Effect of Magnesium Concentration on Lactase Activity: A Comparative Analysis of Glucose Production

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Abstract

This experiment studied the effect of varying magnesium concentrations (0 mM, 1 mM, 3 mM, and 5 mM) on the activity of the lactase by focusing on glucose concentration as an indicator of lactase activity. Magnesium is known to function as a cofactor that enhances the lactase activity. The experimental setup included four different magnesium concentration groups and measurements of glucose concentration after a certain amount of time to determine the impact of magnesium on lactase activity. A two-way ANOVA was performed to evaluate lactase activity across the groups, considering two independent variables: time and magnesium concentration. The results indicated that lower magnesium concentrations optimized lactase activity, while higher concentrations inhibited the enzyme and slowed lactose hydrolysis.

Introduction

The digestion of lactose, a critical process for many organisms, relies on the enzyme lactase. Lactase facilitates the breakdown of lactose, a disaccharide present in milk, into its simpler components, glucose and galactose. With nearly 70% of the population being deficient in intestinal lactase, the consumption of lactose-containing dairy products can trigger gastrointestinal symptoms such as nausea, abdominal pain, and diarrhea (Forsgård, 2019). This condition is known as lactose intolerance which is when the body doesn't produce enough lactase to break down or digest lactose. Lactose is a sugar found in milk and other various dairy products. Magnesium is recognized as an essential cofactor for lactase, however, its optimal concentration for maximizing enzyme activity is not well-defined.

Magnesium plays a critical role as a cofactor in lactase activity, influencing its ability to hydrolyze lactose into glucose and galactose. In previous studies, it has been found that increasing magnesium concentrations can enhance the catalytic efficiency of lactase by stabilizing the enzyme-substrate complex and optimizing reaction conditions (Swoboda & Massey, 1971). However, the relationship between magnesium levels and enzyme efficacy is not linear; excessively high concentrations may inhibit lactase activity by altering the

enzyme's conformation or competing with essential ions (Haque & Abdel-Aal, 2017). Experiments analyzing lactase activity at increasing magnesium concentrations over time have revealed that moderate magnesium levels enhance enzymatic performance, but further increases can diminish its efficacy.

In this study, the main focus is on how varying magnesium concentrations affect lactase efficiency in hydrolyzing lactose alongside the resulting glucose concentration in the presence of lactose. To accomplish this, an experiment was done by combining a lactose solution with lactase and magnesium. The control and experimental groups used different solutions of magnesium and were placed in a water bath and the glucose concentration, as an indicator, was measured at 2 minute intervals for 10 minutes. This study hypothesizes that as the concentration of magnesium increases, the activity of lactase will also increase, but this relationship will plateau after a specific concentration of magnesium has been reached. The findings of this study could inform strategies for optimizing lactose hydrolysis through tailored magnesium concentrations, potentially improving treatments for lactose intolerance and enhancing dairy processing in the food and beverage industry.

Methods

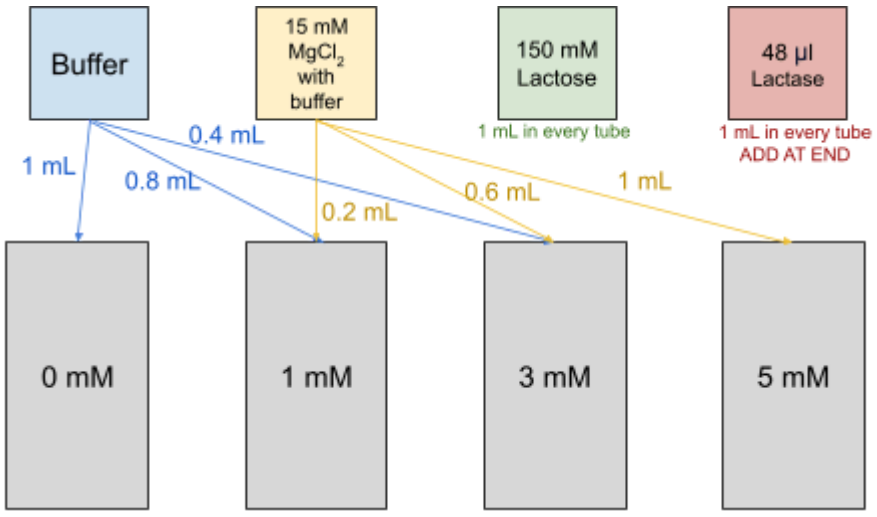

Figure 1: Preparations of solutions with 0 mM, 1 mM, 3 mM and 5 mM magnesium for lactase activity experiment, using Tris buffer, 15 mM $MgCl_2$, 150 mM lactose and lactase (added last).

The 0 mM magnesium solution was prepared by adding 1 mL of Tris buffer solution to a test tube containing 1 mL of 150 mM lactose solution. This step was repeated for a total of three test tubes to create replicates. The 1 mM magnesium solution was prepared by mixing 0.2 mL of 15 mM $MgCl_2$ with 0.8 mL of Tris buffer solution, followed by the addition of 1 mL of 150 mM lactose solution. Similarly, the 3 mM magnesium solution was prepared by combining 0.6 mL of 15 mM $MgCl_2$ with 0.4 mL of Tris buffer solution and the 5 mM solution contained 1 mL of 15 mM $MgCl_2$, and then 1 mL of lactose solution was added to both groups. For all test solutions, 1 mL of lactase buffer solution was added at the end and the reaction started immediately after adding (Fig. 1).

For each magnesium concentration, three test tubes were prepared as replicates. The reaction began upon adding 1 mL of the lactase buffer solution to the test tube, which was then vortexed for 1-2 seconds and placed in a 37°C water bath to simulate the human internal temperature. A timer was started for 2 minutes, and at 30-second intervals, the same procedure was repeated for the second and third test tubes. After 2 minutes, the solution in the first test tube was mixed thoroughly using a 1000 µL micropipette for three cycles to ensure homogenization. A 20 µL sample was then pipetted onto parafilm, and the glucose concentration was measured using the OneTouch® Ultra® 2 glucose meter. The water bath lid was closed immediately after sampling to maintain temperature stability.

This procedure was repeated at 2-minute intervals for a total of 10 minutes for all test tubes and replicates. Glucose concentrations were recorded for each time point and averaged across replicates for each magnesium concentration.

The data collected was analyzed using a two-way ANOVA test to evaluate the impact of varying magnesium concentrations on lactase activity and determine statistical significance. Graphs illustrating glucose concentration over time were generated to represent trends in lactase activity.

Results

This study aimed to explore how varying magnesium concentrations impacted the performance of lactase with glucose concentration (mmol/L) across 5 time intervals lasting 2 minutes each. Four comparisons of data were performed by Two-way ANOVA, with a significance level of 0.05. For the first comparison (two-way ANOVA, $F(2, 39) = 251.53$, $p < 2 \times 10^{-16}$), which is among the 3 experimental groups, the null hypothesis is that there is no significant difference among three groups. The null hypothesis was rejected since the p-value is smaller than the significance level, meaning there is enough evidence to suggest a statistically significant difference among three experimental groups. Further comparisons between each experimental group and the control group were conducted. The 1 mM Mg group showed significant higher lactase activity compared to the control (two-way ANOVA, $F(1, 26) = 104.553$, p-value = 1.33×10^{-10}), and 5 mM Mg group exhibited a significant decrease compared to the control (two-way ANOVA, $F(1, 26) = 168.44$, p-value = 7.24×10^{-13}). However, the 3 mM group did not show significant increase or decrease from the control (two-way ANOVA, $F(1, 26) = 0.804$, p-value = 0.378). This data suggested that 1 mM Mg group and 5 mM Mg group had significantly increased and decreased lactase activity compared to the control, while the 3 mM Mg group had no significant difference.

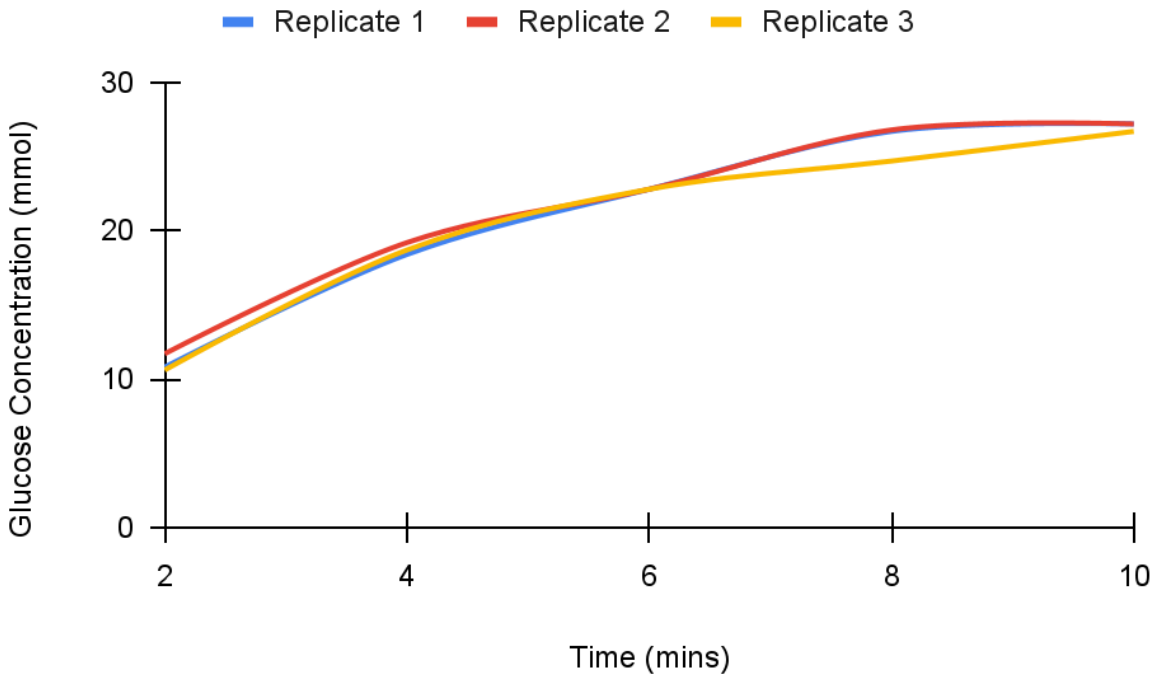

Figure 2. Glucose concentration with 1 mM magnesium of 3 replicates over 10 minutes with 5 2-minute intervals

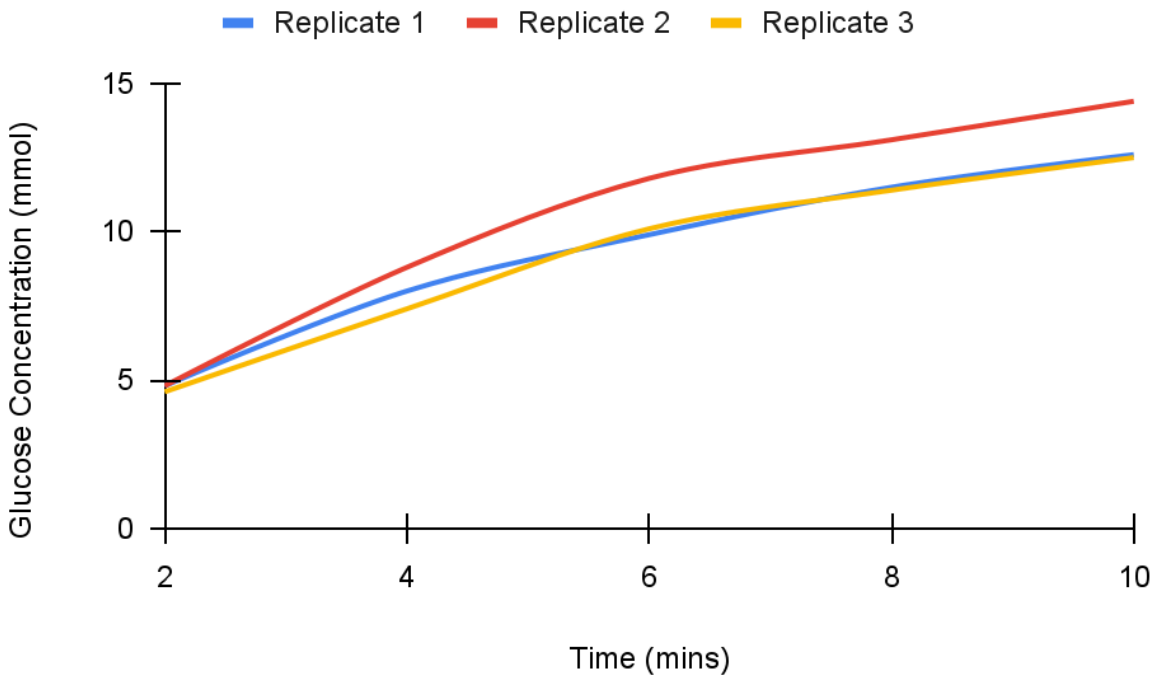

Figure 3 . Glucose concentration with 3 mM magnesium of 3 replicates over 10 minutes with 5 2-minute intervals

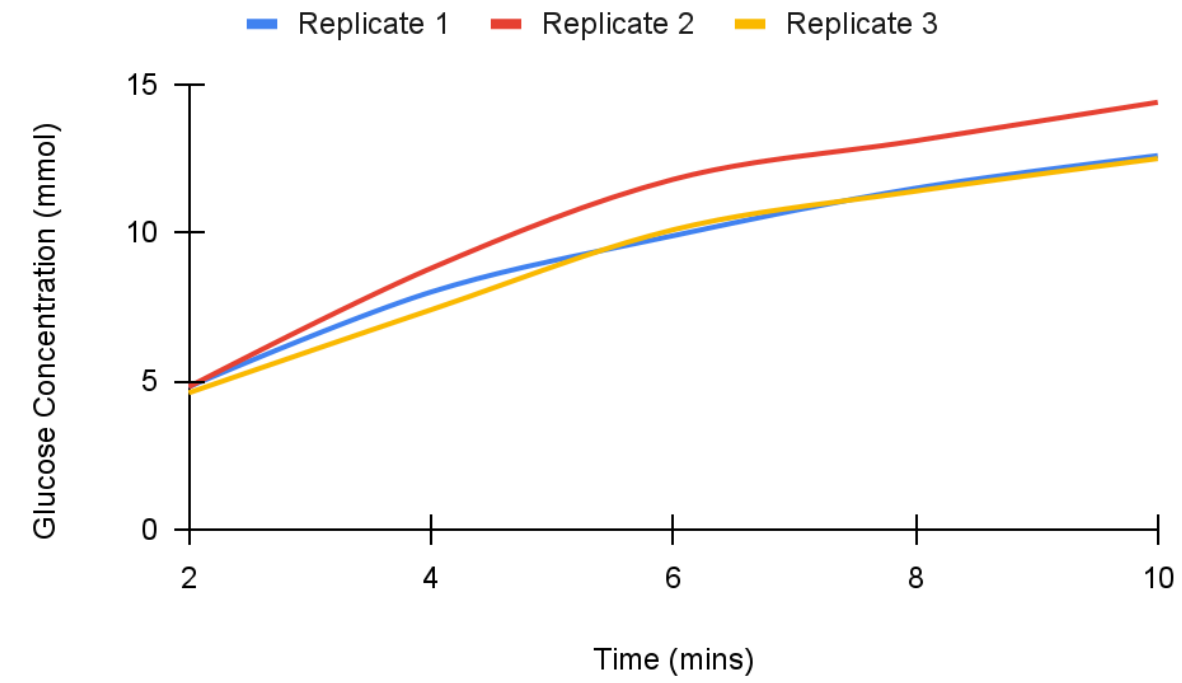

Figure 4. Glucose concentration with 5 mM magnesium of 3 replicates over 10 minutes with 5 2-minute intervals

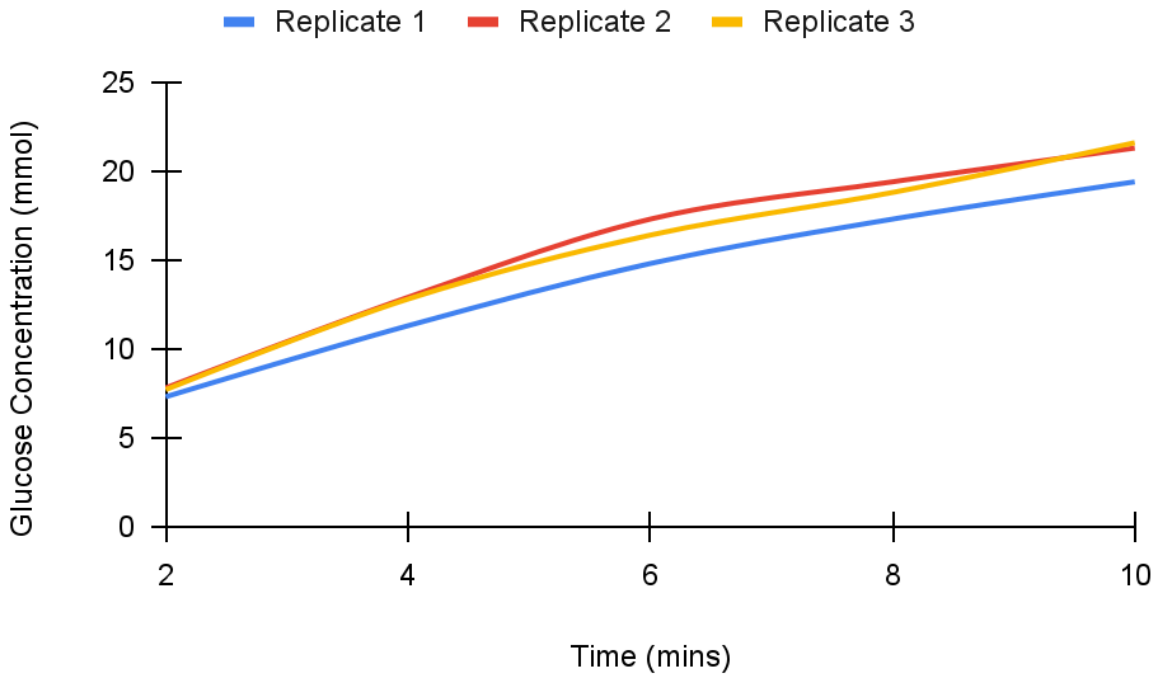

Figure 5. Glucose concentration with 0 mM magnesium (control group) over 10 minutes with 5 2-minute intervals

Discussion

This study evaluates how varying concentrations of magnesium influence lactase activity, hypothesizing that as magnesium concentration increases, lactase activity would also increase, but this relationship would plateau after a certain concentration. The results indicate that lactase activity initially increases with magnesium concentration, but then decreases at higher concentrations, with no observable plateau.

Four comparisons were performed based on the results in two-way ANOVA, comparison among 3 experimental groups, control vs. 1 mM Mg group, control vs. 3 mM group, and control vs. 5 mM group. This statistical method was used in our experiment to analyze the effects of both magnesium concentration and time on lactase activity, allowing us to assess how these two independent factors interact in influencing glucose production. The significant differences observed among the 3 experimental groups suggest that magnesium had varied effects on lactase activity. 1 mM group showed a statistically higher lactase activity compared to the control, suggesting that the concentration of magnesium is most favorable among the three concentrations.The results of the 3 mM Mg group, which did not significantly differ from the control, implied that 3 mM magnesium would reach a plateau (Fig.3). The 5 mM Mg group exhibited lower lactase activity than the control, indicating that overlimit magnesium concentration had an inhibitory effect.

At 1mM magnesium (Fig. 2), lactase activity was the most optimal, as indicated by the highest glucose concentrations measured over 10 minutes, reaching ~27.2 mmol/L. At 3 mM magnesium (Fig. 3), the activity declined, reaching a peak glucose concentration of around 19.9 mmol/L. At 5mM magnesium (Fig. 4), the activity was significantly inhibited, with the highest glucose concentration being around 12.6 mmol/L. The control with 0mM magnesium (Fig. 5), showed higher glucose production than 3mM and 5mM treatments, reaching approximately 21.3 mmol/L by the 10 minutes mark. These results suggest that

magnesium functions as a cofactor for lactase, enhancing activity at lower concentrations, while inhibiting it at higher levels.

High concentrations of magnesium may interfere with enzyme function by altering the active site, competing with substrate binding or disrupting enzyme conformation. If magnesium concentrations exceed the optimal point, the overabundant magnesium can disrupt the enzyme's optimal conformation and lead to a reduction in lactase function (Demirhan, 2008). Magnesium can also bind to allosteric sites, which can change the shape of lactase, and may hinder lactose access to the active site. High concentration of magnesium can also alter the ionic strength, which affects the three-dimensional structure of lactase. Structural changes of lactase may disrupt the active site, causing the lactase to be less effective (Loeffler, 1979).

Several sources of error could have influenced the results. Measurement variability was a primary concern as glucose concentrations were recorded immediately after mixing the enzyme and substrate solutions.Variations in the mixing process could have introduced inconsistencies, as the majority of the mixing was performed manually with a pipette, while only the initial mixing was conducted using a vortex machine. Additionally, glucose measurements were taken shortly after manual mixing and pipetting onto parafilm, and the slight differences in timing between replicates could have affected the results. Temperature control was another potential source of error. The water bath was opened constantly to mix the solutions and measure glucose concentrations, leading to minor temperature fluctuations. Since lactase activity is highly sensitive to temperature, these fluctuations may have influenced enzymatic performance. Finally, handling variability, such as the simultaneous management of three test tubes and the use of separate timers, may have introduced timing inconsistencies across replicates.

Additionally, prior to obtaining reliable data, there were complications with the glucose concentration measurements due to inaccurate concentrations of lactose and lactase. The experiment started with 15 mM of lactose. Insufficient lactose concentration led to a lack of substrate for lactase to break down into glucose. Additionally, the experiment began with 16 fccnlu of lactase, which was not enough to effectively break down the lactose as it was being diluted due to the addition of other solutions. These two initial obstacles resulted in the OneTouch Ultra®2 meter repeatedly showing "Error 4" (Table 2 in Appendix), indicating that there was not enough lactose in the solution for glucose detection and insufficient lactase to break down the lactose. By increasing the lactose concentration to 150 mM and the lactase to 48 fccnlu, glucose values were detectable.

Future research could address these limitations and expand on the findings of the study. Testing a broader range of magnesium concentrations could provide more precise results into the concentration range at which lactase activity peaks and declines. For real-life applications, this experiment gives a better understanding on how magnesium affects lactase activity and can be used to optimize the production of lactose-free dairy products. Also, it might be used as part of a supplement for individuals with lactose intolerance, helping digestion of dairy products.

Conclusion

This study demonstrates that magnesium concentration influences lactase activity, with optimal activity observed at 1mM. However, at higher concentrations, lactase activity was inhibited, which contrasted with our hypothesis that predicted a plateau after a certain concentration. Glucose concentration was measured, and despite some variability in measurements, the results likely reflect magnesium's role as a cofactor at low levels while suggesting potential inhibition of substrate binding at higher levels. These findings provide

valuable insights into applications such as lactose-free dairy production and emphasize the importance of identifying optimal magnesium concentrations for enzymatic efficiency.

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Appendix

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> summary(model_all)
                  Df Sum Sq Mean Sq F value   Pr(>F)
time               1  828.1   828.1  423.16  < 2e-16 ***
concentration      2  984.4   492.2  251.53  < 2e-16 ***
time:concentration  2   51.5    25.7   13.15 4.31e-05 ***
Residuals          39   76.3     2.0
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> summary(model_1mM)
                  Df Sum Sq Mean Sq F value   Pr(>F)
time               1  773.3   773.3 292.897 1.13e-15 ***
concentration      1  276.0   276.0 104.553 1.33e-10 ***
time:concentration  1    6.9     6.9   2.627    0.117
Residuals          26   68.6     2.6
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> summary(model_3mM)
                  Df Sum Sq Mean Sq F value Pr(>F)
time               1  602.9   602.9 480.858 <2e-16 ***
concentration      1    1.0     1.0   0.804  0.378
time:concentration  1    0.4     0.4   0.306  0.585
Residuals          26   32.6     1.3
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> summary(model_5mM)
                  Df Sum Sq Mean Sq F value   Pr(>F)
time               1  426.1   426.1  333.12 2.41e-16 ***
concentration      1  215.5   215.5  168.44 7.24e-13 ***
time:concentration  1   20.5    20.5   16.05  0.00046 ***
Residuals          26   33.3     1.3
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
>
```

Figure 1: Two-way ANOVA test comparing the effects of magnesium concentration on lactase activity

Table 1: Glucose concentrations were measured at 2-minute intervals over 10 minutes for varying magnesium concentrations

1 mM $MgCl_2$	Replicate 1 (mmol/L)	Replicate 2 (mmol/L)	Replicate 3 (mmol/L)
2 minutes	10.8	11.7	10.6
4 minutes	18.4	19.2	18.7
6 minutes	22.8	22.8	22.8
8 minutes	26.7	26.8	24.7
10 minutes	27.2	27.2	26.7
3 mM $MgCl_2$	Replicate 1 (mmol/L)	Replicate 2 (mmol/L)	Replcate 3 (mmol/L)
2 minutes	8.3	7.5	6.8
4 minutes	12.6	11.8	12.3

1 mM $MgCl_2$	Replicate 1 (mmol/L)	Replicate 2 (mmol/L)	Replicate 3 (mmol/L)
2 minutes	10.8	11.7	10.6
4 minutes	18.4	19.2	18.7
6 minutes	22.8	22.8	22.8
8 minutes	26.7	26.8	24.7
6 minutes	15.1	15.7	15.7
8 minutes	18.7	18.7	17.6
10 minutes	19.9	19.2	20.7
5 mM $MgCl_2$	Replicate 1 (mmol/L)	Replicate 2 (mmol/L)	Replicate 3 (mmol)/L
2 minutes	4.8	4.8	4.6
4 minutes	8	8.8	7.4
6 minutes	9.9	11.8	10.1
8 minutes	11.5	13.1	11.4
10 minutes	12.6	14.4	12.5
Control (0 mM $MgCl_2$)	Replicate 1 (mmol/L)	Replicate 2 (mmol/L)	Replicate 3 (mmol/L)
2 minutes	7.3	7.8	7.7
4 minutes	11.3	12.9	12.8
6 minutes	14.8	17.3	16.4
8 minutes	17.3	19.4	18.8
10 minutes	19.4	21.3	21.6

Table 2: Glucose concentrations were measured using 5 mM Mg to test the initial impact of higher magnesium concentrations on lactase activity

Trials	Initial	2 mins	4 mins	6 mins	8 mins	10 mins
1	Error 4					
2	N/A	< 1.1mmol/L	1.9 mmol/L	3.1 mmol/L	3.9 mmol/L	4.3 mmol/L
3	21.1mmol/L	21.1mmol/L	20.2 mmol/L	20.3 mmol/L	-	-
4	N/A	18.1mmol/L	21.2 mmol/L	-	-	-
5	N/A	21.6 mmol/L	-	-	-	-
6	N/A	21.4 mmol/L	-	-	-	-
7	N/A	Error 4	-	-	-	-
8	N/A	Error 4	Error 4	-	-	-