12-2020

Finding the Balance The Effects of α-cyclodextrin, 2-hydroxypropyl-β-cyclodextrin, and cholesterol Bacteroides vulgatus and Clostridium bolteae

Bethany Weaver
Olivet Nazarene University, mmlbs2@gmail.com

a

FINDING THE BALANCE: THE EFFECTS OF α-CYCLODEXTRIN, 2-HYDROXYPROPYL-β-CYCLODEXTRIN, AND CHOLESTEROL ON *BACTEROIDES VULGATUS* AND *CLOSTRIDIUM BOLTEAE*

By

Bethany G. Weaver

Pence Boyce Summer Research

To my Father whose struggle with high cholesterol inspired me to search for treatments for atherosclerosis

ACKNOWLEDGEMENTS

This research was made possible by the Pence-Boyce summer research program, the Honors program, and Catalyst. I am grateful to have also received funding from the Department of Biological sciences, the Department of Chemistry and Geosciences. I would like to thank Dr. Himes for all his work as my research mentor. I would also like to thank Dr. Sharda and Dr. Heyen for their contributions. I want to extend a thank you to my peers, from the Honors program and from the Pence-Boyce summer research program, for all the encouragement.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Atherosclerosis is a cardiovascular disease that is characterized by the hardening of arteries through the formation of cholesterol plaques. Cyclodextrins could potentially treat atherosclerosis by shrinking plaques. These cyclic oligosaccharides can make complexes with cholesterol but have also shown toxic side effects. This study looked for potential negative effects of cyclodextrins and cholesterol on gut bacteria. It was hypothesized that *Bacteroides vulgatus* will have decreased growth when grown in broth with cholesterol. In contrast, *Clostridium bolteae* will have decreased growth when grown in broth with cyclodextrins. Due to the fact that these bacteria are anaerobic, *Clostridium bolteae* and *Bacteroides vulgatus*, were grown using GAM broth under CO_2. Data was collected by using a spectrophotometer to measure changes in bacterial growth throughout the growth cycle. Each bacteria was treated with one of three chemicals at one of three concentration to make a total of 9 different conditions. α-cyclodextrin, 2-hydroxypropyl-β-cyclodextrin, and cholesterol with concentrations of 1 mM, 10 mM, and 100 mM. The slopes of the log phase of bacterial growth were compared using a two tailed T-Test with $\alpha=0.05$. Growth of *Clostridium bolteae* was significantly inhibited for most of the cyclodextrin trials and all the cholesterol concentrations. *Bacteroides vulgatus* growth was inhibited by 100 mM concentrations of cholesterol and α-cyclodextrins. Interestingly, *Bacteroides vulgatus* growth was significantly increased when grown with 1 mM and 10 mM concentrations of cholesterol. Continued research is needed to determine when cholesterol concentrations become harmful to *Bacteroides vulgatus*. *Clostridium bolteae* needs further research to understand the 10 mM α-cyclodextrin trial that did not show significant inhibition. The results demonstrate that cyclodextrins are associated with inhibited growth for these two

gut bacteria but expansion of this test to other gut bacteria is key for a deeper understanding of the impact that cyclodextrins would have on the gut microbiome as a whole.

INTRODUCTION

Atherosclerosis is a cardiovascular disease that has one of the highest mortality rates in the world. It is characterized by the formation of cholesterol-filled plaques that are chronically inflamed and continue to develop until they occlude the artery or break off causing a stroke or heart attack. Statins are the current standard treatment for atherosclerosis and for the precursor to atherosclerosis, high cholesterol. These drugs lower the levels of unhealthy lipoproteins to slow the progression of plaques (Lusis 2000). However, there remains a gap in medical treatment for a way to reduce inflammation and remove cholesterol from plaques to lower the risk for heart attacks and strokes. Cyclodextrins are oligosaccharides that have been found to regress atherosclerosis by decreasing inflammation and shrinking plaques (Zimmer et al. 2016). Cyclodextrins are known to extract cholesterol from membranes. The possible mechanism for this removal was shown using a computer simulation; it demonstrated that cyclodextrins form dimers to pull out cholesterol molecules, forming 2:1 cyclodextrin-cholesterol complexes (LóPez et al. 2011). Cholesterol is key to the structural integrity of cell membranes; it is possible that cyclodextrins are causing changes in cell membranes of gut bacteria that is impacting their growth. The present study was designed to test the impact of cyclodextrins on the growth of gut bacteria.

2-Hydroxypropyl-β-cyclodextrin (HPβCD) has been shown to solubilize cholesterol and have minimal cytotoxic effects on human cells (Szente et al. 2018). HPβCD is being studied in clinical trials for the treatment of Niemann-Pick C, a fatal hereditary disease that can be characterized by the buildup of cholesterol in lysosomes (Singhal et al. 2018). HPβCD promotes the removal of cholesterol from these lysosomes

through a similar mechanism to atherosclerotic plaque regression. HPβCD is a frontrunner in atherosclerosis research because of its success in Niemann-Pick C clinical trials (Singhal et al. 2018). Another commonly studied cyclodextrin for atherosclerosis treatment is α-cyclodextrin (αCD). αCD was found to inhibit inflammatory pathways better than HPβCD (Pilely et al. 2019). In one study, αCD was also found to decrease obesity by improving lipid metabolism in mice. Interestingly, these mice were fed high fat diets which negatively affected their gut microbiome; then other mice were fed the same diet with αCD and had another large change in bacterial composition as shown in the graph below. There was a decrease in the *Bacteroides* genus in mice fed a high fat diet (HFD) but they rebounded in mice that were treated with αCD along with their HFD. On the other hand, *Clostridium* cluster XIVa was increased in mice that were fed a HFD but drastically decreased when αCD was added.

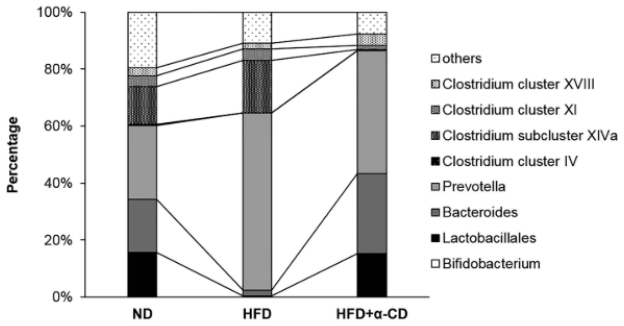

Figure 1. This graph depicts the change in gut bacteria population in mice. The three columns compare mice fed a normal diet (ND), mice fed a high fat diet (HFD) and mice fed a HFD and treated with αCD (Nihei et al. 2018).

These results raise the following questions: what exactly is impacting the growth of these bacteria? Is it the high cholesterol levels, introduction of cyclodextrin or both? Cyclodextrins are not absorbed so they must be fermented by the gut bacteria. This means there is direct contact between the bacteria and these molecules (Amar et al.

2016). A study done of human erythrocytes and a Caco-2 cell line showed that αCD was cytotoxic to these cells; however, once a complex was formed with cholesterol the cytotoxicity of αCD decreased. This same study proposed that αCD could also form complexes with phospholipids (Roka et al. 2015). The mechanism for cyclodextrin cytotoxicity has not been confirmed but is speculated to be related to the removal of cholesterol from membranes and potentially removal of phospholipids as well, which could be dangerous for the bacteria of the human microbiome. This goal of the experiment is to observe any major cytotoxic effects that cyclodextrins may have on human gut bacteria and test the theorized protective qualities of the cyclodextrin-cholesterol complexes.

The human microbiome has become the focus of research for a variety of diseases because of its connectivity to human health. Atherosclerosis is no exception; distinct changes in the human gut microbiome have been noted in patients with atherosclerosis (Jie et al. 2017). Analysis of these changes of bacteria found that inflammation increased when certain strains of bacteria were decreased *in vivo* (Yoshida, Naofumi Emoto, Takuo Yamashita 2018). *Clostridium bolteae* and *Bacteroides vulgatus* are anaerobic bacteria found in the human gut microbiome (Maier et al. 2018). Each has their own unique properties and benefits for overall health. *B. vulgatus* was studied specifically in mice with induced atherosclerosis and was found to have anti-inflammatory effects (Yoshida, Naofumi Emoto, Takuo Yamashita 2018). This is an important piece of information because the genus, *Bacteroides*, was found to be less prevalent in the gut of mice that were fed a high fat diet. On the other hand, *C. bolteae* is a member of *Clostridium* cluster XIVa which was found to be increased in mice being fed a high fat diet (Nihei et al.

2018). These changes can be seen in the figure above. *Clostridium* bacteria have been found to both be proinflammatory and to help regulate inflammation depending on the species (Barnes and Powrie 2011). A healthy balance of gut bacteria is key to a healthy host. It is vitally important to be aware of how different treatments affect microbiome health to ensure that they are as beneficial as possible and do not have unforeseen side effects. Cyclodextrins could be damaging these bacterial membranes by removing cholesterol from them, or through a different mechanism, but either way it is important to see if these bacteria are being negatively affected by cyclodextrin treatment. The human microbiome plays an important role in atherosclerosis so the health of these bacteria is vital and should be considered when testing possible treatments.

Despite the promising data suggesting that cyclodextrins may help to regress atherosclerotic plaques, the specific, direct effects of cyclodextrins on the gut bacteria *B. vulgatus* and *C. bolteae* are not clear. *In vivo* studies are plagued by complex variables that are difficult to control. Isolating individual bacteria for testing can negate these variables. *B. vulgatus* was decreased in both human and mouse studies when under atherosclerotic conditions, while *C. bolteae* was decreased when αCD was introduced into the diet of the mice. This leads to the central questions being addressed in this current proposal: what are the effects of cyclodextrins on these bacterial strains, and does co-treatment with cholesterol change those effects? Cholesterol and cyclodextrins form complexes with a dimer of cyclodextrins pulling a cholesterol molecule in and this complexation may combine the potentially harmful chemicals into a harmless complex. Based on the changes that occur *in vivo* the following hypotheses were developed:

1. *Bacteroides vulgatus* will have decreased growth when grown in broth with cholesterol. 2. *Clostridium bolteae* will have decreased growth when grown in broth with cyclodextrins.

METHODS

Materials

Clostridium bolteae – ATCC

Bacteroides vulgatus – ATCC

αCD 98% (100g) – Fischer Scientific

HPβCD mw ~ 1540 (100g) – Fischer Scientific

Cholesterol 92.5% (25g) – Sigma Aldrich

Gifu Anaerobic Broth (500g) – Thomas Scientific

Falcon Tubes (12mL)

Single use sterile pipets (1mL, 5mL & 10mL)

Equipment

UV/VIS spectrophotometer

Shaking water bath incubator

Carbon Dioxide gas tank

Methods

Bacteroides vulgatus was revived from frozen stock in gifu anaerobic broth (GAM) in a shaking water incubator. Growth curves were determined using the same procedure for each trial: two bacterial falcon tubes were made containing 10 mL of fresh broth and 0.5 mL of bacteria containing broth. These tubes were made anaerobic by filling them with carbon dioxide gas then immediately sealing them. 1 mL samples were taken every hour, alternating between two tubes. Every time a tube was opened it was filled with carbon dioxide again. The absorbance of these samples were immediately measured at 560 nm using the UV/Vis spectrophotometer. The absorbance and time of each sample were recorded to create the growth curve. Untreated samples were tested every hour for 6 hours followed by two additional tests with two hour gap. This was found to accurately capture the growth curve of *B. vulgatus* and was used for the experimental trials as well. αCD, HPβCD, and cholesterol were all tested individually at three different concentrations: 1mM, 10mM and 100mM. Three trials were run for each treatment to limit random error. Combined trials were run for these four different concentrations: 100mM αCD +100mM cholesterol, 100mM αCD +50mM cholesterol, 100mM HPβCD +100mM cholesterol, and 100mM HPβCD +50mM cholesterol. Each combination was run with two trials each.

Clostridium bolteae was rehydrated from a freeze-dried stock in GAM broth in a shaking water bath then frozen stocks were made the same way as previously stated. All methods were the same for *C. bolteae* apart from the times that samples were taken. The absorbances were recorded at these times: 0.5hr, 1hr, 2hr, 2.5hr, 3hr, 4hr, 5hr, 6hr.

Growth curves were by measuring the slope of the log phase growth where the linear upward slope was found. The slopes of each trial were compared to the slopes of the control with a two-tailed T-test. The data is displayed as means +/- standard deviation and P values of less than 0.05 will be used as the threshold for determining statistical significance.

RESULTS

Bacteroides vulgatus and *Clostridium bolteae* have nine different growth curves for the nine treatments. The standard growth curve follows a sigmoidal shape with a linear growth period. The slope of the linear growth was found using a line of best fit. The slopes were compared to a standard control slope. Figure 2 shows all three alpha cyclodextrin treatments for *Bacteroides vulgatus* along with a control curve. The right graph shows the curves after they have been cropped to only display the linear growth period. Of the three alpha cyclodextrin treatments on *Bacteroides vulgatus* only 100 mM was shown to significantly decrease growth.

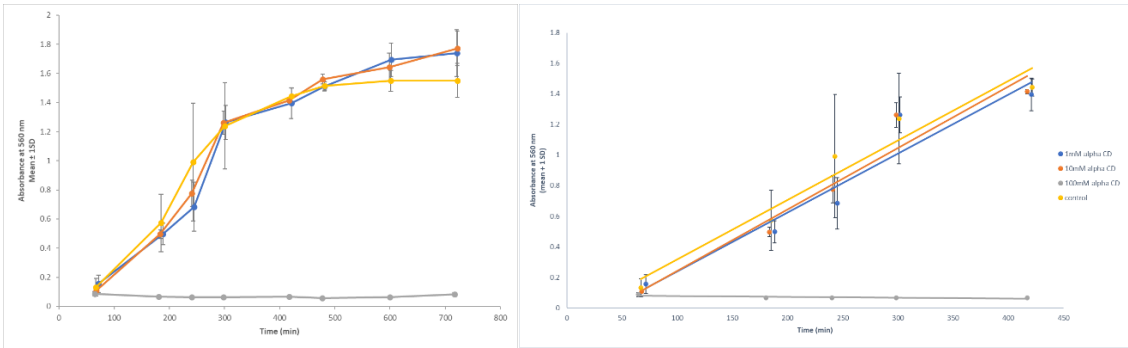

Figure 2. The effects of alpha cyclodextrin on *Bacteroides vulgatus* growth. These two graphs display the same set of data. The data displayed were from mean values with error bars showing 1 standard deviation. The second graph has been cropped to only display the linear segment of growth. The line of best fit was found for this segment then compared using a two-tailed T-test.

Bacteroides vulgatus had decreased growth with two treatments and two other treatments had increased growth. 1 mM and 10 mM cholesterol showed a significant increase in growth rate. 100 mM concentrations of cholesterol and αCD were shown to inhibit growth. Figure 3 depicts the growth curves for all four treatments along with a standard growth curve.

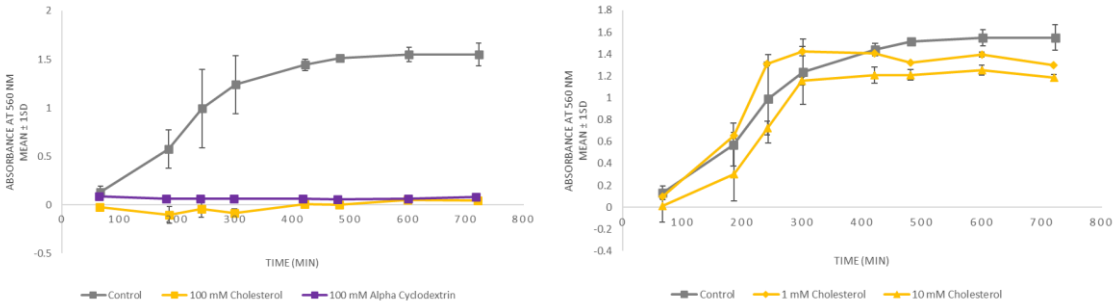

Figure 3. These graphs are depicting all the results that were statistically significant for *Bacteroides vulgatus*. T-test was run with alpha = 0.05 to compare the slopes of the log phase.

Clostridium bolteae had a statistically significant decrease in growth for eight of the nine treatments. All HPβCD and all cholesterol along with 1 mM and 100 mM αCD were shown to inhibit *Clostridium bolteae* growth. Theses eight treatment have a growth curve that is displayed in figure 4.

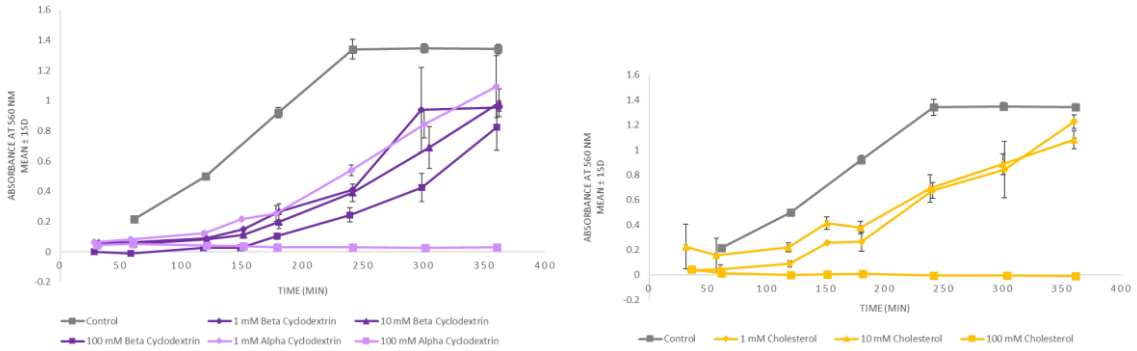

Figure 4. These graphs are depicting all the results that were statistically significant for *Clostridium bolteae*. T-test was run with alpha = 0.05 to compare the slopes of the log phase growth. All the concentrations shown were inhibiting growth.

Table 1 includes all the mean slope values with one standard deviation along with their corresponding p values. The minimum significant p value is 0.05. There are twelve statistically significant treatments between both of the bacteria tested.

Table 1. Results from *Bacteroides vulgatus* and *Clostridium bolteae* under varying conditions. Slopes from the log phase of graphs for each trial were analyzed by a two- tailed t-test, p values are shown below. There were varied levels of significance, values with p≤0.05 are marked with *, p≤0.01 is marked with **, and p≤0.001***.

		Bacteroides vulgatus		*Clostridium bolteae*	
Treatment	Concentration	Slope ± 1SD	T.TEST p value	Slope ± 1SD	T.TEST p value
Control		0.0034 ± 0.00036		0.0052 ± 0.0002	
Alpha Cyclodextrin	1mM	0.00353 ± 0.00029	0.6433	0.00403 ± 0.00058	0.02973*
	10mM	0.00367 ± 0.000058	0.2746	0.0052 ± 0.00035	1
	100mM	-0.000053 ± 0.000021	0.00007785***	-0.000087 ± 0.000058	0.000001363***
Beta Cyclodextrin	1mM	0.0034 ± 0.0001	1	0.00423 ± 0.0004	0.0206*
	10mM	0.00357 ± 0.000058	0.4734	0.00413 ± 0.00042	0.01613*
	100mM	0.00303 ± 0.000058	0.157	0.00357 ± 0.00071	0.01849*
Cholesterol	1mM	0.00603 ± 0.00015	0.000311***	0.00463 ± 0.00012	0.01316*
	10mM	0.0048 ± 0.00066	0.03166*	0.00353 ± 0.00029	0.001194**
	100mM	-0.00022 ± 0.000144	0.00008642***	-0.00005 ± 0.00001	0.000001407***

DISCUSSION

Bacteroides vulgatus was treated with nine different trials. Of those nine trials, four were statistically significant and this can be seen in table 1. Although five of the nine trials showed no significant change in growth, these are still important trials. 2-hydroxypropyl-β-cyclodextrin is specifically designed to be less toxic, the added hydroxypropyl groups decrease the affinity for cholesterol which has been shown to lower the toxicity (Szente et al. 2018). The data for *Bacteroides vulgatus* is consistent with this purpose, even at 100 mM concentration the growth of this bacteria was not significantly inhibited. α-cyclodextrin is unsubstituted but this does not mean there are no toxic effects. On the contrary the toxic effects can be seen in the trial when *Bacteroides vulgatus* was treated with 100 mM α-cyclodextrin and it was significantly inhibited. This inhibition is not present at lower concentrations. This is consistent with the previous study that α-cyclodextrin is slightly more toxic than 2-hydroxypropyl-β-cyclodextrin but both are still being studied as treatments for atherosclerosis (Szente et al. 2018). The hypothesis for *Bacteroides vulgatus* was that it would be inhibited when grown in broths with cholesterol. This was based on study by Jie et al. on patients with atherosclerosis where their gut microbiome was analyzed for consistent changes occurring in most atherosclerotic patients. The Bacteroides genus was decreased in general, and it was suspected that these bacteria grow poorly under high fat conditions that usually associated with atherosclerosis. This was supported by the study done by Nihei et al. on mice. Cholesterol was tested because of the limited ability to replicate a high fat diet in vitro. This hypothesis is supported at 100 mM cholesterol concentrations where *Bacteroides vulgatus* was significantly inhibited. Surprisingly, 1 mM and 10 mM

concentrations of cholesterol significantly increased the growth of *Bacteroides vulgatus.* There was more increased growth in the 1 mM than the 10 mM then at 100 mM the growth becomes inhibited. The mechanism for this increased growth in unknown. The data presented for the lower concentrations of cholesterol contradict the predicted trend. This may be to due to the fact that the studies were using high fat diets while this study looked at cholesterol alone (Jie et al. 2017; Nihei et al. 2018). There is more research to be done to see where the concentrations of cholesterol transitions from beneficial to harmful. There is also a need for this study to be replicated to see if similar results are being found. This is only one species in the Bacteroides genus and it would be beneficial to learn if the entire genus reacts similarly to these conditions. It is difficult to draw large conclusions based off a study on a specific bacteria when the gut microbiome is a diverse population. The diversity of the microbiome is key to its function and a possible unwanted side effect of cyclodextrins could be the destruction of that diversity.

Clostridium bolteae was treated with the same nine trials as *Bacteroides vulgatus*. This bacteria was more susceptible to inhibition; it was significantly inhibited by eight of the nine trials. Interestingly, the only trial that did not show any significant changes in growth was 10 mM α-cyclodextrin. All other data was consistent with previous studies. Similar to *Bacteroides vulgatus*, 100 mM concentrations of cholesterol and α-cyclodextrin are inhibiting growth significantly. The slopes are nearly zero for these two trials as it is seen in table 1. The lower concentrations of α-cyclodextrin and cholesterol, with the exception of 10 mM α-cyclodextrin, make intuitive sense. The lower the concentration, the lower the significance in growth inhibition. 2-hydroxypropyl-β-cyclodextrin is expected to be less toxic than α-cyclodextrin. In 1 mM concentrations the

two significance values are very similar and 10 mM concentrations are complicated. When comparing the 100 mM concentration the expected result can be clearly seen. 100 mM α-cyclodextrin has a slope of nearly zero while 100 mM 2-hydroxypropyl-β-cyclodextrin still shows growth. This is consistent with the idea presented earlier that 2-hydroxypropyl-β-cyclodextrin is less toxic than α-cyclodextrin (Szente et al. 2018). The hypothesis for *Clostridium bolteae* was that there would be inhibited growth when grown in both with cyclodextrins. This is supported by results from a study on mice that were treated with cyclodextrins, and they had decreased Clostridium populations (Nihei et al. 2018). In general, this is supported with the data from this study, except for 10 mM α-cyclodextrin. It is key to note that *Bacteroides vulgatus* was unaffected by 2-hydroxypropyl-β-cyclodextrin but *Clostridium bolteae* had significant growth inhibition at all concentrations. This supports that hypothesis that *Clostridium bolteae* is specifically inhibited by cyclodextrins even the one deemed safer than the others 2-hydroxypropyl-β-cyclodextrin. *Clostridium bolteae* also showed significant growth inhibition for cholesterol at all concentrations. In Jie's study on atherosclerotic patients the Clostridium genus was increased in these patients, unlike the Bacteroides genus. There is not research supporting the decreased growth of *Clostridium bolteae* in cholesterol. *Bacteroides vulgatus* is not entirely representative the Bacteroides genus but the Clostridium genus is even more varied (Barnes and Powrie 2011). There is a need for further research on other members of the Clostridium genus to explain the increase of the population under atherosclerotic conditions. There is a possibility that *Clostridium bolteae* decreases but other species thrive. This points to the importance of expanding the study to include a large variety of species of gut bacteria.

CONCLUSION

The results from these two bacteria show that cyclodextrins can inhibit gut bacteria although further research should be done to see if this inhibition will be universally detrimental to the gut microbiome. The results indicate that there is some dose dependence on the inhibition. The health of gut microbiome composition should be monitored in future clinical research. Further research is needed to fully understand the general impact that cyclodextrins would have on the gut microbiome. This study was limited by the small number of bacteria tested along with limited concentration range. The maintenance of an anerobic environment was difficult to control and while it was every attempt was made to control the environment, oxygen contamination could contribute to the results. Overall, this research supports cyclodextrins having adverse side effects on gut bacteria while raising new questions for future work.

REFERENCES

Amar MJA, Kaler M, Courville AB, Shamburek R, Sampson M, Remaley AT. 2016. Randomized double blind clinical trial on the effect of oral α-cyclodextrin on serum lipids. Lipids Health Dis. 15:115. doi:10.1186/s12944-016-0284-6. [accessed 2019 Sep 8]. http://lipidworld.biomedcentral.com/articles/10.1186/s12944-016-0284-6.

Barnes MJ, Powrie F. 2011. The Gut's Clostridium Cocktail. Science (80-.). 331:289–290. doi:10.1126/science.1201291. [accessed 2019 Sep 15]. www.sciencemag.orgSCIENCEVOL.

Jie Z, Xia H, Zhong S-L, Feng Q, Li S, Liang S, Zhong H, Liu Z, Gao Y, Zhao H, et al. 2017. The gut microbiome in atherosclerotic cardiovascular disease. Nat. Commun. 8:845. doi:10.1038/s41467-017-00900-1. [accessed 2019 Sep 8]. www.nature.com/naturecommunications.

LóPez CA, De Vries AH, Marrink SJ. 2011. Molecular Mechanism of Cyclodextrin Mediated Cholesterol Extraction. PLoS Comput Biol 7:1002020. doi:10.1371/journal.pcbi.1002020. [accessed 2019 Sep 1]. www.ploscompbiol.org.

Lusis AJ. 2000. Atherosclerosis. Nature 407:233–241.

Maier L, Pruteanu M, Kuhn M, Zeller G, Telzerow A, Erin anderson E, rita Brochado A, conrad Fernandez K, Dose H, Mori H, et al. 2018. Extensive impact of non-antibiotic drugs on human gut bacteria. Nature 555:623–627. doi:10.1038/nature25979. [accessed 2019 Sep 3].

https://search.proquest.com/docview/2020725614/fulltextPDF/49F1ABBC2A084BC9PQ/1?accountid=12974.

Nihei N, Okamoto H, Furune T, Ikuta N, Sasaki K, Rimbach G, Yoshikawa Y, Terao K. 2018. Research Communication Dietary a-cyclodextrin modifies gut microbiota and reduces fat accumulation in high-fat-diet-fed obese mice. Biofactors:336–347. doi:10.1002/biof.1429. [accessed 2019 Aug 29]. http://content.ebscohost.com/ContentServer.asp?T=P&P=AN&K=29733482&S=R&D=mnh&EbscoContent=dGJyMNLe80SeqLc4v%2BvlOLCmr1GeqK5Sr6q4SbaWxWXS&ContentCustomer=dGJyMPGuslC3rLZIuePfgeyx44Dt6fIA.

Pilely K, Bakke SS, Palarasah Y, Skjoedt M-O, Bartels ED, Espevik T, Garred P. 2019. Alpha-cyclodextrin inhibits cholesterol crystal-induced complement-mediated inflammation: A potential new compound for treatment of atherosclerosis. Atherosclerosis 283:35–42. doi:10.1016/J.ATHEROSCLEROSIS.2019.01.034. [accessed 2019 Aug 26]. https://www.sciencedirect.com/science/article/pii/S0021915019300589.

Singhal A, Szente L, Hildreth JEK, Song B. 2018. Hydroxypropyl-beta and -gamma cyclodextrins rescue cholesterol accumulation in Niemann–Pick C1 mutant cell via lysosome-associated membrane protein 1. Cell Death Dis. 9:1019. doi:10.1038/s41419-018-1056-1. [accessed 2019 Sep 8]. http://www.nature.com/articles/s41419-018-1056-1.

Szente L, Singhal A, Domokos A, Song B. 2018. Cyclodextrins: Assessing the Impact of Cavity Size, Occupancy, and Substitutions on Cytotoxicity and Cholesterol

Homeostasis. Molecules 23:1–15. doi:10.3390/molecules23051228. [accessed 2019 Aug 29]. www.mdpi.com/journal/molecules.

Yoshida, Naofumi Emoto, Takuo Yamashita T. 2018. Bacteroides vulgatus and Bacteroides dorei Reduce Gut Microbial Lipopolysaccharide Production and Inhibit Atherosclerosis. Circulation 138:2486–2498. doi:10.1161/CIRCULATIONAHA.118.033714. [accessed 2019 Sep 15]. http://trace.ddbj.nig.

Zimmer S, Grebe A, Bakke SS, Bode N, Halvorsen B, Ulas T, Skjelland M, De Nardo D, Labzin LI, Kerksiek A, et al. 2016. Cyclodextrin promotes atherosclerosis regression via macrophage reprogramming. Sci. Transl. Med. 8:333ra50. doi:10.1126/scitranslmed.aad6100. [accessed 2019 Aug 26]. http://www.ncbi.nlm.nih.gov/pubmed/27053774.