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# Effects of Magnesium Deprivation on the Flora of the Gastrointestinal Tract

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# Effects of Magnesium Deprivation on the Flora of the Gastrointestinal Tract

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**Abstract**

Crohn's disease, a pathological condition characterized by gastrointestinal (GI) inflammation and mucosal changes [5], is often associated with hypomagnesemia resulting from changes in the GI mucosa and an increased GI transit rate [1,5]. Furthermore, certain types of bacteria are also implicated in the formation of GI neoplasia and carcinogenesis [4]. A variety of physiologic changes such as an increase in the systemic stress response, severity of the inflammatory response, or an exaggerated immune response [2] is known to occur in Magnesium ($Mg^{2+}$) deficient mice and rats. To understand to what extent $Mg^{2+}$ deficiency could contribute to the aggravation of such diseases by inducing specific changes in the GI microbiome, this study will focus on the effect of a $Mg^{2+}$ deficient diet on the GI flora of Black-6 mice. Mice were fed diets containing known concentrations of $Mg^{2+}$ for 1 week before being placed in a metabolic cage for 24 hours. After 24 hours the mice were sacrificed, blood, feces, and urine samples were collected, and total food consumption, urine excretion and feces excretion was determined. Bacterial analysis of feces, collected via dissection of the colon, revealed that a decrease in the amount of dietary $Mg^{2+}$ consumed is associated with an increase in the number of Lactic Acid Bacteria (LAB) and *Bifidobacterium* Colony-Forming Units (CFU) found per gram of feces. Based on the average amount of $Mg^{2+}$ excreted in the feces and urine of the mice on each diet (Regular, 2% Mg oxide, 2% Mg citrate, 1% Mg citrate, 0.5% Mg citrate, or Mg free) it was also determined that the $Mg^{2+}$ in the 2% Mg citrate diet is absorbed into systemic circulation more efficiently than the $Mg^{2+}$ in the other diets tested. Further research is still required to determine if the changes in GI flora associated with a lower dietary $Mg^{2+}$ consumption are due to systemic hypomagnesemia or due to decreased luminal availability of $Mg^{2+}$ in the GI tract, but based on

the results of this study, it can be concluded that decreased dietary Magnesium consumption has a notable effect on the flora of the GI tract.

## Introduction

Research has shown that a decrease in extracellular $Mg^{2+}$ concentration in mice, due to dietary $Mg^{2+}$ intake, can result in a variety of physiologic changes such as an increase in the systemic stress response, an exaggerated immune response, and an increase in the severity of the inflammatory response [2]. In addition, research has found that $Mg^{2+}$ deficiency causes changes in the stomach mucosa and GI transit rate of rats [1]. Since Crohn's disease is an inflammatory condition characterized by changes in the GI mucosa [5], a better understanding of the factors which may potentially contribute to the manifestation of physiologic symptoms is imperative in the understanding and potential treatment of this disease and similar conditions. A study on the relationship between $Mg^{2+}$ deficiency and serum electrolyte levels ($Mg^{2+}$, Calcium ($Ca^{2+}$), Potassium ($K^{+}$), and Phosphate ($P_i$)), revealed an initial decrease in $Ca^{2+}$ and $P_i$ serum levels and an increase in $K^{+}$ serum levels, which became normalized after 1 week of dietary $Mg^{2+}$ deprivation [3]. Because of this, it is unlikely that changes in the electrolyte balance due to $Mg^{2+}$ deprivation could justify all the observed physiologic responses in mice with long term $Mg^{2+}$ deficiency. Additional research has shown that dietary $Mg^{2+}$ deprivation results in a notable change in the GI flora in mice (B. Ortega, personal communication with researcher, January 30, 2017). Since chronic inflammation and certain types of bacteria have been implicated in the formation of GI neoplasia and carcinogenesis [4], it is necessary to determine potential causes for a change in GI flora which may lead to the development of these conditions. The focus of this

research will therefore be to determine the effect of dietary $Mg^{2+}$ concentration on the GI flora of mice.

## Specific Aims

*Specific aim #1*

Determine the effect of different dietary $Mg^{2+}$ supplements on serum $Mg^{2+}$ concentration and $Mg^{2+}$ excretion. Due to the known physiologic changes, and potential pathologic conditions associated with $Mg^{2+}$ deficiency [1-2], it is important to determine which dietary $Mg^{2+}$ supplements are most effective for recovering affected individuals from $Mg^{2+}$ deficiency, and for maintaining a normal serum $Mg^{2+}$ concentration.

*Specific aim #2*

Determine if limiting the available $Mg^{2+}$ in feces contributes to the observed changes in the GI microbiome. Changes in the bacteria of the GI tract are associated with pathologic conditions such as neoplasia and carcinogenesis [4]. Therefore, it is important to determine if the amount of $Mg^{2+}$ available in the feces is a contributing factor for changes in the bacteria of the GI tract.

## Materials/Methods

*Animal Environment and Husbandry*

Adult (8-10 week-old), internally bred black-6 mice [C57BL/6J (Harlan, Madison, WI USA)], were housed in an indoor, temperature-controlled (23-25 ˚C) room with a 12-hour light:dark cycle, and a humidity of 55-75%. Mice of both genders were included in the study with males having an initial weight of 18.9-26.5 g and females having an initial weight of 15.6-

21.2 g. The mice were assigned to experimental groups based on age, litter, and gender with a similar number of males and females being assigned to each experimental group. Males and females were housed separately in wire topped static cages with other littermates of the same gender to minimize aggression and fighting. A small amount of bedding [Sani-chips manufactured by Teklad] was placed in the bottom of each cage, shredded paper was provided as nesting material, and a cylindrical section of PVC tube was added for enrichment.

*Diet Preparation*

$Mg^{2+}$ free Rodent Diet [manufactured by Teklad] was blended into powder and sifted to ensure uniformity, and 500 g were mixed with 10.26 g of dehydrated Mg citrate ($C_6H_6MgO_7$) dissolved into ultra pure water ($_{dd}H_2O$) containing 4 drops of green food-grade coloring. The $C_6H_6MgO_7$ solution was added to the 500 g of powderized food and mixed, while adding as little $_{dd}H_2O$ as needed to maintain a dough-like consistency, to form a 2% Mg citrate food mixture. The mixture was then pressed into pellets and dried on an aluminum-lined tray overnight before being placed in a plastic bag in the refrigerator. This procedure was also used to produce a 1% Mg citrate diet containing 5.13 g of $C_6H_6MgO_7$ per 500 g of $Mg^{2+}$ free food (red food-grade coloring), and a 0.5% Mg citrate diet containing 2.56 g of $C_6H_6MgO_7$ per 500 g of $Mg^{2+}$ free food (yellow food-grade coloring). This procedure was also used to produce a 2% Magnesium Oxide (MgO) diet, by first grinding 1.65 g of MgO in a mortar containing a small portion of the powdered $Mg^{2+}$ free food until there was no visible clumping. The ground mixture was then added to the remainder of the 500 g of powdered $Mg^{2+}$ free food, 4 drops of blue food-grade coloring were added, and the diet was completed following the standard procedure as described above.

*Preparation of Anesthesia*

A 4% Avertin anesthesia solution was prepared by adding 1 g of 2,2,2-Tribromoethanol to 1 mL of 2-methyl 2-butanol. The container was wrapped in aluminum foil to protect the solution from light, and a stir bar was used to vigorously mix the solution. After the 2,2,2-Tribromoethanol had fully dissolved, 1 mL of the resulting solution was added dropwise to 40 mL of 150 mM NaCl solution which was being vigorously mixed with a stir bar. The container was then wrapped in aluminum foil, to protect the solution from light, and the solution was continuously mixed until all components were completely dissolved to form a clear solution absent of any white precipitate. The resulting 4% Avertin anesthesia solution was then transferred to an amber glass bottle, to protect it from light, and placed in the refrigerator for later use.

*Experimental Protocol*

A predetermined number of male and female Black-6 mice were placed on a specified diet (regular [18% Protein Rodent Diet manufactured by Teklad], 2% MgO, 2% Mg citrate, 1% Mg citrate, 0.5% Mg citrate, or $Mg^{2+}$ free) with deionized water (DIW), (both the assigned experimental diet and DIW were provided ad libitum), for 1 week. After 1 week the mice were weighed and placed in metabolic cages (See Figure 1). Each mouse was provided with a known mass of the assigned diet and DIW. After 24 hours the mice were removed from the metabolic cages and weighed. The mass of food and water remaining in each metabolic cage was recorded. The total mass of feces produced by each mouse in 24 hours was determined and the collected samples were placed in the refrigerator for further analysis. A pipette was used to determine the total volume of urine produced by each mouse in 24 hours and a 1 mL aliquot from each urine sample was transferred to a microfuge tube. The urine samples were placed in an accuSpin Micro 17 and centrifuged at 13,000 RPM for 3 min, the supernatant from each sample was transferred

to a clean microfuge tube and the samples were stored in the freezer for further analysis. After all data concerning the mass, consumption, and excretion of each mouse had been recorded, the mice were anesthetized by administering 4% Avertin to effect, as evidenced by the absence of the pedal reflex, via intraperitoneal (IP) injection (300-500 µL). The mice were then dissected via ventral incision, blood samples were collected from each mouse via intracardiac (IC) puncture, and fecal samples were obtained aseptically via dissection of the cecum and colon. All collected blood and feces samples were immediately placed in clean microfuge tubes. After sitting undisturbed, at room temperature, for 20 minutes to allow for clotting, each blood sample was centrifuged at 6,000 RPM for 10 minutes and a pipette was used to transfer the serum to a clean microfuge tube which was placed on ice. After all samples had been collected, the serum samples and aseptically obtained feces samples were stored in the refrigerator for further analysis. Aseptically obtained feces samples were later submitted for bacterial analysis.

**Figure 1**: C57BL/6J mice were placed in metabolic cages so the 24-hour urine and feces could be collected, and the water and food consumption could be measured.

*Sample Ashing and Suspension*

A sample of the 24-hour feces collected from each mouse was placed into clean, dry crucibles of known mass. After the mass of each sample was determined, the crucibles were placed in an incinerator set to run at 650 ˚C for 12 hours (See Table 1 for full incinerator parameters). After 12 hours, the crucibles containing the ashed samples were removed from the incinerator, the mass of each ashed sample was determined, the crucibles were covered with parafilm, and the samples were stored in a dark, dry location. The samples were later prepared for $Mg^{2+}$ analysis by placing the crucibles containing each sample on an orbital shaker, removing the parafilm, and adding 3 M HCl (prepared from 12 M HCl stock solution) in 100 μL increments until each sample was completely suspended (200-500 μL HCl per sample). A pipette was then used to transfer each sample to a clean microfuge tube. This procedure was also used to 3 prepare samples of each experimental diet (regular, 2% MgO, 2% Mg citrate, 1% Mg citrate, 0.5% Mg citrate, and $Mg^{2+}$ free), for $Mg^{2+}$ analysis.

| **Table 1:** Parameters used for Incineration of Samples | | |
|---|---|---|
| Parameters Tested | Setting | (Mode 1) |
| Working setpoint: | 650 | (wkG.SP) |
| Working Output: | 0.0 | (wrk.OP) |
| Units: | ˚C | |
| Setpoint 1: | 650 | (sp1) |
| Setpoint Rate: | off | (sp.RAT) |
| Setpoint Ramp Units: | m In | (RAMPU) |
| Alarm 1 Setpoint: | 675 | (A1.DHI) |
| Alarm 2 Setpoint: | 1125 | (A2.HI) |
| Alarm 3 Low Setpoint: | 0 | (A3.LO) |
| Autotune: | off | (A. Tune) |
| Proportional Band: | 7 | (PB) |
| Integral Time: | 130 | (TI) |
| Derivitive Time: | 22 | (TD) |
| PV Offset: | 0.0 | (PV.OFS) |
| Customer ID: | 184 | (ID) |
| Timer Configuration: | dwell | (TM.CFG) |
| Timer Status: | rEs | (T.Stat) |
| Set Time Duration: | 11:00 | (DWELL) |
| Timer Resolution: | Hour | (TM.RES) |
| Timer Start Threshold: | off | (THRES) |
| Timer End Type: | off | (END.T) |

*Analysis of Magnesium Concentration*

1 mL of 50 mM $MgCl_2$ was prepared from a 2 M $MgCl_2$ stock solution. The 50 mM $MgCl_2$ solution was then used to prepare 10 Magnesium standards ranging in concentration from 0.0 mM to 2.0 mM. Fresh Calmagite working solution was prepared by combining 10 mL of Buffer Solution, 10 µL of 75 mM Calmagite water solution, and 8 µL of EGTA Stock Solution in a conical vial (See Table 2). 10 µL of each urine sample, suspended feces sample, or suspended food sample (7.5 µL when performing a $Mg^{2+}$ assay on serum samples) including the 10 standards, was added to a cuvette containing 1 mL of working solution. The cuvettes were then covered with parafilm and inverted multiple times before being allowed to sit for 4 min.

After 4 minutes the cuvettes were read in a spectrophotometer set at a wavelength of 520 nm, and the results were recorded for analysis. Samples with an optical density which was found to be outside of the range created by the standard curve, were diluted and retested until the optical density fell within that of the standard curve. The dilution factor range required for the analysis of urine samples was 1 (no dilution) to 100 with samples collected from mice on a 2% Mg citrate diet requiring the most amount of dilution. The dilution factor range required for the analysis of suspended feces samples was 1 to 50 with samples collected from mice on a regular diet, 2% Mg citrate diet, and 2% MgO diet all requiring the most amount of dilution. The dilution factor range required for the analysis of suspended food samples was 10 to 200 with samples collected from mice on a regular diet, 2% MgO diet, 2% Mg citrate diet, and 1% Mg citrate diet, all requiring the most amount of dilution. For the $Mg^{2+}$ assays performed on urine, feces, and food, samples collected from mice on a $Mg^{2+}$ free diet required the least amount of dilution. No dilution was necessary for the analysis of serum samples. Once it was determined that the $Mg^{2+}$ assay readings for the experimental samples were within range of the standard sample readings, the $Mg^{2+}$ assay results for the 10 standards were graphed to form a Magnesium Standard Curve.

**Table 2:** Preparation of Working Solution for $Mg^{2+}$ Assays

| Reagent | Composition |
|---|---|
| 75 mM Calmagite Solution | 1000X Calmagite stock in $_{dd}H_2O$ |
| Buffer Solution | 0.2 M 2-amino-2-methyl-1-propanol in $_{dd}H_2O$<br>Adjust to pH 12.7 with KOH |
| EGTA | 250 mM EGTA Stock Solution (pH 8) |

## Results

In order to investigate the effect of individual $Mg^{2+}$ supplements on mice $Mg^{2+}$ metabolism, animals were assigned to one of six diets for the duration of the experiment as

follows: 11 mice (6 males, 5 females) were assigned the regular diet, 5 mice (3 males, 2 females) were assigned the 2% MgO diet, 12 mice (7 males, 5 females) were assigned the 2% Mg citrate diet, 6 mice (3 males, 3 females) were assigned the 1% Mg citrate diet, 6 mice (3 males, 3 females) were assigned the 0.5% Mg citrate diet, and 7 mice (4 males, 3 females) were assigned the Mg free diet. After 24 hours in a metabolic cage the mice were sacrificed and the average serum $Mg^{2+}$ concentration (See Figure 2), the average amount of $Mg^{2+}$ consumed in the food, and the average amount of $Mg^{2+}$ excreted in the urine and feces (See Figure 3), was determined for each experimental diet. Stored serum samples were analyzed within 24 hours to prevent alterations in the $Mg^{2+}$ concentration of the samples. Stored urine samples were slowly warmed to room temperature, ashed feces samples were suspended, and all urine and feces samples were vortexed before the $Mg^{2+}$ analysis was performed on each sample. The feces samples aseptically obtained from the cecum and colon were submitted, and the bacterial analysis was performed by Dr. Pelletier's lab. All statistical analysis was performed using MiniTab 17 statistical software. (Minitab Inc., State College, PA, USA).

*Effect of Magnesium Supplements on* $Mg^{2+}$ *Concentration*

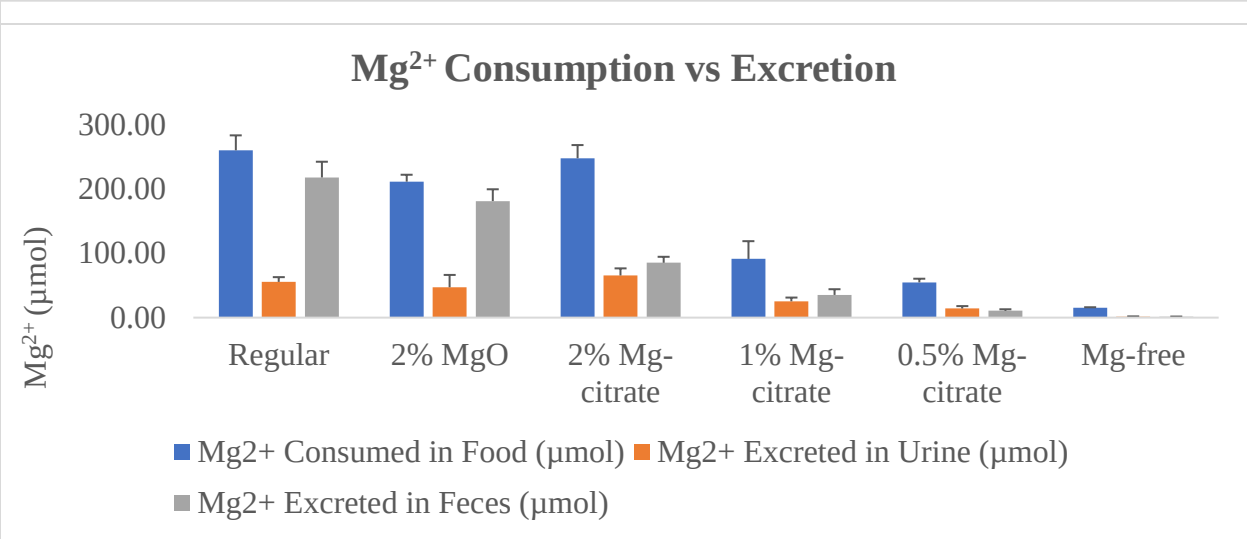


**Figure 3**: Mice were provided with a specified diet (Regular, 2% MgO, 2% Mg Citrate, 1% Mg Citrate, 0.5% Mg Citrate, or Mg Free) and placed in metabolic cages for 24 hours. After 24 hours the feces and urine were collected, and the amount of food consumed was determined. The average amount of $Mg^{2+}$ excreted in the feces and urine in 24 hours was determined for each diet. The average $Mg^{2+}$ concentration of each food was determined by performing a $Mg^{2+}$ analysis on 3 ashed samples of each diet. Based on the results of the $Mg^{2+}$ concentration assays it was determined that mice on a 2% Mg-citrate diet excreted more $Mg^{2+}$ in urine and less $Mg^{2+}$ in feces when compared to mice on a 2% MgO diet.

By comparing the results of the serum $Mg^{2+}$ assays (See Figure 2) to the normal serum $Mg^{2+}$ concentration of 1.1-1.4 mM [4], it was noted that only samples collected from mice on the regular and 2% MgO diets fell within the normal range. Due to significant variation ($p<0.05$) between trials which compared the serum $Mg^{2+}$ concentration of 2 groups of mice on the same diet, it was determined that the serum concentration results were subject to an unknown experimental error, potentially involving the $Mg^{2+}$ assay, which was likely resulting in low serum $Mg^{2+}$ concentration values. Because of this, assay tests were conducted to determine potential sources of the experimental error.

In order to determine if the type of sample used to run the assay (serum or plasma) was affecting the experimental results, an assay test was conducted in which blood samples were collected from 3 male mice (housed in a regular cage and provided a regular diet and DIW for 1

week prior). 200 µL of each blood sample was placed in a microfuge tube containing 3 µL of Heparin and gently mixed, to prevent clotting. The remainder of each blood sample was placed in a microfuge tube and allowed to clot as usual. The standard serum collection protocol was used to centrifuge and collect each sample and a $Mg^{2+}$ assay was performed using 10 µL of each sample. By comparing the results of the assay, it was determined that although there was no statistical difference ($p>0.05$) in the $Mg^{2+}$ concentration reading of serum when compared to plasma, the serum concentration was much higher on average.

In order to determine if the metabolic cage, the use of DIW, the sample size used to perform the $Mg^{2+}$ assay, or the serum clotting time was affecting the experimental results, 3 additional assay tests were conducted. In the first assay test, 5 mice (3 male, 2 female) were placed in metabolic cages for 24 hours and 5 mice (3 male, 2 female) were kept in the regular cages. Both groups of mice were provided with regular food and DIW for the duration of the experiment. After 24 hours, serum samples (with a clot time of 20 minutes) were collected. In the second assay test, 5 mice (3 male, 2 female) were placed in metabolic cages for 24 hours and 5 mice (1 male, 4 female) were kept in the regular cages. Both groups of mice were provided with regular food and tap water for the duration of the experiment. After 24 hours, serum samples (with a clot time of 30 minutes) were collected. In the third assay test, serum samples (with a clot time of 25 minutes) were collected from 4 female mice, housed in a regular cage with regular diet and DIW for 1 week prior. In order to further test for potential error, all 3 $Mg^{2+}$ assays were performed using 7.5 µL and again using 10 µL of each serum sample. By comparing the results of the assays, it was determined that there were no significant differences between the mice in the metabolic cages and the mice in the regular cages, the mice on DIW and the mice on

tap water, serum samples with different clotting times (20 minutes, 25 minutes, or 30 minutes), or sample volume used to perform the assay.

A $Mg^{2+}$ analysis was performed on the feces and urine collected from the mice on each diet and the average amount of $Mg^{2+}$ excreted in the feces and urine in 24 hours was determined (See Figure 3). The average $Mg^{2+}$ concentration of each food was determined by performing a $Mg^{2+}$ analysis on 3 ashed samples of each diet. The total $Mg^{2+}$ consumed was calculated by multiplying the average $Mg^{2+}$ concentration of each diet by the amount of the diet consumed in 24 hours (See Figure 3). Based on the results of the $Mg^{2+}$ concentration assays it was determined that mice on a 2% Mg-citrate diet excreted more $Mg^{2+}$ in urine and less $Mg^{2+}$ in feces when compared to mice on a 2% MgO diet (See Figure 3).

*Effect of Limiting Available $Mg^{2+}$ on the GI Microbiome*

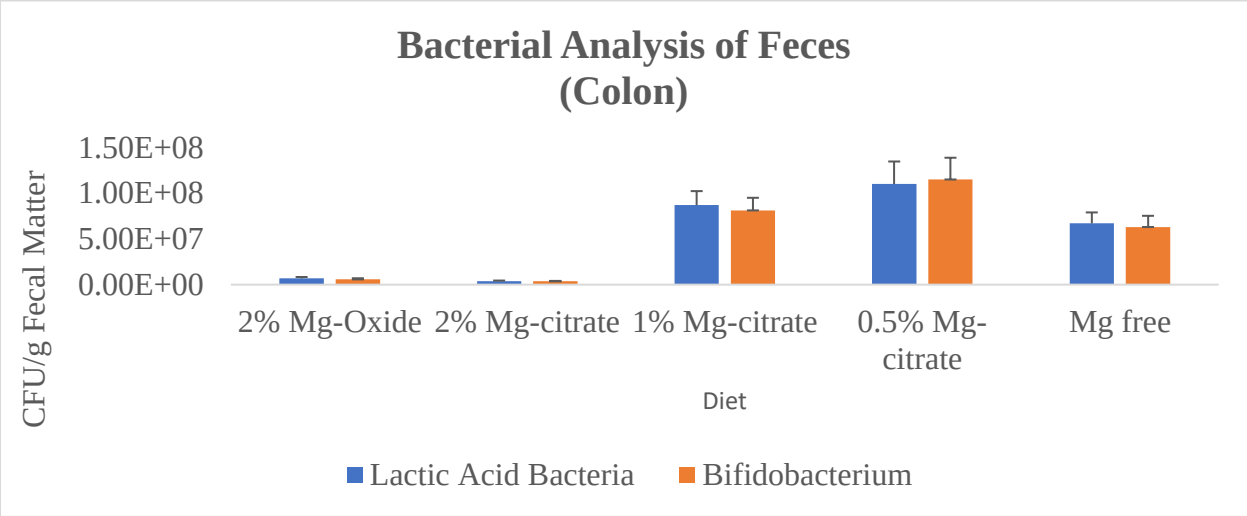


**Figure 4**: Mice were provided with a specified diet (Regular, 2% MgO, 2% Mg Citrate, 1% Mg Citrate, 0.5% Mg Citrate, or $Mg^{2+}$ Free) and placed in metabolic cages for 24 hours. After 24 hours the mice were sacrificed, feces samples were collected aseptically from the colon, and the samples were submitted for bacterial analysis. Based on the results of the bacterial analysis (performed by Dr. Pelletier's lab), it was determined that mice on a 2% Mg citrate diet have a population of Lactic Acid Bacteria and *Bifidobacterium* similar to that of mice on a 2% MgO diet. It was also determined that a decrease in Mg citrate concentration generally resulted in an increase in the total number of both LAB and *Bifidobacterium* CFU per gram of feces.

A bacterial analysis of the feces samples, aseptically obtained via dissection of the colon and submitted for analysis, was performed by Dr. Pelletier's lab. The results of the bacterial analysis showed that mice on a 2% Mg citrate diet have a population of LAB and *Bifidobacterium* similar to that of mice on a 2% MgO diet (See Figure 4). It was also found that a decrease in Mg citrate concentration generally resulted in an increase in the total number of both LAB and *Bifidobacterium* CFU per gram of feces.

## Discussion

*Effect of Magnesium Supplements on $Mg^{2+}$ Concentration*

Due to low serum $Mg^{2+}$ concentrations (when compared to the normal range of 1.1-1.4 mM [4]), and significant variation between groups of mice on the same diet, $Mg^{2+}$ assay tests were performed to determine potential sources of experimental error. The results of the $Mg^{2+}$ assay which compared serum to plasma, showed no statistical difference in the $Mg^{2+}$ concentrations of the 2 sample types, yet the serum $Mg^{2+}$ concentration was much higher than the plasma $Mg^{2+}$ concentration on average. Due to this it was determined that serum samples are superior to plasma samples for the analysis of $Mg^{2+}$ concentration and therefore, this aspect of the experiment did not require modification. Based on the results of statistical analysis of the different assay tests performed, it determined that, although there was no statistical significance between the assays conducted with different sample volumes (7.5 µL or 10 µL), the serum $Mg^{2+}$ concentration reading for each sample was higher on average when 10 µL of serum sample was used instead of 7.5 µL, and therefore the minimum sample size when performing the $Mg^{2+}$ assay should be 10 µL. It was also determined that it was unlikely for the source of the experimental error to be the metabolic cage, the use of DIW, or the serum sample clot time, and therefore, it

was determined that the source of the experimental error was the $Mg^{2+}$ assay itself. Because of this, it is recommended that the experiment be repeated with an alternate means of measuring $Mg^{2+}$ such as atomic absorption.

The results of the $Mg^{2+}$ concentration assays performed on the 24-hour excrement showed that mice on a 2% Mg-citrate diet excreted more $Mg^{2+}$ in urine (43.45% of excreted $Mg^{2+}$) and less $Mg^{2+}$ in feces (56.55% of excreted $Mg^{2+}$) when compared to mice on a 2% MgO diet (see Figure 3) which excreted 20.67% of the total Mg excreted in urine and 79.33% in feces. Nutrients excreted in urine are absorbed by the body and filtered through the kidneys before excretion, and nutrients excreted in feces are eliminated without being absorbed. Because of this it was determined that, in mice, the $Mg^{2+}$ of 2% Mg citrate has increased absorption when compared to the $Mg^{2+}$ of 2% MgO. By comparing the results of a $Mg^{2+}$ assay performed on 3 ashed samples of each experimental diet (including the regular diet) to the calculated $Mg^{2+}$ concentration of each diet, it was determined that the $Mg^{2+}$ assay was only detecting an average of 72.46% ± 2.07 of the total $Mg^{2+}$ in the sample. This supports our previous conclusion that the experimental error encountered was directly related to the $Mg^{2+}$ assay test performed, and therefore an alternative means of determining the $Mg^{2+}$ concentration of samples, such as atomic absorption, should be considered for future experiments.

*Effect of Limiting Available $Mg^{2+}$ on the GI Microbiome*

The results of the bacterial analysis performed by Dr. Pelletier's lab indicate that the Magnesium content of the diet does influence the bacterial flora of the colon with the growth of LAB and *Bifidobacterium* favored at a dose below 1% Mg in the diet. The most likely reason for this is that LAB and *Bifidobacterium* are more efficient at extracting $Mg^{2+}$ from ingesta passing through the colon and therefore proliferate, while less $Mg^{2+}$ efficient forms of bacteria are unable

to obtain the $Mg^{2+}$ required to sustain a normal population, resulting in a notable change in the flora of the GI tract. The feces samples obtained from mice on a $Mg^{2+}$ free diet were found to contain more total LAB and *Bifidobacterium* CFU per gram when compared to the samples obtained from mice on 2% Mg citrate diet. Despite this, the feces samples obtained from mice on a $Mg^{2+}$ free diet were found to contain less total LAB and *Bifidobacterium* CFU per gram when compared to the samples obtained from mice on a 1% Mg citrate or 0.5% Mg citrate diet. Because of this, it was concluded that LAB and *Bifidobacterium*, despite being able to proliferate under low $Mg^{2+}$ conditions, still require an undetermined amount of $Mg^{2+}$ to do so. Potential areas of future research include repeating the experiments while utilizing atomic absorption to obtain more accurate and reliable values, and testing additional diets to determine if other compounds are more efficient for recovering mice from $Mg^{2+}$ deficiency. In addition, LAB and *Bifidobacterium* should be cultured using a low $Mg^{2+}$ media in order to verify the ability of these bacterial strains to proliferate under low $Mg^{2+}$ conditions, and mice on a $Mg^{2+}$ deficient diet should be given $Mg^{2+}$ injections to verify if the changes in total LAB and *Bifidobacterium* found in the colon are influenced by the level of $Mg^{2+}$ found within the intestinal lumen or if the changes are caused by systemic $Mg^{2+}$ levels. Additional research should also be performed to determine what, if any, changes in other types of bacteria (in addition to LAB and *Bifidobacterium*) naturally found in the GI tract are associated with dietary $Mg^{2+}$ deficiency.

## Conclusion

Dietary Mg citrate was found to have better $Mg^{2+}$ absorption when compared to MgO and it was therefore determined that Mg citrate should be considered as a more effective means for $Mg^{2+}$ supplementation. In addition, it was determined that a decrease in dietary $Mg^{2+}$

consumption results in significant changes to the GI microbiome, and is associated with a significant increase in the Lactic Acid Bacteria and *Bifidobacterium* found in the GI tract.

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