrected degree of freedom and interpret F-values, and hence an adjusted p-value. ANOVA repeated measure was used to compare the mean heartburn scores during the 120-minute testing time and incremental area-under-the-curve (iAUC) data of four trials. The level of statistical significance is $P < .05$. Mean and standard deviation (SD) will be stated as the results of variable analysis.

# CHAPTER 4

# RESULTS

## Descriptive Characteristics

Forty people responded to the recruitment survey; 31 people met inclusion criteria; 15 people responded to the interview appointment, and signed the consent. The consent form (Appendix B) was translated into Spanish because many of the participants' first language was Spanish and they were not fluent in English. Out of the 15 participants, subject #8 was disqualified because she smoked cigarettes. 14 people were randomly assigned to different sequences of four treatments, each with at least one week apart. Subject #5 did not show up at the first trial and dropped out; subject #7 and #10 had to be discontinued with their trials because of surgeries unrelated to the study. Data were collected from 11 participants (3 males, 8 females) who finished all four trial arms; however four participants were excluded from the data calculation because they did not display heartburn symptoms in all four trials (n=3), or did not follow the protocol (n=1). Hence, data from seven participants (1 male, 6 females) were used to assess the effect of vinegar on the alleviation of heartburn symptoms using the five-point Likert scale (Appendix D). Due to missing data, data from only six participants was available for the visual analogue scale (VAS) assessment (Appendix D).

Table 2 displays baseline demographic profiles of the seven participants whose data were used for hypothesis testing. The age of the participants averaged 39.6 $\pm$ 12.2 years (range: 21 to 51 years old). Body Mass Index (BMI) was 29.4 $\pm$ 4.2 kg/m$^2$, (range: 23.1 to

35.2 kg/m$^2$). Three out of seven participants were overweight (BMI= 25 to 29 kg/m$^2$), and three out of seven participants were obese (BMI ≥ 30 kg/m$^2$).

Table 2
*Participants demographic profiles (n=7)*

| Variables | Number | Mean ± SD[1] | Min-Max |
|---|---|---|---|
| Gender (M/F) | 1/6 | | |
| Age (year) | | 39.6 ± 12.2 | 21 - 51 |
| Height (cm) | | 161.6 ± 9.5 | 150 - 175 |
| Weight (kg) | | 76.5 ± 10.4 | 55.5 - 86.6 |
| BMI (kg/m$^2$) | | 29.4 ± 4.2 | 23.1 - 35.2 |
| Fat (%) | | 36.8 ± 7.7 | 25.1 - 43.8 |
| FFM (kg) | | 48.0 ± 7.0 | 40.6 - 61.6 |
| Waist circumference (cm) | | 92.4 ± 10.3 | 74.5 - 101.5 |

[1]SD = Standard deviation

**Heartburn Study**

Table 3 displays the heartburn scores of seven participants from baseline (Time 0) to 120 minutes on the five-point Likert scale: Scale 1 representing no heartburn to scale 5 representing very severe heartburn. All subjects were asked to consume the chili in 15 minutes, and the timer was started just before the participants started eating the chili. Table 4 contains the heartburn scores from the VAS. Participants recorded their severity of heartburn at each designated time by putting a mark on a 10 centimeter continuous horizontal line ranging from no pain to unbearable pain. For the purpose of equal time spans in the graphs among heartburn scores, heartburn scores before minute-15 and at minute-20 and minute -25 were not included in the data analysis. Therefore all the heartburn scores recorded in the graphs were at 15-minute intervals during the 120-minute recording

period (Figures 1 and 2). Incremental area-under-the-curve (iAUC) for heartburn scores was calculated using the method recommended in a 2004 article by Wolever. iAUC data from both scales (n=7) met Shapiro-Wilk normality test. Sphericity assumption was met for both questionnaire heartburn scores and iAUC Likert scale data. iAUC VAS data violated Sphericity assumption, and Greenhouse-Geisser, which has middle range of conservation, was used to adjust the p-value. ANOVA repeated measure was used to compare the four trial arms of the mean heartburn scores during the 120 minutes testing time and the iAUC heartburn score of each treatment (Table 3, 4, 5). Even though the graph shows a lower heartburn score with vinegar after the chili and antacid trials during the first hour period, and a lower heartburn score with antacid trial during the second hour period, there is no statistically significant difference among the mean and iAUC heartburn scores among different trials (Likert scale questionnaire p= .259, VAS questionnaire p= .659, iAUC Likert scale p= .184, iAUC VAS p= .326). The Bar graphs show there is much standard deviation (SD) overlaps among iAUC heartburn scores in different trials, which indicates no significant difference in heartburn alleviation with different treatments (Figure 3 and 4).

Table 3
*Heartburn score questionnaire five-point Likert scale[1] (Mean ± SD) (n=7)*

| Time (Minutes) | Control | Vinegar In | Vinegar Out | Antacid |
|---|---|---|---|---|
| 0 | 1.14 ± 0.4 | 1.14 ± 0.4 | 1.14 ± 0.4 | 1.14 ± 0.4 |
| 15 | 2 ± 0.8 | 2.29 ± 1.0 | 1.86 ± 0.7 | 2 ± 0.8 |
| 30 | 2.43 ± 0.8 | 2.43 ± 1.1 | 2.29 ± 0.8 | 2.14 ± 0.7 |
| 45 | 2.57 ± 0.5 | 2.43 ± 1.1 | 2 ± 0.8 | 2 ± 0.8 |
| 60 | 2.57 ± 1.0 | 2.71 ± 1.0 | 2 ± 0.8 | 2 ± 0.8 |
| 75 | 2.71 ± 1.3 | 2.71 ± 1.0 | 2.29 ± 0.8 | 2 ± 0.8 |
| 90 | 2.71 ± 1.3 | 3.14 ± 0.7 | 2.57 ± 1.0 | 2.14 ± 1.1 |
| 105 | 2.71 ± 1.3 | 3 ± 1.0 | 2.86 ± 1.2 | 2.14 ± 1.1 |
| 120 | 3 ± 1.2 | 3 ± 1.0 | 2.71 ± 1.1 | 2.14 ± 1.1 |

| | |
|---|---|
| P Value | .259 |
| Effect Size | .444 |

[1] Scale 1-5, (1) None, (2) Mild, (3) Moderate, (4) Severe, (5) Very Severe.
[2] P value obtained through ANOVA repeated measure test.

Table 4
*Heartburn score questionnaire VAS[1] (Mean ± SD) (n=6)*

| Time (Minutes) | Control | Vinegar In | Vinegar Out | Antacid | |
|---|---|---|---|---|---|
| 0 | 0.1 ± 0.2 | 0.35 ± 0.9 | 0.13 ± 0.3 | 0.6 ± 1.3 | |
| 15 | 2.67 ± 1.9 | 2.37 ± 2.0 | 2.68 ± 2.4 | 2.85 ± 2.1 | |
| 30 | 3.6 ± 1.1 | 2.62 ± 2.8 | 3.1 ± 2.8 | 3.05 ± 2.2 | |
| 45 | 3.45 ± 1.4 | 3 ± 2.8 | 2.57 ± 2.5 | 2.43 ± 2.2 | |
| 60 | 3.55 ± 2.2 | 3.65 ± 2.4 | 2.57 ± 2.5 | 2.52 ± 2.1 | |
| 75 | 3.93 ± 2.9 | 3.75 ± 2.5 | 3 ± 2.6 | 2.33 ± 2.1 | |
| 90 | 4.02 ± 3.1 | 4.73 ± 2.0 | 4.2 ± 2.6 | 2.62 ± 2.8 | |
| 105 | 4.42 ± 3.2 | 4.63 ± 2.0 | 4.98 ± 3.0 | 2.65 ± 2.8 | |
| 120 | 4.63 ± 3.2 | 4.97 ± 1.9 | 5.08 ± 3.2 | 2.58 ± 2.7 | |
| P Value[2] | | | | | .659 |
| Effect Size | | | | | .232 |

[1] Visual analogue scale 0-10, 0 = no pain, 10 = unbearable pain.
[2] P value obtained through ANOVA repeated measure.

Table 5
*Heartburn score iAUC[1] (Mean ± SD)*

| iAUC | Control | Vinegar In | Vinegar Out | Antacid | P Value[2] | Effect size |
|---|---|---|---|---|---|---|
| Likert Scale[3] | 161.79 ± 117.4 | 175.36 ± 109.8 | 130.36 ± 94.0 | 105.36 ± 87.9 | .184 | .425 |
| VAS[4] | 389.18 ± 209.5 | 393.75 ± 267.5 | 336.96 ± 237.8 | 230.13 ± 218.2 | .326 | .611 |

[1] iAUC = incremental area-under-the-curve. [2] P value obtained through ANOVA repeated measure. Greenhouse-Geisser adjustment is used for VAS p value.
[3] n=7. [4] Visual analogue scale n=7, except antacid trial n=6

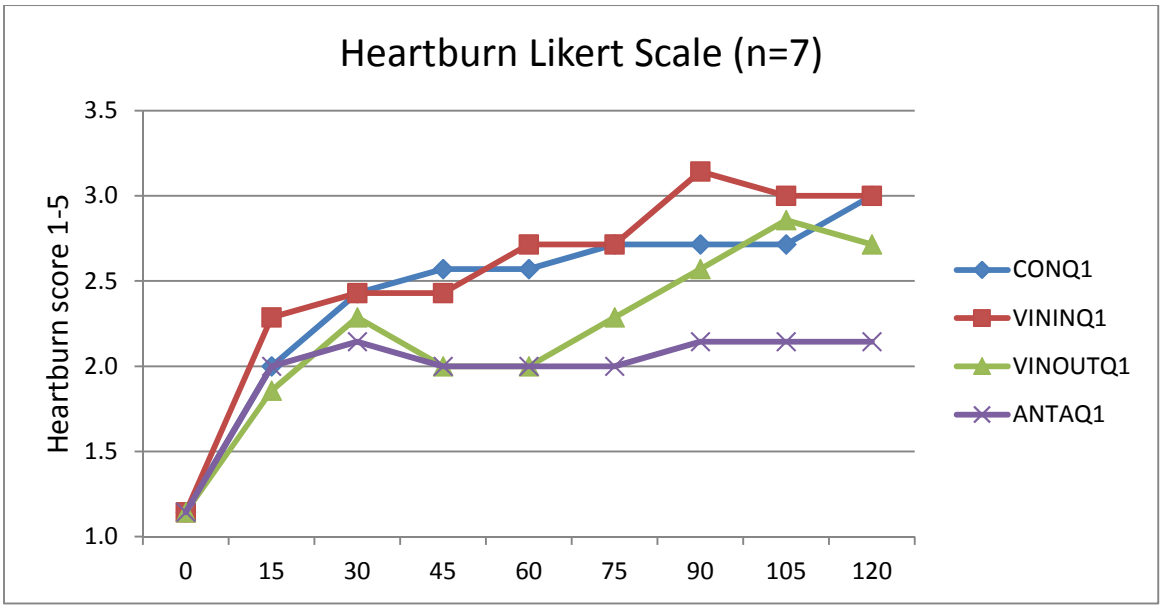


*Figure 1*. Postprandial incremental heartburn scores after chili meals using a five-point Likert scale (1. None, 2 Mild, 3 Moderate, 4 Severe, 5 Very Severe) under four experimental conditions: control, vinegar added to the chili, diluted vinegar after chili meal and antacid after chili meal. Inlay chart depicts mean heartburn score for each experimental condition at each time point. (Horizontal axis, unit = minutes).

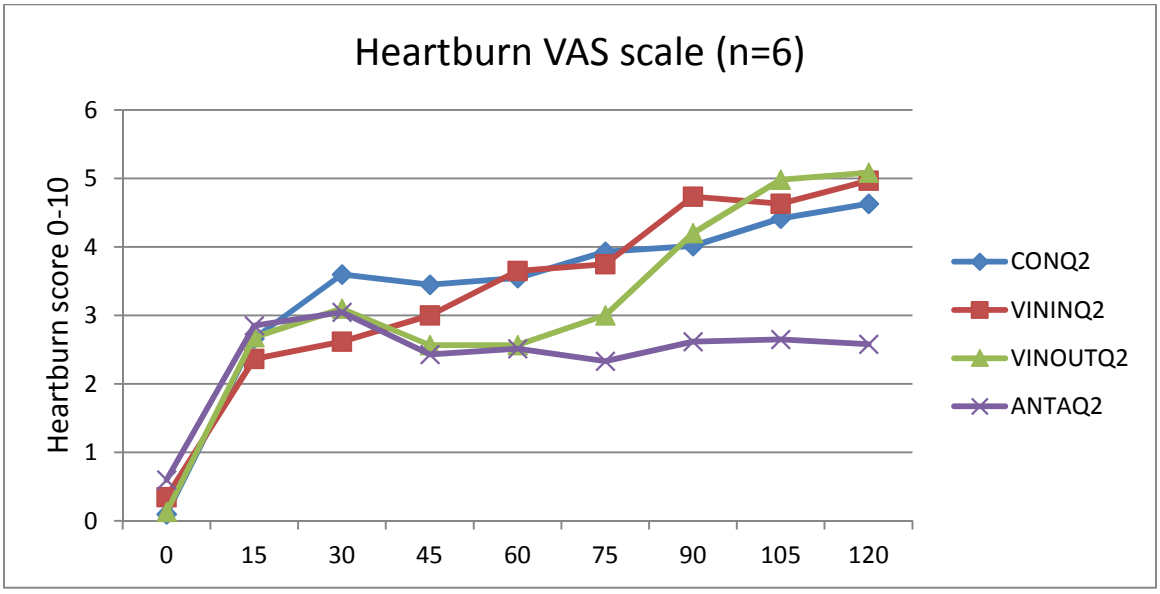

*Figure 2.* Postprandial incremental heartburn scores after chili meals using a 10-centimeter visual analogue scale (0 = no pain, 10 = unbearable pain) under four experimental conditions: control, vinegar added to the chili, diluted vinegar after chili meal and antacid after chili meal. Inlay chart depicts mean heartburn score for each experimental condition at each time point. (Horizontal axis, unit = minutes).

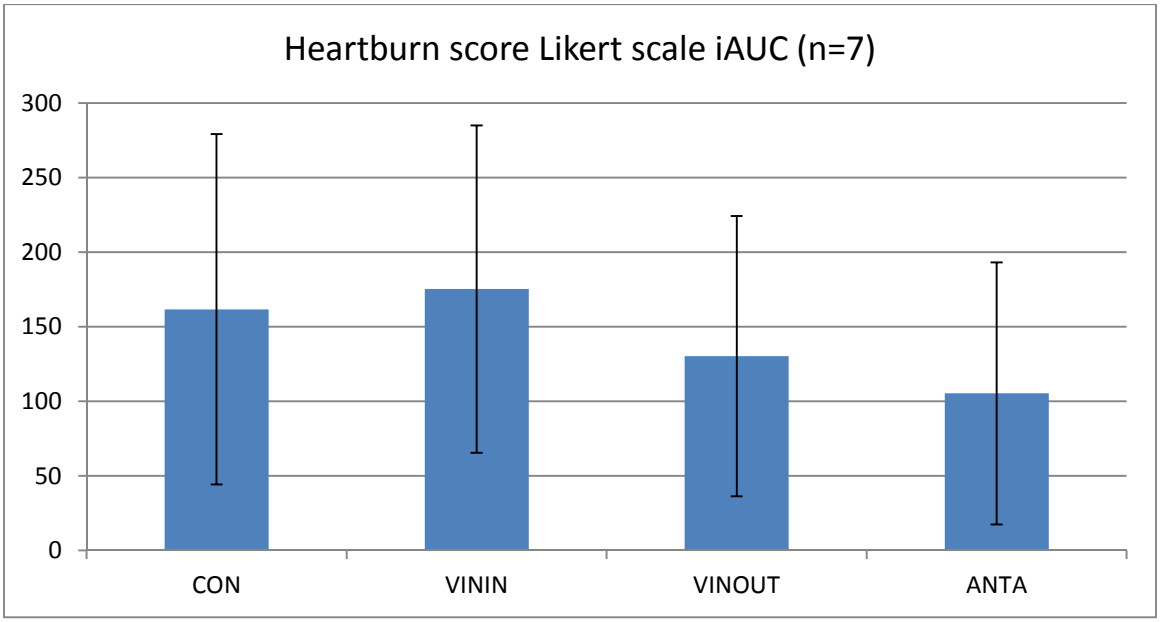


*Figure 3.* Postprandial incremental heartburn scores after chili meals using a five-point Likert scale under four experimental conditions: 1. Control (CON), 2.Vinegar added to the chili (VIN), 3.Diluted vinegar after chili meal (VINOUT) and 4.Antacid after chili meal (ANTA). Inlay chart depicts iAUC heartburn score (mean ± SD) for each experimental condition (P=.184).

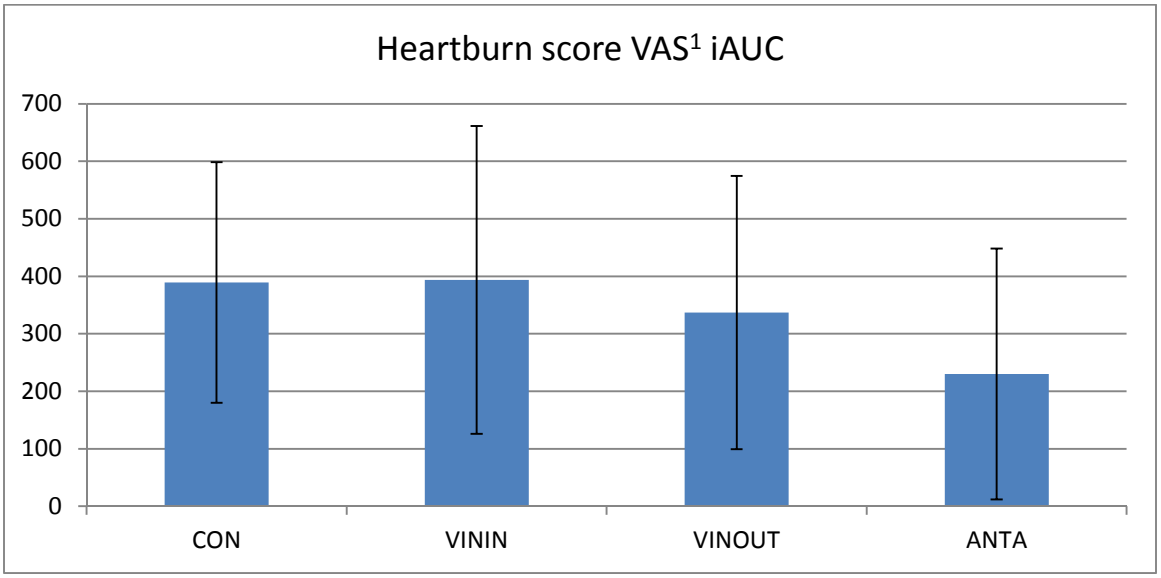

*Figure 4*. Postprandial incremental heartburn scores after chili meals using a visual analogue scale under four experimental conditions: 1. Control (CON), 2.Vinegar added to the chili (VIN), 3.Diluted vinegar after chili meal (VINOUT) and 4.Antacid after chili meal (ANTA). Inlay chart depicts iAUC heartburn score (mean ± SD) for each experimental condition (P=.326). [1]Visual analogue scale n=7, except antacid trial n=6

**Antacid Responding Circumstance**

After carefully reading the graphs of each individual participant's mean heartburn data (Appendix F) during four trail-arms, an interesting phenomenon was noticed. Some participants responded well to antacid, but not as much for the others. Hence, seven participants were divided into two groups: Antacid non-responder and antacid responder. The criterion to assign a participant to the antacid responder group was that his/her mean heartburn data scored below three on the VAS during the whole 120-minute span of the individual's antacid trial. Except #14 who was missing VAS data in antacid trial day, so the Likert scale heartburn number no larger than 2 was used to assign her the antacid responder group. The decision of using mean heartburn score less than three on the VAS as the criterion was made by perceiving that when a participant marked mild heartburn on the Likert scale (2 out of 5) generally corresponding to the VAS at around or less than 3 cm on a 10 cm line.

Likert scale iAUC data from antacid non-responder group (n=3) met Shapiro-Wilk normality test, but not VAS iAUC data. ANOVA repeated measure was used to compare the difference of the mean heartburn scores during the total 120 minutes testing time and the iAUC heartburn score of each treatment (Table 5, 6, 7). All of the data in this group violated Sphericity assumption, and Greenhouse-Geisser adjustment was used to obtain the p-values. Effect size was not available for any of data in this group due to not enough data.

Table 6

*Heartburn score questionnaire Likert scale[1] (Mean ± SD) - Antacid non-responder (n=3)*

| Time (Minutes) | Control | Vinegar In | Vinegar Out | Antacid |
|---|---|---|---|---|
| 0 | 1.33 ± 0.6 | 1.33 ± 0.6 | 1.33 ± 0.6 | 1.33 ± 0.6 |
| 15 | 2.33 ± 0.6 | 2.0 ± 1.0 | 2.0 ± 1.0 | 2.67 ± 0.6 |
| 30 | 2.67 ± 0.6 | 2.33 ± 1.5 | 2.33 ± 1.2 | 2.67 ± 0.6 |
| 45 | 2.67 ± 0.6 | 2.0 ± 1.7 | 2.0 ± 1.0 | 2.67 ± 0.6 |
| 60 | 2.67 ± 2.2 | 2.33 ± 1.5 | 2.0 ± 1.0 | 2.67 ± 0.6 |
| 75 | 3.0 ± 2.9 | 2.33 ± 1.5 | 2.33 ± 0.6 | 2.67 ± 0.6 |
| 90 | 3.0 ± 3.1 | 3.0 ± 1.0 | 3.0 ± 1.0 | 3.0 ± 1.0 |
| 105 | 3.0 ± 3.2 | 3.33 ± 0.6 | 3.33 ± 1.2 | 3.0 ± 1.0 |
| 120 | 3.33 ± 3.2 | 3.33 ± 0.6 | 3.0 ± 1.0 | 3.0 ± 1.0 |
| P-value[2] | | | | .807 |
| Effect Size | | | | N/A |

[1] Scale 1-5, (1) None, (2) Mild, (3) Moderate, (4) Severe, (5) Very Severe. [2] P-value obtained through ANOVA repeated measure. Greenhouse-Geisser adjustment is used.

Table 7

*Heartburn score questionnaire VAS[1] (Mean ± SD) - Antacid non-responder (n=3)*

| Time (Minutes) | Control | Vinegar In | Vinegar Out | Antacid |
|---|---|---|---|---|
| 0 | 0.2 ± 0.4 | 0.7 ± 1.2 | 0.27 ± 0.5 | 1.2 ± 1.7 |
| 15 | 2.6 ± 1.2 | 1.83 ± 1.7 | 2.43 ± 2.6 | 4.27 ± 1.9 |
| 30 | 3.57 ± 0.7 | 2.3 ± 3.6 | 3.4 ± 3.0 | 4.5 ± 1.9 |
| 45 | 3.2 ± 1.4 | 2.3 ± 4.0 | 2.3 ± 2.3 | 4.03 ± 1.4 |
| 60 | 3.37 ± 3.1 | 3.23 ± 3.7 | 2.33 ± 2.3 | 4.2 ± 0.6 |
| 75 | 4.23 ± 4.4 | 3.3 ± 3.8 | 2.4 ± 2.2 | 3.77 ± 1.6 |
| 90 | 4.37 ± 4.6 | 4.63 ± 3.0 | 4.6 ± 2.3 | 4.3 ± 2.8 |
| 105 | 4.43 ± 4.7 | 5.23 ± 2.4 | 5.43 ± 2.6 | 4.33 ± 2.8 |
| 120 | 4.53 ± 4.5 | 5.93 ± 1.6 | 5.13 ± 2.6 | 4.3 ± 2.8 |

| | |
|---|---|
| P Value[2] | .837 |
| Effect Size | N/A |

[1] Visual analogue scale 0-10, 0 = no pain, 10 = unbearable pain. [2] P value obtained through ANOVA repeated measure. Greenhouse-Geisser adjustment is used.

Table 8

*Heartburn score iAUC[1] (Mean ± SD) - Antacid non-responder (n=3)*

| iAUC | Control | Vinegar In | Vinegar Out | Antacid | P Value[2] | Effect size |
|---|---|---|---|---|---|---|
| Likert Scale | 170 ± 191.5 | 136.67 ± 177.1 | 129.17 ± 130.5 | 163.08 ± 113.4 | .776 | N/A |
| visual analogue scale | 409.83 ± 328.8 | 307.25 ± 429.8 | 356.33 ± 226.0 | 341.08 ± 245.0 | .761 | N/A |

[1] iAUC = incremental area-under-the-curve.
[2] P value obtained through ANOVA repeated measure. Greenhouse-Geisser adjustment is used for both scales.

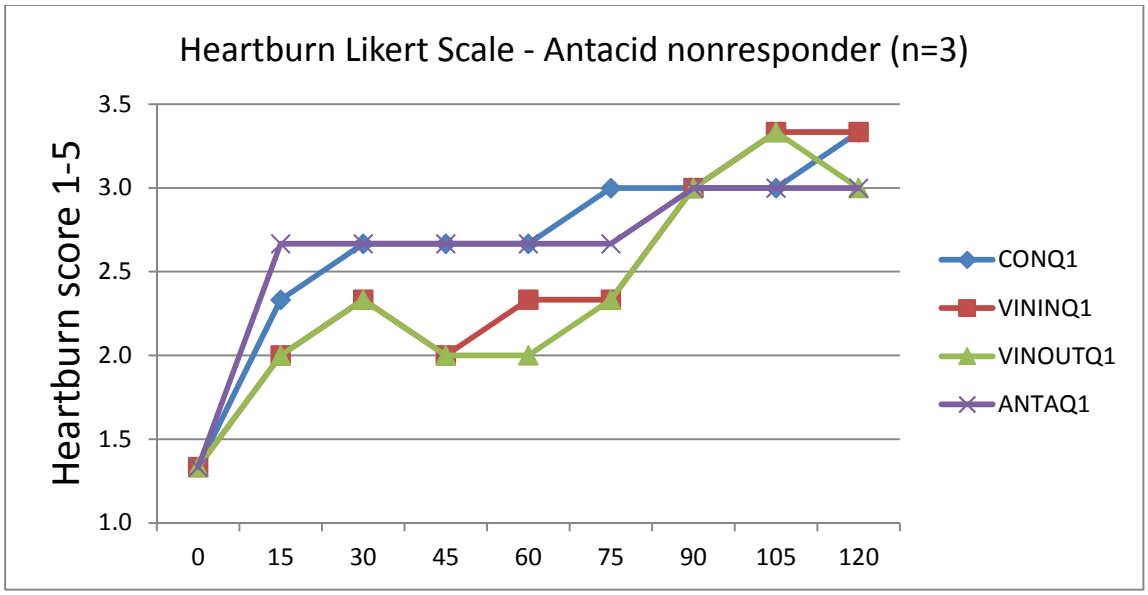


*Figure 5*. Postprandial incremental heartburn scores after chili meals using a five-point Likert scale (1. None, 2 Mild, 3 Moderate, 4 Severe, 5 Very Severe) under four experimental conditions: control, vinegar added to the chili, diluted vinegar after chili meal and antacid after chili meal. Inlay chart depicts mean heartburn score for each experimental condition at each time point. (Horizontal axis, unit = minutes).

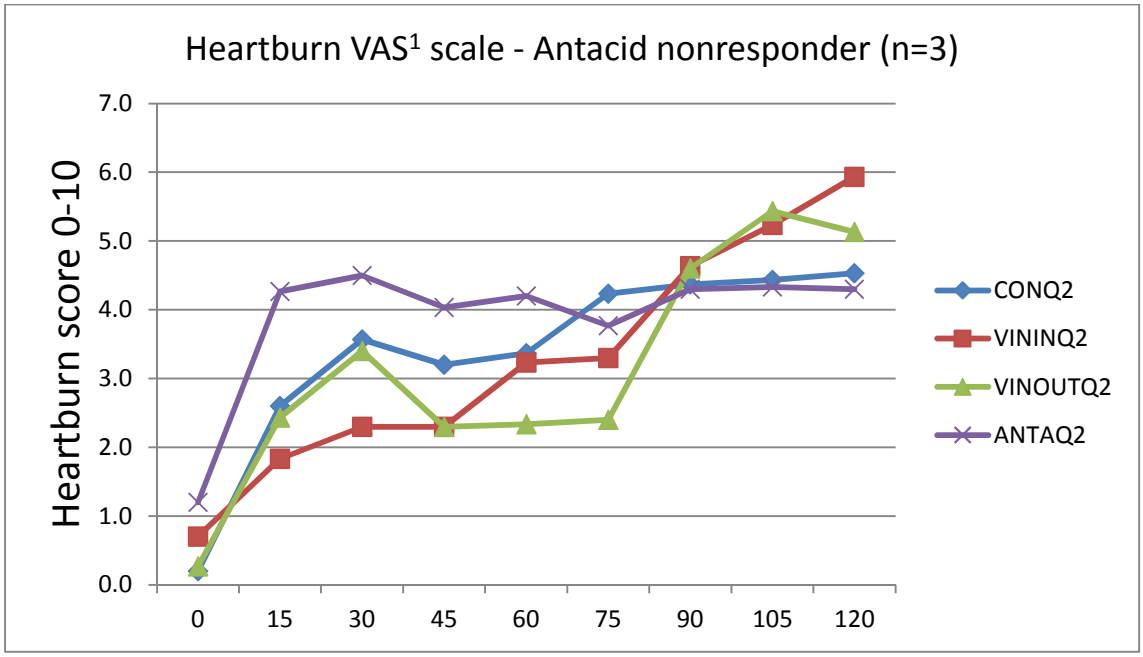


*Figure 6*. Postprandial incremental heartburn scores after chili meals under four experimental conditions: control, vinegar added to the chili, diluted vinegar after chili meal and antacid after chili meal. Inlay chart depicts mean heartburn score for each experimental condition at each time point. (Horizontal axis, unit = minutes). [1]Visual analogue scale 0-10, 0 = no pain, 10 = unbearable pain.

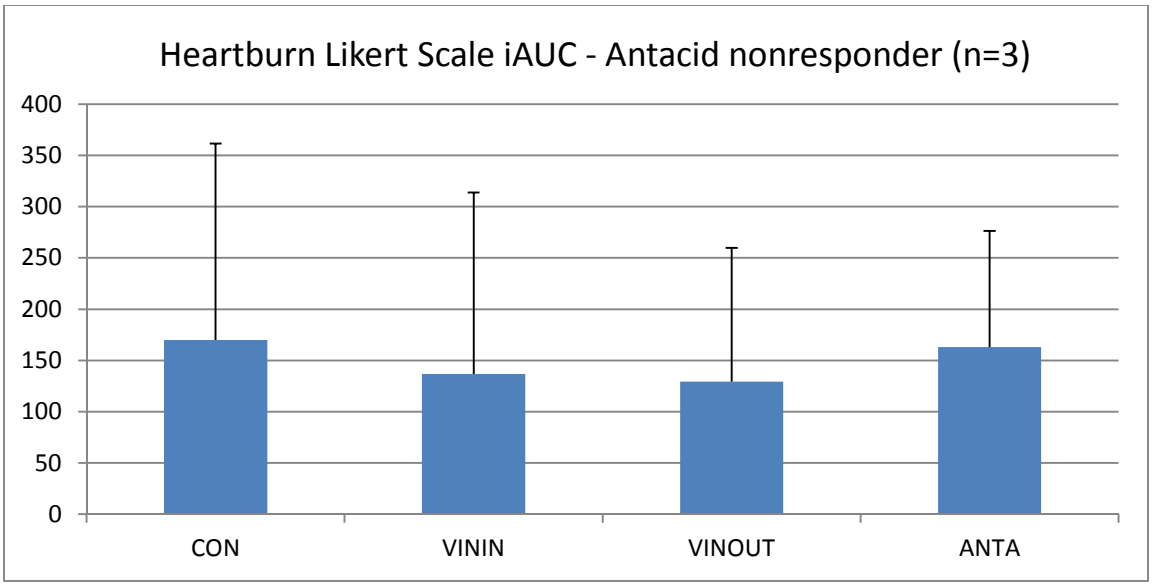


*Figure 7*. Postprandial incremental heartburn scores after chili meals using a five-point Likert scale under four experimental conditions: 1. Control (CON), 2.Vinegar added to the chili (VIN), 3.Diluted vinegar after chili meal (VINOUT) and 4.Antacid after chili meal (ANTA). Inlay chart depicts iAUC heartburn score (mean ± SD) for each experimental condition (P=.776).

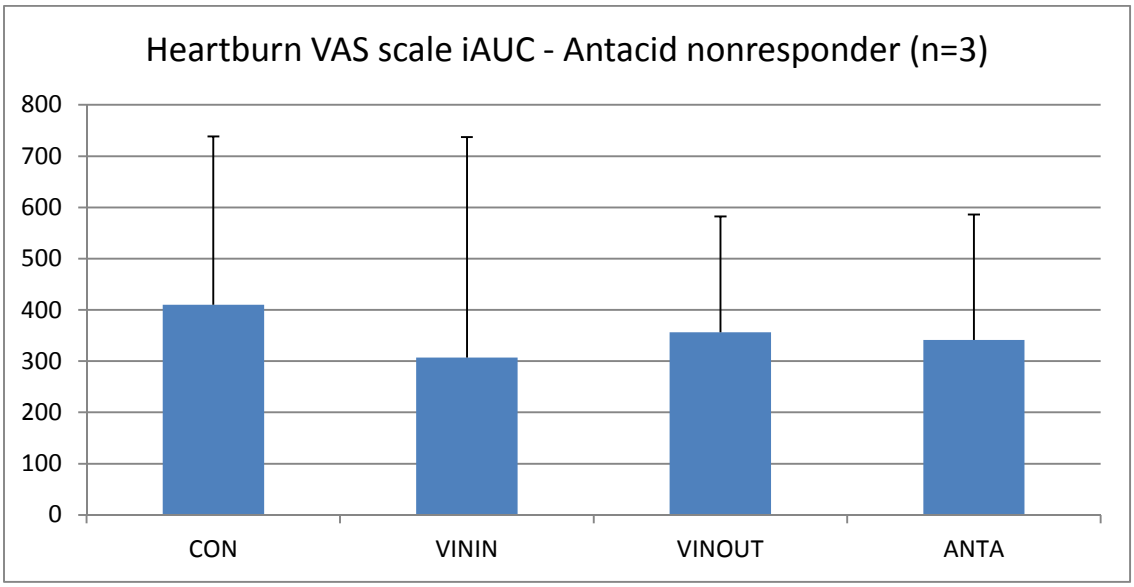


*Figure 8*. Postprandial incremental heartburn scores after chili meals using a visual analogue scale under four experimental conditions: 1. Control (CON), 2.Vinegar added to the chili (VIN), 3.Diluted vinegar after chili meal (VINOUT) and 4.Antacid after chili meal (ANTA). Inlay chart depicts iAUC heartburn score (mean ± SD) for each experimental condition (P=.761).

Both iAUC data from antacid responder group (n=4) met Shapiro-Wilk normality test. ANOVA repeated measure was used to compare the difference of the mean heartburn scores during the total 120 minutes testing time and the iAUC heartburn score of each treatment (Table 9, 10, 11). Only Likert scale iAUC data met Sphericity assumption, and Greenhouse-Geisser adjustment was used to obtain the p-values for the rest of data in this group. Likert scale iAUC p= .017, and the significance was heartburn scores between vinegar added to the chili trial and antacid trial. The effect size from the iAUC Likert scale data was .936. Another significant p-value was mean heartburn score data for Likert scale p= .034 with effect size .967. VAS iAUC and mean heartburn scores effect size were unavailable, and iAUC p= .203, and mean heartburn score p= .205.

Table 9

*Heartburn score questionnaire Likert scale[1] (Mean ± SD) - Antacid Responder (n=4)*

| Time (Minutes) | Control | Vinegar In | Vinegar Out | Antacid | |
|---|---|---|---|---|---|
| 0 | 1 ± 0 | 1 ± 0 | 1 ± 0 | 1 ± 0 | |
| 15 | 1.75 ± 1.0 | 2.5 ± 1.0 | 1.75 ± 0.5 | 1.5 ± 0.6 | |
| 30 | 2.25 ± 1.0 | 2.5 ± 1.0 | 2.25 ± 0.5 | 1.75 ± 0.5 | |
| 45 | 2.5 ± 0.6 | 2.75 ± 0.5 | 2 ± 0.8 | 1.5 ± 0.6 | |
| 60 | 2.5 ± 0.6 | 3 ± 0 | 2 ± 0.8 | 1.5 ± 0.6 | |
| 75 | 2.5 ± 0.6 | 3 ± 0 | 2.25 ± 1.0 | 1.5 ± 0.6 | |
| 90 | 2.5 ± 0.6 | 3.25 ± 0.5 | 2.25 ± 1.0 | 1.5 ± 0.6 | |
| 105 | 2.5 ± 0.6 | 2.75 ± 1.3 | 2.5 ± 1.3 | 1.5 ± 0.6 | |
| 120 | 2.75 ± 1.0 | 2.75 ± 1.3 | 2.5 ± 1.3 | 1.5 ± 0.6 | |
| P Value[2] | | | | | .034 |
| Effect Size | | | | | .967 |

[1]Scale 1-5, (1) None, (2) Mild, (3) Moderate, (4) Severe, (5) Very Severe. [2]P value obtained through ANOVA repeated measure. Greenhouse-Geisser adjustment is used.

Table 10

*Heartburn score questionnaire VAS[1] (Mean ± SD) - Antacid Responder*

| Time (Minutes) | Control | Vinegar In | Vinegar Out | Antacid | |
|---|---|---|---|---|---|
| 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 | |
| 15 | 2.73 ± 2.3 | 2.9 ± 2.5 | 2.93 ± 2.8 | 1.43 ± 1.3 | |
| 30 | 3.63 ± 1.6 | 2.93 ± 2.5 | 2.8 ± 3.2 | 1.6 ± 1.4 | |
| 45 | 3.7 ± 1.6 | 3.7 ± 1.3 | 2.83 ± 3.3 | 0.83 ± 1.4 | |
| 60 | 3.73 ± 1.4 | 4.07 ± 0.6 | 2.8 ± 3.2 | 0.83 ± 1.4 | |
| 75 | 3.63 ± 1.4 | 4.2 ± 0.5 | 3.6 ± 3.3 | 0.9 ± 1.6 | |
| 90 | 3.67 ± 1.6 | 4.83 ± 0.7 | 3.8 ± 3.4 | 0.93 ± 1.6 | |
| 105 | 4.4 ± 2.0 | 4.03 ± 1.8 | 4.53 ± 3.9 | 0.97 ± 1.7 | |
| 120 | 4.73 ± 2.1 | 4 ± 2.0 | 5.03 ± 4.4 | 0.87 ± 1.5 | |
| P Value[2] | | | | | .205 |
| Effect Size | | | | | N/A |

[1] Visual analogue scale 0-10, 0 = no pain, 10 = unbearable pain. N=6. [2] P value obtained through ANOVA repeated measure. Greenhouse-Geisser adjustment is used.

Table 11

*Heartburn score iAUC[1] (Mean ± SD) - Antacid Responder*

| iAUC | Control | Vinegar In | Vinegar Out | Antacid | P Value[2] | Effect size |
|---|---|---|---|---|---|---|
| Likert Scale[3] | 155.63 ± 54.8 | 204.38 ± 24.0 | 131.25 ± 79.4 | 68.25 ± 64.5 | .017 | .936 |
| visual analogue scale[4] | 373.69 ± 122.3 | 458.63 ± 83.2 | 349.44 ± 280.4 | 119.17 ± 148.5 | .203 | N/A |

[1] iAUC = incremental area-under-the-curve.

[2] P value obtained through ANOVA repeated measure. Greenhouse-Geisser adjustment is

used for VAS p value. [3] Likert scale n=4. [4] VAS n=4, except Antacid trial n=3.

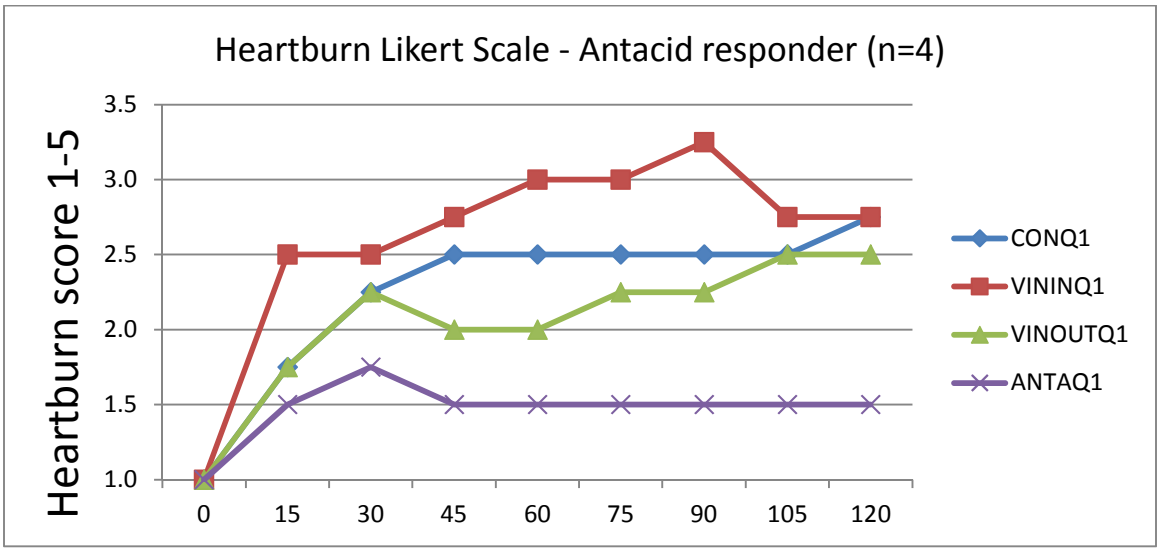


*Figure 9*. Postprandial incremental heartburn scores after chili meals using a five-point Likert scale (1. None, 2 Mild, 3 Moderate, 4 Severe, 5 Very Severe) under four experimental conditions: control, vinegar added to the chili, diluted vinegar after chili meal and antacid after chili meal. Inlay chart depicts mean heartburn score for each experimental condition at each time point. (Horizontal axis, unit = minutes).

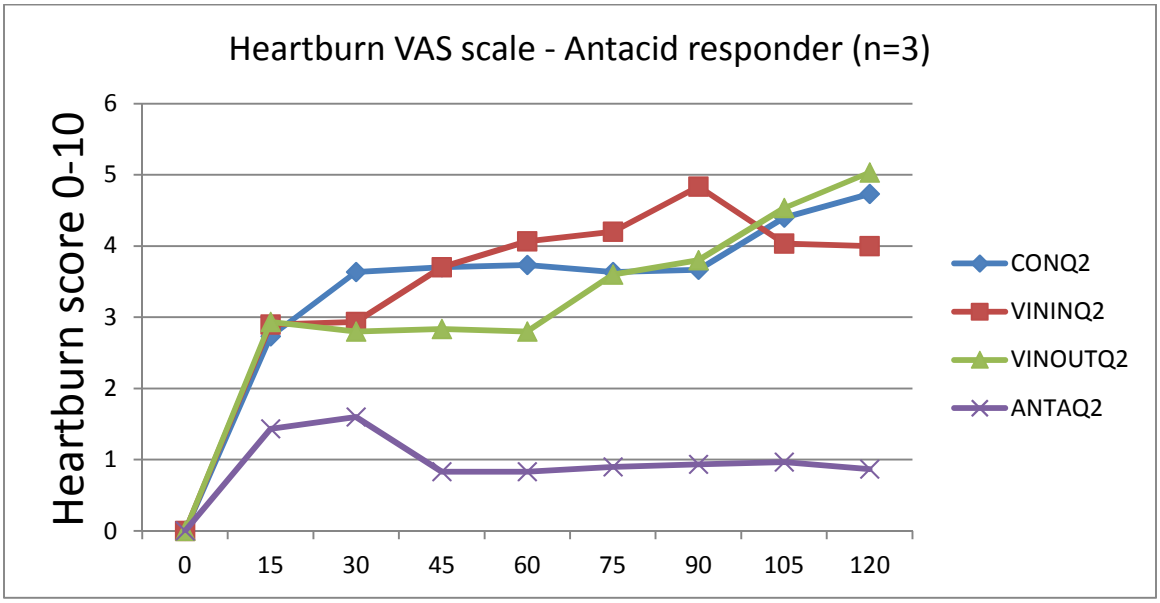


*Figure 10*. Postprandial incremental heartburn scores after chili meals using a 10-centimeter visual analogue scale (0 = no pain, 10 = unbearable pain) under four experimental

conditions: control, vinegar added to the chili, diluted vinegar after chili meal and antacid after chili meal. Inlay chart depicts mean heartburn score for each experimental condition at each time point. (Horizontal axis, unit = minutes).

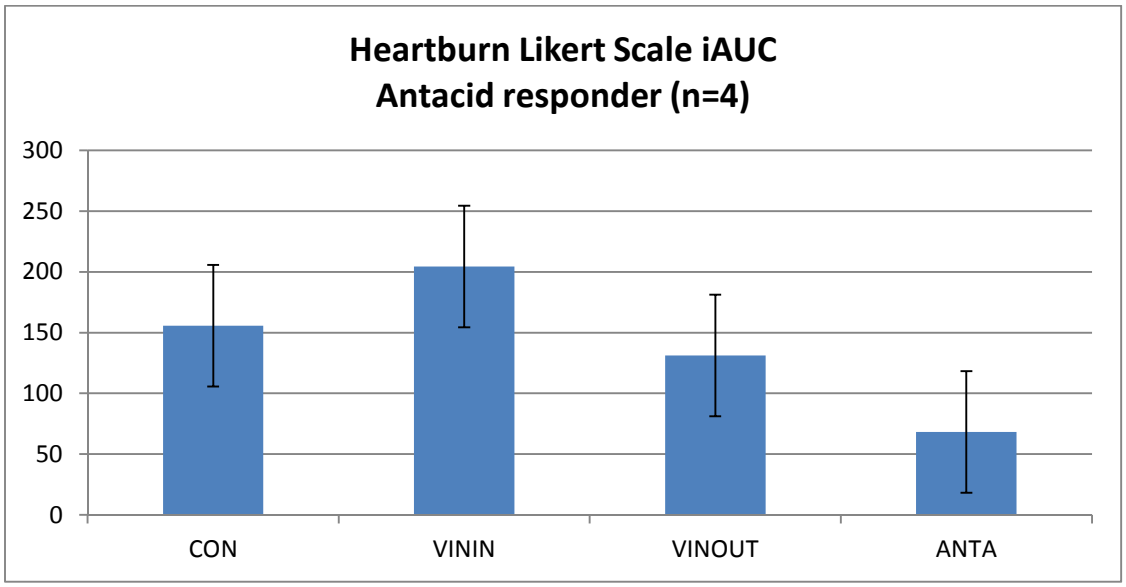


*Figure 11.* Postprandial incremental heartburn scores after chili meals using a five-point Likert scale under four experimental conditions: 1. Control (CON), 2.Vinegar added to the chili (VIN), 3.Diluted vinegar after chili meal (VINOUT) and 4.Antacid after chili meal (ANTA). Inlay chart depicts iAUC heartburn score (means ± SD) for each experimental condition (P=0.246).

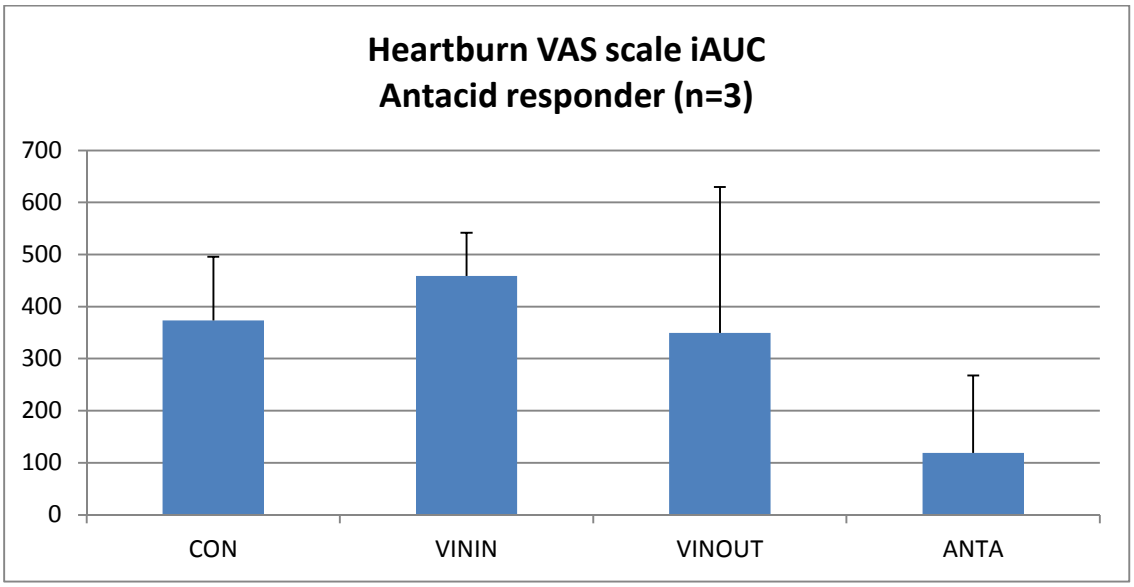


*Figure 12.* Postprandial incremental heartburn scores after chili meals using a visual analogue scale under four experimental conditions: 1. Control (CON), 2.Vinegar added to the chili

(VIN), 3.Diluted vinegar after chili meal (VINOUT) and 4.Antacid after chili meal (ANTA). Inlay chart depicts iAUC heartburn score (means ± SD) for each experimental condition (P=0.163).

# CHAPTER 5

# DISCUSSION

People with GERD have different etiologies and therefore a wide range of the symptom manifestations. Many previous studies have investigated various possible underlying causes of the GERD symptoms, with different approaches and suggestions to reducing the heartburn that occurred. This study was to investigate how the ingestion of organic vinegar with mother, acetic acid, affects the intensity of heartburn related to GERD. Even though the result shows no statistically significant difference among each treatment trial (vinegar added to the chili meal, vinegar after chili meal or antacid) and the placebo trial, the effective size indicated that according to the result data, there was a large difference among each intervention.

## Effect Size

Due to little available data for analysis, effect size was used to interpret data along with p value using ANOVA repeated measure. Type II error might be made when sample size is too small, and a significant change from the intervention could have a non-significant p-value. According to Levine and Hullett in their 2002 article, with null hypothesis, both the sample size and the scale of the change due to the interventions affect the p-values. Therefore, a measurement fairly independent from the sample size is necessary to observe the effectiveness of the intervention (Levine & Hullett, 2002) when usable data from the participants was not corresponding to the original calculated recruitment size (Appendix C),

either too much or too little. According to Cohen et al. in their 2001 article, the formula used would be leading more toward partial eta-squared than eta-squared. Effect size when using ANOVA ($\eta^2$), the strength of the relationship between the factor and the dependent variable or the degree of the difference between groups, is as follows: Small= 0.02, Medium= 0.13 and Large= 0.26 (Cohen, Miles & Shevlin, 2001). Effect size in this study was analyzed by Multivariate Tests effect: partial eta squared.

**Interesting Data Findings**

Some interesting data were observed from studying each individual participant's heartburn rating during four trial arms. Among seven participants, one treatment might have worked very well for a few people, but the same treatment caused more heartburn in others. This phenomenon was observed in all three treatments. For people who responded well to antacid, vinegar added to the chili might cause more heartburn than the chili alone in the placebo trial. Likert scale mean heartburn scores showed significant p-value of .026 between antacid treatment and vinegar added to the chili treatment in the antacid responder group. Oddly, in the same group, when diluted vinegar was ingested after the chili meal, it appeared to lower the heartburn intensity during the first hour comparing to the placebo trial although the effect was not as pronounced as antacid. For people who did not respond to antacid, according to the VAS graph, antacid might cause more heartburn than the chili alone in the placebo trial. Interestingly, among three participants in the antacid non-responder group, one responded very well with diluted vinegar, but vinegar added to the chili caused the worst heartburn among four trials. Yet, another responded better when vinegar was in the chili, while the last participant responded well to both vinegar treatments. This phenomenon might be due to the fact that the participants were in a different

subcategory or had a different etiology of GERD, and it also explained the huge overlap of the SD in the result. Another interesting observation was that, for people who responded to vinegar treatment, the effect only lasted 60 to 75 minutes, and then the heartburn intensity might increase afterwards.

Another interesting outcome was discovered after noticing lower heartburn scores in the placebo treatment when a few participants had CON trial at their fourth week. The sum of the mean heartburn scores (n=8) was decreasing as the study progressed, regardless of the treatment sequences. The data of the participant, who did not follow the protocol and drank her after-meal beverage at the 60-minute mark instead of the 15-minute mark, was included in this data analysis because the comparison was focused on the mean heartburn score of the test day, not the time of each trial. Therefore, even though she applied the treatments later than the protocol, her mean heartburn score of the day still could be used for comparison with her other three trials. The current study used cross-over design, which does not address "learned effect" from the participants during the trials. A brain processing for the perceived heartburn threat after the first week of treatment might explain this outcome. Siwiec et al. has demonstrated that the amygdala and hippocampus were involved with the esophageal afferent signal processing (Siwiec et al., 2015). The amygdala and hippocampus are part of the default mode network, within which part of its tasks is detecting a threat and readying the body for an automatic response. The participants who joined the study had agreed to ingest heartburn inducing chili for four trials, on the same day of the week, and at the same time of the day. In real life, we do not operate in this manner. The heartburn inducing chili meal, which was presented to the participants, had a more parallel meaning to a hole in the road on the way to work than a spontaneous meal, which may or may not

induce heartburn. Further research is needed to investigate this learned effect and the suitability of the cross-over design in the subjective based study.

**Considerations for Future Study Design**

There were several opportunities to make improvements for the next study design. First, we had a surprisingly high prevalence of the Spanish speaking population in our participants, and there were not many studies targeting heartburn symptoms in the Hispanic population. It would be beneficial to have an ethnic question added to the medical history questionnaire, and the comparison among different ethnicity could be performed as well.

Second, the heartburn assessment methods in the current study replicated the study Collings et al. did in 2002, investigating how antacid chewing gum affected heartburn and pH in the esophagus (Collings et al., 2002). During the trials, most of the participants would mark the heartburn score on the Likert scale sheet, and then marked a corresponding position on the VAS line where the heartburn intensity was on the Likert scale sheet. Understandably, when two pieces of paper put side by side with similar orientation to mark the same symptom, it is very easy to make marks at the same location on each paper. The problem with this approach was that the marks on the VAS lines were not being mindfully considered. Furthermore, they were confined by the dots on the Likert scale sheet, instead of being treated as a continuous line. The purpose of a continuous line was to distinguish the different degree of each status of heartburn on the Likert scale. For example, a mild heartburn could range from 0.2 to around 3.5 cm on a VAS line, and as time changes, the heartburn symptom might change its intensity in that range despite the fact that it would still be a mild heartburn on the Likert scale. Unfortunately, the VAS graphs did not show the

details of the change we were looking for, except for the graphs from a few participants. After carefully reading the data, it might be valuable to only use VAS assessment for heartburn symptoms, or having one scale in portrait orientation and the other one in landscape placement to break up the automatic transferred thinking pattern.

Third, when working with the participants who do not speak the same language, a translator who understands the details in the study protocol is necessary. Trying to explain the protocol with different languages might create confusion and disturb the trial progression. Besides, it was hard to know if the participants understood the conversation.

Fourth, a fair design flow for all treatments should have liquid antacid and diluted vinegar drank right at the beginning of the meals, so they would start to work almost the same time as the vinegar added to the chili trial. In our current study design, vinegar added to the chili started 15 minutes earlier than the other two treatments.

Additionally, pinching the nose could be a reinforced protocol instead of a suggestion to reduce the strong smell of vinegar especially when drinking the diluted vinegar. Pinching the nose and reducing the pungent smell could also reduce the possibility of aspiration of vinegar into a wrong pipe.

There was an issue during the recruitment phase. We would like to recruit only people who would get heartburn symptom from eating our chili meal, so a sample was provided on the interview day. However, we could not let the volunteer try our chili without signing the consent form, and therefore, we needed to recruit the volunteer before we knew if he/she would have heartburn symptoms after eating chili. Using the actual amount of chili in the trial as a sample might also be helpful. During the trials, some participants could

not finish the whole bowl while a male participant mentioned the amount was too little to induce heartburn for him.

**Possible Rationale for Data Interpretation**

**Antacid responder.** The possible explanation for how vinegar caused more heartburn for some participants, especially the ones who responded to antacid well, is the following. For people who have damages to their esophageal epithelium because of prolonged acidic reflux, chili could pass the stratified squamous layer and irritates the mucosa layer below. While vinegar is a weak acid, it could still create more irritation to these participants than the chili alone. The pH for the chili in the control trial was 4.76, and in the VININ trial, the chili pH was 4.4. Contradictorily, diluted vinegar seemed provide some heartburn relive for this group. The possible reason could be diluted vinegar had more protons available than the one mixed in the chili. Diluted vinegar in VINOUT trial pH was 3.02. Dutta et al. demonstrated that acid infusion in the upper third part of the esophagus significantly increased saliva secretion (Dutta et al., 2010). While most of the damaged esophageal epithelium was located at distal part of the esophagus, the saliva might have neutralize the content, and increased primary peristalsis from swallowing extra saliva might help to clear the esophagus as well. Especially Bontempo et al. mentioned that during esophageal acidification, the primary peristalsis activated by deglutitions had increased duration and amplitude and decreased propagation velocity (Bontempo et al., 1994). Therefore, ingestion of vinegar might provide a better esophageal clearance for GERD patients whose etiology is not hyposensitive esophageal mucosa to acid stimulation, such as patients with asymptomatic ERD.

**Antacid non-responder.** Even though not statistically significant, we observed that participants, who did not respond to antacid well, had lower heartburn intensities during the first 60 minutes in at least one vinegar trial when comparing to the placebo. Before we hypothesize the mechanisms which might happen in the esophagus or the stomach after ingestion of organic vinegar with mother, that could help reduce the sensation of heartburn, it is helpful to review the results and proposals of previous research that are highly related to the association among the esophagus, reflux episode, acid infusion and nervous system in the esophagus. As earlier described in the subcategories of GERD section, people with NERD or FH had very high refractory rates of PPI treatment. Their reflux content could be neutral or the heartburn sensation could occur even without the existence of reflux episode respectively. Zerbib et al. mentioned that anti-reflux therapy, such as PPI, worked much better with patients who had a normal esophageal acid exposure, and their heartburn symptoms and reflux episodes correlated closely (Zerbib et al., 2012). In the esophagus section, we pointed out the distinguishing function of chemonociception vagal afferents and mechanical stimuli perception spinal afferents. Dutta et al. and Bontempo et al. showed acid infusion in the human esophagus increased salivation secretion and the frequency of dry and wet swallowing (Dutta et al., 2010; Bontempo et al., 1994). Furthermore, in the brain processing section, Siwiec et al suggested that abnormal highly active sensory afferents in the esophagus could be an important underlying factor for NERD patients (FH included) (Siwiec et al., 2015). Corazziari et al. also showed that acid infusion affected secondary peristalsis, and although a pH of 5 to 7 could elicit secondary peristalsis in the lower esophageal body, an infusion of a pH of 4 or below, significantly reduced the volume needed to produce esophageal motor activity in normal participants (Corazziari, Pozzessere, Dani, Anzini & Torsoli, 1978). Therefore, theoretically, in this study, the diluted vinegar in

VINOUT trial, at pH 3.02, could produce esophageal secondary peristalsis. However, more research is needed to evaluate the change of the vinegar pH at the upper third and the bottom third of the esophagus, and how it might affect GERD patients.

Since this current study does not have any intraluminal esophageal data available, this hypothesis is only an inference and will need further research to investigate. Generally people who do not respond to antacids well, might either have a neutral reflux content or highly active afferents in the esophagus or both. Therefore, while the spinal afferent senses the distention of the esophagus or perceives something in the esophagus, the vagal afferent detects no acid from its chemonociceptors. It has been suggested that vagal afferent (acid sensitive) could elicit secondary peristalsis; increase salivation secretion; increase frequency of dry and wet swallowing, which induces the primary peristalsis from somatic striated skeletal muscle. The mechanisms mentioned prior have two purposes, to neutralize the content in the esophagus and clear the esophagus by pushing the content down to the stomach. Without the protons from the acid (a noxious chemical), these mechanisms could not happen, however, the spinal afferents continue sending a sensory signal of discomfort. By ingesting organic vinegar with mother, it would start the esophageal primary peristalsis. It might provide protons that are needed in the esophagus to activate the vagal afferents and consequent mechanisms. It might also stimulate gastric juice secretion in the stomach because of high amount of microbes gained during the fermentation process, and hence a prominent proton source. It is debatable that vinegar is a weak acid, and its protons do not disassociate from its salt easily. The hypersensitive afferents in some NERD and FH patients might react to weak acid differently than normal people, and their vagal afferents might be able to be stimulated with less proton or less acidic content. Additionally, for some

patients with FH diagnosis, heartburn sensation could occur without the actual reflux episode. While there might not be something to clear in their esophagus, the consequent mechanisms due to activated vagal afferents might still provide a sense of relive to the spinal afferents in the esophagus.

# CHAPTER 6

# CONCLUSION

This is the first study to investigate the association between acid and heartburn symptoms related to GERD from a beneficial point of view. The acid chosen for the study was organic apple cider vinegar with mother. In summary, the results of our study support our second null hypothesis - compared to the placebo trial, the vinegar trials do not show significant alleviation of the heartburn sensation.

After seven participants further divided into antacid responders and non-responder groups, antacid showed significant alleviation of the heartburn sensation when compared to vinegar added to the chili trial in the antacid responder group. This result only supports part of our first null hypothesis because there was no significant difference between diluted vinegar after chili and antacid trials. The results also resemble those of previous studies on GERD which proposed the gap theory, which states that the content in the esophagus might leak through damaged esophageal epithelium lining and therefore cause heartburn. Interestingly, the graph data changed after the participants were divided into two different groups, according to their antacid responding circumstance, which supports the conclusions from previous research that GERD patients have varied pathophysiology and the same symptoms might have diverse underlying etiologies. Although not statistically significant,

ingestion of organic vinegar appeared to alleviate heartburn symptoms for some individuals, however, the effect seemed to last only for the first 60 to 75 minutes. The effect size through ANOVA from the available data showed either there was a great association between the treatment and the heartburn score or there was a large degree of the difference among trials.

Further research is needed to target the population in the same GERD subcategory and investigate if this approach would bring a more similar heartburn response among the participants to each treatment in the current study. A great deal of ongoing research continues investigating the complicated etiologies of GERD, and it might not be feasible to try to identify the GERD etiology of each participant. It would be interesting to investigate the underlying reason for the upsurge of heartburn symptoms at the second hour for several individuals. More research is necessary to confirm the organic apple cider vinegar's effect on antacid responders and non-responders.

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# APPENDIX A

# INSTITUTIONAL REVIEW BOARD APPROVAL

ASU Knowledge Enterprise Development

APPROVAL: EXPEDITED REVIEW

Carol Johnston
SNHP - Nutrition
602/827-2265
CAROL.JOHNSTON@asu.edu

Dear Carol Johnston:

On 12/17/2014 the ASU IRB reviewed the following protocol:

| | |
|---|---|
| Type of Review: | Initial Study |
| Title: | Is Apple Cider Vinegar Effective for Reducing Heartburn Symptoms Related to Gastroesophageal Reflux Disease? |
| Investigator: | Carol Johnston |
| IRB ID: | STUDY00001974 |
| Category of review: | (2)(a) Blood samples from healthy, non-pregnant adults, (7)(b) Social science methods, (7)(a) Behavioral research |
| Funding: | None |
| Grant Title: | None |
| Grant ID: | None |
| Documents Reviewed: | • consent, Category: Consent Form;<br>• protocol, Category: IRB Protocol;<br>• heartburn measure 2, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions);<br>• heartburn measure 1, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions);<br>• recruitment flyer/script, Category: Recruitment Materials;<br>• Health history, Category: Screening forms;<br>• screening survey, Category: Screening forms;<br>• recipes, Category: Technical materials/diagrams; |

The IRB approved the protocol from 12/17/2014 to 12/16/2015 inclusive. Three weeks before 12/16/2015 you are to submit a completed "FORM: Continuing Review (HRP-212)" and required attachments to request continuing approval or closure.

If continuing review approval is not granted before the expiration date of 12/16/2015 approval of this protocol expires on that date. When consent is appropriate, you must use final, watermarked versions available under the "Documents" tab in ERA-IRB.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103).

Sincerely,

IRB Administrator

cc:

Zoe Yeh
Lillian Padgitt-Cobb

# APPENDIX B

# CONSENT FORM

**DIET STRATEGIES TO IMPROVE REDUCE HEARTBURN SYMPTOMS RELATED TO GERD**

## INTRODUCTON

The purposes of this form are (1) to provide you with information that may affect your decision as to whether or not to participate in this research study, and (2) to record your consent if you choose to be involved in this study.

## RESEARCHERS

Dr. Carol Johnston, a Nutrition professor at Arizona State University Downtown Campus, and Zoe Yeh, Nutrition Masters student, have requested your participation in a research study.

## STUDY PURPOSE

The purpose of the research is to examine the effects of common food items to relieve heartburn symptoms following the consumption of a chili and chocolate milk meal. The chili recipe is expected to evoke symptoms of heartburn, and participants will be asked to record symptoms of heartburn for a 2-hour period post meal.

## DESCRIPTION OF RESEARCH STUDY

You have indicated to us that you are 18 years of age or older, a non-smoker and generally healthy; however, you have experienced heartburn symptoms related to GERD three or more times per week for the preceding three months or longer. You have not been diagnosed with erosive reflux disease or other chronic diseases such as diabetes or heart disease, you are not a vegetarian, and (if female) you have not recently been pregnant or lactating (past 6 months). Participants will be asked to maintain their usual diet and physical activity level throughout the trial with the exception of the day prior to testing. This study will initially involve the completion of a brief medical history questionnaire to demonstrate the absence of medical conditions that may impact the study. Your weight, height, and girth will be measured at this time. This first meeting will take about 15 minutes. There are four additional visits (e.g., the test days) that will last about 2 hours each and are scheduled about a week apart. The procedures on test days are identical. On the day prior to testing you are asked to avoid heavy exercise (normal activities such as walking to work or walking the dog is ok). You will be asked to eat a normal breakfast and lunch of your choice. The evening before the test day is also a meal of your choice, but we will ask you to eat the same dinner meal on the days prior to testing (4 days total). Following dinner, you will fast overnight and not consume any food or beverage with the exception of water. On test days, you will travel to ASU (the Nutrition labs at the ABC1 Building on the ASU Downtown campus) early in the morning. Your finger will be pricked for a blood sample. You will sit down and consume a test meal (chili and chocolate milk). Your finger will be pricked four more times over the next 2 hours. You may drink water during these two hours but you are not to consume any food and you cannot lay down. There will be several short questionnaires to complete at scheduled times during these 2 hours to assess the degree of heartburn. Otherwise, you may read, study, or work on the computer at the test site. Once testing is complete, you may proceed with your normal activities.

Finger pricks will be conducted under sterile conditions using disposable, retractable lancets, and the level of glucose in blood will be recorded.

**RISKS**
You will likely develop symptoms of heartburn following the ingestion of the test meal. Evoking symptoms of heartburn will allow us to assess the efficacy of a natural treatment for heartburn. We will have TUMS available to relieve symptoms if desired once the 2-hour test period is over. Bruising of the skin or a feeling of faintness is possible during the finger pricks. Disposable retractable lancets will be used and sterile conditions will be used.

**BENEFITS**
There is no direct benefit for participating in this trial. If desired, you will be provided with study results and your personal blood data at the end of the study.

**NEW INFORMATION**
If the researchers find new information during the study that would reasonably change your decision about participating, then they will provide this information to you.

**CONFIDENTIALITY**
All information obtained in this study is strictly confidential unless disclosure is required by law. The results of this research study may be used in reports, presentations, and publications, but your name or identity will not be revealed. In order to maintain confidentiality of your records, Dr. Johnston will use subject codes on all data collected, maintain a master list separate and secure from all data collected, and limit access to all confidential information to the study investigators.

**WITHDRAWAL PRIVILEGE**
You may withdraw from the study at any time for any reason without penalty or prejudice toward you. Your decision will not incur negative treatment to you by the researchers.

**COSTS AND PAYMENTS**
The all test foods will be given to you during the study free of charge. You will receive a $10 Target card at test visits 1 and 2 and a $15 Target cared at test visits 3 and 4 ($50 total if the study is completed).

**COMPENSATION FOR ILLNESS AND INJURY**
If you agree to participate in the study, then your consent does not waive any of your legal rights. However, in the event of harm, injury, or illness arising from this study, neither Arizona State University nor the researchers are able to give you any money, insurance coverage, free medical care, or any compensation for such injury. Major injury is not likely but if necessary, a call to 911 will be placed.

**VOLUNTARY CONSENT**
Any questions you have concerning the research study or your participation in the study, before or after your consent, will be answered by Dr. Carol Johnston; 500 N. 3rd Street Phoenix, AZ 85004; 602-827-2265.

If you have questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk, you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Research Compliance Office, at 480-965 6788.

This form explains the nature, demands, benefits and any risk of the project. By signing this form you agree knowingly to assume any risks involved. Remember, your participation is voluntary. You may choose not to participate or to withdraw your consent and discontinue participation at any time without penalty or loss of benefit. In signing this consent form, you are not waiving any legal claims, rights, or remedies. A copy of this consent form will be given to you.

Your signature below indicates that you consent to participate in the above study.

| _____ | _____ | _____ |
|---|---|---|
| Subject's Signature | Printed Name | Date |

| _____ | _____ |
|---|---|
| Contact phone number | Email |

**INVESTIGATOR'S STATEMENT**

"I certify that I have explained to the above individual the nature and purpose, the potential benefits, and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have provided the subject/participant a copy of this signed consent document."

Signature of Investigator_____ Date_____

**ESTRATEGIAS DE DIETA PARA AYUDAR A REDUCIR Y MEJORAR LOS SINTOMAS DE ACIDEZ RELACIONADOS CON ERGE**

## INTRODUCCION

El proposito de este formulario es: (1) proporcionarle información que pueda afectar su decisión en cuanto a si debe o no participar en este estudio de investigación, y (2) registrar su consentimiento si decide participar en este estudio.

## INVESTIGADORES

La Dra. Carol Johnston, Profesora de Nutrición de la Universidad Estatal de Arizona Downtown Campus, y Zoe Yeh, estudiante de la maestría en Nutrición, han solicitado su participación en un estudio de investigación.

## PROPOSITO DEL ESTUDIO

El propósito de la investigación es examinar los efectos de alimentos comunes para aliviar los síntomas de acidez tras el consumo de chili y una bebida de leche chocolatada. Se espera que la receta de chili produsca los síntomas de la acidez estomacal, y se pedirá a los participantes que registren los síntomas de acidez por un período posterior a la comida de 2 horas.

## DESCRIPCION DEL ESTUDIO DE INVESTIGACION

Usted nos ha indicado que tiene 18 años de edad o más, es no fumador y generalmente goza de buena salud; sin embargo, usted ha experimentado los síntomas de acidez relacionados con la ERGE (Enfermedad por reflujo gastroesofágico) tres o más veces por semana durante los pasados tres meses o más. No ha sido diagnosticado con la enfermedad de reflujo erosiva u otras enfermedades crónicas tales como la diabetes o enfermedades del corazón, usted no es vegetariano, y (si es mujer) no ha estado recientemente embarazada o lactando los últimos 6 meses. Se les pedirá a los participantes que mantengan su dieta y nivel de actividad física habitual durante todo el estudio, con la excepción del día antes de la prueba. Este estudio inicialmente implicará la realización de un cuestionario breve de su historial médico para demostrar la ausencia de condiciones médicas que pudiesen afectar el estudio. Su peso, altura y su circunferencia de cintura se medirán en esta etapa. Esta primera reunión durará unos 15 minutos. Hay cuatro visitas adicionales (por ejemplo, los días de prueba) que durarán alrededor de 2 horas cada una y están programadas con una semana de diferencia. Los procedimientos en los días de prueba son idénticos. El día antes de la prueba se le pedirá que evite el ejercicio pesado (actividades normales como caminar al trabajo o pasear al perro está bien). Se le pedirá comer un desayuno normal y el almuerzo de su elección. La noche anterior al día de la prueba es también una comida de se su elección, pero se le pedirá que coma la misma comida de la cena en los días previos a la prueba (4 días en total). Después de la cena, deberá ayunar durante la noche y no consumir ningún alimento o bebida con excepción de agua. En los días de prueba, deberá viajar a ASU (los laboratorios de Nutrición en el Edificio ABC1 en el Downtown Campus de ASU) temprano en la mañana. Su dedo será pinchado para obtener una muestra de sangre. Posteriormente va a sentarse y consumir una comida de prueba (chili y leche con chocolate). Su dedo será pinchado cuatro veces más durante las siguientes 2 horas. Usted puede tomar agua durante estas dos horas, pero no podrá consumir ningún alimento y no podrá recostarse. Habrá varios cuestionarios cortos para completar durante estas 2 horas para evaluar el grado de acidez. El resto del tiempo, usted puede leer, estudiar o trabajar en su computadora personal en el lugar de la

prueba. Una vez que se complete la prueba, puede continuar con sus actividades normales.
Los pinchazos en los dedos se llevarán a cabo en condiciones estériles utilizando agujas retráctiles desechables, y se registrará el nivel de glucosa en la sangre.

**RIESGOS**
Es probable que usted desarrolle síntomas de acidez estomacal después de comer de la comida de prueba. Provocar los síntomas de la acidez estomacal nos permitirá evaluar la eficacia de un tratamiento natural para la acidez estomacal. Tendremos TUMS disponibles para aliviar los síntomas si se desea una vez que el periodo de prueba de 2 horas ha terminado. Si es necesario, vamos a detener las pruebas y permitir el uso de un antiácido. Leves moretones en la piel o una sensación de desmayo son posibles de ocurrir durante los pinchazos en los dedos. Se utilizarán agujas retráctiles desechables en condiciones estériles.

**BENEFICIOS**
No hay ningún beneficio directo por participar en este estudio. Si usled desea, se le proporcionarán los resultados del estudio y sus datos personales de sangre al final del estudio.

**NUEVA INFORMACIÓN**
Si los investigadores encontrasen nueva información durante el estudio que pudiera cambiar razonablemente su decisión acerca de la participación en este estudio, entonces van a proporcionarle esta información a usted.

**CONFIDENCIALIDAD**
Toda la información obtenida en este estudio es estrictamente confidencial a menos que su difusión sea requerida por la ley. Los resultados de este estudio de investigación pueden ser usados en informes, presentaciones y publicaciones, pero su nombre o identidad no serán revelados. A fin de mantener la confidencialidad de sus registros, la Dra. Johnston utilizará un código numérico en todos los datos recogidos, mantendrá una lista maestra separadamente y segura de todos los datos recogidos, y limitará el acceso a toda la información confidencial a sólo los investigadores del estudio.

**PRIVILEGIO DE RETIRADA**
Usted puede retirarse del estudio en cualquier momento y por cualquier razón sin pena ni perjuicios hacia usted. Su decisión no provocará trato negativo hacia usted por parte de los investigadores.

**COSTOS Y PAGOS**
Todos los alimentos de la prueba serán dados durante el estudio de forma gratuita. Usted recibirá una tarjeta de Target de $ 10 en las visitas de ensayo 1 y 2 y una tarjeta de Target por $15 en las visitas de las pruebas 3 y 4 ($ 50 en total si se completa el estudio).

**INDEMNIZACIÓN POR ENFERMEDAD Y LESIONES**
Si acepta participar en el estudio, su consentimiento no implicará una renuncia a ninguno de sus derechos legales. Sin embargo, en caso de daño, lesión o enfermedad que surgiera de este estudio, ni la Universidad Estatal de Arizona, ni los investigadores son capaces de darle cualquier cobertura de dinero, seguros, asistencia médica

gratuita, o compensación alguna por dicha lesión. Una lesión grave no es probable, pero si es necesario, se hará una llamada al 911.

**CONSENTIMIENTO VOLUNTARIO**

Cualquier pregunta que tenga sobre el estudio de investigación o su participación en el estudio, antes o después de su consentimiento, será contestada por la Dra. Carol Johnston; 500 N. 3rd Street Phoenix, AZ 85004; 602-827-2265.

Si usted tiene preguntas acerca de sus derechos como sujeto/participante en esta investigación, o si usted siente que ha sido puesto/a en situación de riesgo, puede ponerse en contacto con el Presidente de la Junta de Revisión Institucional de Sujetos Humanos, a través de la Oficina de Cumplimiento de Investigaciones de ASU, en 480-965 6788.

Este formulario explica la naturaleza, las demandas, los beneficios y los riesgos del proyecto. Al firmar este formulario, usted acepta a sabiendas de asumir cualquier riesgo involucrado. Recuerde que su participación es voluntaria. Usted puede optar por no participar o retirar su consentimiento y dejar de participar en cualquier momento sin penalidad o pérdida de beneficios. Al firmar este formulario de consentimiento, usted no renuncia a ningún reclamo, derechos legales o remedios. Una copia de este formulario de consentimiento le será entregada a usted.

Su firma indica que usted da su consentimiento para participar en el estudio arriba mencionado.

| _____ | _____ | _____ |
|---|---|---|
| Firma del Sujeto | Nombre | Fecha |

| _____ | _____ |
|---|---|
| Teléfono de Contacto | Email |

**DECLARACIÓN DEL INVESTIGADOR**

"Certifico que he explicado a la persona arriba mencionada de la naturaleza y el propósito, los beneficios potenciales y los posibles riesgos asociados con la participación en este estudio de investigación, y he respondido a las preguntas que se han planteado, y he sido testigo de la firma anterior. Estos elementos de Consentimiento Informado se ajustan a la garantía dada por la Universidad Estatal de Arizona a la Oficina de Protección de Investigaciones Humanas para proteger los derechos de los sujetos humanos. He proporcionado al sujeto/participante una copia de este documento de consentimiento firmado ".

Firma del Investigator_____ Fecha_____

# APPENDIX C

## SAMPLE SIZE CALCULATION

SAMPLE SIZE

The data used to calculate the sample size for this research was based on the results of two studies that measured similar outcome variables. The results used from these two studies were describing how effectively antacids treat the symptoms of heartburn for patients with gastroesophageal reflux disease (GERD), and this research is to test how effective vinegar can alleviate heartburn symptom related to GERD.

Study A (Hartono,Juanda L. 2011).

Patients were interviewed and those who experienced heartburn and acid regurgitation more than once a week, for the preceding 3 months, were considered for the study. Both symptomatic erosive reflux disease (ERD) and non-erosive reflux disease (NERD) patients in this study followed a treatment of Rabeprazole, a proton pump inhibitor, 20 mg twice daily for two-week. The baseline reflux symptom scores (RSS) were assessed prior to and after the treatment. The results of the medication response were shown in the table below.

**Symptomatic response to PPI**

| | Symptomatic ERD (n = 24) | NERD (n = 34) | p value |
|---|---|---|---|
| RSS pre-treatment ± SD | 15.17 ± 9.09 | 16.79 ± 10.69 | 0.716 |
| RSS post-treatment ± SD | 1.79 ± 3.67 | 6.24 ± 6.47 | < 0.001 |
| Mean %Δ RSS ± SD | 89.08 ± 21.67 | 58.53 ± 32.54 | < 0.001 |

The NERD group Mean %Δ RSS ± SD value was used to calculate sample size, and there were 34 subjects in this group. Minimal detectable difference in means was 59 and SD was 33. The significance level was set at .05 and a power of 0.8. Using the established formula for cross-over study and quantitative measurement, based on the study A data, a total of 8 subjects are needed for this research.

Study B (Collings,KL 2002).

Study B was a single-blind, four treatment cross-over design. 26 subjects who have had heartburn symptom for two month or longer were recruited for the study. Two subjects did not have complete data, so only 24 subjects' data were recorded in the result. Self-assessment of heartburn severity - 100-point visual analogue scale (VAS) were taken every 5 minutes after the initiation of the treatment for the first 15 minutes, and at 15-min intervals thereafter for the total period of 120 minutes. Four groups in this study were Placebo, chewable antacid (two tablets of calcium carbonate 500 mg ), lower dose antacid gum (two gums each containing 300 mg of calcium carbonate ), and higher dose antacid gum (two gums each containing 450 mg of calcium carbonate). Percent of mean change and standard deviations (SD) were not given in the table or the text in the study, so the percent of mean change number was obtained through the result figure provided. The result of lower dose antacid gum group was used for calculations. Mean change = [50 (mean of the highest score of VAS heartburn severity) - 21 (mean of the lowest score of VAS heartburn severity)] / 50 = 0.58 = 58%. SD was not given by the study, so the largest possible number 58 is used for the calculation. The significance level was set at 0.05 and a power of 0.8. Using the established formula for cross-over study and quantitative measurement, based on the study B data, a total of 18 subjects are needed for this research.

Based on the calculated sample size of the two studies, approximately 13 subjects are needed to optimize the data of this cross-over research for its representative accuracy and its application to the general public. After anticipating a 20% drop-out rate, a total of 15 subjects are needed for the recruitment. See the table below.

| Author | Year | Symptomatic Response Mean% change | N per Group | Calculated n per group | Age Range | Subject State | Test |
|---|---|---|---|---|---|---|---|
| Hartono et al. | 2011 | 59 | 34 | 8 | 50.79 ± 16.02 | NERD | Mann-Whitney |
| Collings et al. | 2002 | 58 | 24 | 18 | 18-60 | GERD | ANOVA |
| AVERAGE | | 58 | 29 | 13 | | | |

# APPENDIX D

## CHILI RECIPE

## INGREDIENTS

- 1 1/2 pounds lean ground beef
- 8 ounces smoked sausage, such as andouille or apple chicken sausage
- 1 cup chopped onion
- 1 red or green bell pepper, chopped
- 2 cloves garlic, minced
- 1 can (4 ounces) mild green chili peppers
- 2 to 3 tablespoons diced jalapeno peppers, or to taste
- 2 cans (14.5 ounces each) diced tomatoes or fire-roasted tomatoes, undrained
- 1 can (8 ounces) tomato sauce
- 1 can (15 ounces) black beans, drained and rinsed
- 1 teaspoon grill seasoning blend
- 2 to 3 tablespoons chili powder
- 1/2 teaspoon ground cumin
- 1/4 teaspoon ground black pepper
- 1 teaspoon salt, or to taste

## PREPARATION

In a large skillet or saute pan, brown the ground beef and sausage with onion until meat is no longer pink. Add the bell pepper and garlic and cook, stirring, for about 1 minute longer. Transfer to the slow cooker and add remaining ingredients; cover and cook on LOW for 6 to 8 hours.

Serves 6 to 8.

# APPENDIX E

## HEARTBURN ASSESSMENT SCALES

Please mark the circle below based on your heartburn severity at the indicating timing on the left side of column.

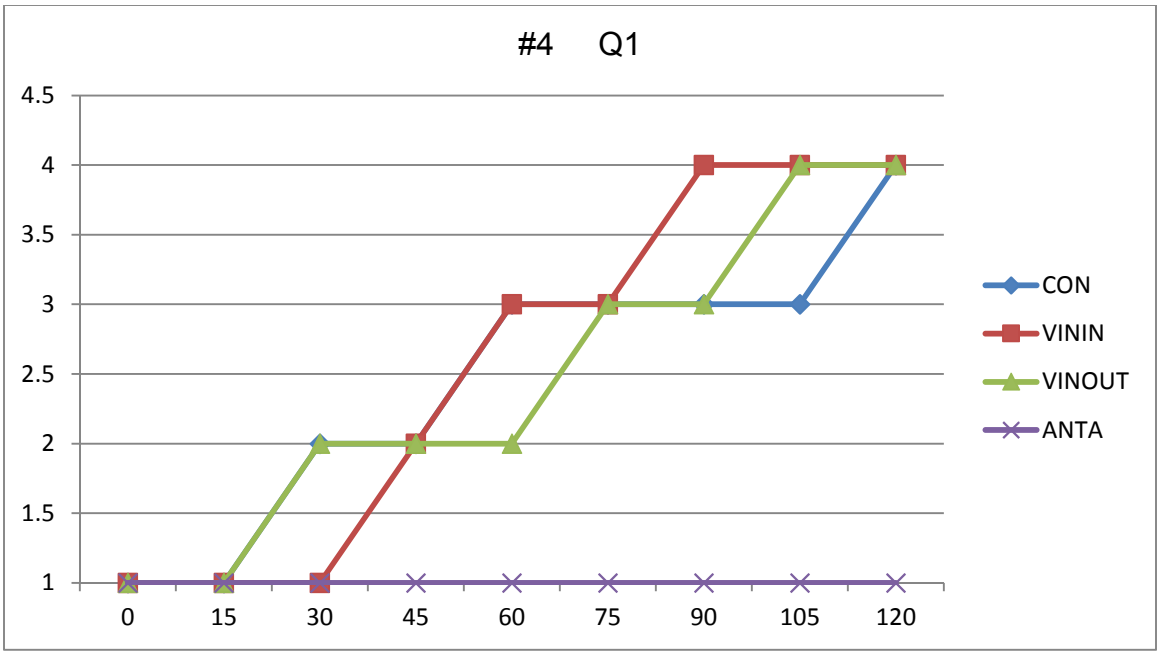
| | None (1) | Mild (2) | Moderate (3) | Severe (4) | Very Severe (5) |
|---|---|---|---|---|---|
| Before the meal | ❍ | ❍ | ❍ | ❍ | ❍ |
| Mark this line if your heartburn starts before 15 minute marking | ❍ | ❍ | ❍ | ❍ | ❍ |
| 15 minutes since the starting of the meal | ❍ | ❍ | ❍ | ❍ | ❍ |
| 20 minutes since the starting of the meal | ❍ | ❍ | ❍ | ❍ | ❍ |
| 25 minutes since the starting of the meal | ❍ | ❍ | ❍ | ❍ | ❍ |
| 30 minutes since the starting of the meal | ❍ | ❍ | ❍ | ❍ | ❍ |
| 45 minutes since the starting of the meal | ❍ | ❍ | ❍ | ❍ | ❍ |
| 1 hour since the starting of the meal | ❍ | ❍ | ❍ | ❍ | ❍ |
| 1 hour and 15 minutes since the starting of the meal | ❍ | ❍ | ❍ | ❍ | ❍ |
| 1 hour and 30 minutes since the starting of the meal | ❍ | ❍ | ❍ | ❍ | ❍ |
| 1 hour and 45 minutes since the starting of the meal | ❍ | ❍ | ❍ | ❍ | ❍ |
| 2 hours since the starting of the meal | ❍ | ❍ | ❍ | ❍ | ❍ |

Please mark "X" which indicates the severity of your heartburn on the line according to each recording time. Thank you!

| | | |
|---|---|---|
| **Before the meal** | No Pain | Unbearable Pain |
| Mark this line if your heartburn starts **before 15- minute** marking time | No Pain | Unbearable Pain |
| **15 mins** since the starting of the meal | No Pain | Unbearable Pain |
| **20 mins** since the starting of the meal | No Pain | Unbearable Pain |
| **25 mins** since the starting of the meal | No Pain | Unbearable Pain |
| **30 mins** since the starting of the meal | No Pain | Unbearable Pain |
| **45 mins** since the starting of the meal | No Pain | Unbearable Pain |
| **1 hour** since the starting of the meal | No Pain | Unbearable Pain |
| **1 hour and 15 mins** since the starting of the meal | No Pain | Unbearable Pain |
| **1 hour and 30 mins** since the starting of the meal | No Pain | Unbearable Pain |
| **1 hour and 45 mins** since the starting of the meal | No Pain | Unbearable Pain |
| **2 hour** since the starting of the meal | No Pain | Unbearable Pain |

# APPENDIX F

## INDIVIDUAL HEARTBURN GRAPHS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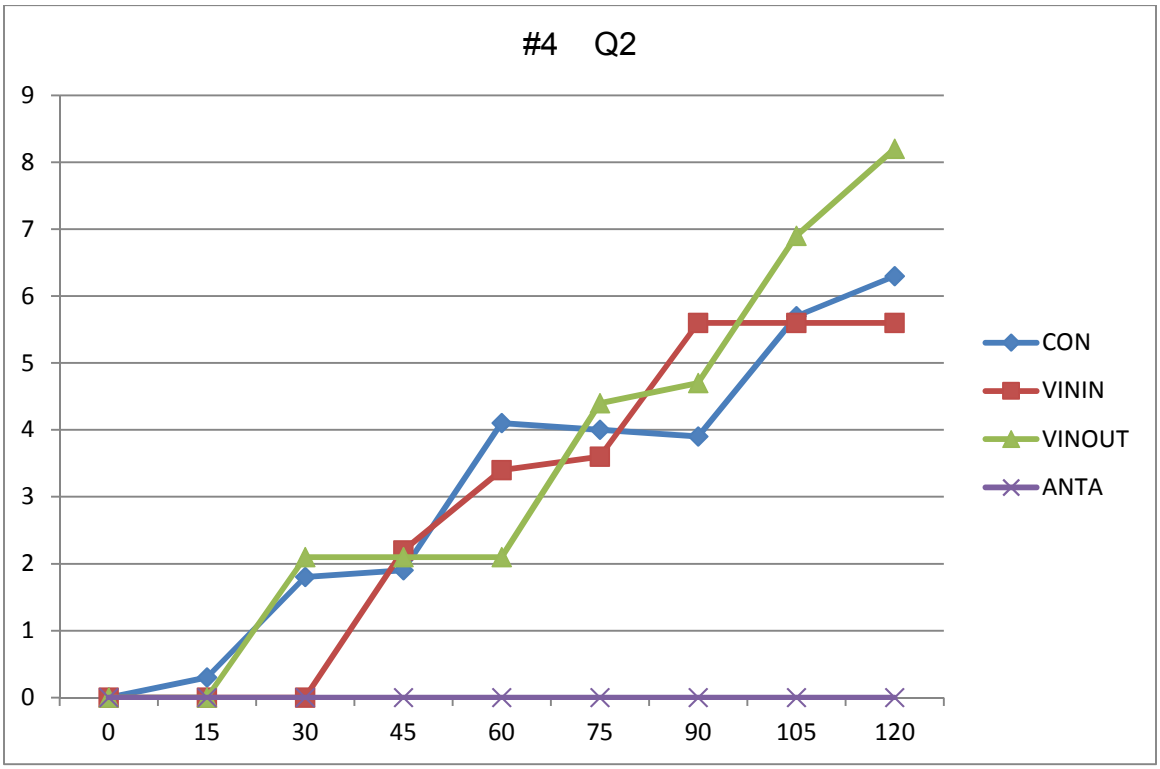
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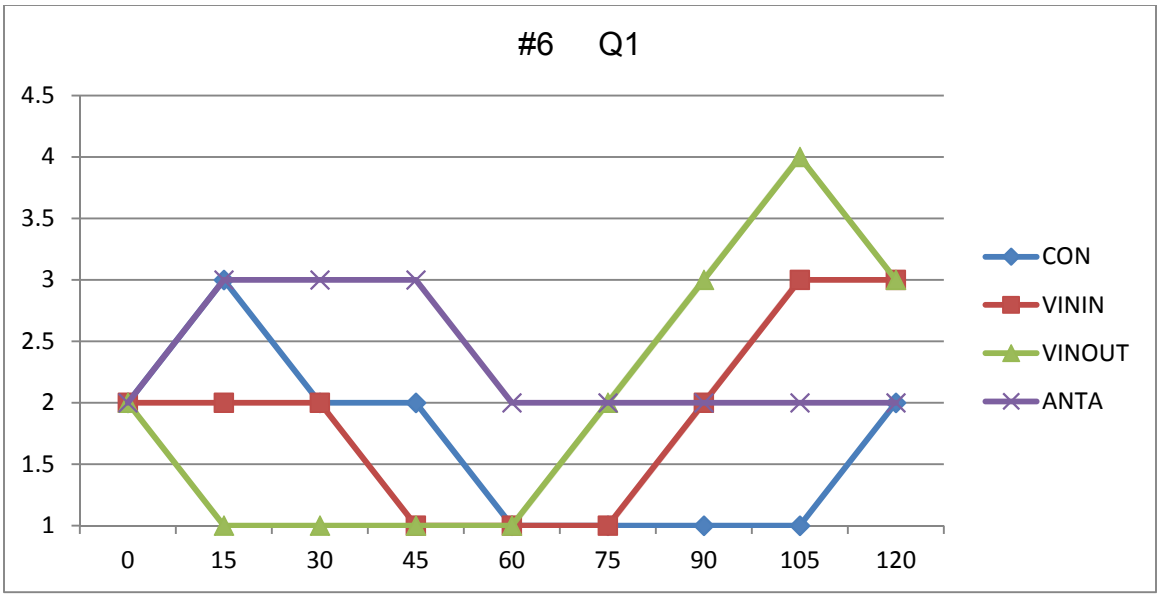
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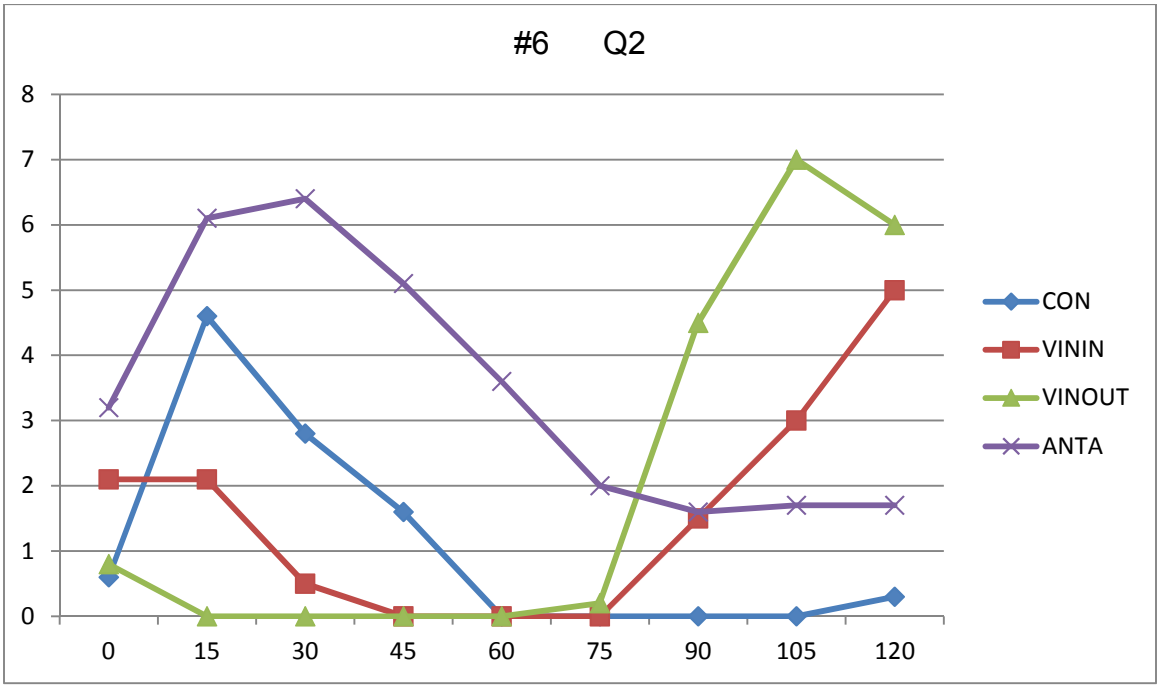
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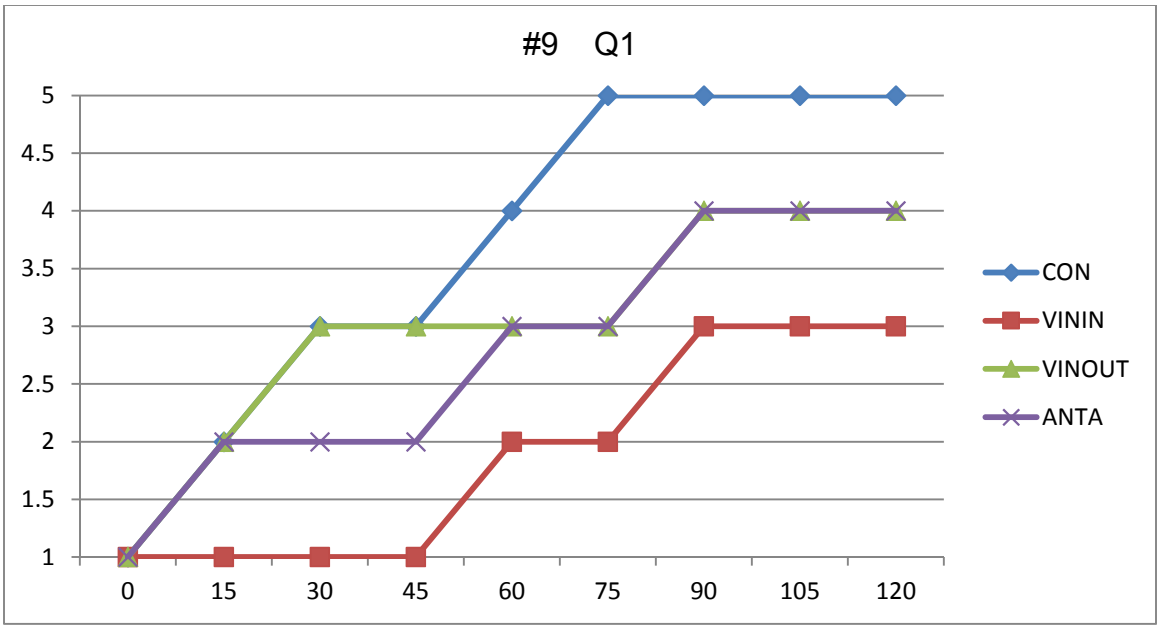
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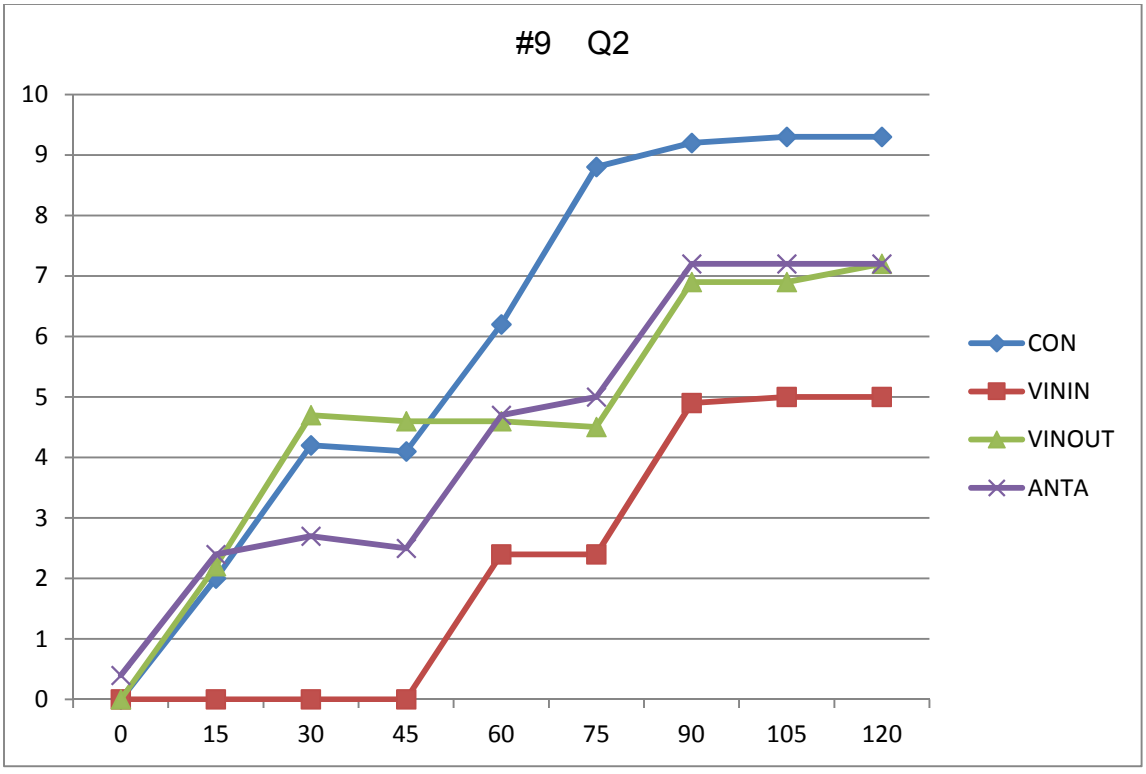
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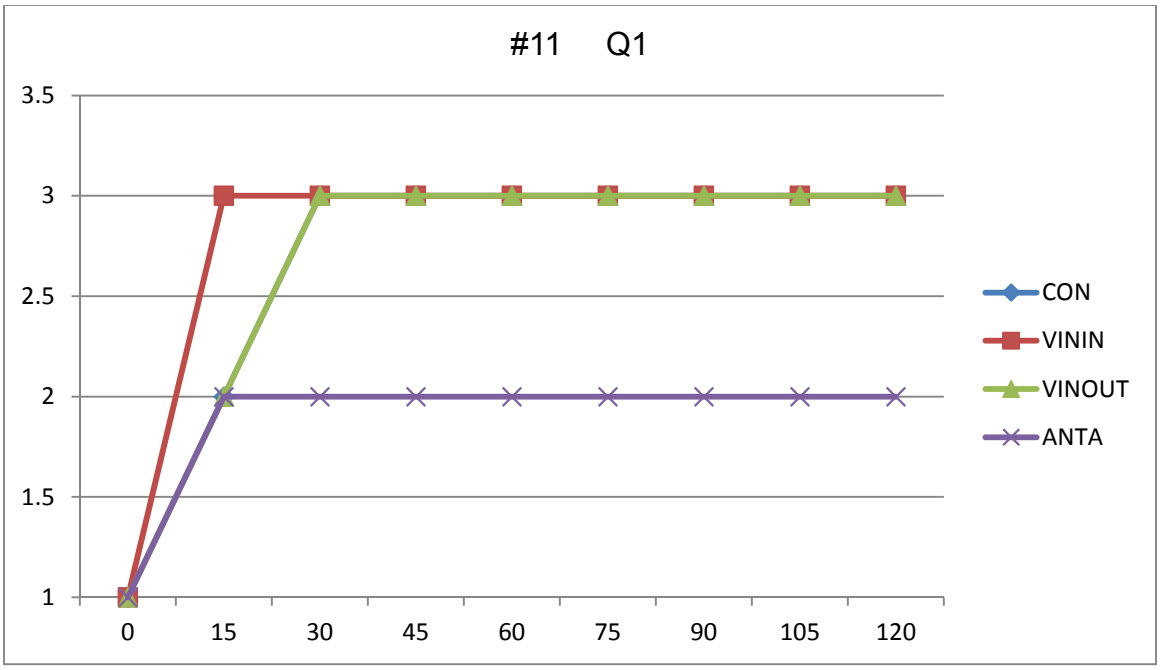
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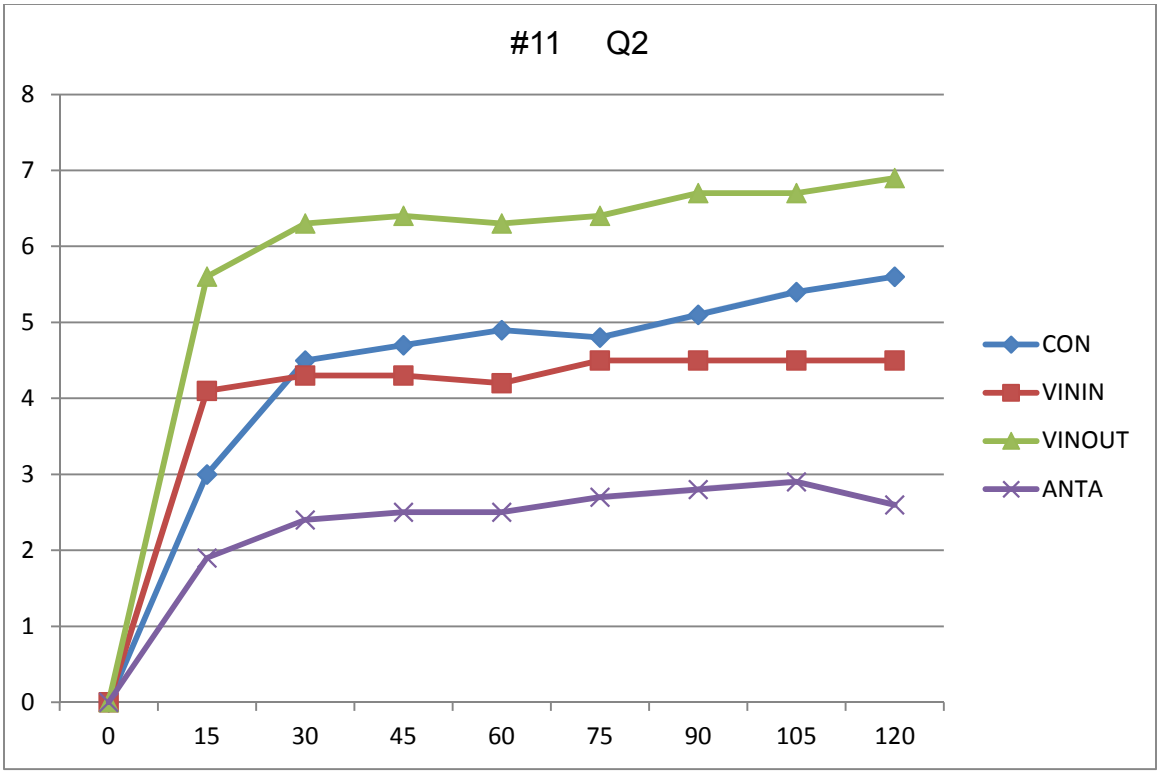
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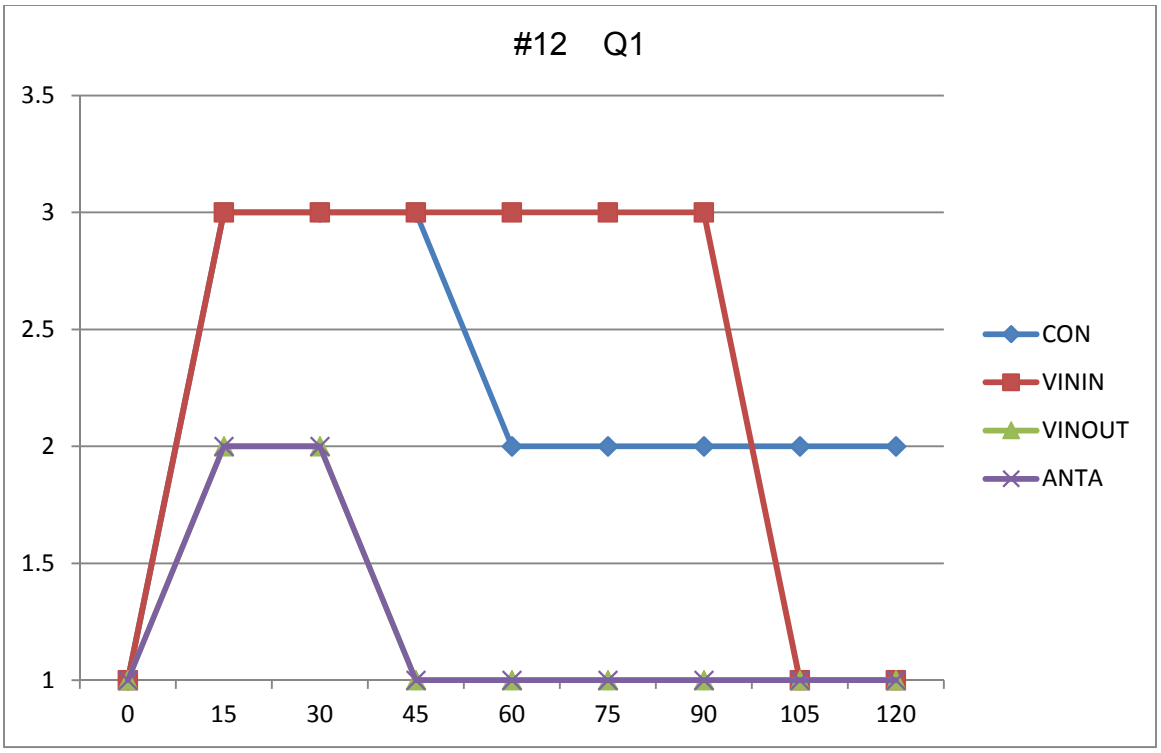
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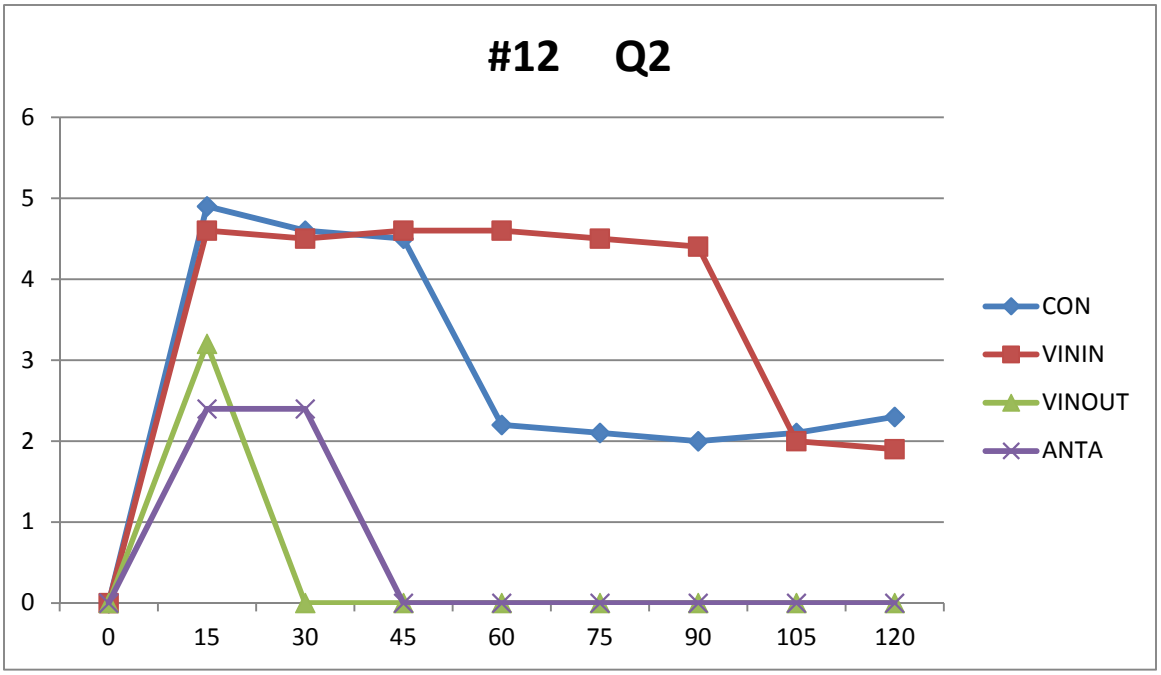
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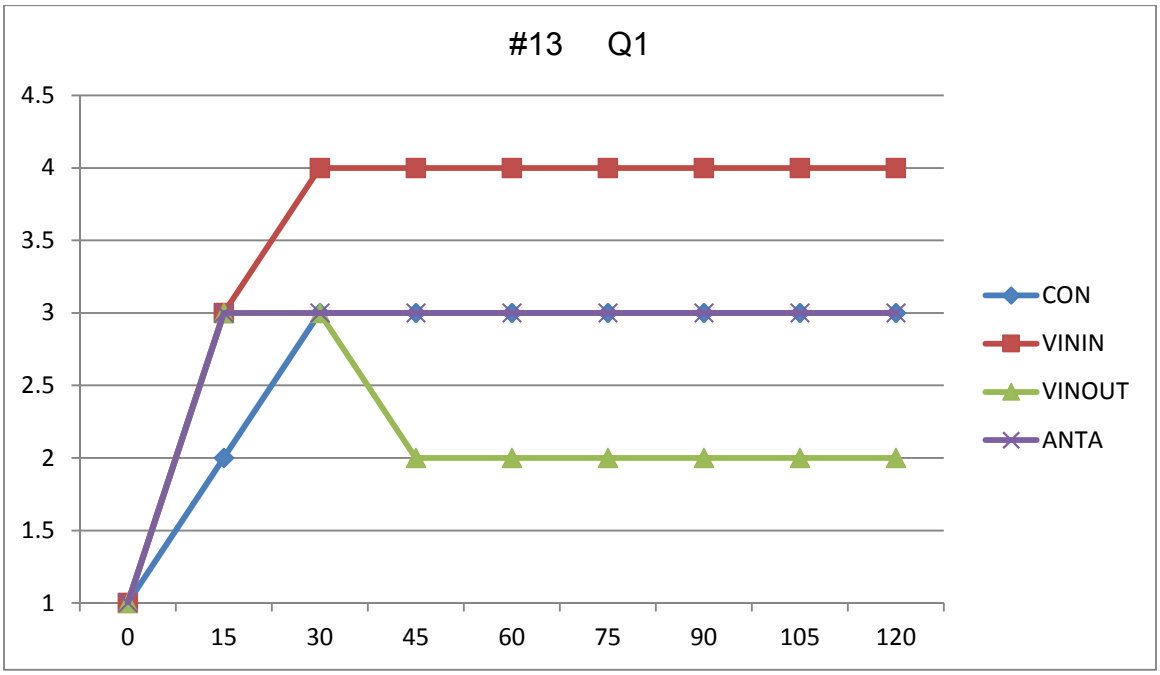
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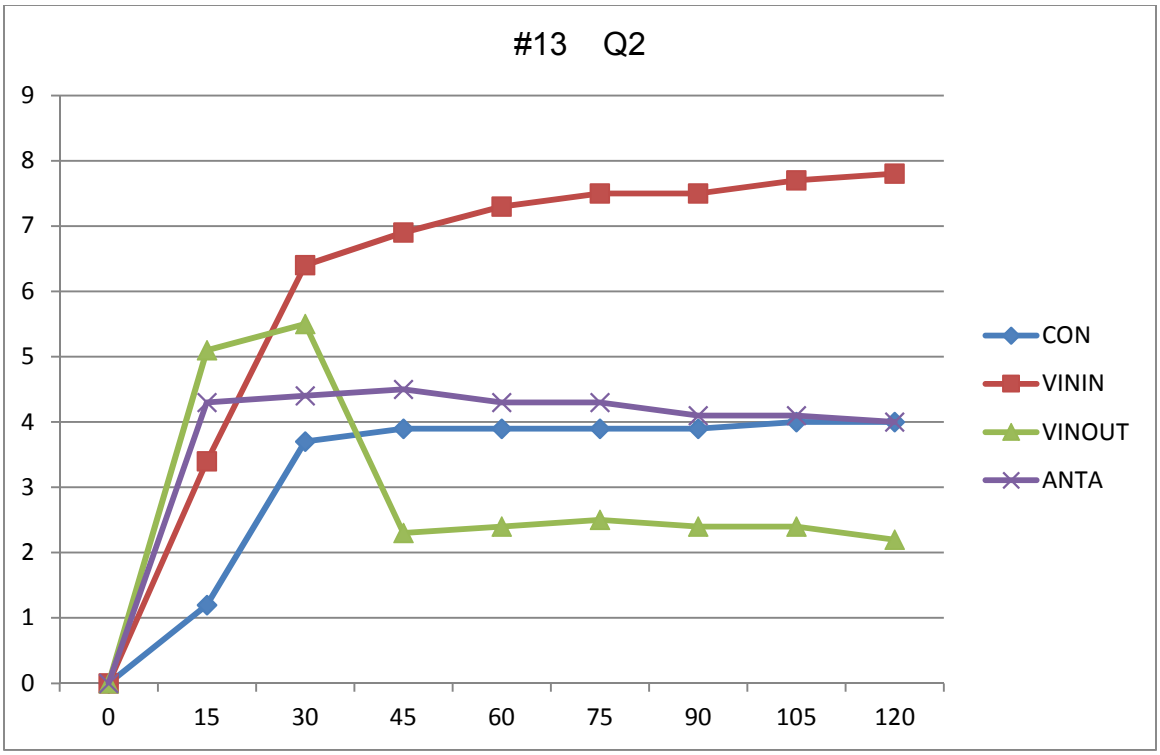
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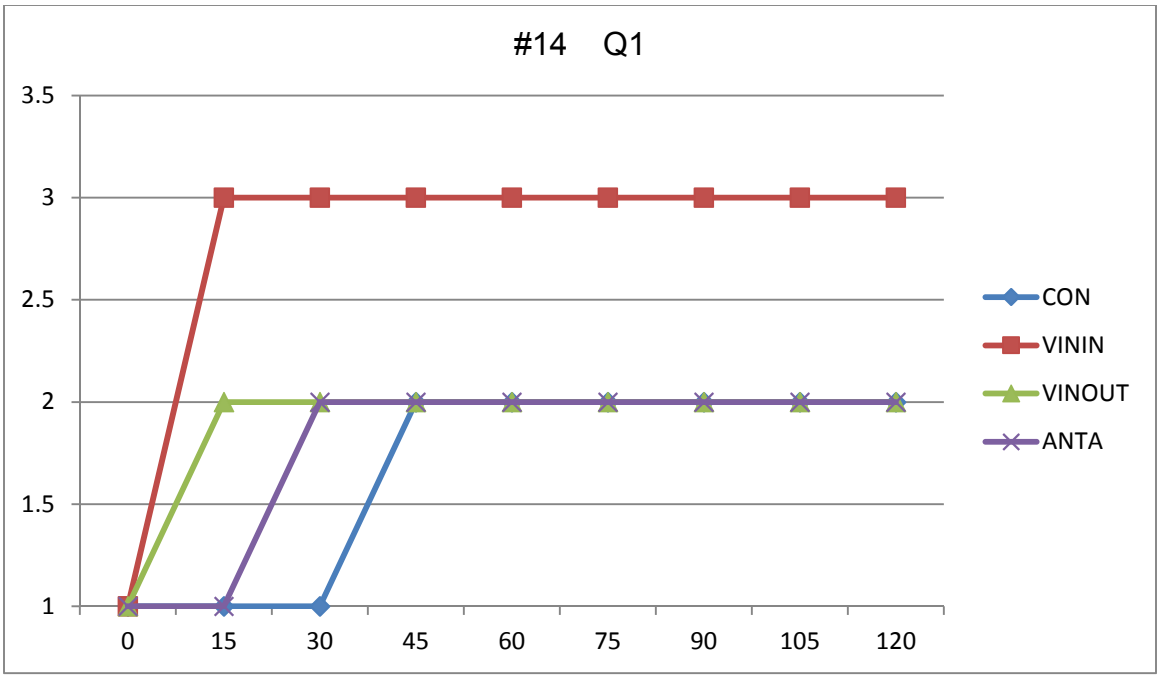
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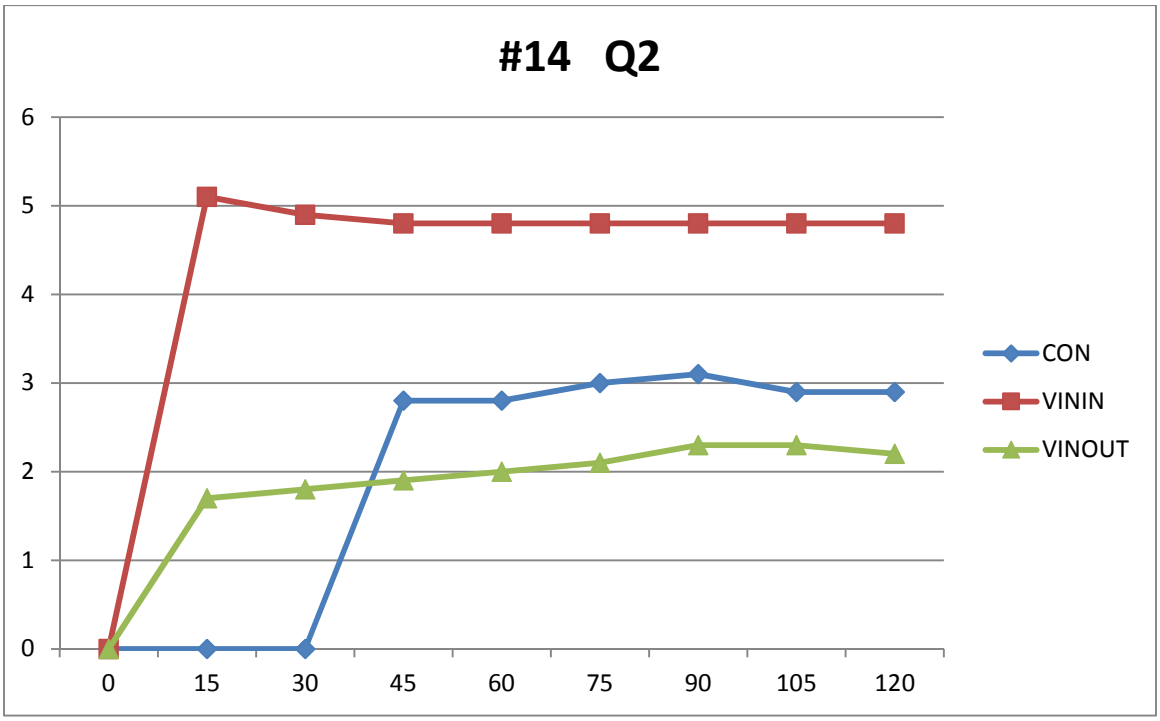
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