JOURNAL OF
EMERGING INVESTIGATORS

Article

Impacts of the gut microbiota on arginine synthesis

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SUMMARY

The presence of the amino acid arginine–crucial for tissue deposition and protein synthesis–impacts fetal development. Lower maternal arginine availability is correlated with lower birth weight. Certain microbial species are associated with specific geo-localities and diets, and some bacteria synthesize arginine. Thus, there may be diverse gut microbiota populations in pregnant women from different regions that influence arginine synthesis. In this study, we tested bacteria geographically representative of regional diets and capable of arginine synthesis. We hypothesized that arginine flux in the presence of the *Bacillus megaterium* would be significantly higher than in the presence of *Lactobacillus acidophilus, Escherichia coli*, and *Clostridium sporogenes*. We inoculated control plates and 4 sets of plates treated with one bacterial species (corresponding to four regional diets) with a 20% citrulline solution–an arginine precursor compound–and incubated. Their pH was evaluated during the 1-week incubation period to determine the arginine synthesis rate. The changes in the pH of *C. sporogenes* plates and the *B. megaterium* plates were significantly different from the other groups ($p<0.0001$). Their higher pH suggests greater synthesis of arginine. Abundances of *B. megaterium* and *C. sporogenes* are associated with Western and Mediterranean diets, respectively. This suggests that both diets offer means of gastrointestinal microbiota modulation to an environment conducive to arginine synthesis.

INTRODUCTION

Fetal nutrition depends on three primary elements: maternal nutritional intake, nutrient availability in the maternal circulation, and placental ability to transport substrates from the maternal circulation to the fetal circulation (1). Maternal nutrition is crucial in determining placental size, morphology, and nutrient transporter abundance (1). Proper maternal nutrition also contributes to fetal growth and development. Hence, suboptimal maternal nutrition is one of the most significant contributing factors to intrauterine growth restriction (IUGR) (1). Along with neonatal health and conditions, nutrition also impacts immediate postnatal health. IUGR causes 50% of nonmalformed infant deaths and also impacts fetal propensity for genetic and metabolic disorders as well as chronic diseases later on in life (2).

The nutritional presence of the amino acid arginine impacts fetal growth and development (1). Pregnancy requires increased tissue deposition from mothers to fully develop a fetus; arginine is crucial for this process because it is used to synthesize new proteins (3). Lower maternal arginine availability is strongly associated with lower birth weight (3). Arginine also serves as the precursor to other vital fetal compounds including nitric oxide, creatine, and polyamines, all of which contribute to cell differentiation and proliferation in the earliest stages of fetal development (3). Decreased levels of arginine in maternal diets are associated with IUGR and fetal resorption. Fetal growth restriction (FGR) occurs in approximately 3%-7% of pregnancies worldwide, and in developing countries, the prevalence of FGR is 6 times higher (2).

The complex ecosystem of microorganisms residing in the gastrointestinal tract, often referred to collectively as gut microbiota, aids the fulfillment of human nutrition requirements, digestive processes, and disease prevention (4). Diet and nutrition are two of the most influential factors in both the quantity and composition of these gut microbiota (4). Dietary stipulations often form patterns throughout geographical regions based on agricultural and cultural practices; therefore, certain gut microbiota are associated with populations in specific areas.

Because of this, there may be diverse gut microbiota populations in pregnant women in different regions with distinct impacts on arginine metabolism and synthesis (3). However, some bacterial species such as *Lacticaseibacillus paracasei* and *Bacteroides vulgatus* have arginine auxotrophies—the inability to synthesize their supply of this specific nutrient (5). Microbial species tested in this study needed to be both geographically representative and capable of arginine synthesis. The current void in the literature indicates a need for further research to investigate the impacts of altering the gut microbiota–potentially through diet–on arginine availability in pregnant women. Arginine can be produced from citrulline by the enzymes argininosuccinate synthetase and argininosuccinate lyase in the cytosol. Some bacteria can then use the twin-arginine translocation (Tat) pathway to secrete arginine (6,7).

For this comparative study that examines arginine synthesis facilitated by the arginine precursor compound citrulline in the presence of different bacterial genera, four bacterial species were selected: *Bacillus megaterium, Lactobacillus acidophilus, Escherichia coli*, and *Clostridium sporogenes*. These species are representative of predominant species found in typical gut microbiota compositions of people from Western Europe and the U.S., East Asia, West Africa, and the Mediterranean region, respectively, though many of these species would be found in varying concentrations across

https://doi.org/10.59720/23-305

regions. The chosen species were ideal because they were readily accessible as well as culturable, aerobic organisms whose growing conditions were most easily replicated.

Bacillus subtilis is one of the most studied bacteria for examining the arginase pathway due to its effective gene regulation (8). Out of the four species in our study, *B. megatherium* has the most similar biochemical profile to *B. subtilis* because it is from the same genus, thus we hypothesized that *B. megatherium* would potentially have greater arginine flux than the species *L. acidophilus, E. coli,* and *C. sporogenes* (8). Indeed, our study found that *B. megaterium* and *C. sporogenes* had higher pH values after the incubation period; the shifts in pH toward a higher, less acidic value may indicate greater de novo synthesis of arginine from the precursor compound citrulline.

RESULTS

In this experiment, 10 control agar plates and 4 sets of 10 agar plates treated with one bacterial species each (corresponding to four regional diets) were inoculated with a 20% citrulline solution–an arginine precursor compound–and incubated. To determine the rate of arginine synthesis over a one-week incubation period, we evaluated the pH of all plates using a hand-held pH probe.

Typically, organisms maintaining homeostasis have a neutral range pH (around 7.0). All control and experimental groups had a near-neutral average initial pH: control (7.31), *B. megaterium* (7.38), *C. sporogenes* (7.75), *E. coli* (7.81), and *L. acidophilus* (7.91). Arginine is a basic amino acid (stable pH range of 10.5 - 12.5). Shifts in pH toward a higher, less acidic value suggest greater de novo synthesis of arginine from the precursor compound citrulline.

We observed that the most notable spike in these pH values occurred within 24 hours of incubation; after this period, the pH values stayed comparatively constant for the rest of the incubation period (**Figure 1**). Even after just 24 hours, the pH of plates treated with *C. sporogenes* and those treated with *B. megaterium* universally rose significantly higher than the pH of the control and other experimental groups ($p<0.0001$, Kruskal-Wallis test, **Figure 2**). After one week, a notable increase in bacterial growth was evident in all four experimental groups (**Figure 3**). However, the change in pH in the plates treated with *C. sporogenes* and in those treated with *B. megaterium* was nearly or greater than double the change in the control group. The pH of plates treated with L. acidophilus did not rise significantly higher than the pH of the control group ($p>0.05$, Kruskal-Wallis test); *L. acidophilus* was the only experimental group with an insignificant difference in pH increase from the control group after the 1-week incubation period.

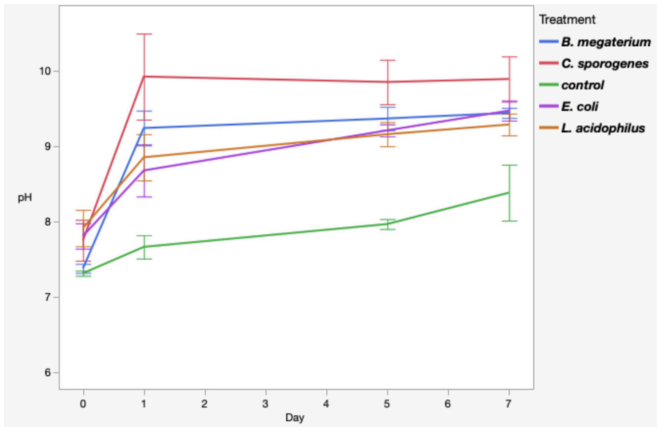

Figure 1. Mean pH throughout one-week incubation period. Mean pH value ± standard deviation of the four bacterial cultures and control. 10 control agar plates inoculated with 20% citrulline solution and four sets of 10 experimental agar plates inoculated with 20% citrulline solution and 1.5 mL of one bacterial culture each (*B. megaterium, L. acidophilus, E. coli,* or *C. sporogenes*) were tested for 1 week, with pH values evaluated every 24 hours.

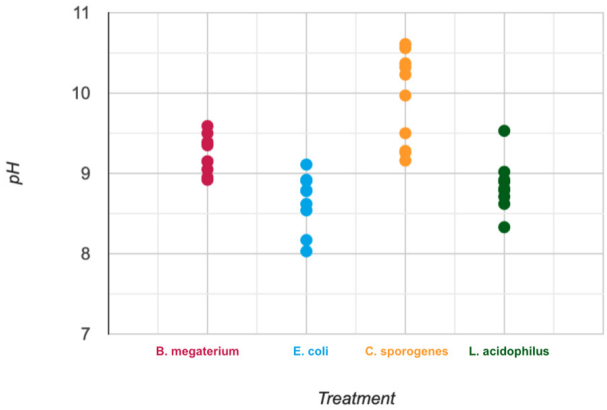

Figure 2. pH of all bacterially treated plates after 24 hours of incubation. pH of all 10 plates [experimental groups inoculated with one bacterial species each (*B. megaterium, L. acidophilus, E. coli,* or *C. sporogenes*)] evaluated after first 24-hour interval.

DISCUSSION

Certain microbial species are associated with specific geo-localities and dietary patterns, and gut bacteria are synthesizers of arginine, a critical aspect of maternal nutrition. As such, there may be diverse gut microbiota populations in pregnant women from different regions that influence arginine synthesis. In this study, we tested the pH levels in plates cultured with *B. megaterium, L. acidophilus, E. coli,* and *C. sporogenes* and inoculated with a 20% citrulline solution as an indicator of arginine synthesis. We found that *B. megaterium* and *C. sporogenes* had significantly higher pH values after the incubation period.

Arginine is a basic amino acid. These shifts in pH toward a higher, less acidic value are a departure from controlled growth conditions and may suggest greater de novo synthesis of arginine from the precursor compound citrulline. Additionally, upon initial inoculation with citrulline, the plates treated with *B. megaterium* had the closest average pH to that of the control group. By contrast, at the end of the incubation period, *B. megaterium* had one of the most disparate pH levels compared to the control group. The transformation from most to one of the least similar to the control may suggest an extreme level of arginine synthesis accounting for the stark contrast.

The most significant spike in these pH values occurred within the first 24 hours of incubation; after this period, the pH values stayed comparatively constant for the rest of the incubation period. This suggests that the bacterial species were able to rapidly synthesize arginine from citrulline within 24 hours of incubation and then mainly worked to maintain

https://doi.org/10.59720/23-305

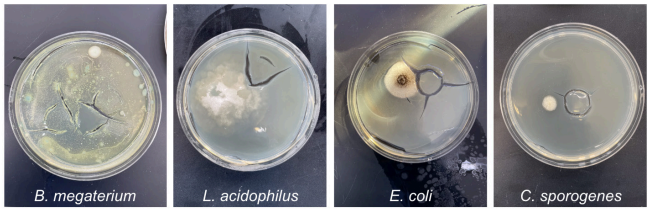

Figure 3. Bacterial growth on treated plates after one-week incubation period. Four sets of 10 experimental agar plates inoculated with 20% citrulline solution and 1.5 mL of one bacterial culture each (*B. megaterium, L. acidophilus, E. coli, and C. sporogenes*, respectively) after 1 week, with pH values evaluated every 24 hours.

these levels of arginine flux for the duration of the incubation period. *L. acidophilus* was the only experimental group with an insignificant difference in pH increase from the control group after the 1-week incubation period. This lack of a significant increase in pH suggests that *L. acidophilus* is the least effective arginine synthesizer in the study.

An abundance of *B. megaterium* is associated with the intestinal microbiota of populations adhering to a traditional Western diet (8). An abundance of *C. sporogenes* is associated with the intestinal microbiota of populations adhering to a traditional Mediterranean diet (9). Hence, both the Western and Mediterranean diets offer potential natural means of modulation of the gastrointestinal microbiota into an environment conducive to arginine synthesis.

In this study, we are limited by only assessing pH changes as a read of arginine production. It is possible that the pH changes may also have been caused by other factors including the presence of a greater amount of a bacterial species or varying production of acidic metabolites among the different bacterial species. Additionally, the best negative control for this experiment would be to measure the pH change of a bacterial strain of the same species but that cannot synthesize arginine due to a mutation.

Future studies could evaluate the efficacy of biochemical modulation of the gut microbiota in regard to arginine synthesis. Additionally, this experiment could be replicated with other bacterial species to examine the effects of other microorganisms on arginine synthesis. This would also offer an opportunity to investigate bacterial species correlated with other dietary stipulations. Another direction for this research would be to focus solely on the dietary catalysts for arginine synthesis by studying arginine metabolism in organisms after the consumption of specific foods rich in arginine or precursor compounds, effectively investigating a more universally-accessible application.

Arginine is a crucial determinant of proper maternal nutrition; as such, lower maternal arginine availability is strongly associated with lower birth weight (2). These results concerning arginine metabolism and the possibilities for dietary gut microbiota modulation could reduce the occurrence of growth-related fetal deaths.

MATERIALS AND METHODS

Bacterial strains and culturing

The four bacterial cultures—*B. megaterium, C. sporogenes, E. coli*, and *L. acidophilus*—were lyophilized stocks from Carolina Scientific. To reactivate the cultures with a rehydration solution, we aseptically removed 1.0 mL of rehydration medium from the test tube and added it to the lyophilized culture in the vial. Then, we mixed gently with the sterile pipet, avoiding air bubbles. Next, we removed the rehydrated culture from the vial and transferred the rehydrated culture back into the tube containing the remaining rehydration medium. Finally, we incubated the reactivated cultures at 37ºC for 24 hours.

Agar plate inoculation

Using a high-precision scale to determine the mass of the citrulline powder, 100 mL of a 20% citrulline solution was prepared. All plates were streaked with 1 mL of the 20% citrulline solution using a micropipette. Except for the control group, which was not inoculated with any bacterial species, we placed three drops of reactivated culture at one edge and then tilted the dish so that the inoculum dripped down the surface. Then, we laid it flat to incubate at 37ºC. A nutrient beef broth agar media was used for all the plates that was known to support the growth of all four tested species. The use of glucose agar was intentionally avoided because glucose can have an acidification effect on bacterial cultures; in contrast, beef broth agar is not known to have any notable impact on the pH of cultures (10).

pH evaluation

After 24 hours of incubation, all plates were removed from the incubator. To measure the pH of the plates, the pH electrode for emulsions and semi-solid samples (HI1612D) from Hanna Instruments was used due to its capacity to evaluate the pH of various states of matter. The electrode was inserted into the semi-solid agar, and then the pH value was recorded. A photo of each plate was taken and added to a Google Drive album. The photos were labeled with the experimental group, plate number, and incubation duration (*B. megaterium*–#1– 24 hours).

After the data were recorded and the pictures were taken, the plates were returned to the incubator.

This process was repeated after 120 hours of incubation and after 168 hours of incubation. The total incubation period was one week.

Statistical analysis

All data were added to a spreadsheet. Once compiled, the data were statistically analyzed for normal distribution and equal variances using a Shapiro-Wilk Test. Because the data were nonparametric and included more than two groups of the independent variable, the data were then analyzed using a Kruskal-Wallis Test run by SPSS Statistics.

Received: October 27, 2023
Accepted: March 5, 2024
Published: August 15, 2024

REFERENCES

1. Belkacemi, Louiza, et al. “Maternal Undernutrition Influences Placental-fetal Development.” *Biology of* Reproduction, vol. 83, 2010), 325-331. https://doi.org/10.1095/biolreprod.110.084517
2. Wu, Guayao, et al. “Maternal Nutrition and Fetal

https://doi.org/10.59720/23-305

Development." *The Journal of Nutrition*, vol. 134, 2004, 2169-2172. https://doi.org/10.1093/jn/134.9.2169
3. Kao, Christina, et al. "The Microbiome, Intestinal Function, and Arginine Metabolism of Healthy Indian Women are Different From Those of American and Jamaican Women." *The Journal of Nutrition*, vol. 146, 2016, 706-713. https://doi.org/10.3945/jn.115.227579
4. De Filippo, Carlotta, et al. "Diet, Environments, and Gut Microbiota: A Preliminary Investigation in Children Living in Rural and Urban Burkina Faso and Italy." *Frontiers in Microbiology*, vol. 8, 2017, 1-14. https://doi.org/10.3389/fmicb.2017.01979
5. Zengler, Karsten, and Livia Zaramela. "The social network of microorganisms – how auxotrophies shape complex communities." *Nature Reviews Microbiology*, vol. 16, June 2018, 383-390. https://doi.org/10.1038/s41579-018-0004-5
6. Wijnands, Karolina, et al. "Arginine and Citrulline and the Immune Response in Sepsis." *Nutrients*, vol. 7, 2015, 1426-1463. https://doi.org/10.3390/nu7031426
7. Frobel, Julia, et al. "Twin-arginine-dependent translocation of folded proteins." *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 367, 2012, 1029-1046. https://doi.org/10.1098/rstb.2011.0202
8. Xiong, Lifeng, et al. "Arginine Metabolism in Bacterial Pathogenesis and Cancer Therapy." *International Journal of Molecular Sciences*, vol. 17, 2016, 363-380. https://doi.org/10.3390/ijms17030363
9. Nagpal, Ravinder, et al. "Gut microbiome-Mediterranean diet interactions in improving host health." *F1000Research*, vol. 8, 2019, 1-18. https://doi.org/10.12688/f1000research.18992.1
10. Agnello, M., et al. "Arginine Improves pH Homeostasis via Metabolism and Microbiome modulation." *Journal of Dental Research*, vol. 96, 2017, 924-930. https://doi.org/10.1177/0022034517707512