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Letter From the Editors

We are STEM + Youth (STEMY), a local, entirely student-run nonprofit organization on a mission to break gender, racial, and socioeconomic barriers through science, technology, engineering, and math (STEM) education. Through our 8 engaging, exciting programs, we aim to spark passions for STEM among disadvantaged and underrepresented students.

STEMY began as an after-school club called the Manual Science Review (MSR), which aimed specifically to help duPont Manual High School students with their science fair projects. After about a year, we saw the tangible impacts that the MSR's programs had on the school—program participants came to enjoy the science research process and grew more interested in STEM—and we wanted to expand beyond just our high school. Officially founded over a year ago, this organization is the result of our drive to change lives by engaging all students, regardless of gender or background, in STEM. We've already directly impacted nearly 1,000 local students from all backgrounds and plan to engage thousands more in new programs this year.

Effective STEM education isn't just valuable to improve academic performance—the right kind of learning influences students' career options, worldview, and mindset. High-quality STEM experiences have been shown to improve problem-solving skills, build confidence, and motivate students to enter high-paying jobs in the future. STEM programs like ours lead participants to view the world through an observational, curiosity-driven lens and have the power to open minds that have been closed by years of learning through rote memorization. Critically, sparking passions for STEM among underrepresented and disadvantaged students can break down established social barriers in STEM fields.

To expose as many local students as possible to the numerous benefits of STEM, we are launching several new programs during the 2018-19 school year. Our initiatives will engage students in exciting, creative STEM activities to build new skills and spark new passions among participants. *Innovation* is one of these many programs. All across this city, there are talented, dedicated high school students who pursue incredible research projects and demonstrate the amazing things students with strong STEM backgrounds can do. We believe that these students deserve the opportunity showcase their efforts, which is why we created Innovation.

Innovation, just like our organization, is created and produced by students and students only. We cultivate strong partnerships among editors and authors and give writers the unique opportunity to publish high-quality, peer-reviewed work as a high schooler. Through this journal, we hope to spread the *innovative* spirits of our authors and their amazing research throughout the community. We believe that Innovation is a unique way to expose Louisville to STEM, and we will continue to produce and publish this journal for years to come.

Feature Article: My Experience With Research

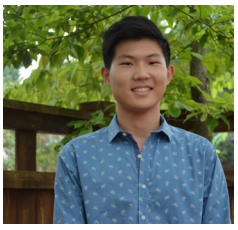
Eddy Zhong

I started doing research for science fair in my freshman year. At first, it seemed like a rather daunting task: I had to learn some very advanced knowledge, come up with an experiment, and work in a college lab. However, I found a topic that I liked and a mentor who was very helpful in guiding me. Because the topic I chose was interesting to me, I had motivation and desire to learn more. Finding a mentor can seem difficult, but I learned that the key is to simply reach out. Personal connections can be very useful, as they can make it easier to contact potential mentors. However, personal connections are by no means necessary for finding a good mentor. Many people I know simply emailed professors who conducted research in their topic area.

My project is a continuation, meaning that I continued working on the same project for multiple years. I built my project from the ground up, starting with the most basic experiments in freshman year. By now, I have spent three years on this project and will continue with this project in my senior year. I conduct research about the impact of beta-glucan particles on the macrophage response to cancer, which is very time-consuming and labor-intensive. Although my research topic required multiple years of work to reach an advanced level, not all projects need to be continuations to be successful. In fact, most people start a new project each year.

Admittedly, doing research is not easy. I have to spend a lot of time during the week and sacrifice many weekends to finish experiments. On occasion, I spend eight straight hours in the lab. When conducting research, I have had many failures. I learned that the most important thing is to not get discouraged and to persevere. One experimental procedure I have to run is called ELISA, which involves adding tiny amounts of liquid to 96 wells on a plate individually. There have been quite a few times when I added the wrong liquid, resulting in invalid data. When experiments don't turn out as expected, I examined the reasons why and learned from them to improve in the future.

Despite the challenges I have faced, I find research to be a very rewarding experience: I am learning valuable skills for the future while contributing to the advancement of science. I will definitely pursue research as an undergrad, and thanks to my work in high school, I will be more than prepared.



About Eddy: Eddy Zhong is a senior at Kentucky Country Day School. He is in his fourth year of conducting immunotherapy research at the University of Louisville, and he attended the Intel ISEF 2018. In his free time, he enjoys playing the piano and traveling abroad.

Influence of Breastfeeding Duration on Cognition of Adolescents

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ABSTRACT

Though the optimum time frame for an infant to be breastfed was at least six months (which UNICEF recommended), from the 71% of women in the United States who breastfed their infant, only 35% actually breastfed for six months. Breastfeeding varies between short and long term benefits for both the mother and the infant. Many of these benefits came from unsaturated fatty acids, such as docosahexaenoic acid (DHA), that are naturally found in breastmilk only. However, breastmilk substitutes, such as formula, lack these nutrients, which affect the neurodevelopment of infants of life. It was hypothesized that if a child is breastfed and the amount and duration a child was breastfed affected the cognitive development of an infant, then those same effects would also influence the cognitive skills children used later in their lives as adolescents.

The data collected was breastfeeding duration (duration the adolescent took formula), cognitive ability measurement (measured by an Intelligent Quotient (IQ) test score), age, grade, ethnicity, socioeconomic status, and gender. Statistical tests were conducted to find a correlation between breastfeeding duration and cognitive ability. There is no direct correlation between breastfeeding duration and cognitive ability, but after confounding variables were adjusted, there was a clear correlation that supported the hypothesis.

Keywords: Breastfeeding duration, cognitive abilities, docosahexaenoic acid, IQ score

INTRODUCTION

The importance of breastfeeding gained the attention of the World Health Organization and United Nations Children's Fund when breastfeeding rates decreased, formula feeding rates increased, and coincidently, morbidity and mortality rates of children increased as well (Walls, 2012). Kathleen Jones (2002) had stated that women should stay home for six months after giving birth to breastfeed, an optimum duration recommended by UNICEF. However, of the 71% of women in the United States who breastfed their infant, only 35% actually breastfed for six months (Godfrey & Lawrence, 2010). Many of the benefits that come with breastfeeding include unsaturated fatty acids that are only naturally found in breastmilk, such as docosahexaenoic acid. Unfortunately, breastmilk substitutes, such as formula, lack these nutrients, which affect the neurodevelopment of infants of life.

Within the ongoing research of breastfed infants and its impacts, studies have neglected an examination of the scientific connection between the intake of breastmilk by infants and their cognitive development, only considering participants younger than the high school age. When studies discussed cognitive development, they referred to the beginning development that shaped the cognition and intelligence of the human during infancy. When researchers used the terms cognitive abilities or cognitive skills, they were used as the competence of cognition and intelligence used by the human. After sources that validate the effect breastfeeding has on cognition were examined, this study produced a true scientific connection between the intake of breastmilk by infants and their cognitive development and included adolescent participants, factors that other researchers in the field lack.

LITERATURE REVIEW

Intelligence, Cognition, and IQ Tests

Researchers have consistently used Intelligence Quotient, or IQ, tests as a stable measure of cognition. The Autumn Group (2016) discussed the various perceived definitions of intelligence. From the various definitions, they all summarized to a common definition of cognitive ability, or the ability to use cognition. As mentioned in previous sources, infants that were not breastfed acquired a lower IQ score compared to infants who were breastfed, indicating a lower cognitive ability.

In a three-year follow-up study by Lee et al. (2016), the authors investigated the association between breastfeeding and cognitive development of infants during the first three years of their lives. Lee et al. (2016) had stated that cognitive development of infants is determined by heredity, psychosocial factors, and their interactions, especially with their mothers. The research showed that infants who were breastfed for nine months or longer had a better cognitive development than infants who were not breastfed for that period of time. Hence, it suggested that not only does breastfeeding influence cognition, as was demonstrated by previous sources, but also that the longer duration of which an infant is breastfed improves the cognitive development in infants. Though duration was significant in Lee's et al. study, and many others in the field, they neglected to show if the participants' cognitive abilities improved due to duration.

Scientific Connection

Among all the benefits, a significant gain was the brain enhancements, especially in the cognitive development of an infant. A study by Bar, Milanaik, and Adesman (2016) stated that there is currently a growing body of research that would demonstrate the effects of breastfeeding on neurodevelopment. Though researchers have found the molecule responsible for the development of cognition, they have failed to provide the scientific connection between the intake of breastmilk by infants and their cognitive development in relation to this macromolecule. An article, however, composed by a family of doctors, known as Dr. Sears (2016), briefly discussed a significant macromolecule that could only be acquired through breastfeeding and its crucial role in the link of breastfeeding and brain development. The macromolecules was an omega-3 fatty acid named docosahexaenoic acid, or DHA, which was a vital nutrient for growth, development, and maintenance of brain tissue.

DHA, and other breastmilk fats such as cholesterol, provided the right substances for manufacturing myelin, the fatty sheath that surrounded the nerve fibers. Myelin works as insulation, which made it possible for nerves to carry information from one part of the brain or body to another. Confirmedly, Pérez-Escamilla (2015) stated that docosahexaenoic acid (DHA) and another PUFA derivative played crucial roles in the proper growth, development, and maintenance of the brain. Lönnerdal (2016) stated that infant formulas have undergone many modifications in previous decades; the performance of formula-fed infants more closely resembles that of breastfed infants. These alterations include a more synthetic docosahexaenoic acid, which justified that breastfeeding was a more beneficial type of infant feeding, compared to formula.

Sears et al. (2016) stated during the first two years of an infant's life, the brain would rapidly grow and create connections from everyday experiences that shape the brain's growth. Neurons reproduced and connected amongst each other, creating these connections. The more infants would have interacted with their environment, the more their brains would have developed more connections. These are the same connections, or interactions, mentioned in Lee et al. (2016), that contributed to the cognitive development. Since breast milk is digested quickly and breastfeeding consisted of intimate interactions, infants are fed more when breastfed rather than when given formula. Consequently, necessary modifications had to be made to formulas. Otherwise, breastfed infants would (and will always) have an increased intake of docosahexaenoic acid and myelination and have amplified experiences due to longer but quickly made interactions, which would have led to the greater number of neurons, greater speed in their multiplication and connections, and faster growth

in the size of the brain (Sears et al., 2016).

In a book by Sternberg and Pretz (2005), Britt Anderson had proposed that the individual differences in the biological basis of human intelligence were the brain size was the nerve conduction velocity (myelination) and the neuron number. He had explained that higher IQ was correlated with larger brain size and with a higher nerve conduction velocity (myelination); stronger myelination had also produced higher speed of neural connection, which had occurred when an infant was breastfed as presented by Sears et al. By the examinations of the sources by Sears et al. (2016) and Sternberg and Pretz (2005), when there is a higher speed of neuron connections, faster growth or a large size of the brain, and higher conduction of myelination, it is due to an increased intake of docosahexaenoic acid. This occurs when an infant has a longer duration of breastfeeding and resulted to a higher IQ, which implicated higher intelligence or cognitive ability; thus, it scientifically demonstrated the effects of breastfeeding duration on cognitive ability.

Since it has been scientifically proven that breastfeeding affects cognitive abilities, the remaining portions of the study will be used to answer the question: To what extent does the duration of breastfeeding influences an adolescent's cognitive ability? Past research that examined the effects of breastfeeding on cognition indicated a strong correlation between infants who were breastfed and higher cognition. This research has integrated the conclusions of prior research with extensive examination of the specific effects of docosahexaenoic acid on cognition. Furthermore, this research will extend former resources to include adolescents.

METHODOLOGY

The quantitative correlational experimental research study analyzed patterns within the data that was collected. In the research conducted by Lee et al., along with many others, a correlation between the two variables were mentioned. The research stated that the positive correlation between breastfeeding and cognitive development may be because breast milk provides the nutrients required for development of the immune brain. In particular, human breast milk may support development of the newborn brain because it has long-chain polyunsaturated fatty acids (LCPUFAs), such as docosahexaenoic acid (DHA). This verified the basics of the scientific connection that was mentioned in this research.

The data that was collected was the breastfeeding duration, or formulated duration, cognitive ability measurement (which came from an IQ score), age, grade, ethnicity, socioeconomic status, and gender of the participants. Adolescents between the ages of 14 and 18 who were breastfed were collected because the breastfeeding duration was needed. The scores of the IQ tests that the participants had taken in the experiment reflected their cognitive ability.

Participants and Procedure

Potential participants that were approached were adolescents between the ages of 14 and 18. The potential participants were approached mainly at a high performing high school's library and an out-of-school community. The potential participants were given a detailed consent form and told a short description of the research and their part in it. The consent form contained the name of the researcher, project purpose, what will be asked to be collected, time requirement, benefits, potential risks, and security of confidentiality. They were then asked if they wanted to become a participant in a study to help answer the research question. It was explained that they needed to write how long they were breastfed, so that the breastfeeding duration variable could be obtained and there would be written documentation of it.

Participants were informed that to gain this information, they would have to ask their mother or some guardian that would know. If this piece of data could not be collected, the adolescent could not be a participant. If they could have obtained this data, the participants were told to take an IQ test on www.iqtest.com before the end of the week. They were told that time was a factor that would have determined their IQ score and that after they had taken the IQ test, they were asked to send their results to the researcher's email when the website asked for it. Potential participants were also contacted, mostly through email or text, to ask for the results of possible confounding variables.

IQ Test and Confounding Variables

Similar to other researchers, this research study used the scores of the participants' IQ tests to determine their cognitive ability. Bar et al. (2016) validated the use of an IQ test; they stated that the other tests that were used had measured academic achievement rather than intelligence. This specific IQ test that the participants had taken was recommended by Psychology Today due to its accurate reading of an individual's intelligence or cognition. Confounding variables had also been collected and adjusted, so the final analysis would have produced an accurate conclusion. Lee et al. (2016) discussed the effects of adjusting to different covariates and how they lead to different results. Furthermore, Bar et al. (2016) stated that after researchers had adjusted for confounding variables, they concluded that breastfeeding was associated with significantly high scores for cognitive development.

Statistical Analysis

After all the data was obtained, confounding variables were adjusted by the stratification method. This specific method was used instead of the multivariate method because of the number of participants, which was a limitation. The stratification method broke down the confounding variables into more specific and relevant subcategories: Lower, Upper, Middle, 9th grade, 10th grade, 11th grade, 12th grade, Foreign, American, Male, and Female. Due to the great variety of ethnicities and possible language barrier for some participants, the confounding variable separated into Foreign, ethnicities laid outside of the United States, and America, ethnicities laid within the United States.

The ages of participants grouped within their grades because there were many different ages within the different grades. Since the adolescent participants acquired different levels of education that affected the IQ scores, it was ideal to group the adolescents into grades. The results were then split into breastfed and formula. For each subcategory, the average and standard deviation was found for both breastfeeding duration and cognitive ability, or cognition for short on the graphs and tables.

Confounding Variables & Participant Limitations

The abundance of confounding variables along with the limited amount of participants were the most significant limitations. In a medical education study by Pourhoseingholi, Baghestani, Vahedi (2012), it stated that there were mostly two options to dealing with confounders (or confounding variables) in the analysis stage: Stratification and Multivariate methods. Since there are also many possible confounding variables, adjusting for different confounding variables with different methods aused a certain limit that caused the results to be slightly askew. In the search of participants, it was exceptionally difficult to find male adolescents that were breastfed. More than half of the male participants that were approached were not breastfed. Thus, there were many more females than males n this data set. Similarly, it was difficult to find females that took formula milk, but there was still a balanced amount of females and males in the control group (the group of participants that took formula milk instead of breastfed).

RESULTS

After all the data from the participants was collected, it was then added into three tables and a graph.

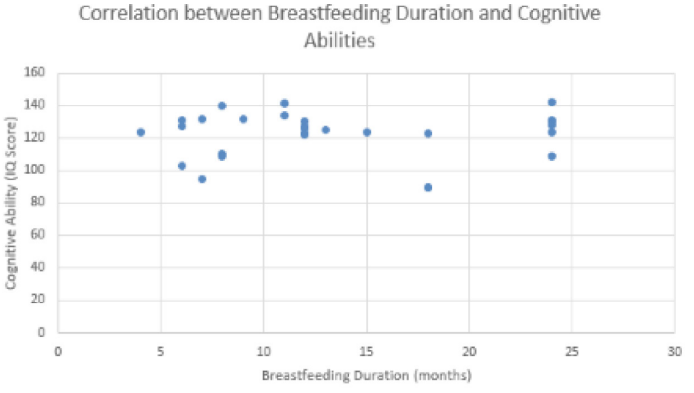
Name	Form	Breast | Form	Duration	Cognition	Age/Grade	Economic	Ethnicity	Gender
C. H.	yes	Breastfed	7	132	16/10	Middle	White	Female
B. M.	yes	Breastfed	24	142	16/11	Lower	Ethiopian	Female
F. E.	yes	Breastfed	24	109	14/9	Middle	S. Sudan	Female
K. G.	yes	Breastfed	11	134	16/10	Middle	Greek	Female
S. N.	yes	Breastfed	6	131	15/9	Lower	Eritrean	Female
F. M.	yes	Breastfed	13	125	16/10	Lower	White	Female
S. Z.	yes	Breastfed	18	90	16/10	Lower	Eritrean	Female
G. T.	yes	Breastfed	12	126	16/11	Middle	White	Female
K. I.	yes	Breastfed	6	103	15/10	Middle	White	Female
M. W.	yes	Breastfed	18	90	16/11	Lower	Ethiopian	Female
A. P.	yes	Breastfed	18	123	16/11	Middle	White	Female
M. M.	yes	Breastfed	24	131	14/9	Middle	Ethiopian	Female
S. T.	yes	Breastfed	24	124	14/9	Middle	Ethiopian	Female
S. M.	yes	Breastfed	4	124	16/11	Middle	White	Female
L. J.	yes	Breastfed	12	130	16/11	Upper	Black	Female
R. P.	yes	Breastfed	15	124	16/11	Upper	Indian	Female
Z. M.	yes	Breastfed	8	110	16/11	Middle	Mix	Female
T. C.	yes	Breastfed	8	109	15/10	Lower	White	Female
L. F.	yes	Breastfed	6	127	16/11	Middle	Cuban	Female
R. C.	yes	Breastfed	8	140	16/10	Middle	Indian	Female
S. L.	yes	Breastfed	11	141	16/11	Upper	Asian	Female
F. M.	yes	Breastfed	12	123	15/10	Middle	Mix	Female
B. R.	yes	Breastfed	24	130	16/10	Upper	Indian	Male
R. G.	yes	Breastfed	24	128	14/9	Middle	Eritrean	Male
M. W.	yes	Breastfed	12	123	16/11	Middle	Black	Male
F. C.	yes	Breastfed	12	127	16/10	Upper	White	Male
A. R.	yes	Breastfed	9	132	17/11	Upper	Iranian	Male
K. M.	yes	Breastfed	7	95	16/10	Middle	Black	Male
I. G.	yes	Breastfed	12	122	15/11	Middle	Mix	Male

Table 1. Data from participants that were breastfed

A. D.	yes	Form	10	114	17/11	Middle	Black	Female
C. F.	yes	Form	18	138	17/11	Middle	White	Female
D. H.	yes	Form	7	122	18/12	Middle	Black	Female
R. M.	yes	Form	12	127	15/9	Upper	White	Female
E. F.	yes	Form	12	133	16/11	Middle	Mix	Male
D. H.	yes	Form	8	107	18/12	Upper	Black	Male
R. W.	yes	Form	48	116	16/10	Upper	Black	Male
C. S.	yes	Form	12	111	17/11	Lower	White	Male

Table 2. Data table from participants that took formula milk.

Within Table 1 and Table 2, each row displayed all of a specific individual's data. Every column had shown all of the data for a specific variable. In the first column of the tables, the participants' initials of their first and last name were displayed. In the second column, it had displayed whether or not the subject has turned in a consent form. The third column had displayed whether the subject was breastfed or formulated. The independent variable that was collected was the time, in months, that the adolescents were breastfed, which was located in the fourth column. The dependent variable, the IQ score, had measured cognitive abilities, which was located in the fifth column under cognition for short. In columns six through nine, the results of possible confounding variables that were adjusted for were displayed. Column 6 had displayed the age and grade; column 7 had displayed the socioeconomic status; column 8 had displayed the ethnicity and the final column, column 9, had displayed the adolescent's gender.



Graph 1. Correlational graph had shown whether there was a correlation between duration and cognition.

			Socioeconomic Status			Grade				Ethnicity		Gender	
			Lower	Middle	Upper	9	10	11	12	Foreign	American	Female	Male
Breastfed	Duration	AVG	14.8	11.5263	13.8333	20.4	11.4545	12.3846	N/A	16.4	10.2143	13.1364	14.2857
		SD	7.56307	7.86955	5.34478	8.04984	5.44727	5.39349	N/A	7.3368	3.66225	6.59217	6.8972
	Cognition	AVG	112.4	109.158	130.667	124.6	118.909	124.154	N/A	124.867	119.429	122.182	122.429
		SD	23.6495	39.9823	5.78504	9.1815	16.8669	13.1773	N/A	16.2958	10.8537	14.6536	12.6076
Formula	Duration	AVG	12	11.75	22.6667	12	48	13	7.5	N/A	15.875	11.75	20
		SD	N/A	4.64579	22.0303	N/A	N/A	3.4641	0.70711	N/A	13.3998	4.64579	18.7617
	Cognition	AVG	111	126.75	116.667	127	116	124	114.5	N/A	121	125.25	116.75
		SD	N/A	10.8128	10.0167	N/A	N/A	13.4907	10.6066	N/A	10.9545	10.0457	11.4419

Table 3. Table that had adjusted the confounding variables by using stratification.

DISCUSSION

General Correlation

From Graph 1, it was shown that, without adjusting to confounding variables, there was no correlation from the data that was available.

Socioeconomic Status' Analysis

It was noticed from Table 3 that the lower class had the highest average duration of breastfeeding with the mean being 14.8 months, while the upper class had the highest average IQ scores with a mean of 130.6; the middle class had the highest standard deviation due to the high variance in the sample. The relationship between lower class and higher class for those that were breastfed and not breastfed, or took formula milk, was noteworthy, due to the fact that lower socioeconomic families would benefit more from breastfeeding their children longer, especially if it meant their children would have greater cognitive abilities. It was also significant to note that the middle class had the shortest duration of breastfeeding and also the lowest IQ score. The lower class participants that were breastfed were feeding longer and still had a higher IQ score than the lower class participants that took formula milk. Within the upper class, even though the control group took formula milk longer, those who were breastfed received a high IQ score.

Ages with Grades

Of the four grade levels, there were no twelfth grade participants who were breastfed as an infant. The ninth grade participants had the highest average duration of breastfeeding, 20.4 months, and the highest average IQ score, 124.6. Since this specific research field is growing, that could have been a variable as to why the younger group was breastfed longer and scored higher.

It infers that today's society recently started to realize the importance of breastfeeding duration. In fact, there were fewer ninth graders who took formula milk and not a single twelfth grader who was breastfed.

Ethnicity

When the varieties of ethnicities were grouped by American, who were White, Black, or a mix of both races, and Foreign, the Foreign had the longest average duration of time spent breastfeeding of 16.4 months and had the highest average IQ score of 124.86. There was not a single foreign adolescent who took formula milk. According to Alexander Leung and Reginald Sauve (2005), "Breastfeeding is universally accepted as the optimal method of infant feeding for the first year of life." Since the study had claimed universally the optimum time was a year, but UNICEF had said it was six months (half that time), it was given that internationally (to Non-Americans) a year, or even more, was natural, which had explained why the Foreign participants had an average that was over a year and the American participants had an average that was less than a year. The American families could have had been affected by other confounding variables that caused them to result to formula, such as work. Jones (2002) had discussed situations similar to such. She had stated that factors, which influence the choice of breastfeeding, included returning to work, which could have been the situation for these specific American families.

Gender

After the data was collected, it was noticed in Table 1 and Table 2 that the most of the participants that were breastfed were female. There were more males that took formula milk than females when they were approached but this was not displayed on the table.

Statistical Analysis

Amongst all participants, breastfed and formulated, the average duration was 13.9 months and IQ score was 121.9. At a confidence interval of 99%, the true average IQ scores of both breastfed and those who took formula milk was found to be between 115.8 and 128.1 with a margin of error of 6.1. Amongst the participants that were breastfed, the average duration was 13.4 months and IQ score was 122.2. A confidence interval of 99% was constructed and it was concluded that the true mean of the breastfed IQ scores were between 115.1 and 128.9 with a margin of error of 6.9. This had demonstrated a somewhat large variance in data due to the small, but sufficient sample size. The standard error of the treatment group was 1.26, while the standard error for the control group was .995 due to the difference in variance. The degrees of freedom for the control group was found to be 7, while the degrees of freedom for the experimental group was found to be 27, due to the massive difference in sample sizes. After running a T-test on the resulting IQ scores from the independent breastfed group and the formula group, the t-value was found to be 0.81817; this signifies a meaningful difference. Using the t-test, the p-value was found to be .21034, which is insignificant at 0.05, the standard alpha level. From these results, it was gathered that the control group and treatment group are not significantly variant from one another.

CONCLUSION

The purpose of the experiment was to evaluate the extent to which breastfeeding duration in an infant's life had influenced his or her cognitive abilities in the adolescent years. The purpose answered the extent to which the hypothesis was supported. It was hypothesized that if a child was breastfed and the amount and duration of which a child was breastfed had influenced the cognitive development of an infant, then those same influences would influence the cognitive skills children would have used later in their lives as an adolescent. The experiment evaluated the extent breastfeeding duration influenced the cognitive abilities of the adolescents, with and without the confounding variables being adjusted.

Without Adjusted Confounding Variables

There was no direct correlation between the independent and dependent variables, breastfeeding duration and cognitive abilities respectively, when there were no adjustments to confounding variables. There were many factors that influenced the cognitive ability of

an adolescent, such as the various confounding variables that were not mentioned and the numerous years the adolescent lived after they stopped breastfeeding. These factors were more likely the cause of why there was no direct correlation between breastfeeding duration and cognitive ability. In conclusion, the results without adjustments to confounding variables had not supported the hypothesis.

With Adjusted Confounding Variables

On the other hand, there was an evident and clearer positive correlation between breastfeeding duration and cognitive ability when there were adjustments made to confounding variables with the stratification method. This wasdisplayed in Table 3, which showed that the duration an adolescent was breastfed, including the adolescents that fed from formula in some cases, still influenced his or her cognitive ability. This positive correlation showed that the longer the adolescent was breastfed, the stronger the cognitive abilities were, and the same for some adolescents that fed from formula; thus, the results that adjusted to confounding variables supported the hypothesis.

Future Practical Applications

If this experiment was extended, the addition of more participants was recommended, especially more male participants that breastfed because there were many more females that breastfed and the results would be balanced and more accurate. In addition, a bigger control group with participants that took formula would be beneficial. If there was more research done on the difference between breastfed infants, infants who took formula milk, and their difference in IQ scores, this would have had a bigger impact on the results. Another recommendation would be to account for more adjusted confounding variables, which would have definitely created a more accurate, positive correlation than the one presented in Table 3.

REFERENCES

Autumn Group. (2016). What is an IQ Score? Retrieved September 21, 2016, from http://www.iqtest.com/whatisaniqscore.html

Bar, S., Milanaik, R., & Adesman, A. (2016). Long-term neurodevelopmental benefits of breastfeeding. Current Opinion In Pediatrics, 28(4), 559-566.

Godfrey, J., & Lawrence, R. (2010). Toward optimal health: the maternal benefits of breastfeeding...Ruth A. Lawrence, M.D. Journal Of Women's Health (15409996), 19(9), 1597-1602. doi:10.1089/jwh.2010.2290

Jones, K. (2002). Product feature. Benefits of breastfeeding and breast pumps. British Journal Of Midwifery, 10(3), 181-182.

Lee, H., Park, H., Ha, E., Hong, Y., Ha, M., Park, H., & ... Kim, Y. (2016). Effect of Breastfeeding Duration on Cognitive Development in Infants: 3-Year Follow-up Study. Journal Of Korean Medical Science, 31(4), 579-584. doi:10.3346/jkms.2016.31.4.579 .

Leung, A. C., & Sauve, R. S. (2005). Breast is best for babies. Journal Of The National Medical Association, 97(7), 1010-1019.

Lönnerdal, B. (2016). Bioactive Proteins in Human Milk: Health, Nutrition, and Implications for Infant Formulas. The Journal Of Pediatrics, 173 SupplS4-S9. doi:10.1016/j.jpeds.2016.02.070.

Pérez-Escamilla, R. (2006). Influence of breastfeeding on psychosocial development.
Encyclopedia on early childhood development, 1-6.

Pourhoseingholi, M. A., Baghestani, A. R., & Vahedi, M. (2012). How to control confounding
effects by statistical analysis. Gastroenterology & Hepatology From Bed To Bench, 5(2), 79-83.

Sternberg, R. J & Pretz, J. (Eds.) (2005). Cognition and intelligence: Identifying the mechanisms of the mind (pp. 88-103). New York: Cambridge University Press.

Sears. (2016). Breastfeeding and Brain Development. Retrieved November 14, 2016, from http://www.askdrsears.com/topics/feeding-eating/breastfeeding/why-breast-is-best/breastfeeding-brain-development

Timby, N., Domellöf, E., Hernell, O., Lönnerdal, B., & Domellöf, M. (2014).
Neurodevelopment, nutrition, and growth until 12 mo of age in infants fed a low-energy, low-protein formula supplemented with bovine milk fat globule membranes: a randomized controlled trial. The American Journal Of Clinical Nutrition, 99(4), 860-868. doi:10.3945/ajcn.113.064295

Walls, D. (2012). The Role of the Childbirth Educator in the WHO International Code of Marketing of Breastmilk Substitutes. International Journal Of Childbirth Education, 27(4), 89-91.

Examining the Sensitivity and Reactivity between Luminol and Bluestar Magnum in Detecting Diluted Blood Concentrations

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ABSTRACT

This study was designed to test two kinds of latent blood reagents: luminol and Bluestar Magnum, to determine their ability to detect diluted blood by evaluating the strength of their chemiluminescence and time duration of the reactions. It was found in a previous study by the researcher that BlueStar Forensic has the advantage when investigating crimes in scenarios where atmosphere temperature is not desirable and light cannot be diminished. The results of this study can spread the awareness of the biased justice system and a better, more accurate method to conduct blood-pattern analysis forensic studies to use in the courtroom. Three dilutions of blood were applied to vinyl tile. Three dilutions were set to sit for 30 minutes before application of luminol or Bluestar. Photographs were taken of each trial. Chemiluminescence intensity was graded by a rating scale. Additionally, the time duration of each reaction was recorded. These data support the hypothesis that Bluestar significantly outperformed luminol in both chemiluminescence and time duration in all blood dilutions. Even in the most diluted solution, Bluestar showed great visibility. Regression and ANOVA tests show that time duration and chemiluminescence were mostly correlated. Luminol's time durations in general were much shorter than Bluestar's. In conclusion, Bluestar Magnum has superior sensitivity and reactivity against luminol and therefore should be used in lieu of luminol when investigating crime scenes.

INTRODUCTION

Visual examination of blood evidence at a crime scene is mandatory and essential to resolve a case. However, there is a possibility that blood is present in amounts that cannot be seen with the naked eye or that the blood might have been cleaned before the officials arrived at the scene. This usually is the case as the criminal(s) tend to clean their victim(s)' blood with heavy-duty cleaning chemicals in an attempt to leave without a trace. Other clean up attempts can include mopping, or washing a blood-soaked garment in the washing machine. Therefore, the blood can become greatly diluted and hard to see to human eyes. But, even with the strongest cleaning chemicals, blood particles will still remain. In these instances, luminol is used to locate trace amounts of blood that would have been otherwise undetected through visual examination ("Luminol (Blood)", n.d.). Luminol exposes blood patterns that could progress the case. These patterns could expose the exit route from the crime scene, drag marks, or the attempt to clean up the blood.

Luminol is a pale-yellow crystalline solid that, when combined with other vital chemicals, will glow a bright blue light when in contact with blood; this helps forensic officials locate and analyze blood patterns to progress the case (Clegg, 2014). Luminol in conjunction with a strong oxidizing agent, such as hydrogen peroxide, and a base, is what is used in the solution sprayed at crime scene; the base is an addition of an alkali, such as sodium hydroxide (Brunning, 2014). Investigators that have used luminol most likely used the Grodsky formula, a commonly used formula for luminol. This formula is comprised of luminol, sodium carbonate, and sodium perborate. This luminol solution then reacts with a catalyst; in most forensic cases, this is the iron in hemoglobin of blood at the crime scene investigations.

Haem is a biochemical structure that structures a vital piece of peroxidase. This structure is similarly present in hemoglobin. In this way, the nearness of hemoglobin, and therefore blood, can be disclosed by exploiting the ability of haem to catalyze luminol's property of chemiluminescence. The Luminol reaction sequence can be seen in Figure 1.

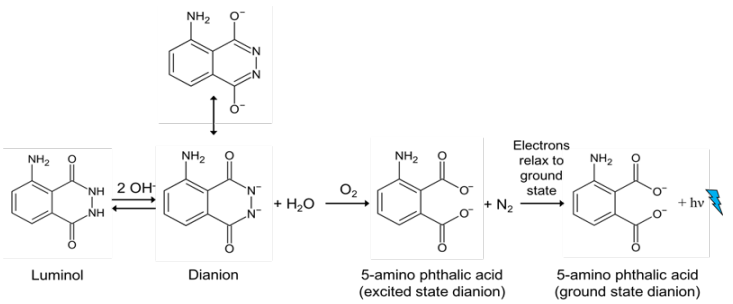

Figure 1. Luminol's Reaction Sequence (Whyte, 2018)

Luminol holds disadvantages that are unfavorable in the work field despite its benefits. The main disadvantage to luminol is that the oxidized products formed when the oxidizing agent in luminol reacts with blood are soluble. Therefore, the original blood stain is not preserved when the luminol is sprayed onto the stain; luminol is predominantly used for the detection of stains as small details, such as fingerprints, cannot be deciphered (Cheyne, 2011). Luminol chemiluminescence can also be triggered by a number of substances other than blood, such as feces, horseradish, bleach, copper containing compounds, and excessive smoke. Other drawbacks of luminol include interferences with DNA/RNA and other presumptive testing, difficulty of photography, the short time duration of the chemiluminescent reaction, having to work in the dark because the glow is very faint, and health and safety issues.

In the early 2000's, a commercial formulation of luminol was created in hopes of overcoming all the drawbacks of luminol to provide a better blood-detecting reagent that is more accurate and reliable. This luminol-based formulation is called BlueStar Forensic. The manufacturer (Roc Imports) claims that it is the most sensitive and long lasting presumptive blood test on the market. BlueStar is prepared and used similarly to luminol. Since BlueStar's release into the market, there has not been an abundance of comparison studies of the two latent blood reagents. The studies that have been conducted conclude that BlueStar is remarkably more beneficial and effective than the original luminol solution as BlueStar consistently emitted brighter, long lasting reactions with significantly less false positives than luminol. These studies also found that BlueStar does not interfere with any other presumptive tests such as RNA and DNA tests, while also preserving the original structure of the bloodstain ("Compare BLUESTAR", 2004).

Currently, forensic analysis evidence is seen as biased, unreliable, and inaccurate in courts, leading to many false convictions (Stern, 2014). According to a 2009 publication by the National Research Council entitled *Strengthening Forensic Science in the United States: A Path Forward*, "the forensic science system, encompassing both research and practice, has serious problems that can only be addressed by a national commitment to overhaul the current structure that supports the forensic science community in this country" ("Committee on", 2009).

This year's study continued to investigate and test the claim that BlueStar Forensic is brighter, better, and more reliable than luminol. It was hypothesized that Bluestar Magnum will outperform luminol in sensitivity and chemiluminescence. The greater the chemiluminescence and time duration, the better crime investigators can locate the trace amounts of blood that can potentially solve a case with accurate evidence.

METHODOLOGY

The blood that was used for this study is synthetic blood purchased from Sirchie. This synthetic blood is chemically similar as it is formulated with ingredients to mimic human blood. The Grodsky luminol formula was used for this experiment. The Grodsky formulation of luminol was purchased from Pioneer Forensic, LLC. Bluestar Magnum, purchased from Crime Scene, was used as the comparing latent blood reagent. The substrate used for this experiment was vinyl tile purchased from the Home Depot. Vinyl tiles are the most common household flooring and therefore would be prevalent in

crime scene investigations. The vinyl tiles were cut into 4 by 4-inch squares. There were 30 trials per blood dilution for each blood reagent. There were three blood dilutions that were tested: 1:1 (undiluted), 1:10, and 1:100. The undiluted solution acted as the control. Each dilution was diluted with distilled water.

The experimentation took place in a basement with already limited light sources, but total darkness allowed easier detection and photography of smaller blood traces. Thus, all windows were closed and all outside light sources were blocked prior to experimentation. Each blood reagent was prepared according to the manufacturer's instructions. Each vinyl square was sampled with one millimeter of the pure or diluted blood for a total of 90 millimeters per blood reagent or 180 millimeters in all. After all the tiles are sampled, the stains were left to sit for 30 minutes. After the 30 minutes, the samples were wiped with a dry paper towel to remove the majority of the stain.

Prior to spraying the solutions, a camera was set up on a tripod facing down to the tile. Each square tile was placed directly below the camera before spraying. Lights were turned off before spraying the corresponding latent blood reagent. The tiles were then sprayed corresponding to their manufacturer's instructions. The Bluestar trials were sprayed at least 1 foot away from the tile; the tiles sprayed with the luminol solution were sprayed in a similar fashion. Time durations were recorded as well. When the reaction was visible, the timer started to record in seconds and the timer stopped once the reaction was no longer visible to the researcher. This process repeated for all 180 tiles. Photographs were reviewed after experimentation to analyze and rate each reaction on a 0-3 rating Scale (see Figure 2 and Table 1).

Reaction Strength Grading	
0	Chemiluminescence not seen
1	Weak chemiluminescence
2	Moderate chemiluminescence
3	Strong chemiluminescence

Table 1. Reaction Strength Grading

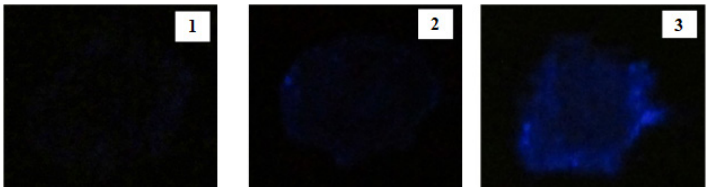

Figure 2. Reaction Scale Examples

RESULTS

As seen in the Figure 3 and Figure 4, Bluestar Magnum significantly outperformed Luminol in all blood concentration in both time duration, and strength of chemiluminescence.

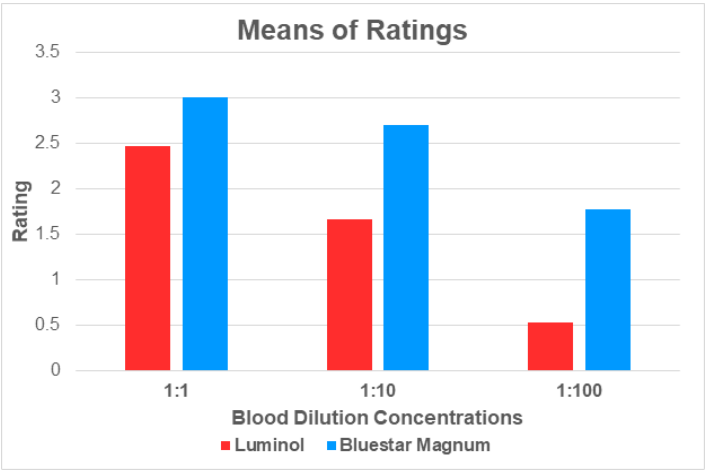

Figure 3. Means of Ratings

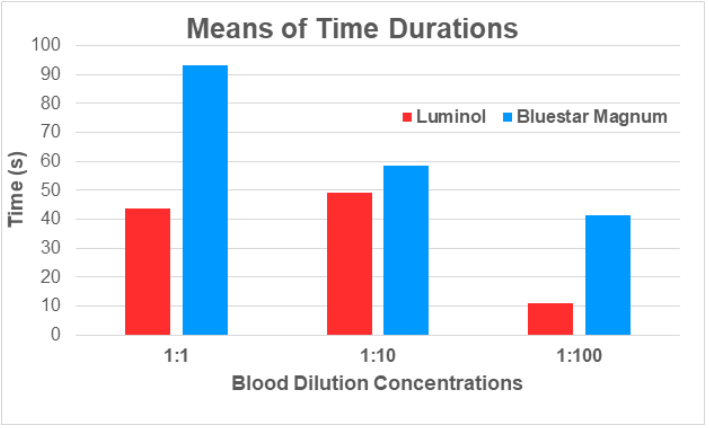

Figure 4. Means of Time Durations

Regardless of blood concentration and light setting, Bluestar showed great visibility. Luminol could not be seen in even a dimly lit room when the lights were briefly turned on.

The regression test (see Figure 5) and other statistics supported that luminol is very inconsistent while Bluestar is consistent in both its

time duration and chemiluminescence. In Table 2, it is seen that the standard deviations of Bluestar are much lower than the standard deviations of luminol.

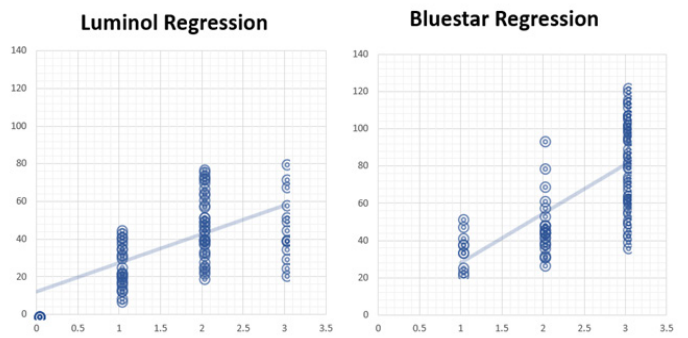

Figure 5. Regression Test Graphs

	Luminol			Bluestar Magnum		
	1:1	1:10	1:100	1:1	1:10	1:100
Rating	2.47	1.67	0.533	3.00	2.70	1.76
Time (s)	43.9	49.1	11.0	93.4	66.0	41.2
Std. Dev. (Rating)		0.937			0.69	
Std. Dev. (Time)		22.8			21.7	

Table 2. Means and Standard Deviations for Rating and Time

A two-way ANOVA was performed to see if the interactions were statistically significant. All relationships had a p-value < 0.005 (See Table 3).

Two-Way ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Sample	39.2	1	39.2	173.7046	5.91E-28	3.895458
Columns	77.54444	2	38.77222	171.809	6.44E-42	3.047906
Interaction	3.9	2	1.95	8.640917	0.000264	3.047906
Within	39.26667	174	0.22567			
Total	159.9111	179				

Table 3. Two-Way ANOVA Results

A simple regression test was performed to see the how time duration and chemiluminescence correlated for both blood reagents. The regression test run for luminol showed that luminol is very inconsistent with its results as there was little visible correlation between the two variables. Bluestar's regression test revealed that it was much more consistent as the correlation between the variables was much more prominent; the higher the rating of chemiluminescence, the longer the duration of the reaction.

CONCLUSION

The data and statistics collected support the hypothesis. The two-way ANOVA calculation shows that the relationships between reagent and luminosity, temperature setting and luminosity, and both reagent and temperature setting all had statistically significant differences ($p < .0005$) (see Table 3). Table 2 and Figure 5 suggests that Bluestar is much more stable and consistent as opposed to luminol.

As seen throughout the figures and tables, a constant recurring trend was that a higher concentration of blood yields a higher chemiluminescence and longer reaction time duration. A higher concentration of blood affects the amount of emitted light due to the rate of the reaction. A higher concentration of blood indicates a higher presence of iron. As more iron catalyzes the luminol or Bluestar solution, it emits more photons of blue light, reacting sooner if a catalyst is added. Time duration of the reaction increased as the strength of reaction increased. This can be due to the number of photons being emitted. As more photons are being emitted over a period of time, the photons will relax in a prolonged manner.

St. Louis University performed a similar study comparing luminol and Bluestar Magnum to each other. This study mainly focused on how each reagent would affect DNA preservation for genetic profile determinations; however it also tested how varying blood dilutions would affect the strength and time duration of a reaction on various substrates, including vinyl tile. The scale used to rate the strength of reaction at St. Louis University is identical to the scale used in this study. The St. Louis study concluded that Bluestar Magnum is superior to luminol due to Bluestar's chemiluminescence and time duration results (Lautz, J. & Webb, S., 2006).

Identifying a more accurate and effective blood reagent leads to the decline of the amount of falsely imprisoned — and even executed — suspects. By conducting this study, awareness of the biased justice system and a better, more accurate method to conduct blood-pattern

analysis forensic studies to use in the courtroom can be spread.

To conclude, Bluestar Magnum is far superior than luminol and should be used in lieu to acquire accurate evidence for use in court. Bluestar Magnum is more efficient, easier to prepare, and just as easy to use. Forensic investigators should utilize Bluestar when on the scene. Further experimentation should be done to other substrates.

REFERENCES

Brunning, A. (2014). Crime Scene Chemistry – Luminol, Blood & Horseradish. Compound Interest. Retrieved from http://www.compoundchem.com/2014/10/17/luminol/

Cheyne, M. (2011). Illuminating Latent Blood. Retrieved from https://www.abacusdiagnostics.com/Illuminating_Latent_Blood.pdf.

Clegg, B. (2014). Luminol. Chemistry in its element. Retrieved from https://www.chemistryworld.com/podcast/luminol/7272.article

Committee on Identifying the Needs of the Forensic Sciences Community, National Research Council. (2009). Strengthening Forensic Science in the United States: A Path Forward. pp. 22. Retrieved from https://www.ncjrs.gov/pdffiles1/nij/grants/228091.pdf.

Compare BLUESTAR® FORENSIC to other reagents. (2004). BlueStar Forensic. Retrieved from http://www.bluestar-forensic.com/gb/compare.php

Lautz, J., & Webb, S. (n.d.). (2006). A Comparison On The New Formula of BlueStar Latent Bloodstain Reagent and Its Effects on DNA Typing/Amplification. Saint Louis University Clinical Laboratory Sciences Department,1-18. Retrieved January 15, 2018, from http://www.bluestar-forensic.com/pdf/en/Lautz_Webb_Bluestar_Magnum_effects_on_DNA.pdf

Luminol (Blood). (n.d). Minnesota Department of Public Safety. Retrieved from https://dps.mn.gov/divisions/bca/bca-divisions/forensic-science/Pages/forensic-programs-crime-scene-luminol.aspx

Luminol Material Safety Data Sheet. (1998). Aldon Corporation.

Whyte, D., & Lohner, S. (2018, May 5). Crime Scene Chemistry—The Cool Blue Light of Luminol. Retrieved from https://www.sciencebuddies.org/science-fair-projects/project-ideas/Chem_p078/chemistry/crime-scene-luminol-blood

Shermer, M. (2015). Can We Trust Crime Forensics? Scientific American. Retrieved from https://www.scientificamerican.com/article/can-we-trust-crime-forensics/.

Stern, M.J. (2014) Forensic Science Isn't Science. Retrieved from http://www.slate.com/articles/health_and_science/science/2014/06/forensic_science_is_biased_and_inaccurate_but_juries_believe_it_and_convict.html.

Three-Dimensional Visualization of Computed Tomography Scans

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ABSTRACT

The Computed Tomography (CT) scan has led to groundbreaking advancements in the medical field; however, there is still a need for improvement in the way CT scans are displayed. Conceptualizing CT images is a difficult task for physicians as it requires extensive use of the brain's working memory due to the complex three-dimensional structures of human anatomy. A proposed solution to this problem is to present medical images in a three-dimensional format. In doing so, the physician would no longer be burdened with the additional cognitive load imposed during the mental conversion of a two-dimensional image into a three-dimensional representation within working memory. A unique technological solution to achieve this uses holography. Within this research, holograms that depicted CT scans were created using an app called Holapex Holography. A quasi-experimental study was conducted where radiologists were presented with medical images in a traditional format via textbook and through holography. Cognitive load analysis was performed to determine if a difference in cognitive effort was experienced while viewing the holograms. An efficiency study was conducted to evaluate hologram performance and user experience. The results indicated that the holograms presented a significant performance improvement over traditional textbook handouts.

INTRODUCTION

Imaging has been a focus of the medical sphere since engineer Godfrey Hounsfield and physicist Allan Cormack invented the computed tomography (CT) scan in 1972. CT scans are defined as diagnostic medical tests that produce multiple images of the body (RSNA & ACR, 2016). These images can be viewed on a computer monitor or printed on film. CT scans have profoundly changed the practice of healthcare, as foreign bodies or any other abnormal occurrence within a patient can be detected without having to make a single incision. Most importantly, a CT scan helps reveal and assess internal injuries quickly enough to save a patient's life (Wampole et al., 2013).

If a foreign body is detected in a patient, physicians go on to determine the next steps. While planning for operations with foreign bodies, physicians typically mentally translate the two-dimensional CT scans into a three-dimensional mental picture of the patient's anatomy (Wampole et al., 2013). Visualization is critical to understanding and reporting image data; however "the complexities of the human anatomy and its variation from one individual to the next, combined with the increasing number of imaging methods and representations required to fully characterize its structure and function," make visualization a formidable challenge (Rojas, 2014). In 2015, thirty-eight percent of patients with foreign bodies were misdiagnosed by the initial treating physician (Matsuda, 2017), supporting that detection by imaging is problematic despite advances in techniques. In light of this issue, significant literature discusses how CT scans could be better presented to the physician, patient, and hospital to minimize error and maximize positive outcomes.

LITERATURE REVIEW

Benefit for the Physician

The amount of benefit for the physician by changing the manner CT scans are presented can be measured through the lens of cognitive load. Cognitive load is the amount of information imposed upon the working memory of the brain (Hackett, 2013). The Cognitive Load Theory (CLT) relies on the model of human information processing, which occurs through three types of memory: sensory, working, and long-term. Working memory has significant limitations in terms of size, holding only seven items at a time. The central tenet of CLT is that working memory can be overloaded with information, resulting in decreased cognitive performance. For example, learning the names of individual muscles, bones, nerves, etc., does not impose a high cognitive load, but manipulating these into usable units for understanding spatial and functional relationships requires an extensive use of cognitive load (Hackett, 2013).

CT Scan Display Methods

Two professors, Yuji Sakamoto and Yoshinao Aoki, from the Graduate School of Engineering at Hokkaido University, believe that displaying CT scans three-dimensionally is a more efficient way to display image data (2007). Research worldwide has supported this idea with Matthew Wampole in the U.S. stating three-dimensional visualization is the first step into new methods of anatomical analysis as classic methods for viewing imaging data reduce the three-dimensional structure of the human body to two-dimensional representations, resulting in significant loss of information (2013). Hadi Sasani (2017), from Turkey, concluded that three-dimensional imaging leads to lower information loss and increase definition of anatomical structures; Navid Farahani (2017), from Cyprus, goes on to say that three-dimensional imaging could also enhance the study of disease processes, especially those involving structural changes in which spatial relationships are relevant. These varying sources all support the idea that three-dimensional CT imaging is preferable to two-dimensional CT imaging due to the fact that emulating the three-dimensional structure of a patient retains more information about the patient.

3-D Display Methods

Many different methods for three-dimensional display have been proposed. Currently, there is no clear "best" three-dimensional imaging method; rather, each of the available methods involves tradeoffs in image size, accuracy, and cost (Matsuda, 2017). Thus, determining which three-dimensional imaging method is most appropriate often depends on the medical question under investigation. Despite this, Sakamoto and Aoki anticipate digital holographic technology to be the most practical method since it allows differences in viewing angle, appearance, and focus to be captured in a manner that satisfies human perception without a need for any special observation devices (2007). Studies by the Air Force Research Laboratory and the US Research Development and Engineering Command show that the use of digital holograms improved time and accuracy in identifying tactical and terrain-related, therefore, features a significantly faster generation of routes when compared to conventional two-dimensional maps and imagery (Hackett, 2013). In support of this view, Andrew Doblas's experiments, published in 2016, show that digital holographic microscopy allowed rapid detecting, from a single drop of blood, of differences between erythrocytes (red blood cells that contain hemoglobin) of diabetic patients and healthy patients. These varying sources have conclusions that reason for the testing of digital holograms to increase efficiency in the medical field because of digital holograms' optimal viewing.

The Gap: Cell-Phone Use in Medicine

There are a number of devices that have been tested to display CT scans three-dimensionally; however, the reason these devices have not become widespread is that they either one, cost large sums of money to obtain, or two, require a whole new set of skills to utilize. There is a gap in research on devices that use digital holographic technology and sufficiently surpass these downfalls. According to the CTO of IBM, Paul Bloom, "we see three-dimensional

technology moving into the cell phone, which will have the ability to transmit information off the cell phone to create a three-dimensional hologram" (Sivakumar et al., 2012). Cell phones are the most ubiquitous screens in both the developed and developing worlds, and are attractive candidates for conversion into medical devices. Some work has already been directed towards this end, with several recent papers discussing the use of cell phones as diagnostic devices. Researchers at UCLA have constructed a modified lensless cell phone that enables holographic digital microscopy, while researchers at UC Berkeley have constructed a complex objective attachment that transforms a cell phone into a microscope (Smith, 2011).

Monetary Benefits

Today's technological advances to the hologram provide some outstanding advantages to not only everyday consumers but also large business corporations and governments (Sivakumar et al., 2012). To a panel of investors, Tom Greenberg explained that, because incisions can be perfectly located from holographic techniques, the incision can be made smaller and damage less tissue (Dalglish, 1994). That means that a patient's hospital stay and the related cost can be reduced considerably. With health care costs increasing throughout the world, there is a pressing need for reducing the cost and complexity of biomedical devices (Dalglish, 1994). Additionally, with growing demand for high-quality health care, portable devices that can transmit relevant data to remote experts are likely to have a large impact on the quantity and quality of care (Smith, 2011).

Additional Work Needed

Although a new approach to 3-D medical imaging had been deduced, additional studies were required to see if this technology, made for entertainment, had applications in the medical field by testing if it fulfills the goal of reducing extraneous cognitive load to ultimately reduce time and mental resources wasted processing image information. Additional research was directed towards the utilization of cell phones as medical instruments and identifying how the altered visualization affected the radiologists themselves since radiologists are the physicians that will most often view CT scans.

METHODOLOGY

The chosen method was a quasi-experimental method, where a total of fourteen radiologists from Mid-Western area hospitals viewed two and three-dimensional head CT scans in order to compare the two types of scans and provide feedback on their application and affect on cognitive load. Radiologists were chosen because they are the physicians who most frequently view CT scans in a hospital; thus they can provide the most insight on the two imaging types. Since total or partial color blindness has a 1.3% prevalence in the US population and could result in limitations for viewing in three-dimensional based methods, radiologists were chosen that had no known visual problems such as color blindness (Sasani, 2017). Within the parameters of a quasi-experimental method, the data collection was split up into three sections: a pre-test, an intervention with two parts, and a post-test.

Pre-Test

In order to collect personal information and measure the prior medical imaging knowledge of each participant, the radiologists completed a pre-test that included nine practice questions for a CT registry exam. The pre-test was used to account for any outliers in experimental data that was due to differences in prior knowledge. The insight to create a pre-test was obtained from reading the results of a similar study that U.S. Army researcher Matthew Hackett conducted in 2013 (Hackett, 2013).

Intervention

The second part of the study was the intervention. A total of 4 head CT scans were chosen from the medical textbook *Brain CT Scans in Clinical Practice*. Head CT scans that were chosen had brain abnormalities where physical symptoms were not present until the condition is well underway. These types of conditions were chosen so that the time that it takes to decipher a scan has more relevance to the goal of the study. The selection of CT scans included a control scan

in which the brain was normal. All scans used were unidentified. Three different combinations of the four scans were created and radiologists were given one combination for two-dimensional viewing (intervention 1) and another combination for three-dimensional viewing (intervention 2). In intervention 1, the two-dimensional CT scans were printed on glossy paper, similar to that of a textbook, and filled an 8.5 by ll inch space. The scans were stapled together in a booklet to ensure that the radiologists viewed each scan one at a time.

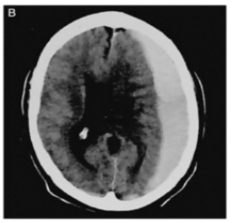

Figure 1. Acute subdural haematoma

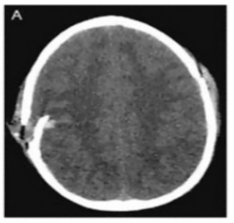

Figure 2. Depressed skull fracture

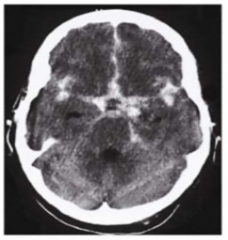

Figure 3. Subarachnoid hemorrhage

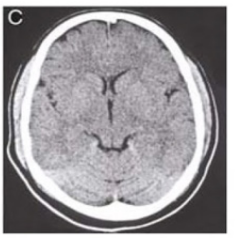

Figure 4. Normal

To display the CT scans in a three-dimensional manner, the scans were uploaded to an holography app called Holapex Hologram. Once the scans were uploaded to the app, the app configured the image in four different directions on the screen. Then, the external Holapex device was placed in the middle of the screen. The external Holapex device was created by taking a sheet of .5 cm plastic and cutting four trapezoids with 1 and 6 cm bases and 3.5 cm sides. The sides were hot glued together so that a 1x1cm and a 6x6 cm square were made from the openings. The narrower side was placed on the phone and the hologram was projected inside the external device. Radiologists could rotate the hologram to get a full view.

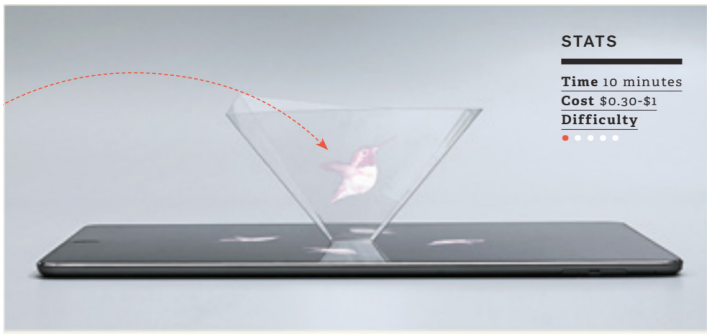

Figure 5. Holography Device

When viewing the three-dimensional scans, the radiologists were seated in a closed off, dimly lit room for a viewing experience that is similar to how radiologists view two-dimensional scans in the workplace. With a similar setting for visualization, more of the outlying variables can be controlled; thus, the two types of visualization can be more accurately compared. The time it took for each radiologist to accurately diagnose the four scans was recorded using a tenth of a second accuracy stopwatch.

Post-Test

The third part of the study was the post-test. The post-test was used to collect self-reported cognitive load analysis and to get radiologist's feedback on the two imaging types. This data was collected using two Likert scale evaluations from 1-5, and three open-ended questions. A Likert scale was used so that the feedback could be easily compared for evaluating cognitive load amongst the group of radiologist. Both quantitative (Likert scale) and qualitative (open-ended) data was collected. The information from the post-test fills another gap in the body of knowledge since there lacks information on how radiologists personally feel about two-dimensional and three-dimensional medical imaging devices.

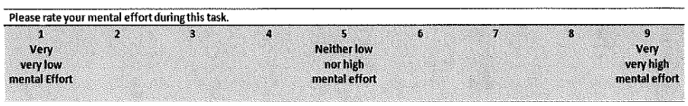

Figure 6. Nine-Point Rating Scale for Cognitive Load Measurement

RESULTS

Pre-Test Results

On the pre-test, there were background information questions about the institution at which participants have or will complete their residency; as shown in Figure 7, most of the participants have or will get their post-graduate schooling from the University of Louisville.

Figure 7. Residency Location

The pre-test also included nine practice questions for a CT registry exam. This part of the pre-test was used to measure the prior medical knowledge of each participant. Figure 8 is a graph of the number people who missed a certain number of questions. It can be seen that most people missed 3 out of the 9, which equates to a 66.67% and is passing. Another important takeaway from the pre-test is that all but one participant had been exposed to some form of 3-D CT scan in the past.

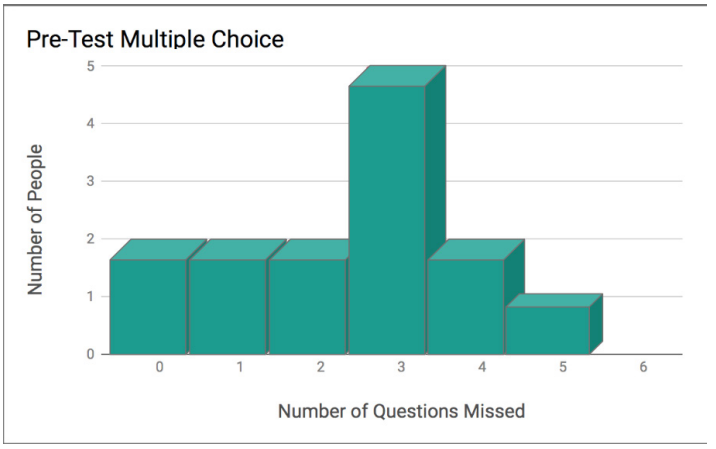

Figure 8. Pre-Test Multiple Choice

Intervention Results

Figure 9 is a graph of the comparison between the time that it took the radiologists to view the 3-D and 2-D CT scans in relation to their year of residency graduation. It varies year to year whether or not the 3-D CT scan took more or less time for diagnoses, but through a two-sample t-test, it was found that the difference in the diagnosing time was not significant at the .05 significance level. This suggests, that in terms of time, the two imaging types may have the same efficiency.

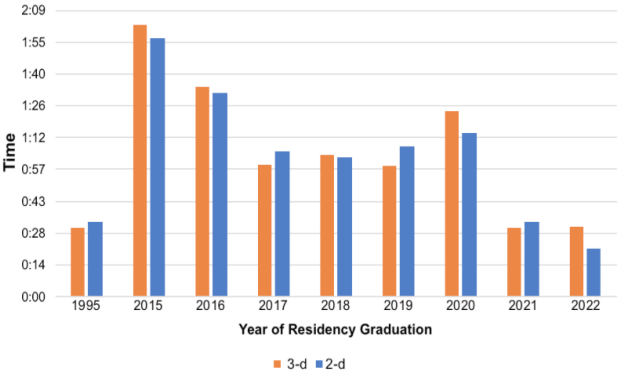

Figure 9. 3-D vs. 2-D Diagnosis Time

When the pre-test data is superimposed onto the 3-D vs. 2-D diagnosis time, shown in Figure 10, it is clearer what the trend of the graph represents. The radiologists that took less time to diagnose scans also missed fewer questions; it can be seen that those radiologists are nearing the end of their residency, thus they are in the midst of studying for various tests. Moving away from that population, the diagnosis time and the number of questions missed increases.

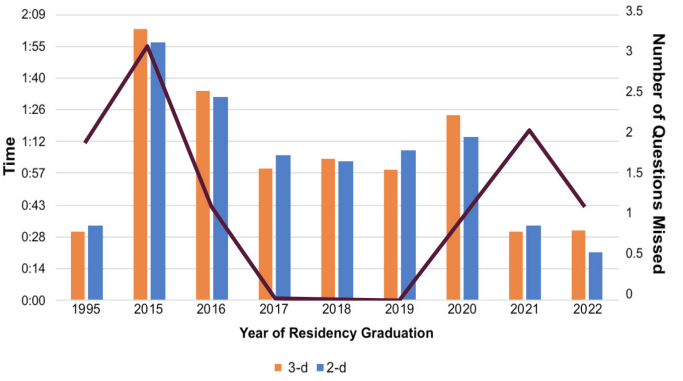

Figure 10. Intervention and Pre-Test Results Comparison

Post-Test Results

The responses to the Likert scale evaluations in the post-test resulted with an insignificant difference in the amount of cognitive load imposed when viewing the 3-D and 2-D scans. However, the answers to the open-ended questions in the post-test suggested the opposite. 86% of the 14 radiologists said they would choose the 3-D image device; however, 100% of the 86% included the qualifier "if" in their answer. Some of the most common responses were that the radiologists wished it had the same capabilities as the CT software that they currently use. They would also use the 3-D device if the hologram was larger, which is valid since the hologram was displayed from a cell-phone while radiologists usually view scans on a 20-inch screen.

The reason the radiologists had primarily given for choosing the 3-D CT scan over the 2-D scans was that it made it easier for them to visualize what was occurring. They could skip the step of mental translation and move straight ahead to diagnosing. The answers to the open-ended questions refute the initial findings from the Likert scale evaluations and help provide evidence for the new understanding.

CONCLUSION

Holograms presented a significant performance improvement over traditional 2-D scans. There are a number of possible reasons for this improvement. The first is the "wow-factor." Medical holograms are a novel technology, which may have garnered additional interest and focus from participants; this inherent curiosity with the technology may have caused them to study the material more intently.

The next possibility pertains back to the Cognitive Load Theory. The creation of such a mental image would be a direct process towards learning about the item. Since medical holograms have the advantage of imitating the 3-D structure of the human anatomy, holograms can be directly associated to what is occuring, while a 2-D image requires a 3-D mental translation within the working memory. Many anatomical structures are difficult to conceptualize, such as the spatial relationships between various blood vessels and valves. Medical holograms provide additional 3-D data to understand these relationships.

The cognitive load measurement showed trends which indicate that the medical holograms may result in decreased cognitive load. The benefits of decreased cognitive load include more efficient diagnosing outcomes and the ability to utilize the working memory for other cognitive tasks (Hackett, 2013). By freeing resources in the working memory, attention can be focused on other equally important items such as the next steps for the physician. While cognitive load is important, the post-test includes performance in its calculation, providing a more thorough metric for assessing utility. The post-test showed trends of high efficiency for the holograms. In this case, the medical holograms provided a mix of relatively low cognitive load with high performance.

The post-test results indicate that the overall user opinion of the holograms is very promising. All participants were able to see all the intended views and were able to easily use the holograms. No users reported any issues regarding eye strain or difficulty reading the scans. These early usability trends indicate that for radiologists who have or will complete their residency in Louisville or at an institution with the same curriculum as the University of Louisville may have a higher acceptance of the technology.

There were a few limitations with this project that should be accounted for in future directions. Although the doctors viewing the scans were limited to radiologists, there are specialties within radiology; radiologists that specialize in chest or abdominal CT scans may have taken longer to diagnose the scan. In the future, this could be accounted for by including a question in the pre-test. Although there was some variation in the places where the radiologists had received their residency, having more participants and increasing the variation would allow the conclusion to be further generalized. The last limitation and future direction is the device used. Taking the feedback from the radiologists and creating an app like the Holapex app that has the capabilities that CT software currently includes would increase usability. Also, displaying the scans on a larger tablet rather than a cell phone would allow for more optimal visualization.

Future work is necessary to validate the results of this early experiment, including a

larger sample size and additional medical image types rather than just head CT scans. Other technologies for visualizing CT scans in 3-D would serve as excellent comparison studies. Replicating this method but using another scan type, such as an MRI, would show the range of use for this 3-D visualization method. These additional experiments could be analyzed in relation to the cognitive load metrics gathered from self-reporting, ensuring both data sets indicate similar trends. Based upon the results, holograms showed improved diagnosing efficiency, possible reduced cognitive load during CT scan diagnosing, and warrants additional research to fully understand its potential benefits.

REFERENCES

Alhayki, Z., & Shah, Z. (2016). Use of Tangible Holograms in Education & Communication. International Journal of Research and Analytical Reviews, 3(1), 24-27.

Brain Aneurysm Statistics and Facts. (n.d.). Retrieved November 04, 2017, from https://www.bafound.org/about-brain-aneurysms/brain-aneurysm-basics/brain- aneurysm-statistics-and-facts/

Buhmann, J. M., Malik, J., & Perona, P. (1999). Image recognition: Visual grouping, recognition, and learning. Proceedings of the National Academy of Sciences, 96(25), 14203-14204.

Dalglish, B. (1994). Health for profit: the private sector is aching to get into medicine. Maclean's, 107(45), 44.

Doblas, A., Roche, E., Ampudia-Blasco, F. J., Martinez-Corral, M., Saverda, G., & Garcia- Sucerquia, J. (2016). Diabetes screening by telecentric digital holographic microscopy. Journal of Microscopy, 261(3), 285-290.

Farahani, N., Braun, A., Jutt, D., Huffman, T., Reder, N., Zheng, L., & ... Pantanowitz, L. (2017). Three-dimensional Imaging and Scanning: Current and Future Applications for Pathology. Journal Of Pathology Informatics, 81-10.

Hackett, M. (2013). Medical holography for basic anatomy training. Army Research Lab Orlando FL.

Igbaseimokumo, U. (2009). Brain CT Scans in Clinical Practice. Dordrecht, London: Springer. Matsuda, S., Yoshimura, H., Yoshida, H., Ryoke, T., Yoshida, T., Aikawa, N., & Sano, K. (2017). Usefulness of Computed Tomography Image Processing by OsiriX Software in Detecting Wooden and Bamboo Foreign Bodies. Biomed Research International, 1-5.

Muskingum University. (n.d.). Learning Strategies Database - Visual Imagery. Retrieved May 13, 2013 from http://www.muskingum.edu/-cal/database/general/encoding5.html.

Radiological Society of North America (RSNA) and American College of Radiology (ACR). (2016, June 8). Computed Tomography (CT) - Head. Retrieved November 15, 2017, from https://www.radiologyinfo.org/en/info.cfm?pg=headct

Rojas G.M., Gálvez M., Potler N.V., Craddock R.C., Margulies D.S., Castellanos F.X. Stereoscopic Three-Dimensional Visualization Applied to Multimodal Brain Images: Clinical Applications and a Functional Connectivity Atlas. Front Neurosci 2014; 8: 1-14.

Sakamoto, Y., & Aoki, Y. (2007). Autostereoscopic visualization of volume data using computer-generated holograms. Electronics & Communications in Japan, Part 3: Fundamental Electronic Science, 90(11), 31-39.

Sasani, H. et.al. (2017). Comparison of 3D Reformat Computed Tomography Images Using Different Softwares: Do They Have an Incremental Value in 3D Imaging?. Biomedical Research (0970-938X), 28(1), 379-384.

Sivakumar, G. P., Boovarahan, N. C. A., & Venkatesan, P. (2012). Display of real-world objects and videos on a mobile device using holographic projection. International Journal of Advanced Research in Computer Science and Electronics Engineering (IJARCSEE), 1(8), 67-71.

Smith, Z.J., Chu, K., Espenson, A.R., Gryshuk, A., Molinaro, M., Dwyre, D.M., Lane, S., Matthews, D., Wachsmann-Hogiu, S. (2011) Cell phone-based platform for biomedical device development and education applications. Plos ONE 6(3), 1-11.

Wampole, M. E., Kairys, J. C., Mitchell, E. P., Ankeny, M. L., Thakur, M. L., & Wickstrom, E. (2013). Consistent Surgeon Evaluations of Three-Dimensional Rendering of PET/CT Scans of the Abdomen of a Patient with a Ductal Pancreatic Mass. Plos ONE, 8(9), 1-9.

Glucose-Lowering Effect of a Novel Insulin (SCI-1) and its Thermal Stability Compared to Insulin Lispro (Humalog)

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ABSTRACT

Insulin is a hormone that is secreted by the beta cells of the pancreas. The hormone is essential for survival and controls blood glucose levels. Insulin consists of two chains, A and B chains, which are connected by disulfide bonds. Lispro insulin (Humalog) is a fast-acting, two-chain insulin that is clinically prescribed for patients. Single Chain Insulin connects the A chain and B chains together by adding an amino acid linker.

In preliminary experiments, Single Chain Insulin-1 (SCI-1) was shown to be active in lowering blood glucose and, unlike Lispro insulin, it exhibited thermal stability when heated above 45°C. The focus of this study is to compare glucose-lowering effects of Lispro insulin and SCI-1. In addition, the thermal stability of Lispro insulin and SCI-1 will be compared at higher temperatures.

Streptozocin-induced diabetic rats were injected subcutaneously with Lispro insulin or SCI-1. The tip of the tail was snipped with a sharp razor blade to obtain blood samples. During the first hour, blood glucose was measured at ten minute intervals using a standard glucometer. Subsequent measurements were made less frequently for the 5-6 hour duration of the experiment. The data obtained from the experiment were then graphed and analyzed.

In studies discussed below, SCI-1 had a more prolonged glucose-lowering effect than Lispro insulin. SCI-1 was found to be a biphasic insulin, hich is fast-acting in the first stage and long-acting in the second. Compared to Lispro insulin, SCI-1 showed more heat stability. SCI-1, heated for 110 hours at 75°C, retained most of its activity; in contrast, the glucose-lowering effect of Lispro insulin was decreased by approximately 50% when heated for only 5 hours at 75°C.

In conclusion, SCI-1 appears to function in a biphasic manner and is much more heat stable in comparison to Lispro insulin. Thermal stability of insulin may be of great importance during its shipping and storage.

INTRODUCTION

Diabetes is a disease commonly referred to as a state with high blood glucose levels. There are two types of diabetes: type 1 and type 2. Type 1 diabetes is an autoimmune disorder where the body produces little or no insulin. It occurs when autoantibodies attack the pancreatic beta cells that synthesize insulin; it is most often diagnosed at a juvenile age. Type 2 diabetes is a chronic condition where the body develops resistance to insulin. This type of diabetes is generally diagnosed in adults and is commonly genetic but is also heavily influenced by environmental factors, such as obesity and lack of exercise (2007). Obesity results in a greater resistance to insulin, and when the body cannot make the sufficient amount of insulin needed to maintain normal glucose levels, blood glucose levels rises.

Insulin is a hormone produced by the pancreas and helps regulate blood glucose levels. It is released by the beta cells of the pancreatic islets of Langerhans in response to a rise in blood glucose, which may occur during eating a meal (especially containing carbohydrates). Insulin works by activating GLUT4 transporters in cells in responsive tissues; this stimulates the uptake of glucose from the blood which in turn decreases blood glucose levels (2009).

Active insulin consists of two chains, the A chain and the B chains that are linked together by disulfide bonds. The A chain is comprised of 20 amino acids whereas the B chain consists of 31, making a total of 51 amino acids as seen in Figure 1 (Weiss, 2014). Insulin is stored as hexamers in the beta cells. Insulin hexamers are a unit of six

insulin molecules and two zinc atoms.

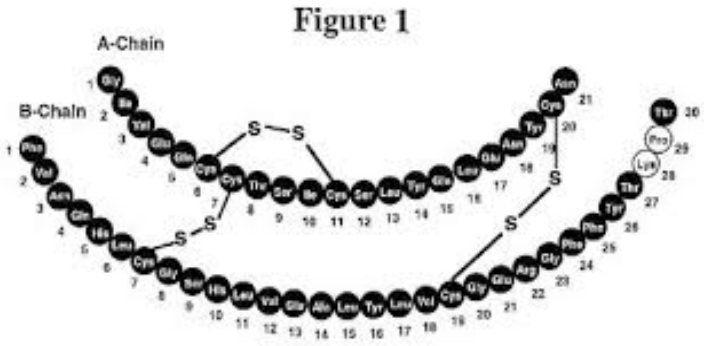

Figure 1. Amino acid makeup of mature human insulin

Lispro insulin (Humalog) is a fast-acting insulin that is clinically used to treat people with diabetes. It is a synthetic insulin that acts in a similar fashion as human insulin. Lispro insulin is made by switching the amino acids in the 28th and 29th position on the B chain of human insulin (2005). The advantage of Lispro insulin is that the amino acid alteration makes the hexamer less stable and the released insulin rapidly enters the blood plasma phase.

Precursor insulin, also called proinsulin, is the immature form of insulin that is made by the pancreas. In proinsulin, the A chain and B chains of proinsulin are connected by a C-peptide, consisting of 35 amino acids, which is later cut off by enzymes in beta-cells to form active insulin (Figure 1). The amount of C-peptide can show how much insulin is being produced (Vezzosi, Bennet, Fauvel, & Caron, 2007). Previous studies have shown that proinsulin, while less active than mature insulin, is more stable when heated at higher temperatures.

Single chain insulins synthesized in the laboratory connect the A chain and the B chain by adding six to eight amino acids as linkers instead of the normal C-chain as seen in Figure 2. Single Chain Insulin-1 (SCI-1) is an insulin that has a 6-amino acid C-peptide (amino acids Glu, Glu, Gly, Pro, Arg, and Arg). In preliminary experiments, SCI-1 was active in lowering blood glucose. In addition, similar to other SCI insulins tested in the lab, it was found to exhibit stability when heated at 45 degrees Celsius (Liu, Ramos-Castañeda, & Arvan, 2003).

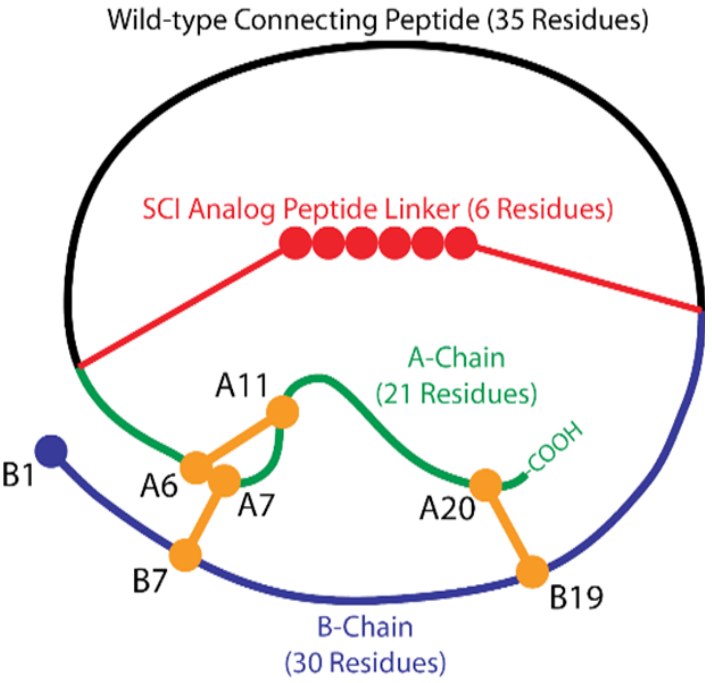

Figure 2. Diagram of proinsulin, mature insulin, and SCI. SCI-1 contains the following sequence in the connecting peptide (Glutamic acid, Glutamic acid, Glycine, Proline, Arginine, and Arginine).

The focus of this research is to examine the glucose-lowering effects of injecting SCI-1 subcutaneously compared to Lispro insulin. In addition, the experiment will verify whether SCI-1 shows thermal stability. The issue of thermal stability of insulin is of importance in its shipping and storage. The issue has recently been highlighted due to a publication showing that some insulin vials purchased in the United States and Europe may be less active that the required 100 units per ml, perhaps due loss of activity during shipping and storage (Carter & Heinemann, 2017).

METHODOLOGY

Diabetic male Lewis rats were used for the course of these experiments. Diabetes was induced by administering streptozocin. Streptozocin (STZ) is a chemical that is used to induce diabetes in rats by giving them an intraperitoneal injection of the substance. STZ works by killing the pancreatic beta cells that produce insulin, in turn making the rats diabetic. After the STZ injection, each rat is kept in quarantine for 72 hours to confirm that they are indeed diabetic by checking their blood glucose

using a standard patient glucometer. Rats were used after three weeks of rest.

The insulins tested were SCI-1 and Lispro insulin which were injected in equal amounts (moles) subcutaneously; Lispro insulin was used as a control. The subcutaneous injections were made in the excess skin along the back of the neck. To measure the rat's blood glucose, the tip of their tail was snipped using a sharp razor at the beginning of the experiment to obtain small blood samples (10-20 microliters each time). Clinical glucometers were used to measure blood glucose levels. For the first hour of each experiment, blood glucose measurements were taken every ten minutes. Then for the second hour and third hour, they were taken every 20 minutes, and every 30 min for the fourth hour. Finally, samples were taken once every hour for the remaining duration of the experiment.

The thermal stability of the insulins was tested in a 75°C incubator. The insulins tested, SCI-1 and Lispro insulin, were placed in glass vials and were lightly agitated for the indicated duration of time. They were then subcutaneously injected into diabetic rats to measure the effect of heating the insulin on its glucose-lowering effect.

RESULTS

SCI-1 and Lispro, were injected subcutaneously at 0.5 Units/300g rat, and a similar volume of diluent was used as a control. Subcutaneous Injections of heated lispro and heated SCI-1 were given at 0.5 Units/300g rat.

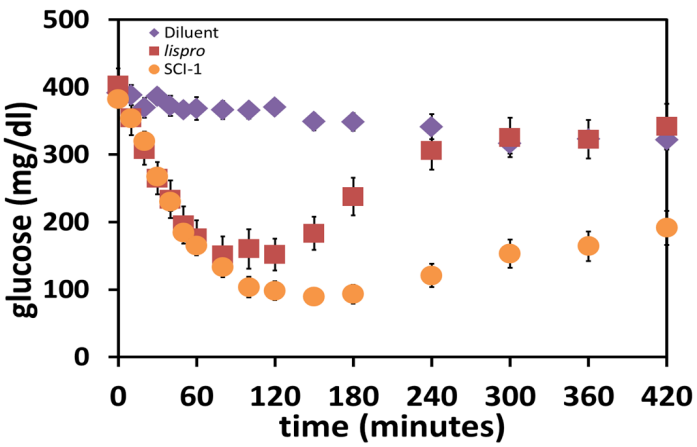

Figure 3. Glucose-Lowering Effect of SCI-1 vs. Lispro vs. Diluent

Figure 3 shows the effects of SCI-1, Lispro insulin, and diluent on blood glucose levels of diabetic rats. For the first hour after the subcutaneous injection, SCI-1 lowers blood glucose similarly to Lispro insulin. The second hour is where the effect of Lispro insulin decreases and blood glucose levels rise towards baseline. However, SCI-1 remains active for the remainder of the experiment and is very effective in decreasing blood glucose levels for an extended period of time. Hence, SCI-1 was shown to be a biphasic insulin where in the first stage was fast-acting and in the second it was long-acting.

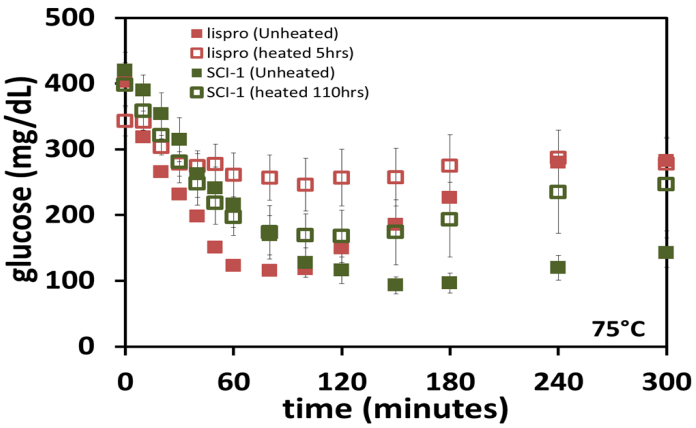

Figure 4. Heated and Unheated Lispro Insulin vs. Heated and Unheated SCI-1

Figure 4 shows the thermal stability of SCI-1 and Lispro insulin by displaying the glucose-lowering effect of each insulin when agitated at 75°C for various periods of time. According to the figure, Lispro insulin lost approximately 50% of its activity after exposure to 75°C for 5 hours. In marked contrast, SCI-1 retained most of its activity after more than four and a half days of exposure to 75°C.

CONCLUSION

The experimental data suggests SCI-1 has a more prolonged glucose-lowering effect than Lispro insulin. As seen in Figure 3, SCI-1 and Lispro insulin lower blood glucose at almost the same rate for the first hour. After the first hour, Lispro insulin's activity starts to dwindle and blood glucose levels rise. SCI-1 activity, however, remains for the rest of the experiment. SCI-1 insulin appears to act in a biphasic manner with

an action profile that is similar to mixed insulin formulations such as 70/30 or 75/25 insulins used by patients.

SCI-1 also appears to be significantly more heat stable than Lispro insulin, as seen in Figure 4. SCI-1 heated for 110 hours at 75°C still retains much of its glucose-lowering effect compared to when it was unheated. However, when Lispro insulin was heated for only 5 hours at 75°C, it lost approximately half of its activity. The decrease in its glucose-lowering effect is thought to be due to the unstable nature of two-chain insulins. In experiments not shown, SCI-1 heated and agitated at 55°C for more than 30 days remained 100% active. The thermal stability of SCI-1 may prove to be of clinical importance.

Further studies are needed to determine the mechanism for SCI-1 being long-acting and having a significant impact on lowering blood glucose. The prolonged actions of SCI-1 could be due to it being injected subcutaneously and the insulin getting trapped in the layers of the skin or because the signaling to the insulin receptors is more persistent. The mechanism is not yet known, but with further research, it could be determined. In addition, future studies could create an insulin with even more thermal stability and a longer shelf life to make it more efficient to ship and store.

ACKNOWLEDGEMENTS

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REFERENCES

Prediabetes & Insulin Resistance. (2009, August 01). Retrieved December 17, 2017, from https://www.niddk.nih.gov/health-information/diabetes/overview/what-is-diabetes/prediabetes-insulin-resistance

Weiss, M. (2014, February 01). Insulin Biosynthesis, Secretion, Structure, and Structure-Activity Relationships. Retrieved December 17, 2017, from https://www.ncbi.nlm.nih.gov/books/NBK279029/

Types of Diabetes. (2007). Retrieved December 17, 2017, from https://dtc.ucsf.edu/types-of-diabetes/

Insulin Lispro. (2005). Retrieved December 17, 2017, from https://www.drugbank.ca/drugs/DB00046

Vezzosi, D., Bennet, A., Fauvel, J., Caron, P. Insulin, C-peptide and proinsulin for the biochemical diagnosis of hypoglycemia related to endogenous hyperinsulinism. Eur J Endocrinol 2007;157(1):75-83

Liu, M., Ramos-Castañeda, J., & Arvan, P. (2003, April 25). Ming Liu. Retrieved December 17, 2017, from http://www.jbc.org/content/278/17/14798.full

Carter, A.W., and Heinemann, L. J. Insulin concentration in Vials Randomly Purchased in Pharmacies in the United States: Considerable Loss in the Cold Supply Chain Diabetes Science and Technology, 1-3, 2017.

The Effects of Time and Temperature on the Amount of Nicotine Inhaled from E-Cigarette Usage

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ABSTRACT

The purpose of this year's experiment was to investigate the relationship between time and temperature and the concentration of vaporized nicotine. It was hypothesized that the longer the time allowed, and the higher the temperature, the more nicotine vaporized.

A TA Instruments Q1000 Differential Scanning Calorimetry (DSC) with High Pressure Cell and vacuum pump was used was used to measure vapor pressures. An Agilent 6890N Network Gas Chromatograph and Agilent 7694 Headspace Sampler was used to determine the vapor phase compositions of the 50/50 glycerol/1, 2-propanediol mixtures with 0%, 1.2%, and 2.4% nicotine.

Nicotine concentration in the vapor phase is much higher than its concentration in the liquid phase in the studied temperature range due to its higher vapor pressure compared to 1, 2-Propanediol. Nicotine concentration in the vapor phase decreases with the increase of temperature. The heats of vaporization were found to be 52.7 kJ/mol for nicotine and 58.0 kJ/mol for 1, 2-Propanediol. The activation energies were found to be 74.1kJ/mol for nicotine and 66.0 kJ/mol for 1, 2-Propanediol. Positive heats of vaporization indicate endothermic processes of vaporization (energy absorbed).

INTRODUCTION

Before the advent of advanced scientific technologies capable of analyzing substances in detail, tobacco-smoking was touted as a cure for 36 different health problems ("History of Tobacco," n.d.). However, as more research was conducted, it was found that nicotine, the main addictive substance in tobacco, is a highly toxic poison. In its pure form, nicotine is a clear liquid with a distinguishable odor, and upon application, it can cause burning sensations, irritation, vomiting; in higher doses, it can cause increased blood viscosity, convulsions, lung cancer, and ultimately death (Mishra et al, 2015). Thus, it should come as no surprise that various ways to help people become free of their nicotine addictions emerged. One of these ways is the electronic cigarette, commonly called e-cigarette. E-cigarettes work by heating e-liquid into a vapor that is inhaled by the user. Some benefits e-cigarettes have over traditional cigarettes include emitting no secondhand smoke, not staining the teeth, and usually being allowed in non-smoking areas. E-cigarettes are also notable because e-liquid can contain varying amounts of nicotine, or even none at all, depending on user preference. Therefore, e-cigarettes can potentially be used to wean the user off of nicotine over time ("E-Cig vs Tobacco," n.d.).

The effects of glycerol ($C_3H_8O_3$) on the flash point and vapor pressure of propylene glycol ($C_3H_8O_2$) were investigated in a previous study. It was concluded that increased levels of glycerol increased the flash point and lowered the vapor pressure of propylene glycol. While glycerol and propylene glycol are extremely important and make up roughly 95% of e-liquid, they mainly serve as carriers, making up the "cloud" of vapor that is inhaled ("E-Cig vs Tobacco," n.d.). The concentration of glycerol and propylene glycol do

not reveal information about the concentration of the addictive agent found in most e-liquid: nicotine. This study focused on the nicotine in the e-liquid. For example, just because the e-liquid is advertised as containing 1% nicotine doesn't necessarily mean that the user actually inhales a nicotine concentration of 1% from the vapor every time. The actual concentration of nicotine inhaled each smoke could depend on a wide variety of factors, including but not limited to the marketed concentration of nicotine in the e-liquid, the other components of the e-liquid such as glycerol or propylene glycol, the type of e-cigarette that is used to smoke the e-liquid, and more. The purpose of this research is to determine the effects of time and temperature on the amount of nicotine vaporized in a nicotine solution. In essence, this is the concentration of nicotine an e-cigarette user would be likely to actually inhale.

It was hypothesized that the longer the time allowed and the higher the temperature, the more nicotine would be inhaled. Longer duration of burning and higher temperatures would hypothetically allow for more e-liquid to burn, which is expected to result in more vaporized nicotine. The information obtained from this research would benefit individuals who are trying to quit their addiction to nicotine through the use of e-cigarettes, and want to know how much nicotine they are actually smoking.

METHODOLOGY

Materials

The materials used were 99+% glycerol purchased from ACROS, 99% 1, 2-Propanediol (propylene glycol) purchased from ALDRICH, and pure nicotine from e-cigs.com.

Sample Preparation

Three samples were created in total. The samples all consisted of 50% propylene glycol and 50% glycerol and various concentrations of nicotine. The 50/50 glycerol/1, 2-propylene glycol mixture was created according to Chart A. Chart B shows the composition of the samples.

50/50 Gly/1,2-PG Mixture Preparation			
	Target Mass (grams)	Actual Mass (grams)	Conc.
Glycerin	80	79.99	50.000%
1,2-PG	80	79.991	50.000%

Chart A: 50/50 Glycerol/1, 2-Propylene Glycol Mixture Preparation

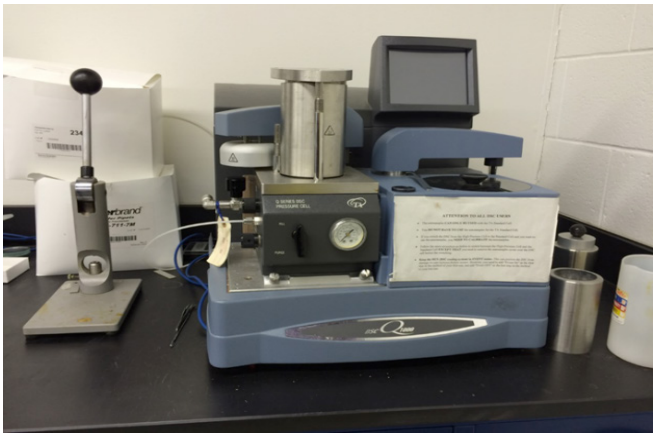
		Target Mass (grams)			Actual Mass (grams)		
Nicotine Concentration		Total		Nicotine	Mixture	Nicotine	Total	Conc.
0.0%	0 mg / 1000 mg	50		0.0	50.0	0	50.0000	0.00%
1.2%	12 mg / 1000 mg	50		0.6	49.4	0.6061	50.0091	1.21%
2.4%	24 mg / 1000 mg	50		1.2	48.8	1.2155	50.0081	2.43%

Chart B: Nicotine Composition of Samples

A Semi-Micro Balance and a pipette were used to attain maximum accuracy in the creation of all samples. The samples were mixed using the SpeedMixer DAC 150 FVZ at 3000 rotations per minute for 1 minute.

Differential Scanning Calorimetry (DSC) Tests

To prepare the samples for the vapor pressure tests, a TA Instruments Q1000 Differential Scanning Calorimetry (DSC) equipped with High Pressure Cell was used. First, a hermetic pan and a hermetic lid with a laser-cut pin-hole were balanced using a Semi-Micro Balance. The resulting mass was zeroed, and the pan was removed from the balance with tweezers. A fine-tip pipette was used to apply a drop of the 0% nicotine sample to the pan, and the mass of the sample was balanced and recorded in the computer attached to the DSC. The tweezers were used to place the hermetic lid on top of the pan, and the two were sealed together using a pan sealer. Then, the sample was placed in the DSC in front of the reference pan, and the securing machine parts were fixed into place. The compressed air cylinder was opened. Then, using the computer, the pressurizing was started. When the desired pressure was reached, the DSC was sealed to maintain the pressure, and the computer was used to start the test. This process was repeated for the two remaining samples.

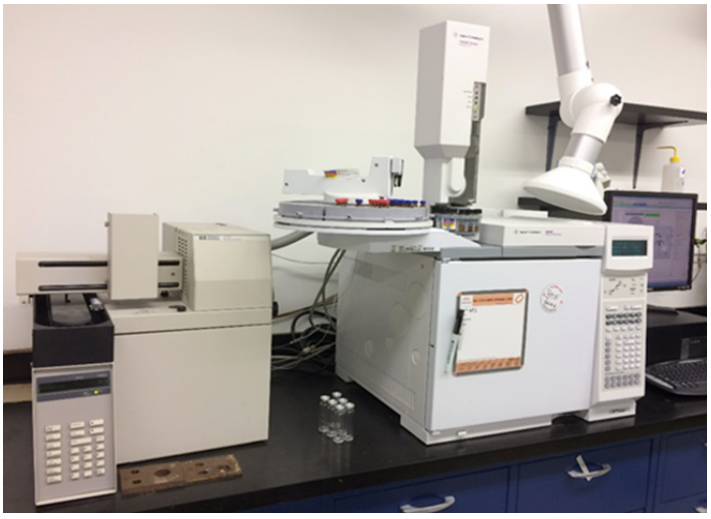


TA Instruments Q1000 Differential Scanning Calorimetry equipped with High Pressure Cell

Each sample was tested at 5 different pressures: high pressure 1, high pressure 2, atmospheric pressure, low pressure 1, and low pressure 2. To achieve extremely high pressures, the High Pressure Cell was used, and to achieve extremely low pressures, a vacuum pump was used.

Gas Chromatography Tests

First, the samples for the gas chromatography (GC) tests were created. An adjustable pipette was used to transfer 1 mL of the 0% nicotine sample from the sample jar into a headspace vial. The cap was put onto the vial and sealed, and then the vial was labeled. 25 vials were made for each sample. This was repeated for the remaining two samples.



Agilent 6890N Network Gas Chromatograph and Agilent 7694 Headspace Sampler

The gas chromatography tests were conducted using an Agilent 6890N Network Gas Chromatograph and Agilent 7694 Headspace Sampler. First, the compressed air, hydrogen, and helium cylinders were opened. Then, the appropriate switches were flipped on to connect the gases to the instruments. The Agilent Technologies 6890N online software was opened to manipulate the machinery. The GC was set up, and the flame ignition was turned on and ignited with a cigarette lighter. Then the GC headspace was set up, the parameters transmitted to the machine, and allowed to equilibrate so that the desired temperature was reached. When this was accomplished, 4 vials were placed in the headspace slots. The method was set up, and the test was started. This was done for all of the samples. The GC parameters are shown below.

Headspace	Agilent 7694 Headspace Sampler		
Oven Temp	100~160 °C	Loop Temp	180 °C
Equil. Time	0.5 ~ 20 min	Agitation	High
Pressurization	0.50 min	Vial Pressure	52 kPa
Loop Fill Time	0.10 min	Loop Equil.	0.05 min
Injection Time	0.20 min	Transfer Line Temp	200 °C

Chart C. Headspace – Gas Chromatograph Parameters 1

GC	Agilent 6890 GC		
Inlet Temp	250 °C	Split Ratio	100 : 1
Column Flow	5.0 ml/min	Flow Mode	Const. Flow
Column	HP-1	30m x 530µm x 0.88µm	
Oven Temp	60°C to 260°C @ 20°C/min; Hold 1min.		

Chart D. Headspace – Gas Chromatograph Parameters 2

Detector	FID
Detector Temp	250 °C
H2 Flow	45 ml/min
Air Flow	400 ml/min
Makeup	He
Const. MU Flow	45 ml/min

Chart E. Headspace – Gas Chromatograph Parameters 3

DATA ANALYSIS

The Antoine equation was used to describe the temperature-dependent vapor pressures. The Differential Scanning Calorimetry was used to obtain the parameters for the Antoine equation, which allowed the Antoine Equation to be used to determine values for conditions that were not tested.

The Ideal Gas Law was used to estimate the mass of the sample in the headspace, which was in turn used to calculate the response factor. The response factor is the ratio of the mass in headspace to the area of the signals from the Gas Chromatograph. The change of vaporized mass with time was used to calculate the vaporization rate, which was then used to calculate the activation energy (the minimum amount of energy that the reactant must possess in order to undergo a specified reaction) through the Arrhenius equation. The Clausius-Clapeyron equation was used calculate the heats of vaporization from the vapor pressures at equilibrium state.

RESULTS

Compound	Formular	Molar Mass	Boiling Point	Antoine Equation Parameters from DSC *			Vapor Pressure @140°C	Mass in HeadSpace @140°C	GC Peak Area	Response Factor
		g/mol	°C	A	B	C	Pa	µg	pA·s	µg/(pA·s)
1,2-Propanediol	$C_3H_8O_2$	76.1	188	5.51	-844	51	12175	5125	17365	0.295
Glycerol	$C_3H_8O_3$	92.1	290	9.99	-4974	326	209	107	229	0.466
Nicotine	$C_{10}H_{14}N_2$	162.2	247	6.83	-4152	620	23491	21080	25587	0.824

* Antoine Equation parameters for 1, 2-Propanediol and Glycerol were adapted from a previous study. The parameters for Nicotine were obtained from this study.

Table 1. Response Factor for GC Measurement

In Table 1, the response factors (the ratio between the produced signals and the quantity of signal-producing analytes) were used to calculate the peak areas of propylene glycol, glycerol, and nicotine.

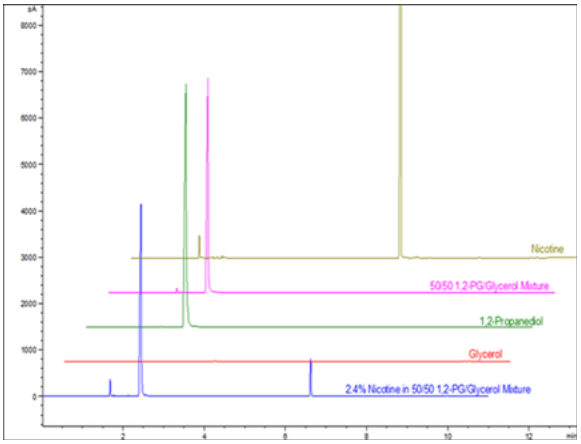


Figure 2. Headspace – Gas Chromatograph Measurement

Notice the strong signals from propylene glycol and nicotine, and the weak signals from glycerol in Figure 2. Glycerol did not play a large role in this research, and was included because it was used last year, and also because its weak signals offset the strong ones of propylene glycol and nicotine.

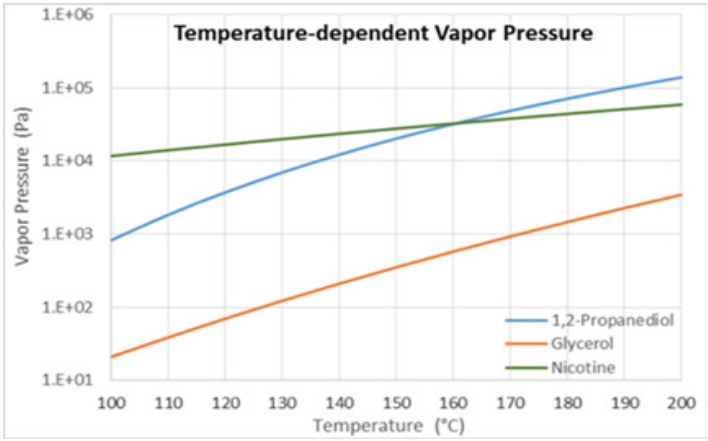


Figure 3. Temperature-dependent Vapor Pressure

As shown by Figure 3, nicotine has a higher vapor pressure than 1, 2-propanediol at temperatures below about 160°C, although it has a higher boiling temperature than 1, 2-propanediol. It can also be seen that glycerol has a much lower vapor pressure than 1, 2-propanediol and nicotine.

1.2% Nicotine in Liquid	Nicotine Concentration (wt.%) in Vapor Phase				
Temp. (°C) \ Time (min)	0.5	1.0	2.0	5.0	20.0
100	19.1%	15.4%	15.1%	16.7%	18.0%
120	23.6%	18.8%	19.0%	19.1%	17.7%
140	21.3%	16.8%	17.3%	15.6%	15.0%
150	18.4%	15.6%	15.9%	14.7%	14.5%
160	17.0%	14.5%	15.0%	14.2%	14.4%

Table 2. Effects of Temperature and Time on Nicotine Concentration in Vapor Phase vs. Liquid Phase (1.2% Nicotine)

2.4% Nicotine in Liquid	Nicotine Concentration (wt.%) in Vapor Phase				
Temp. (°C) \ Time (min)	0.5	1.0	2.0	5.0	20.0
100	32.9%	28.5%	31.1%	34.4%	34.5%
120	46.3%	38.5%	34.5%	36.7%	33.1%
140	44.3%	33.3%	33.3%	30.6%	29.6%
150	39.1%	31.0%	31.9%	28.5%	28.5%
160	35.0%	28.4%	29.1%	28.3%	29.3%

Table 3. Effects of Temperature and Time on Nicotine Concentration in Vapor Phase vs. Liquid Phase (2.4% Nicotine)

Tables 2 and 3 show that the concentration of nicotine in the vapor phase is much higher than its concentration in the liquid phase in the studied temperature range. Nicotine concentration in the vapor phase decreases with the increase of temperature, and does not vary much with time except at very short times.

Figure 8. Effects of Temperature and Time on Sample Color Change: 0% Nicotine in 50/50 PG/Glycerol at 100, 120, 140, and 160°C

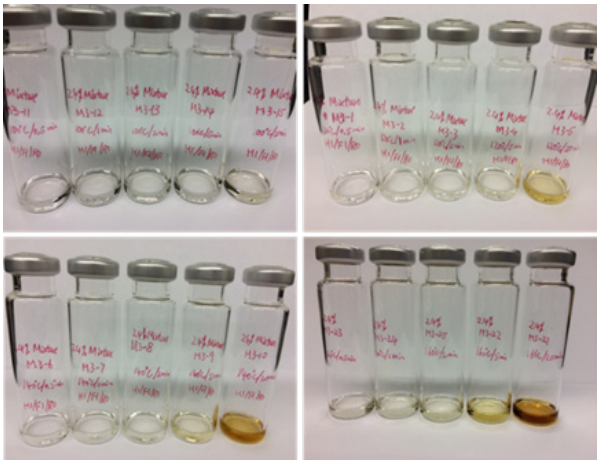


Figure 9. Effects of Temperature and Time on Sample Color Change: 2.4% Nicotine in 50/50 PG/Glycerol at 100, 120, 140, and 160°C

The color change observed in Figures 8 and 9 show the oxidation of nicotine at high temperatures and nicotine concentrations.

	Nicotine Conc. in Liquid	Activation Energy kJ/mol	
Nicotine	1.2%	74.1	70.1 ± 5.7
	2.4%	66.0	
1,2-PG	0.0%	66.1	66.3 ± 0.6
	1.2%	66.9	
	2.4%	65.8	

Table 10. Nicotine Concentration and Activation Energy

	Nicotine Conc. in Liquid	Heat of Vaporization (kJ/mol)		
		This Work	Average	Literature *
Nicotine	1.2%	52.4	52.7 ± 0.5	53.3
	2.4%	53.1		
1,2-PG	0.0%	58.4	58.0 ± 0.5	58.6
	1.2%	58.0		
	2.4%	57.4		

Table 11. Nicotine Concentration and Heat of Vaporization

Table 10 shows the activation energies of various samples used in this research. According to Table 11, positive heats of vaporization indicate endothermic processes of vaporization. The heat of evaporation for nicotine was found to be 52.7 ± 0.5 kJ/mol, which is independent of the nicotine concentration in e-liquid. The heat of evaporation for propylene glycol was found to be 58.0 ± 0.5 kJ/mol. The activation energy for nicotine evaporation was 70.1 ± 5.7 kJ/mol, and the activation energy for propylene glycol was 66.3 ±

0.6 kJ/mol. Data from this work match data from literature very well.

DISCUSSION

The purpose of this research was to determine the effects of time and temperature on the actual amount of nicotine inhaled from e-cigarette usage. This is important because the advertised nicotine concentration might not be the actual nicotine concentration inhaled due to nicotine's chemical properties. It was hypothesized that the more time allowed and the higher the temperature, the more nicotine would be inhaled. The hypothesis was fully supported by the data obtained.

In addition, it was found that at first, the inhaled nicotine concentration would be much higher than the nicotine concentration in e-liquid due to the high vapor pressure of nicotine compared to propylene glycol and glycerol in the studied temperature range. It was also found that nicotine is not stable and might be oxidized at higher temperatures and longer times. It is worth noting that e-liquid includes various impurities (Herrington et al., 2017), which may have affected these results.

In the future, these impurities could be analyzed to determine their effect on nicotine's evaporation rate. Also, since e-liquid comes in various advertised flavors, some of these flavored e-liquids could be experimented with to determine the effects of flavoring, if any, on nicotine evaporation rate.

REFERENCES

Behar, R. Z., Hua, M., Talbot, P. (2015). Puffing Topography and Nicotine Intake of Electronic Cigarette Users. PLOS One. Retrieved from https://doi.org/10.1371/journal.pone.0117222

Bond, M. (2000). Non-Equilibrium Evaporation and Condensation modeled with Irreversible Thermodynamics, Kinetic Theory, and Statistical Rate Theory (Master's thesis). Retrieved from University of Victoria Libraries.

Boudart, M. (1961). RATES OF EVAPORATION OF LIQUIDS. Retrieved from http://www.dtic.mil/dtic/tr/fulltext/u2/255155.pdf

Chickos, J.S. & Acree, W.E. Jr. (2003). Enthalpies of Vaporization of Organic and Organometallic Compounds, 1880–2002. Journal of Physical and Chemical Reference Data, 32(2), 519-878. doi: 10.1063/1.1475333

Chromatography Research and Technology Manager PerkinElmer, Inc. (n.d.). AN INTRODUCTION TO HEADSPACE SAMPLING IN HAS CHROMATOGRAPHY FUNDAMENTALS AND THEORY. Waltham, Massachusetts: PerkinElmer, Inc.

E-Cig vs Tobacco Cigarette FAQ. Retrieved from http://www.vapertrain.com/page/ecvstc

Farsalinos, K.F., Romagna, G., Tsiapras, D., Kyrzopoulos, S., Voudris, V. (2013). Evaluation of Electronic Cigarette Use (Vaping) Topography and Estimation of Liquid Consumption: Implications for Research Protocol Standards Definition and for Public Health Authorities' Regulation. Int J Environ Res Public Health, 10(6), 2500-2514. doi: 10.3390/ijerph10062500.

Forster, M., Liu, C., Duke, M.G., McAdam, K.G., Proctor, C.J. (2015). An experimental method to study emissions from heated tobacco between 100-200°C. Chemistry Central Journal, 9(20). doi: 10.1186/s13065-015-0096-1

Herrington, J.S., Myers, C. & Rigdon, A. (2017). Analysis of Nicotine and Impurities in Electronic Cigarette Solutions and Vapor. Retrieved from http://www.restek.com/Technical-Resources/Technical-Library/Foods-Flavors-Fragrances/fff_FFAN2127-UNV

Lipkind,D., Hanshaw, W., Chickos, J.S. (2009). Hypothetical Thermodynamic Properties. Subcooled Vaporization Enthalpies and Vapor Pressures of Polyaromatic Heterocycles and Related Compounds. J. Chem. Eng. Data, 54(10), pp 2930–2943. doi: 10.1021/je900034d

Mishra, A. et al. (2015). "Harmful Effects of Nicotine." Indian Journal of Medical and Paediatric Oncology, 36(1), 24-31. DOI: 10.4103/0971-5851.151771

Sandy, C., Winsor, H., Armitage, R., Garside, A. (2015). QUALITATIVE ANALYSIS OF E-CIGARETTE LIQUIDS USING GAS CHROMATOGRAPHY/MASS SPECTROMETRY. EU: Agilent Technologies, Inc.

Sleiman, M., Logue, J. M., Montesinos, V.N., Russell, M.L., Litter, M.I., Gundel, L.A. & Destaillats, H. (2016). Emissions from Electronic Cigarettes: Key Parameters Affecting the Release of Harmful Chemicals. Environmental Science & Technology, 50(17), pp. 9644-9651. doi: 10.1021/acs.est.6b01741

St. Charles, F.K., Moldoveanu, S.C. (2016). The Activity and Enthalpy of Vaporization of Nicotine from Tobacco at Moderate Temperatures. Beiträge zur Tabakforschung International/Contributions to Tobacco Research, 27(1). doi: 10.1515/cttr-2016-0003

Thomas Harriot (1560-1621). (1995). Retrieved from http://galileo.rice.edu/sci/harriot.html

University of Dayton. History of Tobacco. Retrieved From http://academic.udayton.edu/health/syllabi/tobacco/history.htm

Vaping News and Lifestyle Blog. (2016, December 1). How to choose e-liquid nicotine levels [Blog post]. Retrieved from https://www.vapourlabs.co.uk/blog/how-to-choose-e-liquid-nicotine-levels.htm

Engineering a Novel Detection System to Prevent Vehicular Heat Stroke

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ABSTRACT

Vehicular heat stroke kills an average of 37 children every year. Technologies to prevent hot car death exist, but are not reliable. The goal of this project is to create a vehicular heat stroke prevention device that is fully operative and accurate. The main components of this device include an Arduino UNO R3, an Arduino GSM Shield 2, a homemade pressure plate, and a TMP36 temperature sensor. The pressure plate determines if a child is present. The TMP36 calculates the ambient temperature. The GSM Shield was activated, and the previous coding was integrated to send an SMS message if pressure was detected and the temperature was greater than 27 °C. The device was tested against the established criteria. It sent an alert 100% of the time when both criteria were met. The device costs approximately 47.5% less than other systems, making this device a reliable and economical device to prevent vehicular heat stroke.

Keywords: vehicular heat stroke, hot car death, Arduino, TMP36, pressure plate

INTRODUCTION

Vehicular heatstroke, also known as hot car death, has taken the lives of over 700 children since 1998 and will continue to take the lives of an average of 37 children every year ("Heatstroke Deaths of Children in Vehicles," n.d.). In 2017, 42 children died because they were left in a hot car. The most recent death was dated as October 31st, a month when the temperature outside is traditionally not high. This demonstrates that vehicular heat stroke can still occur regardless of ambient temperatures; a study by the American Academy of Pediatrics discovered that the temperature inside a car increases by an average of 3.2 degrees Fahrenheit every 5 minutes (McLaren, 2005). Over half of the deaths were children under the age of two, and the majority of the deaths occurred because the child was forgotten by the caregiver. Vehicular heat stroke affects pets as well as children; according to the American Veterinary Medical Association, hundreds of dogs die every year because of vehicular heat stroke ("Pets in Vehicles," n.d.). These deaths are all preventable.

However, the underlying issue with vehicular heat stroke is that parents and pet owners would not believe they would make the mistake of leaving their child or pet in a hot car until a tragedy occurs. There is an evident psychology behind hot car death, as detailed in a report from Dr. Diamond, a psychologist at the University of South Florida; "The failure to remember that a child is in one's car, which can have a tragic outcome, is referred to as "Forgotten Baby Syndrome." While parents typically leave their child in a car accidentally, due to outside factors such as distraction or fatigue (Diamond), pet owners traditionally leave their pet in a car without realizing how quickly the interior temperature of a car can rise ("Pets in Vehicles," n.d.); a study from the San Francisco

State University Department of Earth and Climate Sciences demonstrated that even within the first 10 minutes of a car being turned off, the interior temperature can increase by nearly 20 degrees.

Organizations have been formed to raise awareness about the problem. Although awareness itself has not solved this problem, it has led to the creation of many technological devices that could be of great assistance in the future. The gap within the body of knowledge regarding vehicular heat stroke lies within current technologies; while they do exist, they include extraneous features. This has ultimately decreased their accuracy. For example, SensorSafe Car Seat, a promising system currently available, connects to the vehicle's on-board diagnostics port and sends alerts to a driver when the ignition is turned off. This system, however, "failed to connect, did not connect consistently, or didn't chime at the appropriate times when used with some models from Acura, Dodge, Kia, Land Rover, Mazda, and Subaru," (Thomas, 2016). Another promising model, General Motors Rear Seat Reminder System, activates when the rear door of a car is opened within 10 minutes of turning on the car. When it was tested in a real-life scenario, the results were as follows: "When she [tester] reached her final destination and turned off the car, there was no reminder," (Messer, 2017).

The technologies that exist are therefore unreliable and further improvement is much needed. This leads to the research question of "How can a device to prevent vehicular heat stroke be created that is fully operative and accurate?"

Hyperthermia

It is important to understand the body's mechanisms for temperature control, prior to engineering the device, in order to have a more thorough understanding of the topic. Hyperthermia, the increase in internal body temperature, can have similar tissue responses to hypothermia. Strayer, et. al (2015) describes the physiological effects of hyperthermia. Hyperthermia causes injury to the vascular endothelium, which ultimately results in blisters, edema, and varied vascular permeability. The extent of the injury is dependent on the temperature. Generally, internal body temperatures above 42.5 degrees Celsius can have drastic effects on the body, including vasodilation, altered respiration, and impaired cardiac functions. However, during infections, specific cytokines communicate with the hypothalamus, the region of the brain that controls homeostatic systems, to allow for a higher internal body temperature.

The type of hyperthermia that occurs during abnormally elevated temperatures is known as heatstroke. Heatstroke is defined as when the body's core temperature reaches 104 degrees Fahrenheit or higher (Mayo Clinic, 2015). During heatstroke, the thermal regulatory cooling responses are damaged. Heatstroke is common in the elderly; Chen et. al from the Russian Academy of Sciences state that "With advanced age, the sensitivity of the hypothalamus to various feedback signals begins to decline." Similar to the elderly, heat stroke is very common in children because their central nervous system is not as developed. This further contributes to the problem of vehicular heat stroke. Heat stroke can occur only once, but it can be a chronic disease as well (Strayer, et. al, 2015).

The body's ability to maintain homeostasis is dependent on its ability to transfer heat to its surrounding environment at the same rate as heat is being produced (Tortora, et. al, 2009). When the core body temperature becomes elevated, the body enters a negative feedback loop. The high blood temperature stimulates specific thermoreceptors that send signals to the preoptic area, and the preoptic area then proceeds by stimulating the heat-losing center and deactivating the heat-promoting center. The heat-losing center sends nerve impulses that dilate the blood vessels to make the skin warm, which allows for excess heat to be lost by conduction and radiation. Evaporation also occurs, because sweat glands are stimulated. The combination of these responses allows for the body temperature to return to normal (Tortora, et. al, 2009). Furthermore, physiologist Nakamura claims that this same mechanism "also functions for metabolic regulation and stress-induced hyperthermia," implying that the body is able to go through similar homeostatic processes regardless

of the fashion in which hyperthermia is induced.

Arduino

After an initial understanding is developed, the base hardware and software platform must be chosen prior to engineering the device. Arduino is an open-source platform microcontroller. The simplicity of Arduinos makes them attractive to programmers and engineers around the globe; "Moreover, the simplicity of the Arduino to create, modify and improve projects, as well as its open-source and reduced cost makes it among the most used microcontrollers..." (Araújo, et. al, 2014). Many Arduino boards exist; however the Arduino used in this device is an Arduino UNO Rev3. This Arduino is an improvement from previous Arduinos in its family, and the ATmega16U2 microchip allows for information to be transferred at a faster rate. The Arduino UNO has 14 digital input/output pins. All Arduinos can be programmed using the online IDE, which is coded in C/C++ (Hobbytronics, 2010).

The tactile pressure plate used in this device is a switch, and it functions as a pull-up resistor. A switch controls the connections in an electrical circuit, meaning that it can make the circuit either open or closed. For this device, the plate is a closed circuit until pressure is applied. A pull-up resistor is a type of resistor used in logic circuits that determines the logic level, or a state, of an Arduino digital input. A logic level has a finite number of states the digital signal can exist in. The level can be determined by the difference in the voltage between the signal and the ground state (GND). In this device, the logic level is 2-levels, so the digital signals are equivalent to 0 and 1, in binary. More specifically, this prototype could be classified as an active low signal, where the logical low corresponds to 1, and the logical high corresponds to 0 (Arduino, n.d.).

The TMP36 temperature sensor used in this device is unlike other temperature sensors; instead of using traditional methods, the TP36 relies on the concept that voltage across a diode increases as temperature increases (Adafruit, n.d.). Traditional methods most commonly include transistors which "indicate temperature by a change in electrical resistance," (Recktenwald, 2013). The TMP36, however, calculates the change in voltage, determines an analog signal from the voltage and converts the analog signal into the temperature.

The Arduino GSM Shield 2 enables the Arduino board to send or receive an SMS. It can make or receive a phone call as well. An assumption of this research is that all car owners additionally own a cell phone. To communicate, the shield uses a Quectel radio modem M10. The modem is a GSM/GPRS modem, and these modems are commonly used with a computer to send and receive an SMS message. AT commands must be used in order to communicate with the modem. These modems are very similar to cell phones (developershome.com, n.d.).

After the individual components of the device were evaluated, the device could be created in order to gather methods-generated data and to answer the initial research question and fill the gap in the body of knowledge.

METHODOLOGY

This project utilized a quantitative approach coupled with the engineering design method for the creation of the device and the collection of data. The engineering design methodology can be described by Dr. Tayal, a professor of software engineering at the University at Mullana as, "a decision-making process (often iterative) in which the basic sciences, mathematics, and engineering sciences are applied to convert resources optimally to meet a stated objective." This methodology entails defining a problem, specifying requirements for a solution, creating and testing a solution, and making changes to the solution as needed. The engineering design methodology was the ideal methodology for this project, as it allowed for flexibility during the creation of the device.

As per the engineering design method, the researcher defined the criteria that the device needed to meet in order for it to successfully accomplish the project goal and answer the research question. The overall goal of the design was to maintain simplicity and accuracy. It was determined that the device needed to determine if a child or a pet is in a car, determine if the conditions inside the car are threatening, if the

previous statements were true, the device needed to alert a designated phone number.

The materials that were used for this project include an Arduino UNO R3, an Arduino GSM Shield 2 with integrated antenna, a TMP36 Temperature Sensor, a laptop, the Arduino IDE, a USB type A-B cord, a smartphone with an activated SIM card, M/M and M/F jumper wires, a mini breadboard purchased from Elegoo, plexiglass, 20-gage insulated wires, heavy duty aluminum foil, a wire stripper, and a 9v 2a battery pack with a USB to barrel jack converter. Additionally, software such as Fritzing was downloaded to create circuitry sketches, and a heat lamp was used to test the TMP36 temperature sensor.

First Criterion

The first step in the creation of the device was engineering a homemade tactile pressure plate with the plexiglass. In the context of this research, there was no tactile pressure sensor currently available, so an original pressure plate was constructed with assistance from an expert advisor. Researchers from the Southern Taiwan University of Science and Technology utilized a similar approach in using infrared ray technology to determine if a baby was in a crib, in "An Arduino-Based Resonant Cradle Design with Infant Cries Recognition." Although infrared technology was not applied in this device, the approach was similar theoretically.

The plate goes directly underneath a car seat to determine if a child is present in a car. This pressure plate is a switch, meaning it makes or breaks the connections in an electrical circuit. Furthermore, it functions as a pull-up resistor. In a pull-up resistor, when the circuit is not complete, current flows directly from the voltage input pin to the digital input pin on the Arduino, so the input will be a high signal when it there is no pressure. When pressure is applied, and the circuit is complete, current is able to flow through the complete circuit to the ground state, allowing for the digital input signal to be low.

To construct the plate, two sheets of plexiglass were divided into equal square parts. The plexiglass squares were sanded to eradicate the rougher areas. Then, the 20-gauge wire was cut in cut to two strands approximately one foot in length, and approximately four inches of the insulation were stripped using wire strippers. Two holes were cut in each square to thread the uninsulated portion of both wires. Finally, aluminum foil was taped on top of the squares as a conductive material to complete the circuit, and foam dividers were placed on each corner to separate the plates. This allows for the plate to be flexible but not breakable.

The pressure plate was then wired to a breadboard with an LED for testing. If the plate was constructed correctly, the LED should emit light when pressure is applied as the LED is incorporated into the complete circuit. The average weight of an underweight infant, 3 pounds, was applied to the plate in testing.

Second Criterion

Temperature was used as a way to determine if the conditions inside a car are threatening. This criterion of the project was modeled after Klavis et. al's "The Development of a Classroom Microclimate Monitoring System," in which sensors to monitor temperature and humidity are utilized. The TMP36 temperature sensor was chosen due to its low cost and high accuracy. A breadboard, which expands the electrical capabilities of a device, was needed to support the TMP36. The sensor was wired as per Adafruit Industries (the sensor's creator) by connecting the 5v and GND pins on the Arduino to the corresponding power rails on the breadboard, then wiring the power rails to the TMP36 temperature sensor and wiring an analog input pin on the Arduino to the terminal strip on the breadboard connected to the TMP36 sensor. The sensor was then coded to calculate the temperature based on the analog signal from a voltage reading, and then convert this into the corresponding temperature. The sensor was tested by programming it to display the temperature in degrees Celsius every minute for 30 minutes, as 80% of the temperature rise in a car occurs within the first 30 minutes of it being turned off (McLaren, et. al, 2005). The sensor was additionally placed underneath a heat lamp to ensure it had the ability to detect a significant increase in temperature.

Third Criterion

The next step to this project was to activate the Arduino GSM Shield 2. The GSM Shield is attached to the Arduino and expands the Arduino's capabilities, allowing it to send and receive SMS messages, and send and receive phone calls. The send SMS function was used for this device to alert a caregiver. To activate the shield, the SIM card was placed in the corresponding cartridge. The program created allows the GSM Shield to connect to a mobile network using the SIM card's PIN number. After the Arduino connects to the network, an SMS message can be sent. Figure 1 below depicts the configuration of the final device.

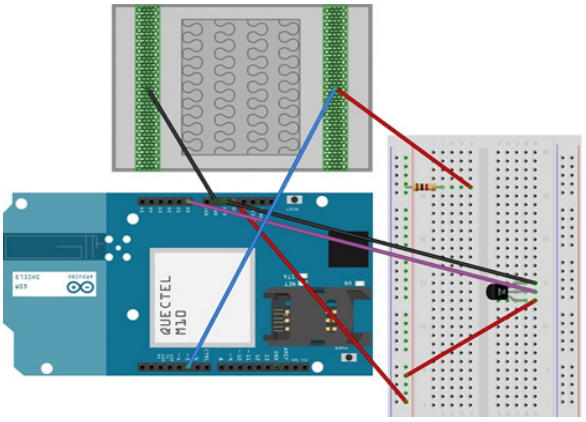

Figure 1. Final Device

Note: Diagram created by researcher with Fritzing software.

Finalization and Integration

The final step in the creation of this device was to integrate the programs for the aforementioned components of this project in order to successfully accomplish the project goal. The code was modified and refined until the device met the design criteria, and the final prototype was tested against the design criteria, as per the engineering design methodology. The sensor was first tested to determine if the device would send an SMS message when both conditions were met. These trials were conducted for a total of 75 times. More tests were conducted, and the device was then tested to ensure SMS messages were sent only when both conditions were met. In these trials, the pressure requirement was fulfilled, but the temperature requirement was not. These trials were conducted for a total of 50 times, and it was noted if an SMS message was sent. Finally, both conditions were met, and the number of seconds it took to send an SMS message were recorded for statistical analysis. The data was recorded, and statistical analyses (mean, specificity, and sensitivity) were calculated based on the methods-generated data.

RESULTS AND DISCUSSION

When the homemade pressure plate was tested to determine its accuracy, it successfully detected pressure 100% of the time in all 50 tests; when three pounds of pressure were applied, the LED lit up 50 times. Moreover, this demonstrates that the homemade pressure plate was constructed correctly. To continue, the TMP36 was tested to determine its accuracy. At room temperature (~25 °C), the voltage output on the TMP36 sensor should be approximately 0.75 volts (Adafruit, n.d.). The sensor used in this device had a voltage output of 0.73 volts at room temperature. This 0.02 difference in the voltage can be attributed to slight differences in room temperature. The sensor was then programmed to display the temperature in degrees Celsius every minute for thirty minutes, as 80% of the temperature increase occurs within the first thirty minutes of a car being turned off (McLaren, et. al, 2005). Additionally, a heat lamp was utilized to determine if the sensor was able to detect an increase in temperature (Fig. 2 below). With minor temperature fluctuations, the temperature began at 18 °C and peaked at 69 °C. This was a 51-degree Celsius change in 30 minutes, thus confirming that the sensor has the ability to recognize major increases in ambient temperatures.

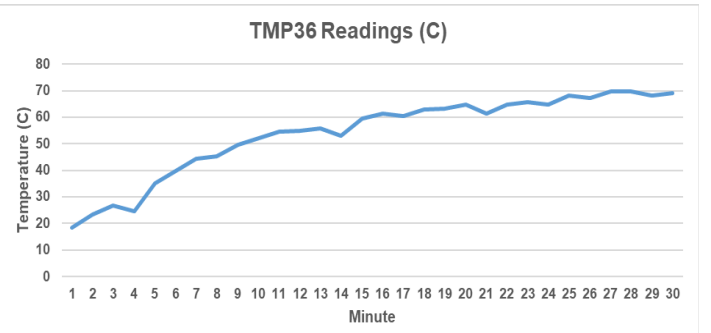

Figure 2. TMP36 Readings (C)

This graphs data for the TMP36 readings

with the heat lamp & depicts the temperature curve, as well.

After the functionality of the individual components was validated, the communications portion could be implemented. The communications portion was entirely software based, and the GSM Shield 2 was activated using a SIM card. The shield then successfully sent an SMS message that was input from the serial monitor on the Arduino IDE to a designated phone number. The code was then modified to send a programmed message to a designated number, and coding for the pressure and temperature components was integrated. Figure 3 depicts the electrical circuitry of the final device.

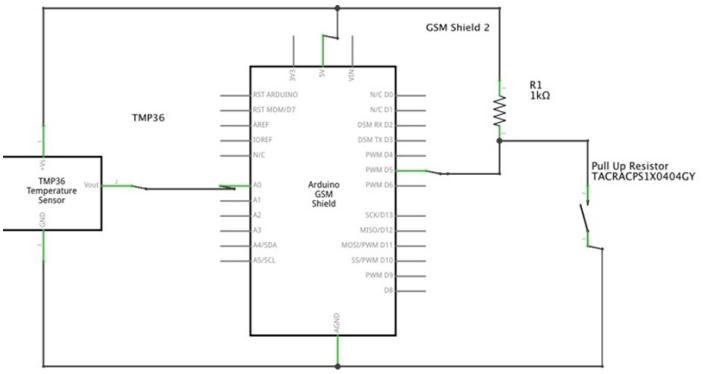

Figure 3. Schematic

Note: Diagram created by researcher with Fritzing software.

Figure 3 is known as a schematic, which is a circuitry diagram. It demonstrates how electricity flows through the device. Power goes directly to the Arduino/GSM Shield complex and is supplied to the other components.

Upon successful construction of the device, the device's accuracy was then determined. After the trials were completed, the data was divided into 30-second intervals for analysis purposes. Figure 4 illustrates the number of trials that fell within each interval.

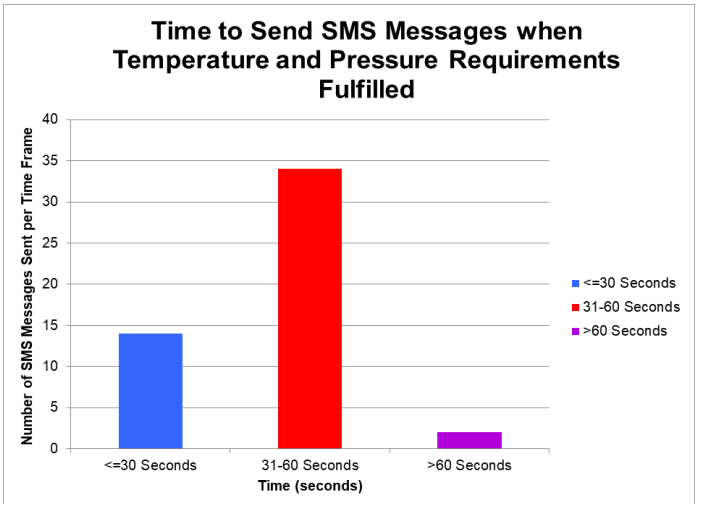

Figure 4. Time to Send SMS Messages when Temperature and Pressure Requirements Fulfilled

This graphs the number of SMS messages that were sent in each 30-second time frame.

Based on the data, the majority of the messages took between 31 and 60 seconds to send an SMS message. The average time for the device to send an SMS message was 33.5 seconds. Other devices take around a minute and a half to send an alert, if an alert is even sent (Messer, 2017). Therefore, this device is approximately 63% faster than other available devices.

68% of the messages fell in the 31-60 second interval. 28% of the messages were sent in less than 30 seconds. Moreover, an SMS message was sent in as little time as 17 seconds. Only 4% of the messages took more than 60 seconds to send, the longest amount of time to send an SMS message being only 64 seconds.

Additional trials were conducted in order to further determine the accuracy of the device. The final device was then tested against the design criteria. The number of SMS messages sent when both criteria were met was recorded. The number of SMS messages sent when only the pressure requirement was fulfilled was also recorded for sensitivity and specificity analyses. Figure 5 portrays the number of SMS messages sent in relation to the criteria met.

	Both Requirements Fulfilled	Only One Requirement Fulfilled
SMS Messages Sent	75	3
SMS Messages Not Sent	0	47

Figure 5. Criteria Met vs SMS

This chart relates the number of messages sent to the requirements that were met. The first requirement was that pressure was detected, and the second requirement was that the temperature in Celsius was greater than 27 degrees, as this is when the risk for heat stroke can develop, according to the National Weather Service.

The device sent an SMS message 100% of the time when both requirements were met. This means the device has a specificity value of 100%. Specificity was calculated by dividing the number of true negative values by the number of true negatives plus true positives (Parikh et. al., 2008). In this project, the true negative value is the number of messages that were not sent, and the true positive value is the number of messages that were sent. There were 0 false negatives (when a condition is indicated as met when it is actually not) with this device; this is critical in the context for which this device will be used. The device has a sensitivity value of 94%. This shows that 6% of the SMS messages sent were false positives, where only one of the criteria was met but an SMS message was still sent. However, in the context of this project, it is acceptable to have a rare false alarm to ensure the safety of the child or pet.

Component	Price
Arduino UNO R3	$22
Arduino GSM Shield 2	$71.50
TMP36 Temperature Sensor	$1.50
Solderless Mini Breadboard	Approx. $4
Homemade Pressure Plate	Approx. $15
Battery Pack + USB to Barrel Jack Converter	Approx. $30
M/M Jumper Wires	Approx. $3
TOTAL	**$147**

Figure 6. Cost Analysis

Figure 6 lists each component of the device with its price. The final device costs approximately $147. Other available systems, such as SensorSafe's SafeMax All-In-One Car Seat, can cost up to $280. Furthermore, the General Motors Rear Seat Reminder System is only available in some GM cars, requiring a user to purchase a new vehicle. Finally, a cost analysis was performed, and it was determined that this device is 47.5% more economical when compared to SensorSafe's care seat, and significantly cheaper when compared to the GM system, further validating the superiority of this device compared to other devices and building on the new understanding.

CONCLUSION

New Understanding

The device successfully met the design criteria by accurately detecting pressure and temperature, as well as sending an alert to a designated phone number 100% of the time when both pressure and temperature requirements were met. The results demonstrate that this device is an accurate, practical, and economic way to prevent vehicular heat stroke, thus building the new understanding. Moreover, the results corroborate the purpose of this project, as this device is operative and accurate. This device additionally fully addresses the shortcomings of other models as detailed in this chart, further contributing to the new understanding and answering the initial research question.

	The Device	SensorSafe Car Seat	GM Rear Seat Reminder System
Price	$147	$250 to $280	Requires new GM car
Speed of Notification	33.5 seconds	Inconsistent (~90 seconds)	Inconsistent
Includes Pets	✓	×	×
Compatible with All Car Models	✓	×	×
Adaptable to All Car Seats	✓	×	×
Adaptable to Multiple Caregivers	✓	×	×

Figure 7. Comparison to Existing Technologies

This chart displays the components of the device compared to SensorSafe's system and GM's system.

Comparison/Contextualization

The device is 47.5% cheaper than other available devices. It is 63% faster and consistent in sending alerts to a designated number. It is also the first available device to address the safety of pets as well as children as it can be placed

underneath both a pet mat and a car seat. It is also compatible with all vehicle models regardless of make and model because it does not need to be integrated into the car's onboard diagnostics port and other advanced car technologies. It also can be used in all car seats and can be upgraded when the child ages and requires a different car seat, ultimately making the device more economical. Finally, it can be easily transferred from car to car depending on the caregiver.

Implications

This problem has intensified to such a point that Congress has to take action, enacting The Hot Car Act of 2017, which directs all new motor vehicles to be equipped with a reminder system that alerts a driver to check the back seat for a child. Although this is a positive step, this is ineffective, as this fails to consider that 87% of passenger vehicles operated are greater than 2 years old. This device would remedy the issues surrounding the Act as it is compatible with all vehicles. Therefore, the researcher would advise doctors to recommend this device to new parents, as it fills the gap in the body of knowledge surrounding the current systems to prevent hot car death.

Limitations

As with all technologies, this device can be improved upon. The biggest limitation of this research is that the researcher was not able to conduct field trials, where the device would be used in a real-life scenario, due to time constraints. Another limitation is that there could potentially be a situation in which the device does not have power. Additionally, if the caregiver's phone is out of power, this device would not accomplish the overall goal of preventing vehicular heat stroke.

Future Directions

The future directions for this research are directly related to the device's limitations. The most immediate next step would be to discuss this device with car seat manufacturers for future patenting and marketing opportunities. The device itself would need some refinement on appearance to make it more applicable for the context in which it will be used. Additionally, a component where an alert is sent if the battery is low would remedy the issue of the device losing power. Another component where an alert is sent to EMS with GPS coordinates of the vehicle after a certain amount of time has elapses and the conditions are met could remedy the issue of the caregiver's phone not receiving the alerts.

REFERENCES

Araújo, André, David Portugal, Micael S. Couceiro, and Rui P. Rocha. "Integrating Arduino-Based Educational Mobile Robots in ROS." SpringerLink. February 20, 2014. Accessed February 2018. https://link.springer.com/article/10.1007/s10846-013-0007-4.

Arduino Pull-Up and Pull-Down Resistors (n.d.). Retrieved February 2, 2018, from Arduino UNO R3. (n.d.). Retrieved September 1, 2017, from https://store.arduino.cc/usa/arduino-uno-rev3

Chao, C., Wang, C., Chiou, J., & Wang, C. (2015). An Arduino-Based Resonant Cradle Design with Infant Cries Recognition. Sensors,15(8), 18934-18949. doi:10.3390/s150818934

Chen, T. T., Maevsky, E. I., & Uchitel, M. L. (2015, January). Maintenance of Homeostasis in the Aging Hypothalamus: The Central and Peripheral Roles of Succinate. Retrieved January 2018, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4313775/

Diamond, D. (n.d.) Theorizing on the Neurobiology of "Forgotten Baby Syndrome". Retrieved from http://psychology.usf.edu/faculty/data/ddiamond/baby-sy.pdf

Evans, S. S., Repasky, E. A., & Fisher, D. T. (2015, June). Fever and the Thermal Regulation of Immunity: The Immune System Feels the Heat. Retrieved January, 2018, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4786079/

Evenflo Company, Inc. (n.d.). Retrieved September 10, 2017, from http://www.evenflo.com/sensorsafe/sensorsafe.html

General Motors Rear Seat Reminder Technology. (n.d.). Retrieved August 25, 2017, from http://gmauthority.com/blog/gm/general-motors-technology/gm-convenience-technology/gm-rear-seat-reminder/

Heatstroke. (2017, August 15). Retrieved September 4, 2017, from http://www.mayoclinic.org/diseases-conditions/heat-stroke/symptoms-causes/syc-20353581

Heatstroke Deaths of Children in Vehicles. (n.d.). Retrieved September 10, 2017, from http://noheatstroke.org/

Kviesis, A., Klavina, A., & Vitols, G. (2017). Development of Classroom Microclimate Monitoring System. doi:10.22616/erdev2017.16.n145

McLaren, C., Null, J., & Quinn, J. (2005, July 01). Heat Stress from Enclosed Vehicles: Moderate Ambient Temperatures Cause Significant Temperature Rise in Enclosed Vehicles. Retrieved September 12, 2017, from http://pediatrics.aappublications.org/content/116/1/e109

Messer, S. (2017, June 14). How 4 Technologies Designed to Prevent Hot Car Deaths Work. Retrieved August 20, 2017, from http://abcnews.go.com/US/technologies-designed-prevent-hot-car-deaths-work/story?id=47991074

Nakamura, Kazuhiro. "Central Circuitries for Body Temperature Regulation and Fever." American Journal of Physiology-Regulatory, Integrative and Comparative Physiology.November 2011. Accessed November 2017. https://www.physiology.org/doi/abs/10.1152/ajpregu.00109.2011.

Null, J. (2003). Study of Excessive Temperatures in Enclosed Vehicles. Retrieved 2017, from http://ggweather.com/heat/heat study.pdf

Parikh, R., A. Mathai, S. Parikh, G. Chandra, and R. Thomas. "Understanding and Using Sensitivity, Specificity and Predictive Values." Indian Journal of Ophthalmology. 2008.Accessed February 2018. https://www.ncbi.nlm.nih.gov/pubmed/18158403.

Recktenwald, Gerald. "Temperature Measurement with a Thermistor and an Arduino." May 2013. Accessed December 2017. http://weblaboratorium.hu/wp-content/uploads/2016/05/thermistorArduino.pdf.

Pets in Vehicles. (n.d.). Retrieved from 2017, from https://www.amva.org/public/PetCare/Pages/pets-inivehicles.aspx

Strayer, D., Rubin, E., & Schiller, A. (n.d.). Rubin's Pathology: Clinicopathologic Foundations of Medicine (6th ed.). Philadelphia.

Tayal, S. P. (n.d.). Engineering Design Process. Recent Advances in Engineering and Technology. doi:2319-7080

TMP36 Temperature Sensor. (n.d.). Retrieved December 21, 2017, from https://learn.adafruit.com/tmp36-temperature-sensor/using-a-temp-sensor

Tortora, & Derrickson. (2009). Principles of Anatomy & Physiology(12th ed.)

The Effect of Vaccination Rates on Measles Infection Rates Utilizing Individual-Based Computer Modeling

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ABSTRACT

Despite the CDC's 2000 declaration that measles has been "eliminated," there have been several recent outbreaks in the United States related to travelers entering communities with decreased vaccination rates. The purpose of this study was to evaluate the effect of decreasing vaccination rates on measles outbreaks using individual-based computer modeling. This computer model, written in C++, ran simulations of vaccination rates from 100% to 85% in a simulated community. It was hypothesized that if the measles vaccination rates were less than 90%, then measles infection rates would rise significantly based on individual-based computer modeling.

The model demonstrated a statistically significant exponential (R^2=0.996) increase in infectious cases with each 2.5% decrease in vaccination rates from 100% to 85% ($p<0.001$). The simulations showed not only an increase in the total number of infected cases but a marked increase in the size of outbreaks as vaccinations rates decrease. The simulation showed a correlation (R^2 = 0.942) between the number of secondary cases produced by the index case and the average number of total cases but there was a wide variation in individual simulations.

Results from this study supported the hypothesis and showed a statistically significant increase in measles cases with each 2.5% decrease in vaccination rates. The data also suggested the greater importance of higher vaccinations rates rather than isolation of infectious cases to control outbreaks.

INTRODUCTION

Measles is a worldwide problem. Even in the United States, where it has been "officially eliminated," there have been outbreaks in recent years. Measles can lead to illness and can result in death. The purpose of this project was to determine what percent of the population needs to be vaccinated to prevent measles outbreaks. Previous formula-based studies have "estimated the herd protection threshold [is] at 92-95% in the USA" (WHO, 2017). This study utilized individual-based population modeling to evaluate the level of measles vaccination needed to prevent outbreaks.

It was hypothesized that if measles vaccination rates were less than 90%, then measles infection rates would rise significantly based on individual-based computer modeling. It is important to know what level of vaccination is needed to prevent future measles outbreaks. There are two basic types of epidemiologic models: formula-based and individual-based. Formula modeling began in the late 18th century with Thomas Malthus (MacFarlane, n.d.). In this type of modeling, formulas are used to represent a population, such as 2^n to represent the doubling of a population with each generation. Modern computers have allowed for the development of another epidemiologic system called individual-based modeling. In this type of modeling, each individual of a population and their characteristics are tracked (Lomnicki, 2011). These models are very data intensive and best for studying small populations.

Several studies have been done looking at modeling of measles outbreaks using various algorithms and formulas (Serres, Gay, & Farrington, 2000). Measles has been modeled using formulas particularly with regard to the level of immunization needed to prevent outbreaks.

One model looking at the outbreak in a partially immunized population was done by Serres, et al. The formula-based model used a formula to determine the spread with each generation. This model provided information regarding risk of spread and estimated size of outbreaks. This formula-based model also estimated the effective reproductive number R_0 (average number of secondary cases produced by a typical case in a particular population) and compared it to calculated R_0 values from outbreaks in the United States and Europe.

This study was different from previous studies in that individual-based modeling was used instead of formula-based modeling, to determine the effect of different measles vaccination rates.

Measles is an infection caused by an RNA virus that is highly contagious (WHO, 2017). Animals do not carry the disease, so humans are the only host. The vaccine became available in 1963 and since that time, cases have plummeted particularly in the Western hemisphere. The measles vaccine is both safe and effective. Still globally, in 2015, there were 35 cases per million people in the population.

While measles has been considered eliminated in the United States, there have been several outbreaks over the past several years related to travel overseas. In 2015, a traveler infected overseas visited Disneyland, resulting in 150 infections (CDC, August 2017). In April 2017, an outbreak started in Minnesota, particularly involving the Somali community, due to low vaccination rates. There were 65 confirmed cases in two months (CDC, July 2017).

METHODOLOGY

The program was written in the C++ programming language on an ASUS Model X550L laptop computer. At the beginning of the program, multiple parameters were set based on census data for Hennepin County, Minnesota, where a measles outbreak occurred in an under-immunized population. The maximum number of people in the simulation was set at 100,000, with number of families per business, school and market obtained by dividing the total number of families by the number of businesses, schools, and stores found in the census data for Hennepin County Minnesota (475,913 households; 555 schools; 39,905 businesses, and 4,184 retail outlets) (2012). The parameters for the population included single adult households and two adult households. Half of the adult households had children less than 18 years old. The number of children in each household with children was determined by a random number generator with numbers from 1 and 5 using census data percentages.

Once the households were established, a program subroutine was created and tracked the individuals in the population. The people were given an age, vaccination status, and immunization status. Probability of transmission during interactions was set at 90% based on CDC data (July 2017). The rate of immunization was the controlled variable. The simulation was repeated 1,000 times for each of the seven different levels of immunization (100, 97.5, 95, 92.5, 90, 87.5, and 85 percent). These levels were chosen because previous studies have "estimated the herd protection threshold at 92-95% in the USA" (WHO, 2017).

The simulation was based on hourly movements of the people. Once the population was moving and interacting for 100 hours, an index infected case was brought into the model. During each hour, people at each location interacted with the other people randomly. If one person had infectious measles but the other did not and was not immune to measles, then a test for transmission was performed. If the infection spread, the new case had a period of incubation of 10 to 14 days based on a random number generator. After the incubation period, the person was infectious.

Once the program was written, the first step in the simulation was determining the probability of disease transmission per contact per hour. The simulation program was run with 0% vaccination rate to simulate a population with no immunity to calculate the R_0. This was repeated 21 times for different transmission rates (30, 12, 10, 8, 5, 7). The runs were started at thirty transmissions per hour based on previous published literature and the other rates were

chosen based on the previous runs to approach the transmission rate closest to 18, the R_0 which corresponds to the published transmission rates (Anderson & May, 1982).

The simulation was run using vaccination rates from 100% to 85% using 2.5% increments. One thousand runs were done at each level of vaccination rate. Analysis was done using Microsoft Excel to look at the number of infected individuals at each level of vaccination to see if there was a statistically significant increase in measles cases as vaccination rates decreased using a t-test.

RESULTS

Finding Transmission Rate as R_0 equals 18

The average R_0 closest to the published data of 18 was a transmission rate of 7 transmissions per hour (Table 1 and Graph 1). The transmission rate was retested for 1000 runs with an average R_0 of 17.8. All further simulations utilized a transmission rate per hour of 7.

Transmission Rate per Hour	R_0
5	15.2
7	18.0
8	20.9
10	21.8
12	24.3
30	34.0

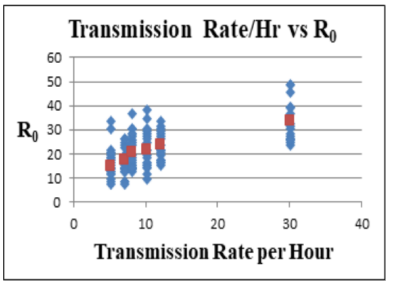


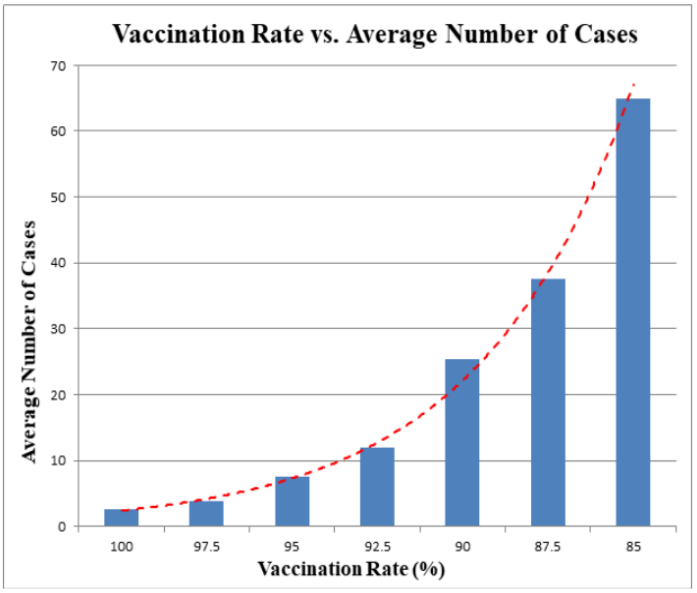
Table 1 and Graph 1. Transmission Rate per Hour

Vaccination Rates Impacting Number of Cases

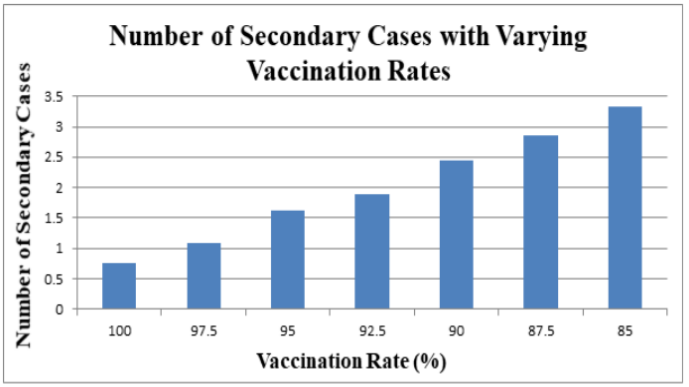
The simulation was run for vaccination rates from 100% to 85% in decrements of 2.5% (Table 2, Graph 2). The rate of immunization decreased from 96% to 81% as the rate of vaccination decreased. There was an exponential increase (R^2= 0.996) in the average number of cases with each decrease in vaccination rates that was statistically significant ($p<0.001$). There were on average more than three times as many cases when the vaccination rate decreased from 95% to 90%. There was a steady increase in the average number of secondary infections (I^2) as the vaccination rates decreased which was also

Vaccination Rate (%)	Average # People Vaccinated	Average # People Immune	Average # People Infected	Infection SD	Infection T-test (p-value)	I_2	I_2 SD	I_2 T-test (p-value)
100	98,734	95,777	2.5	2.5	<0.001	0.76	0.96	<0.001
97.5	96,766	93,869	3.8	4.1	<0.001	1.08	1.07	<0.001
95	93,803	90,995	7.6	7.9	<0.001	1.62	1.40	<0.001
92.5	91,837	89,086	12.0	12.6	<0.001	1.88	1.48	<0.001
90	88,880	86,217	25.3	24.2	<0.001	2.44	1.71	<0.001
87.5	86,909	84,303	37.5	32.7	<0.001	2.86	1.90	<0.001
85	83,955	81,442	65.0	48.8		3.33	2.06	

Table 2. Infection Rates at Different Vaccination Rates



Graph 2. Vaccination Rate vs. Average Number of Cases



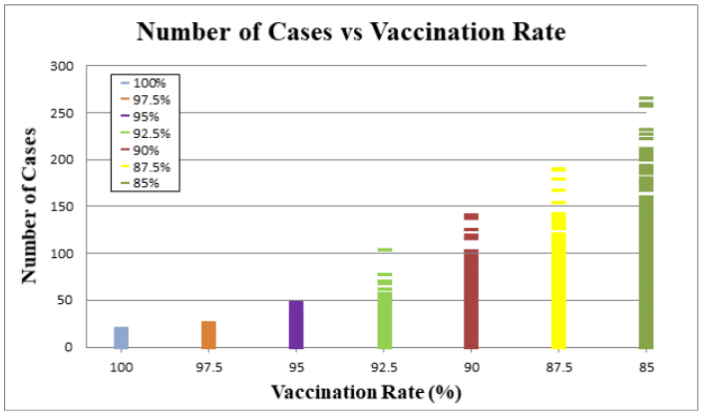
Graph 3. Number of Secondary Cases with Varying Vaccination Rates

Simulations with five or fewer infections were seen in over 50% of cases (557 cases) at 95% vaccination rates but in only 11% (110) of

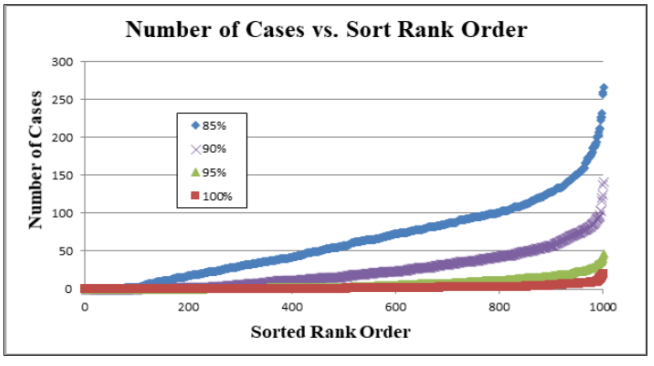
vaccination rates of 85% (Table 3 and Graphs 4 and 5). With high vaccination rates, there were also fewer large outbreaks. Vaccination rates of 95% produced fewer than 10% of stimulations (79) with more than 20 cases and none had 50 cases. Lower rates such as 87.5% vaccination had 62% of outbreaks (616) with more than 20 cases and 30% with 50 or more cases (303). For all the vaccination rates studied, the worst 10% of outbreaks produced 32% of all cases and the worse 20% produced 50% of all cases (Table 4). There was a linear correlation between the mean number of cases compared to I^2 (R^2= 0.942) but with a wide variation in individual simulations (Graph 6 and Table 5).

Vaccination Rate	100%	97.5%	95%	92.5%	90%	87.5%	85%
# Simulations > 5 cases	104	207	443	581	727	804	890
# Simulations > 10 cases	12	81	265	422	645	742	864
# Simulations > 20 cases	1	9	79	201	473	616	787
# Simulations > 50 cases	0	0	0	17	156	303	559
Max # of cases	21	27	48	106	142	191	267

Table 3. Size of Outbreaks for Different Vaccination Rates



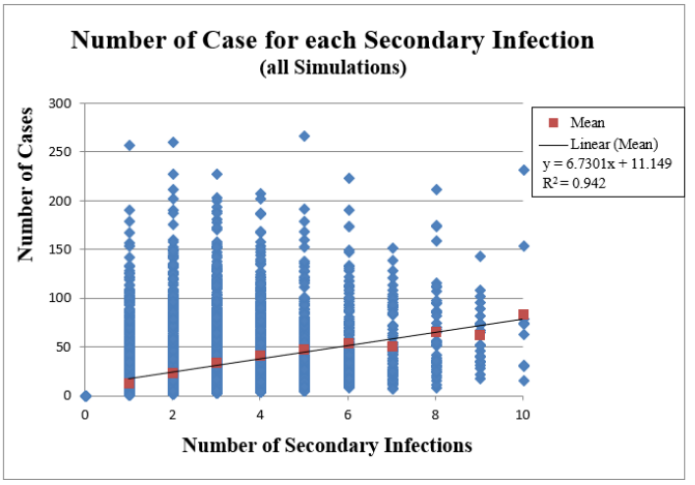
Graph 4. Size of outbreaks increases as vaccination rates decrease



Graph 5. Size of median and worst-case scenario outbreaks increase as vaccination rate decreases

% Vaccinated	Total Number Infected in all Runs	Number Infected in Highest 200 Runs	% of Total	Number Infected in Highest 100 Runs	% of Total
100	2,531	1,295	51 %	842	33 %
97.5	3,828	2,110	55 %	1,387	36 %
95	7,551	4,155	55 %	2,585	34 %
92.5	12,019	6,432	54 %	4,031	34 %
90	25,305	12,936	51 %	7,837	31 %
87.5	37,532	17,815	47 %	10,490	28 %
85	64,959	27,831	43 %	16,350	25 %
TOTAL			51 %		32 %

Table 4. Total Number of Infections at Each Vaccination Level



Graph 6. Linear increase in the mean number of cases as R_0 increases, with wide range of scenarios

Number of Secondary Infections	Average # Cases	Standard Deviation	n
1	13.2	24.1	1809
2	23.8	31.0	1534
3	34.6	36.9	964
4	42.2	36.9	596
5	48.4	37.3	302
6	54.1	38.8	170
7	51.2	34.9	82
8	66.5	46.1	46
9	63.3	31.3	22
10	84.4	65.0	9
11	61.5		4
12	49.0		1

Table 5. Average Number of Cases in a Run Based on Number of Secondary Infections

DISCUSSION

The study began with a calibration phase for the program. There is very little published data regarding the rate of infection transmission per hour per contact. This is not surprising given number of possible variables regarding transmission rates per hour including environmental variables such as location of

contact, type of contact, and air exchange rate. The calibration was done using an estimated R_0 of 18 based on previous field studies. The data produced a probability of transmission of 7% per hour. This is lower than the 30% per hour from some theoretical calculations. Those calculations were based on subjects being in a closed room environment with specific air exchange levels. This is very different than the transmission rate per hour value which includes a general summation of all environments and varying contact interactions in this simulation.

There was a clear exponential increase in the average number of cases as the vaccination rate decreased. Simulations with vaccination rates of 95% or greater had an average of less than 5 cases. Given the varying degree of symptom severity and barriers to accessing the healthcare system, many of these infection episodes would likely not be identified but instead be lost in the community. In contrast, at lower vaccination rates of 87.5% and 85%, outbreaks of more than 50 people were seen in 30% and 56% of the simulations respectively. At lower vaccination rates, the outbreaks are more likely to be identified because of their greater severity.

Reduced vaccination rates produced a steady increase in I^2. The vaccination rate of 95% had an I^2 of only 1.6 compared to vaccination rates less than 90% where the I^2 was greater than 2.5. While the I^2 increased linearly, the number of cases increased exponentially as the vaccination rate decreased. Interestingly, there was a correlation between the I^2 and the average number of cases. An I^2 of one averaged 13 cases while an I^2 of five produced an average 50 cases. There was a large variation, though, in the number of cases at any particular I^2. This would suggest that early isolation of the initial cases in an outbreak is helpful but may not be not consistently effective. This places further importance on community immunization, not just isolation of identified cases to reduce outbreak size.

One key question regarding measles vaccination rates is if there is a certain critical level that controls outbreaks. The study showed a statistically significant exponential increase in infection cases with each 2.5% decrease in vaccination rates. Previous studies have suggested levels of 92-95% for herd immunity in the United States (WHO, 2017). There are several factors that play into the "control" of infections, including the percentage of infected patients who actually develop symptoms and the ability of the community to recognize the presence of an outbreak. It may not be that there is some critical level of vaccination that creates herd immunity, but instead that levels between 92-95% reduce the size and number of outbreaks in the community to a level that they are no longer readily identified. While such a proposition would need a great deal of additional study, the computer model does raise this possibility.

One interesting finding of the simulation is the extent to which a relatively low number of infections produced a large number of cases. The worst 10% of cases produced approximately one-third of all the cases. This simulation, based on pseudo-random processes, produced a skewing of the number of cases from a relatively small number of outbreaks. That makes the study of true super spreader events that occur with complex biological systems and environments that much more difficult to evaluate since random processes also produce skewed data.

There were several potential limitations of the study. The simulation calibration used an R_0 of 18 but there is variation in field research R_0 values and any actual environments used for this measurement can't be equivalent to the 100% susceptible computer simulation population. All individual based computer models are limited by the inability to replicate the complexity of the natural environment and biological organisms such as variety of environments and infection spreading cofactors. The simulation only created a fairly homogeneous population. Most outbreaks in the Western world recently have occurred in specific populations within the community such as the Hennepin County Minnesota outbreak among young Somali immigrants in 2017 and the Amish in Ohio in 2014. The homogeneity of the simulated population limits the model's ability to simulate these different circumstances.

This measles outbreak simulation supported the importance of high vaccination rates to reduce outbreak frequency and size.

The exponential increase in the number of cases as vaccination rates decrease reinforces the importance of community vaccination efforts. The number of identified outbreaks and severity of outbreaks was greater with lower vaccination rates. While the initial I^2 increased with lower vaccination rates, there was marked variation in the number of cases in an outbreak, suggesting the value of community vaccination over case isolation to control measles outbreaks. The study supports the hypothesis that reduced measles vaccination rates significantly increase outbreak size.

REFERENCES

Anderson, R.M. & May R.M. (1982, February 26). Directly Transmitted Infectious Diseases:
Control by Vaccination. Science. Retrieved from https://doi.org/10.1126/science.7063839

Center for Disease Control and Prevention. (2017, July 14). Measles Outbreak – Minnesota April-May. Morbidity and Mortality Weekly Report (MMWR). Retrieved from https://www.cdc.gov/mmwr/volumes/66/wr/mm6627a1.htm

Center for Disease Control and Prevention. (2017, August 23). Measles Cases. Retrieved from https://www.cdc.gov/measles/cases-outbreaks.html

Hennepin County (2012, October). Census 2010 Fact Sheet [Hennepin County Household and Family Types]. Retrieved September 12, 2017 from https://www.hennepin.us/-/media/hennepinus/your-government/research-data/documents/census-2010-households-family-types.pdf

Lomnicki, A. (2011, January 1). Individual-based Models in Population Ecology. Wiley Online Library. Retrieved from http://www.els.net/WileyCDA/ElsArticle/refId-a0003312.html

Macfarlane, A. (n.d.). Thomas Malthus and the Making of the Modern World. Retrieved from http://www.alanmacfarlane.com/TEXTS/Malthus_final.pdf

Microsoft Corporation (2017). Full C and C++ IDE with Visual Studio download [Windows version]. Retrieved from https://www.visualstudio.com/vs/cplusplus/

Serres, G., & Gay, N., & Farrington, C. (2000, June 1). Epidemiology of Transmissible Disease after Elimination. American Journal of Epidemiology, 151(11), 1039-1048. Retrieved from https://academic.oup.com/aje/issue/151/11

World Health Organization (2017, April). Measles Vaccines: WHO Position Paper. Weekly Epidemiological Record, 92(17), 205-228. Retrieved from https://reliefweb.int/sites/reliefweb.int/files/resources/WER9217.pdf

The Effects of Sugar Substitutes and Prebiotics on the Gut Microbiome

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ABSTRACT

The gut microbiome plays an essential role in human health, and our diet has the potential to alter it. Sugar substitutes and food preservatives were tested for their effects on the growth of gut bacteria or its susceptibility to antibiotics. The prebiotic inulin was added to determine if it counteracts harmful effects of these additives. Sublethal concentrations of sugar substitutes and the protective concentration of inulin were determined. A growth curve of E. coli was measured with exposure to sucralose, stevia, ascorbic acid, or sodium nitrate all with or without inulin. Minimum inhibitory concentrations (MICs) and zones of inhibition of antibiotics were determined with exposure to sucralose or preservatives with or without inulin. Exposure to food additives increased the lag times of E. coli an average of 147% and the prebiotic inulin increased them further. The MICs of ampicillin or chloramphenicol with E. coli decreased with exposure to sugar substitutes or preservatives. With additional exposure to inulin, the MICs of ampicillin all increased whereas the MICs of chloramphenicol showed mixed results. The zones of inhibition of antibiotics with either E. coli or L. acidophilus showed a statistically significant increase with exposure to sucralose but decreased when inulin was added. In conclusion, sugar substitutes and food preservatives inhibited the growth of E. coli and acted synergistically with antibiotics. Overall, the addition of inulin appeared protective and counteracted the effects of the food additives on the susceptibility of bacteria to antibiotics. These additives may be altering the gut microbiome when they are consumed.

INTRODUCTION

In a healthy adult, there are over 1,000 species of bacteria and about 10 times more bacterial cells than human cells, according to the Human Microbiome Project. While long thought to play a role in preventing harmful bacteria from attaching to the gastrointestinal tract, new research suggests the gut microbiome plays an essential role in human health, both mental and physical. Alterations in an individual's microbiome can be caused by antibiotics and may result in many disease processes including obesity, heart disease, neurologic disease, mental illness, and allergies. Research has shown that the common food preservatives ascorbic acid, citric acid, and sodium nitrate affect the growth of E. coli and the susceptibility of E. coli to ampicillin and chloramphenicol. This means that the gut microbiome may be altered every time food preservatives are consumed. There are also other food additives that can be both helpful and harmful to the gut microbiome. Sugar substitutes are additives that sweeten food while adding few or no calories. Some studies have shown a link between sugar substitutes and weight gain, but the reason for this is unclear. Another additive is a prebiotic, indigestible fiber that is beneficial to the microbiome. They may be able to help counteract the effects of food preservatives or sugar substitutes on gut bacteria.

The bacteria in our microbiome play a big part in our health. Gut microbes are important in digestion and can allow humans to receive nutrients from things indigestible to humans. There can also be communication between the gut and the brain, called the gut-brain axis. However, imbalances in the gut bacteria, which can be caused by illness or usage of antibiotics, can result in many diseases. These diseases include obesity, diabetes, inflammatory bowel

disease, heart disease, allergies, neurologic disease, and mental illness.

Beneficial bacteria in the gut can help to maintain homeostasis in the gut microbiome. Among these species of beneficial bacteria are Escherichia coli and Lactobacillus acidophilus. E. coli is a Gram-negative facultative anaerobe found in the gut. Most E. coli are normal in the gut, but some virulent strains of E. coli can cause disease. L. acidophilus is a Gram-positive microaerophilic species of bacteria that is considered to be a probiotic, which is bacteria believed to be beneficial to humans. For this reason, L. acidophilus is sold as a probiotic, and it is also in many dairy products.

Antibiotic usage can harm beneficial bacteria as well as pathogenic bacteria, and it can cause alterations to the microbiome. Also, inappropriate usage of antibiotics can cause bacteria to become resistant. It is estimated that by the year 2050, 10 million people will die from antibiotic resistant infections each year, causing more deaths than cancer. The over usage of antibiotics can result in resistance, and about 30% of antibiotics in the U.S. are prescribed unnecessarily, which is 47 million prescriptions. There are 2 kinds of antibiotic resistance: intrinsic resistance and acquired resistance. Intrinsic resistance is the result of the genetic or structural makeup of a microorganism. Intrinsic resistance is naturally occurring, and is predictable, as it is associated with specific groups or species of bacteria. Unlike intrinsic resistance, acquired resistance is unpredictable. It is the result of the altered genetic or structural makeup of a microorganism. Resistance can be acquired through genetic mutations, exchange of genes during horizontal gene transfer, or through a combination.

One commonly used antibiotic is ampicillin. Ampicillin inhibits cell wall synthesis and is a beta-lactam antibiotic from the penicillin family. All antibiotics from the penicillin family have a beta-lactam ring, which is a four-atom ring. The beta-lactam ring binds to penicillin-binding proteins (PBPs), which prohibits the PBPs from cross-linking the cell wall. The most common form of antibiotic resistance to beta-lactams like ampicillin is through the production of beta-lactamase. Beta-lactamase is an enzyme that cleaves the beta-lactam ring, which means that the antibiotic no longer has the ability to bind to the penicillin-binding proteins and cannot harm the bacteria.

Chloramphenicol acts by blocking protein synthesis. It inhibits protein synthesis by binding to the 50S ribosomal subunit in the bacteria. It is used to treat multidrug-resistant bacteria but is not used often because it can cause aplastic anemia in humans. Ciprofloxacin is an antibiotic that inhibits nucleic acid synthesis. It binds to the DNA gyrase and topoisomerase IV enzymes, which prevent supercoiling of DNA. When ciprofloxacin binds to these enzymes, the bacteria are unable to multiply. Resistance can be acquired by a genetic mutation that alters these enzymes, meaning the ciprofloxacin can no longer bind to them, rendering the antibiotic ineffective.

To measure the susceptibility of bacteria to an antimicrobial, a minimum inhibitory concentration (MIC) of the antimicrobial can be determined by exposing bacteria to serial dilutions of an antimicrobial in a 24 well plate. After growing for about 24 hours, the concentration of the antimicrobial that inhibits visible growth of the bacteria is considered the MIC. This is the lowest concentration of an antimicrobial that is lethal to bacteria. If the MIC of an antimicrobial is lower, the bacteria are more susceptible to the antimicrobial because lower concentrations of this antimicrobial are lethal to the bacteria. Another measure of the susceptibility of bacteria to an antibiotic is through a disk diffusion test. This can be tested by inoculating the bacteria on an agar plate and placing antibiotic sensitivity disk in the center of the plate. After about 24 hours of growth, the zone of inhibition can be measured. This is the zone where there is no growth of the bacteria closest to the antibiotic disk because the antibiotic diffuses onto the plate and inhibits the growth of bacteria. With this test, the bacteria are more susceptible to the antibiotic if there is a larger zone of inhibition and more resistant to the antibiotic if there is a smaller zone of inhibition. The bacteria are completely resistant to the antibiotic if there is growth of bacteria all the way up to the disk.

When bacteria are inoculated in new media, the growth of this bacteria can be tracked

over time using spectrophotometric readings, which measure the absorbance of light as it passes through the media. This data can show a bacterial growth curve. From the bacterial growth curve, the time before the bacteria begin to grow (lag time) and the rate of exponential growth (doubling time) can be calculated.

Prior research showed that food preservatives might affect the susceptibility of E. coli to ampicillin and chloramphenicol. Food preservatives are food additives that are used to inhibit the growth of bacteria in food. While they can be used to keep our food safe, they may also be harming the beneficial bacteria in our gut. The legal definition of a food additive is "any substance the intended use of which results or may reasonably be expected to result – directly or indirectly – in its becoming a component or otherwise affecting the characteristics of any food." Food additives are used for many things, including food preservation. Two commonly used food additives are ascorbic acid and sodium nitrate.

Ascorbic acid is naturally present in or added to foods and is sold as a dietary supplement. Ascorbic acid is Vitamin C, which humans cannot produce themselves, so it must be consumed. It is considered GRAS by the FDA, which means generally recognized as safe. However, the Tolerable Upper Intake Level is set at 2,000 mg/day for adults because it has been reported to cause gastrointestinal upset when taken in high doses. Sodium nitrate is a food preservative used in many cured meats. FDA regulations require that less than 500 parts per million be used in food.

Another type of food additive is sugar substitutes, or high-intensity sweeteners. Sugar substitutes are used to sweeten food because they add little or no calories. Two commonly used sugar substitutes are sucralose and stevia. Sucralose, used by the brand Splenda, is a high-intensity sweetener produced from the sugar sucrose. They are very similar in structure, but sucralose is around 600 times sweeter. Sucralose is also heat stable, so it is commonly used in baked goods. Stevia is a high-intensity sweetener extracted from the plant *Stevia rebaudiana*. Stevia is around 200 to 400 times sweeter than sugar. It is classified as GRAS by the FDA. Studies involving sugar substitutes have had varied conclusions. Some have shown sugar substitutes are safe, while others have shown they are unsafe. In some studies, drinking diet sodas have increased the likelihood of obesity and weight gain compared to drinking regular sodas. However, the reason for this is unknown.

On the other hand, prebiotics are indigestible fibers that support the growth of beneficial bacteria in the gastrointestinal tract. Studies have shown that prebiotics can help prevent diabetes, prevent weight gain, help protect against colon cancer, and prevent antibiotic-associated diarrhea, which is diarrhea caused by alterations in the gut flora due to the usage of antibiotics. Inulin is a prebiotic that can be found in some fruits and vegetables like chicory root, bananas, and Jerusalem artichoke.

This project aims to explore the effects of the common food additives sugar substitutes and food preservatives on the gut microbiome and if the prebiotic inulin can counteract their effects. Specifically, the purposes of this project are to determine if (1) sugar substitutes affect the growth of bacteria, (2) sugar substitutes alter the susceptibility of bacteria to antibiotics, (3) prebiotics can counteract the effects of food preservatives or sugar substitutes on the growth of bacteria and (4) prebiotics can counteract the alterations sugar substitutes or food preservatives cause on the susceptibility of bacteria to antibiotics.

It is hypothesized that sugar substitutes will (1) inhibit the growth of bacteria and (2) increase susceptibility of the bacteria to antibiotics because the substitutes might compete with other sugars for the energy source of the bacteria and because the sugar substitute sucralose contains chlorine, which is harmful to the growth of bacteria, and prebiotics will counteract the effects of food preservatives and sugar substitutes on (3) the growth of bacteria and (4) the susceptibility of bacteria to antibiotics because prebiotics support the growth of bacteria in the gut microbiome.

METHODOLOGY

The first step in this experiment was to determine the minimum inhibitory concentrations (MICs) of the sugar substitutes sucralose or stevia with E. coli K-12. This is the lowest concentration of the additive that is lethal to the bacteria. To test this, a stock solution of sucralose or stevia was made by adding 3 g of either to 10 mL of sterile TSA broth. In a 24 well plate, the sucralose or stevia was diluted in sterile TSA broth by a factor of two for six dilutions beginning with the concentration of 0.3 g/mL in the first well. After incubating for 24 hours, the MICs and sublethal concentrations of sucralose were determined by turbidity. The sublethal concentration was the last well with visible growth, and the MIC was the next highest concentration after the sublethal concentration, or the first well without visible growth of the bacteria.

Next, the E. coli was exposed to lethal concentrations of food preservatives or sucralose with and without the prebiotic inulin. The highest concentration of stevia that would dissolve in the media was not lethal to the bacteria, so stevia was not tested in this step. The MICs of the food preservatives were determined during prior research. Solutions of the food preservative ascorbic acid were created by adding either 0.1 g (1xMIC), 0.2 g (2xMIC), 0.3 g (3xMIC), or 0.4 g (4xMIC) of ascorbic acid to 10 mL of TSA broth. These concentrations were repeated and 1g of inulin was added to each tube to perform serial dilutions of inulin 1:2. Solutions of sodium nitrate were created by adding 0.8 g (1xMIC), 1.6 g (2xMIC), 2.4 g (3xMIC), and 3.2 g (4xMIC) sodium nitrate to 10 mL TSA broth, and solutions of sucralose were created by adding 1.5 g (1xMIC) and 3 g (2xMIC) sucralose powder to 10 mL TSA broth. These concentrations were repeated and 1g of inulin was added to each tube to perform serial dilutions of inulin 1:2. 10 µL of E. coli from a 24-hour culture were added to each of the wells, and the 24 well plate was placed in an incubator at 37° C. After 24 hours, the growth of E. coli was determined based on turbidity. The well with the lowest concentration of inulin that had visible growth despite exposure to lethal concentrations of the other food additives was the protective concentration of inulin.

For the remainder of the research, the following sublethal concentrations of sugar substitutes and food preservatives were used: 0.75 g of sucralose in 10 mL of TSA broth, 0.5 g of stevia in 10 mL of TSA broth, 0.05 g of ascorbic acid in 10 mL TSA broth, and 0.4 g of sodium nitrate in 10 mL of TSA broth. For all experiments involving inulin, 0.75 g of inulin in 10 mL of TSA broth was added to these sublethal concentrations of sugar substitutes or food preservatives. This concentration of inulin was found to be a protective concentration, meaning with exposure to this concentration of inulin there was growth of the bacteria despite exposure to the lethal concentrations of the other additives.

A growth curve was then measured using a spectrophotometer. 5 mL of TSA broth were added to two cuvettes (one for a zero and one for a control with just E. coli). Seven additional cuvettes were filled with 5mL of either the inulin solution or the solutions of sublethal concentrations of ascorbic acid, sodium nitrate, sucralose, or stevia, each with and without inulin. 50 µL of E. coli were added to each cuvette except the one used as a zero. Each cuvette was placed in an incubator at 37° C. Spectrophotometric readings were taken every 20 minutes at 546 nm over a period of 12 hours, zeroing the spectrophotometer between each reading.

The MICs of ampicillin and chloramphenicol with E. coli exposed to sublethal concentrations of food preservatives or sugar substitutes with and without inulin were then determined. Solutions of ascorbic acid were made as above. 1 mL of ampicillin (stock concentration of 10 mg/mL) was added to one of each of these two solutions. Serial dilutions 1:2 were made in 24 well plates, varying the concentration of ampicillin but keeping the concentration of ascorbic acid constant. This was repeated with a control and solutions of just inulin as well as sodium nitrate and sucralose with and without inulin. After completing each dilution of ampicillin, this whole step was repeated except 10 µL of chloramphenicol (stock concentration of 34 mg/mL) were added to the solutions instead of ampicillin, and the chloramphenicol was diluted.

10 µL of E. coli were added to each well, and the 24 well plate was placed in an incubator at 37° C. After 24 hours of incubation, the MICs of ampicillin and chloramphenicol were determined based on turbidity. The MIC was the well with the lowest concentration of ampicillin or chloramphenicol that did not have visible growth of the bacteria.

Finally, the zones of inhibition of E. coli and L. acidophilus were each measured with ampicillin, chloramphenicol, and ciprofloxacin. Two tubes were made with 10 mL of TSA broth and 0.75 g of inulin were added to one of these two tubes. Two more tubes were made with 0.75 g of sucralose in 10 mL of TSA broth and 0.75 g of inulin were added to one of these two tubes. 100 µL of E. coli from a 24-hour culture were added to both tubes, and they were incubated at 37° C. After 24 hours, they were plated on 15 MacConkey agar plates each with ampicillin, chloramphenicol, and ciprofloxacin sensitivity disks. These were placed in an incubator at 37° C for 24 hours. The zones of inhibition were then measured in diameter. Four tubes were then made with 5 mL tomato juice yeast extract milk broth. 0.75 g of inulin were added to one, 0.75 g of sucralose to another, and 0.75 g sucralose with 0.75 g inulin to another, and the last was the control. 100 µL of L. acidophilus were added to each tube and they were incubated at 37° C. After 72 hours, the L. acidophilus was plated on 10 MRS agar plates from each tube, with ampicillin, chloramphenicol, and ciprofloxacin sensitivity disks. These plates were incubated at 37° C in a 5% CO_2 enriched incubated for 72 hours and the zones of inhibition were measured in diameter.

RESULTS

	MIC	Sublethal Concentration
Sucralose	0.15 g/mL	0.075 g/mL
Stevia	Not found	Not found
Lowest Protective Concentration of Inulin with Each Additive	0.075 g/mL	

Table 1. Lethal and Sublethal Concentrations of Sugar Substitutes

Lethal and sublethal concentrations of the sugar substitutes were first determined (see Table 1). A minimum inhibitory concentration (MIC) of sucralose was found. The highest concentration of stevia that would dissolve in the media was not lethal to the bacteria, however. Also, a protective concentration of inulin was determined. With exposure to this concentration of inulin, E. coli grew despite exposure to lethal concentrations of the other additives.

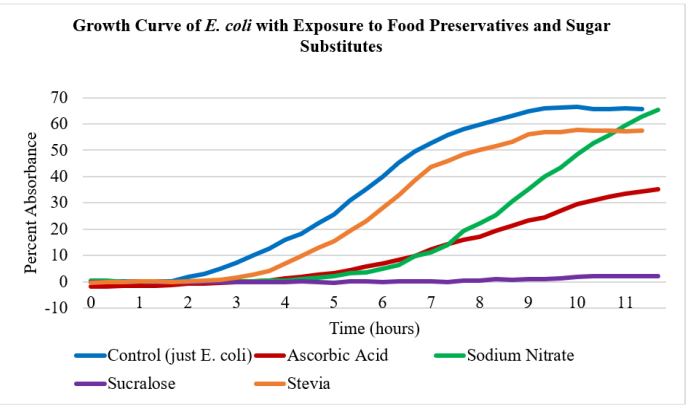


Figure 1. E. coli with Exposure to Food Preservatives and Sugar Substitutes

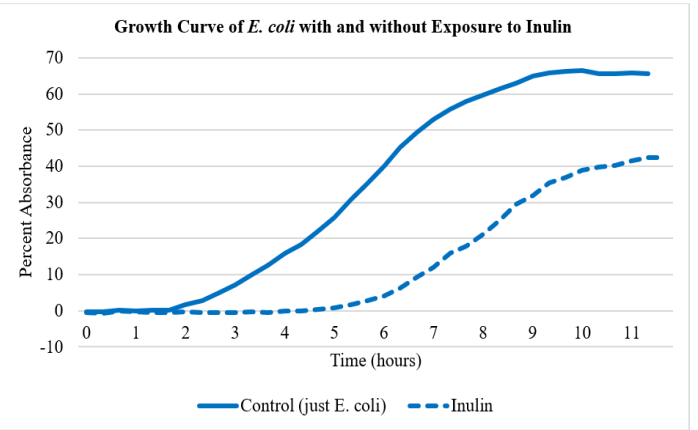


Figure 2. E. coli with and without Exposure to Inulin

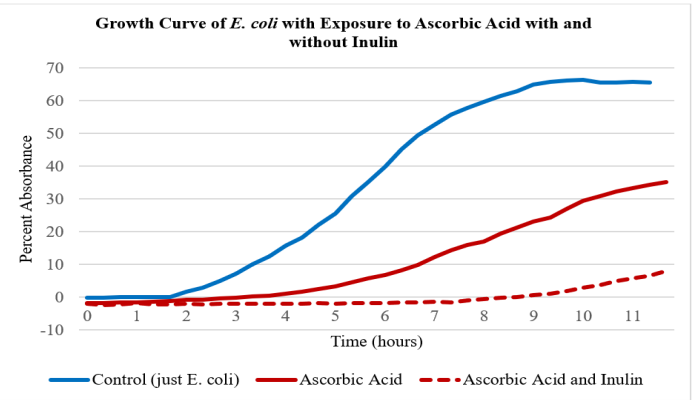


Figure 3. E. coli Exposure to Ascorbic Acid with and without Inulin

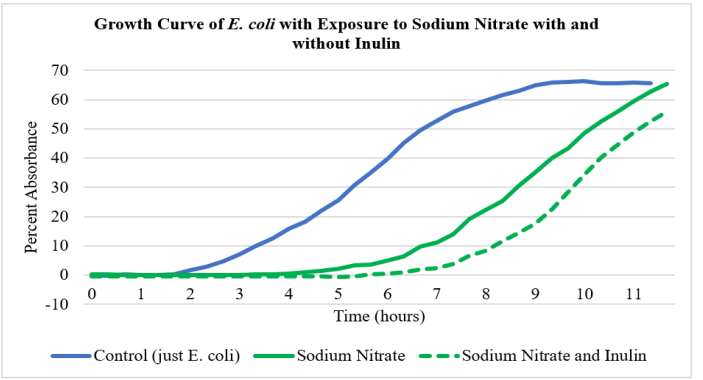


Figure 4. E. coli with Exposure to Sodium Nitrate with and without Inulin

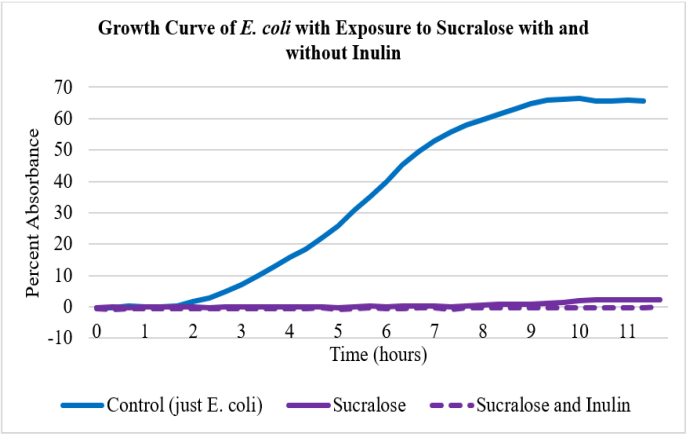


Figure 5. E. coli with Exposure to Sucralose with and without Inulin

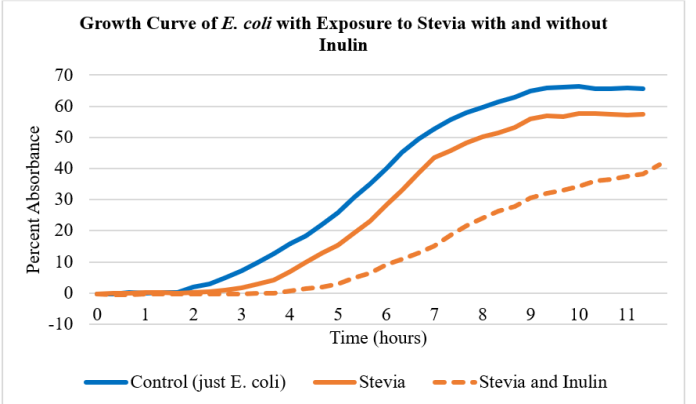


Figure 6. E. coli with Exposure to Stevia with and without Inulin

	Lag Times of *E. coli*				
	Control	Ascorbic Acid	Sodium Nitrate	Sucralose	Stevia
Without Inulin	100 minutes	220 minutes	340 minutes	*	180 minutes
With Inulin	300 minutes	460 minutes	440 minutes	*	300 minutes

Table 2. Lag Times of E. coli

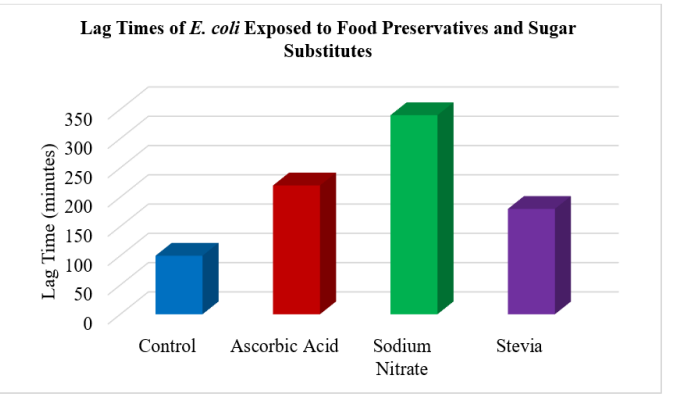


Figure 7. Lag Times of E. coli Exposed to Food Preservatives and Sugar Substitutes

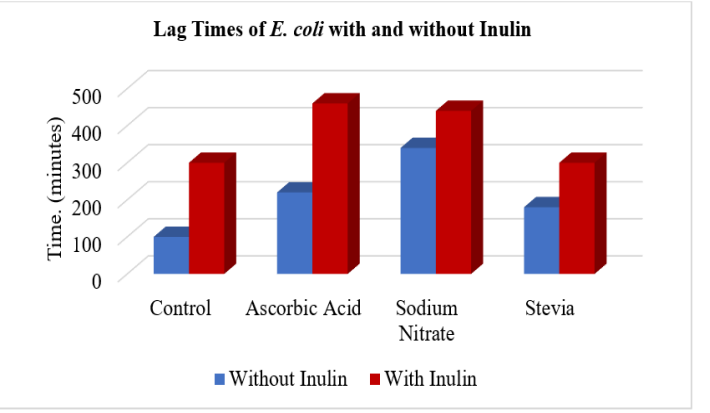


Figure 8. Lag Times of E. coli with and without Inulin

Growth curves of E. coli were measured with exposure to sublethal concentrations of ascorbic acid, sodium nitrate, sucralose, and stevia all with and without inulin (see Table 2 and Figures 1-8). Each of the lag times of E. coli increased with exposure to any of the food preservatives or sugar substitutes compared to the control. (There was a 120%, 240%, and an 80% increase in the lag times with exposure to ascorbic acid, sodium nitrate, and stevia respectively). With the additional exposure to inulin, the lag times of E. coli all increased further. (There was a 200%, 109%, 29%, and a 67% increase in the lag times of E. coli exposed to just inulin compared to the control, ascorbic acid with inulin compared to just ascorbic acid, sodium nitrate with inulin compared to just sodium nitrate, and stevia with inulin compared to just stevia respectively.) After twelve hours of incubation, the lag time of E. coli exposed to sucralose with or without inulin was not able to

be determined, nor was the doubling time of E. coli exposed to ascorbic acid with inulin able to be determined, as there was not enough growth of the E. coli with exposure to these additives.

MICs of Ampicillin				
	Control	Ascorbic Acid	Sodium Nitrate	Sucralose
Without Inulin	7 µg/mL	7 µg/mL	0.3 µg/mL	3 µg/mL
With Inulin	62.5 µg/mL	62.5 µg/mL	12.5 µg/mL	30 µg/mL

Table 3. MICs of Ampicillin

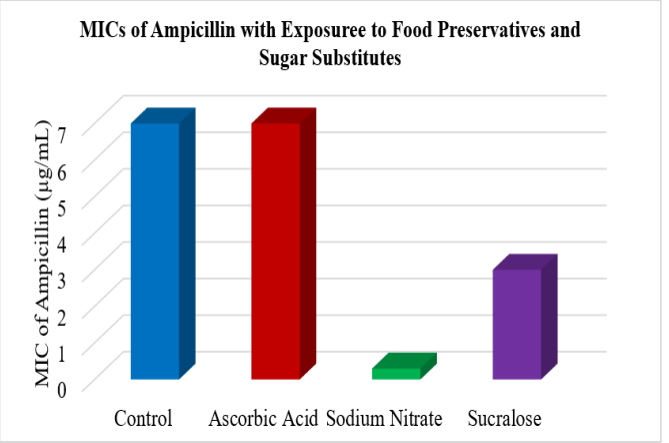


Figure 9. MICs of Ampicillin with Exposure to Food Preservatives and Sugar Substitutes

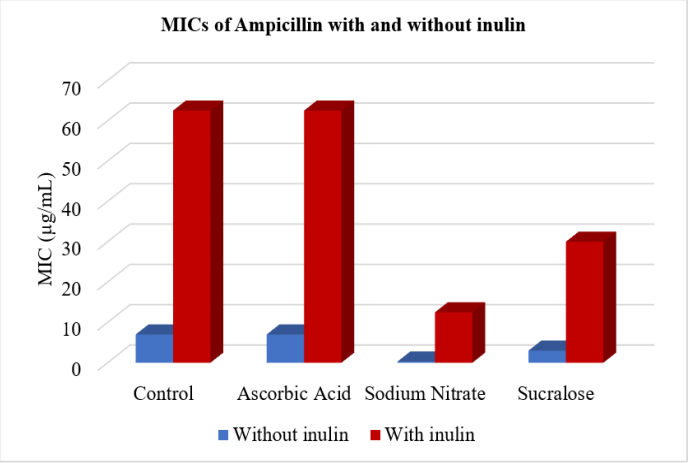


Figure 10. MICs of Ampicillin with and without Inulin

The MICs of ampicillin with E. coli were determined with exposure to food preservatives and sugar substitutes with and without inulin (see Table 3 and Figures 9-10). The MICs of ampicillin with E. coli decreased with exposure to sodium nitrate and sucralose compared to the control. (There was a 95% and a 57% decrease with exposure to sodium nitrate and sucralose respectively). This means the E. coli was more susceptible to ampicillin when also exposed to these additives. The MIC of ampicillin with E. coli was unchanged with exposure to ascorbic acid. However, all of the MICs of ampicillin increased with the additional exposure to inulin, meaning the E. coli was more resistant to ampicillin when also exposed to inulin, and inulin appeared to be protective to the E. coli. (There was a 739%, 739%, 4067%, and 900% increase in MIC with exposure to just inulin, ascorbic acid with inulin, sodium nitrate with inulin, and sucralose with inulin respectively).

MICs of Chloramphenicol				
	Control	Ascorbic Acid	Sodium Nitrate	Sucralose
Without Inulin	17 µg/mL	2.1 µg/mL	1 µg/mL	1 µg/mL
With Inulin	17 µg/mL	2.1 µg/mL	4.2 µg/mL	<0.5 µg/mL

Table 4. MICs of Chloramphenicol

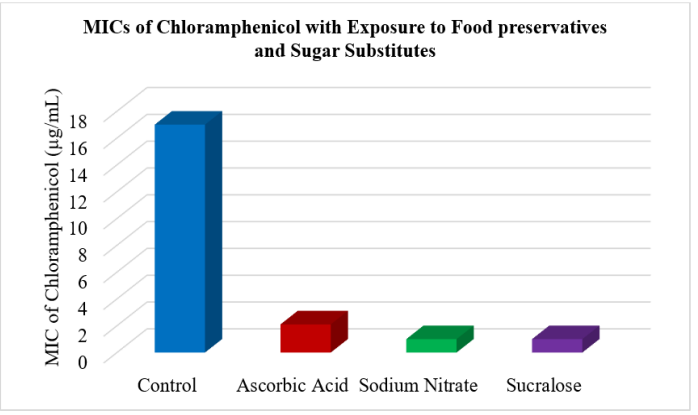


Figure 11. MICs of Chloramphenicol with Exposure to Food Preservatives and Sugar Substitutes

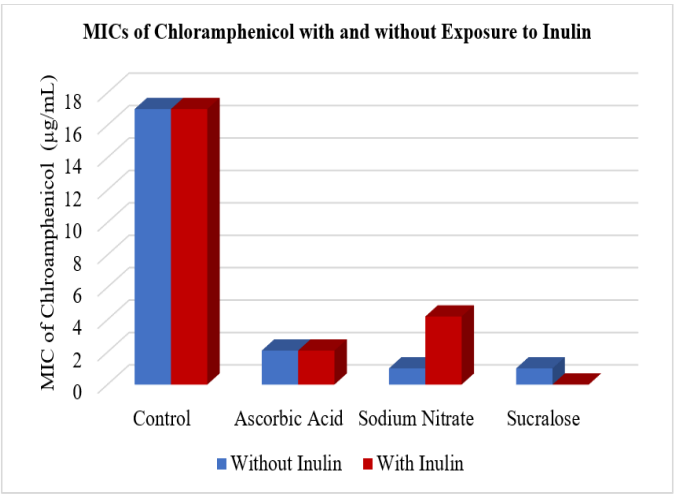
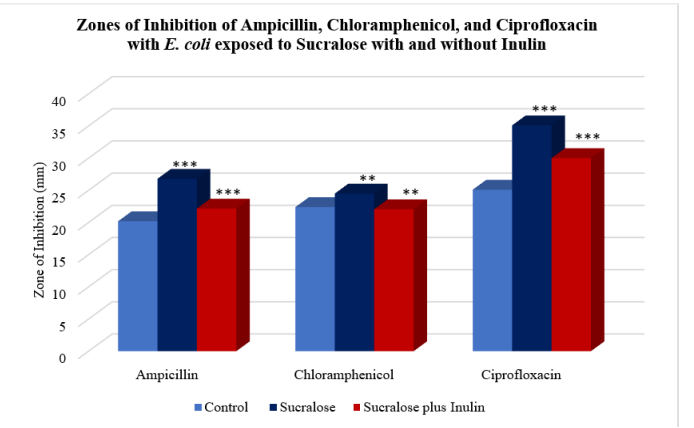


Figure 12. MICs of Chloramphenicol with and without Exposure to Inulin



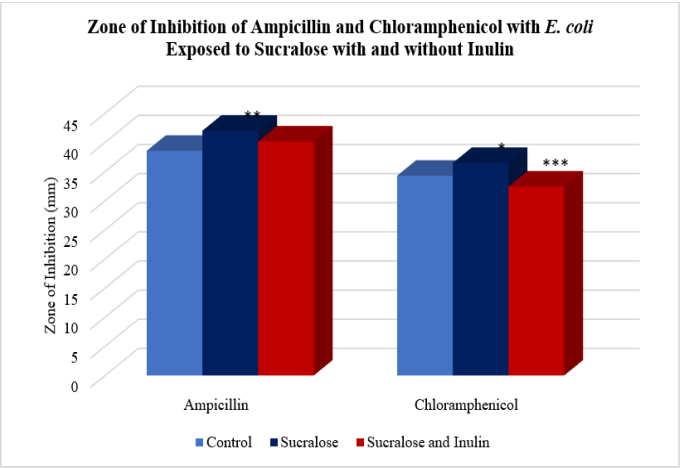
** p<0.01 *** p<0.001

Figure 13. Zones of Inhibition of Ampicillin, Chloramphenicol, and Ciprofloxacin with E. coli Exposed to Sucralose with and without Inulin

The MICs of chloramphenicol with E. coli exposed to food preservatives and sugar substitutes with and without inulin were also determined (see Table 4 and Figures 11-12). The MICs of chloramphenicol all decreased with exposure to food preservatives and sugar substitutes compared to the control. (There was an 88%, 94%, and a 94% decrease with exposure to ascorbic acid, sodium nitrate, and sucralose compared to the control). This again shows that the food preservatives and sucralose increased the susceptibility of E. coli to chloramphenicol. However, only the MIC of chloramphenicol with sodium nitrate increased with the additional exposure to inulin. The MICs of chloramphenicol with the control and ascorbic acid remained the same with the addition of inulin, and the MIC with exposure to sucralose decreased with the addition of inulin.

Average Zones of Inhibition of *E. coli*			
	Ciprofloxacin	Ampicillin	Chloramphenicol
Without Inulin	25.7 mm	20.3 mm	22.4 mm
With Inulin	27.2 mm	22 mm	23.3 mm

Table 5. Average Zones of Inhibition of E. coli



* p<0.025 ** p<0.01 *** p<0.001

Figure 14. Zones of Inhibition of Ampicillin and Chloramphenicol with E. coli Exposed to Sucralose with and without Inulin

Finally, the zones of inhibition of the antibiotic sensitivity disks ampicillin, chloramphenicol, and ciprofloxacin with E. coli were measured with exposure to sucralose with and without the addition of inulin (see Table 5 and Figures 14-15). The average zones of inhibition all increased with exposure to sucralose, suggesting the exposure to sucralose increased susceptibility of E. coli to antibiotics, and the average zones of inhibition all decreased with exposure to inulin in addition to sucralose compared to just exposure to sucralose, suggesting exposure to inulin increased resistance of the bacteria to each antibiotic. A one-tailed t-test was used and the

significance of the difference in these zones of inhibition was tested. The alternate hypothesis that there was a significant difference in the zones of inhibition was accepted with the zones of inhibition of ampicillin ($p<0.001$), chloramphenicol ($p<0.01$), and ciprofloxacin ($p<0.001$) with E. coli exposed to sucralose compared to the control, which were significantly higher than the control. There was also a statistically significant increase the zones of inhibition of ampicillin ($p<0.001$), chloramphenicol ($p<0.01$), and ciprofloxacin ($p<0.001$) with E. coli with exposure to sucralose in addition to inulin compared to just exposure to the sucralose, which were significantly lower compared to just exposure to sucralose. The zones of inhibition of ampicillin ($p<0.01$) or chloramphenicol ($p=0.25$) with L. acidophilus also significantly increased with exposure to sucralose compared to the control, and the zone of inhibition of chloramphenicol ($p<0.001$) significantly decreased with sucralose and the additional exposure to inulin compared to just exposure to sucralose. Again, these results suggest that exposure to sucralose increased susceptibility of bacteria to antibiotics, and additional exposure to inulin with the sucralose increased resistance of bacteria to antibiotics compared to just exposure to sucralose. Also, the L. acidophilus showed complete resistance to the chloramphenicol, regardless of the additive.

CONCLUSION

This project explored the effects of food additives and a prebiotic on gut microbiome, more specifically the effects of sugar substitutes on the growth of gut bacteria and its susceptibility to antibiotics and whether the prebiotic inulin could counteract harmful effects of sugar substitutes or food preservatives on gut bacteria.

The first hypothesis, stating that sugar substitutes would affect the growth of E. coli, was accepted. The lag time of E. coli exposed to stevia was longer than the control, meaning the growth of E. coli was delayed with exposure to stevia. However, an MIC of stevia with E. coli was not found. This could be because stevia might not have antimicrobial properties, or it could be because the MIC of stevia was a higher concentration than would mix in the TSA broth. However, exposure to the sublethal concentration of stevia still delayed the growth of bacteria. An MIC and sublethal concentration of sucralose with E. coli were both found. Therefore, sucralose appears to act similarly to a food preservative or antibiotic in inhibiting the growth of E. coli. These alterations in the growth of E. coli may have been caused by the competition of sugar substitutes as an energy source for the bacteria. The increased lag time with stevia as well as the MIC of sucralose show that sugar substitutes do affect the growth of gut bacteria in vitro, which is concerning as the gut microbiome plays a large role in human health, and some people may consume sugar substitutes daily. This especially may be important when the gut microbiome is already compromised by antibiotics or illness and needs to return to its normal population. While similar results may be expected with the food preservatives as they are added specifically to inhibit the growth of bacteria on food, these results are more unexpected and unintended in sugar substitutes. However, some studies have linked the usage of sugar substitutes to obesity despite the fact that they add very little calories to food, and the reason for this is unclear (Yang, 2010). These results suggest that sugar substitutes inhibit the growth of gut bacteria in vitro, so they may be harming our gut microbiome. As alterations to the gut microbiome have also been linked to obesity, the link between sugar substitutes and obesity may be because of the antimicrobial properties of the sugar substitutes.

The second hypothesis, stating that sugar substitutes would increase susceptibility of E. coli to antibiotics was also accepted. The MICs of ampicillin and chloramphenicol decreased when E. coli was exposed to sucralose compared to the control. This means lower concentrations of the antibiotics killed the bacteria when there was also exposure to food preservatives or sucralose, or E. coli was more susceptible to the antibiotics with exposure to sucralose. This may be because the sucralose worked synergistically with ampicillin and chloramphenicol, as it was also shown to exhibit antimicrobial activity. Also, the disk diffusion tests showed a statistically significant increase in the zone of inhibition of

any antibiotic with either E. coli or ampicillin or chloramphenicol with L. acidophilus when the bacteria was exposed to sucralose compared to the control, meaning exposure to sucralose increased susceptibility of the bacteria to antibiotics. The results of the MICs and zones of inhibition show that sugar substitutes alter the susceptibility of gut bacteria to antibiotics in vitro, meaning they may be altering the susceptibility of gut bacteria to antibiotics every time they are consumed. There is already concern over the effects of antibiotics on the gut microbiome because of the alterations they cause in the gut, and if people are consuming food preservatives or sugar substitutes in addition to antibiotics, this may damage the bacteria further. Also, there was complete resistance of the L. acidophilus to ciprofloxacin regardless of the additive. As L. acidophilus is in many dairy products and is often consumed by most humans, this could be dangerous if the resistance can be transferred to pathogenic species of bacteria.

The third hypothesis, stating that the prebiotic inulin would counteract the effects of sugar substitutes and food preservatives on bacteria, was partially accepted. Inulin was shown to be protective of E. coli with exposure to lethal concentrations of ascorbic acid, sodium nitrate, and sucralose. However, the lag times of E. coli were all longer with exposure to inulin, meaning the growth of bacteria was delayed further with additional exposure to inulin compared to just exposure to the additives. According to Kim and Shin (1996), addition of chicory, which contains inulin, decreased the uptake of glucose in the small intestines of rats. Sucralose is almost identical to sucrose, which is an isomer of glucose, which may be the reason inulin caused these effects in this study. The inulin may have inhibited the uptake of sucralose by the bacteria, allowing E. coli to withstand the lethal concentration of sucralose. However, the inulin may have also inhibited the uptake of the sugar sucrose by the bacteria which could have delayed the growth of the bacteria. While the inulin was protective against the sugar substitutes and food preservatives, there was up to a 240% increase in the lag time of E. coli with additional exposure to inulin. Therefore, if inulin is taken to replenish the gut bacteria after the microbiome has been altered due to illness or the usage of antibiotics, the growth of the bacteria might actually be delayed.

The fourth hypothesis, stating that the prebiotic inulin would counteract the effects of food preservatives and sugar substitutes on the susceptibility of bacteria to antibiotics, was accepted. The MICs of ampicillin all increased with additional exposure to inulin compared to just exposure to the additives, suggesting increased resistance of the E. coli to the antibiotics. The MICs of chloramphenicol with addition of inulin showed mixed results. There was a decrease in the zone of inhibition of any antibiotic with E. coli or ampicillin and chloramphenicol with L. acidophilus with exposure to sucralose plus inulin compared to just sucralose, meaning increased resistance to the antibiotics. This was statistically significant for each antibiotic with either species of bacteria except for chloramphenicol with L. acidophilus. These results show that exposure to sucralose did counteract the effects of sugar substitutes and food preservatives on the susceptibility of bacteria to antibiotics, and inulin may be altering the susceptibility of gut bacteria to antibiotics. While this study is focused on the beneficial bacteria in our gut, there may be similar effects in pathogenic bacteria, and this result is helpful in beneficial bacteria but could be harmful if inulin increases resistance of pathogenic bacteria to antibiotics.

In summary, sugar substitutes and food preservatives appear to inhibit the growth of E. coli and act synergistically with antibiotics. The addition of the prebiotic inulin can exert a protective effect on E. coli against harmful effects of food preservatives or sugar substitutes but may also be delaying the growth of the gut bacteria. The gut microbiome plays an essential role in human health, and it may be altered by consuming these additives. There needs to be more research on the effects of these additives specifically on the gut before it is determined that they are safe to consume.

In the future, to continue this research, other sugar substitutes and prebiotics should be tested for their effects on gut bacteria. It would be interesting to see the effects of these additives on

the virulence of bacteria. Also, this study tested the effects of the additives on gut bacteria in vitro, so more research should be done to see if there are similar effects in vivo.

REFERENCES

Antibiotics: Are you misusing them? (2018, January 18). Retrieved February 4, 2018, from https://www.mayoclinic.org/healthy-lifestyle/consumer-health/in-depth/antibiotics/art-20

045720 Abbeele, P. V., Verstraete, W., Aidy, S. E., Geirnaert, A., & Wiele, T. V. (2013, April 18). Prebiotics, faecal transplants and microbial network units to stimulate biodiversity of the human gut microbiome. Retrieved October 25, 2017, from http://onlinelibrary.wiley.com/doi/10.1111/1751-7915.12049/full

Bull, M. J., & Plummer, N. T. (2014, December). Part 1: The Human Gut Microbiome in Health and Disease. Retrieved October 25, 2017, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4566439/

CDC Features. (2017, December 15). Retrieved February 4, 2018, from https://www.cdc.gov/features/antibioticuse/index.html

Center for Food Safety and Applied Nutrition. (n.d.). Food Additives & Ingredients - Additional Information about High-Intensity Sweeteners Permitted for use in Food in the United States. Retrieved October 25, 2017, from https://www.fda.gov/food/ingredientspackaginglabeling/foodadditivesingredients/ucm397725.htm

Chloramphenicol. (n.d.). Retrieved March 01, 2018, from https://amrls.cvm.msu.edu/pharmacology/antimicrobials/antibiotics-of-veterinary-importance/chloramphenicol

Commissioner, O. O. (n.d.). Consumer Updates - How Sweet It Is: All About Sugar Substitutes. Retrieved October 25, 2017, from https://www.fda.gov/ForConsumers/ConsumerUpdates/ucm397711.htm

Gibson, G. R., McCartney, A. L., & Rastall, R. A. (2007, March 01). Prebiotics and resistance to gastrointestinal infections | British Journal of Nutrition. Retrieved January 10, 2018, from https://www.cambridge.org/core/journals/british-journal-of-nutrition/article/prebiotics-and-resistance-to-gastrointestinal-infections/CE7BC182188DD53BC6EEC551460CA57F

Issa, I., & Moucari, R. (2014, December 21). Probiotics for antibiotic-associated diarrhea: Do we have a verdict? Retrieved January 10, 2018, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4273129/

Jahreis, K., Bentler, L., Bockmann, J., Hans, S., Meyer, A., Siepelmeyer, J., & Lengeler, J. W. (2002, October). Adaptation of Sucrose Metabolism in the Escherichia coli Wild-Type Strain EC3132†. Retrieved October 25, 2017, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC135337/

Kim, M., & Shin, H. K. (1996, September). The water-soluble extract of chicory reduces glucose uptake from the perfused jejunum in rats. Retrieved March 02, 2018, from https://www.ncbi.nlm.nih.gov/pubmed/8814212

LeBel, M. (n.d.). Ciprofloxacin: chemistry, mechanism of action, resistance, antimicrobial spectrum, pharmacokinetics, clinical trials, and adverse reactions. Retrieved January 10, 2018, from https://www.ncbi.nlm.nih.gov/pubmed/2836821

L. (2016, July 21). Spectrophotometry. Retrieved March 02, 2018, from https://chem.libretexts.org/Core/Physical_and_Theoretical_Chemistry/Kinetics/Reaction_Rates/Experimental_Determination_of_Kinetcs/Spectrophotometry

NIH Human microbiome project . (2017) . Retrieved from https://www.hmpdacc.org/hmp/

Quigley, E. M. (2013, September). Retrieved March 02, 2018, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3983973/

Suez J1, Korem T, Zilberman-Schapira G, Segal E, Elinav E. (2015) . Non-caloric artificial sweeteners and the microbiome: findings and challenges. [Abstract] . Gut microbes 6(2), 149–155. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4615743/

S., & Wagner-GrahamMary, A. (n.d.). Molecular Analysis Research at Community College of Philadelphia. Retrieved October 25, 2017, from http://www.dtic.mil/docs/citations/AD1001089

Shreiner, A. B., Kao, J. Y., & Young, V. B. (2015, January). The gut microbiome in health and in disease. Retrieved October 25, 2017, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4290017/

Slavin, J. (2013, April). Fiber and Prebiotics: Mechanisms and Health Benefits. Retrieved December 25, 2017, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3705355/

Tandel, K. R. (2011). Sugar substitutes: Health controversy over perceived benefits. Retrieved October 25, 2017, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3198517/

Tilg, H., & Kaser, A. (2011, June 01). Gut microbiome, obesity, and metabolic dysfunction. Retrieved March 02, 2018, from https://www.jci.org/articles/view/58109

Walsh, F. (2014, December 11). Superbugs to kill 'more than cancer' by 2050. Retrieved February 4, 2018, from http://www.bbc.com/news/health-30416844

Wong, A., Ngu, D. Y., Dan, L. A., Ooi, A., & Lim, R. L. (2015). Retrieved March 02, 2018, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4568587/

Yang, Q. (2010, June). Retrieved March 02, 2018, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2892765/