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Assessment of Dietary Intervention Effects on Food Intake in Mus musculus using Repeated Measures ANOVA

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ABSTRACT

The prevalence of type 2 diabetes, metabolic syndrome, along with obesity that causes disturbances in the body's metabolic processes are the main triggers of chronic liver disease or in scientific language called Non-Alcoholic Fatty Liver Disease (NAFLD), getting out of control. This makes managing this disease an increasingly serious global health challenge. One of the main factors influencing this condition is a high-fat diet and an unhealthy lifestyle. Therefore, evaluation of high-fat diet programs on metabolic parameters such as food intake patterns is important as a preventive measure. This study aims to analyze the differences in food intake levels with seven different types of dietary treatments for 28 days, which were tested on mice (Mus musculus) which have physiological and biochemical characteristics that almost resemble humans. The method used was analysis of variance (ANOVA) for longitudinal data to evaluate the dynamics of food consumption across diet groups and observation periods. The results showed that the type of dietary treatment significantly influenced food intake patterns over time, indicating that diet composition plays a crucial role in shaping eating behavior. These findings highlight the importance of both diet type and treatment duration in influencing consumption patterns. However, since this study has not yet identified the most effective dietary regimen, future research is recommended to investigate diet types with high variability, while considering additional factors such as age, sex, and physiological characteristics, as well as extending the observation period to better understand long-term impacts.

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A. INTRODUCTION

Global health issues continue to be of concern in various medical and public health studies. Metabolic disorders such as metabolic syndrome, diabetes mellitus, obesity and Gaucher disease show an increase in cases year after year. WHO (2024) noted that more than 2.5 billion people are overweight globally. These conditions are closely related to changes in modern lifestyles that tend to be passive and high-fat diets (Fahed et al., 2022). These conditions will eventually inhibit the body's mechanism of processing and distributing nutrients such as proteins, fats, and carbohydrates, which if left untreated can trigger abnormal chemical reactions that result in chronic liver diseases, one of which is NAFLD (Fahed et al., 2022).

NAFLD is a chronic disease caused by excessive fat accumulation in the liver in the absence of significant alcohol consumption. The condition can progress to other systemic disorders, such as fibrosis, cirrhosis and multisystem diseases that affect other organs and metabolic

pathways in the body (Pouwels et al., 2022). Currently, the global distribution rate for this disease has reached 25.24% with the highest findings in the Middle East and South America (Wang et al., 2025). In Indonesia alone, the prevalence even exceeds the global prevalence rate of 30.6%.

The main factor causing the increase in NAFLD is the consumption of high-fat foods over a long period of time. A high-fat diet can trigger insulin resistance and reduce the body's antioxidant enzyme activity. Studies have shown that the prevalence of NAFLD continues to increase as obesity cases soar (Younossi et al., 2023). It is estimated that about 60% of obese individuals are at high risk of developing fatty liver, which can progress to NAFLD if not treated promptly (Huh et al., 2022). The results of the Pouwels et al. study also showed that weight loss through a healthy diet can significantly reduce the severity of NAFLD. The study by Zakir et al. (2022) showed that excess fat consumption leads to accumulation of triglycerides in the liver. A study by Fahed et al. (2022) mentioned that a high-fat diet also decreases insulin sensitivity systemically. Therefore, it is important to evaluate dietary patterns as a form of preventive intervention against chronic metabolic diseases such as NAFLD.

To understand the mechanisms caused by a high-fat diet, animal models such as mice (Mus musculus) are often used in various studies. This is because mice are mammals that have physiological and biochemical characteristics that almost resemble humans, so they can provide a more accurate picture of the problem (Mosaddad et al., 2024). Previous research conducted by Rahmadi (2021), showed that a high-fat diet in rats can cause the development of NAFLD with varying severity, depending on the type of fat given. In particular, a diet containing 60% beef fat was shown to produce the worst liver histology, suggesting that the composition of fat in the diet may be a major factor that increases the risk of NAFLD. This is similar to the results of a study conducted by Agustina (2021) which stated that a low-protein diet in rats can cause significant weight loss and a decrease in serum protein levels, indicating a disturbance in nutritional and health status. These findings strengthen the argument that the use of animal models provides an important insight into the physiological effects of diet.

Although there are many studies on the effect of diet on NAFLD, most of them are limited to static or cross-sectional analyses that do not capture the dynamics of changes in eating behavior. Therefore, a longitudinal approach is important as it allows researchers to observe how the feeding behavior of mice changes over time and how this response is affected by specific dietary treatments. This approach is also able to identify critical moments when significant changes occur, making it more appropriate to design dietary intervention strategies. Martínez-de-Quel et al. (2021) emphasize that longitudinal studies provide a deeper understanding of the long-term effects of interventions, especially in the context of diet and metabolism. This distinguishes this study from previous studies because the main focus is not only on comparisons between diet groups, but also how the interaction between time and diet dynamically affects the feeding behavior of mice.

To observe changes in food intake over time, the appropriate statistical method was Repeated Measures ANOVA. This method was chosen because it is able to evaluate the mean differences between groups over multiple measurement times on the same subject. The advantage of this method is that it considers the correlation between times within the same subject, making it more efficient than the usual ANOVA analysis that assumes independent

observations (Chaka & Njuho, 2022). In addition, repeated measures ANOVA is able to identify the main effects of time, treatment, as well as the interaction between the two, which is particularly relevant for evaluating the effectiveness of long-term dietary interventions. Therefore, repeated measures ANOVA is an appropriate method in modeling longitudinal changes in this study.

Based on background, this study aims to analyze the differences in food intake levels of mice (Mus musculus) in seven different dietary treatment groups over a period of 4 weeks or 28 days. This study will evaluate whether there are significant differences in food consumption patterns between groups as well as changes over time. The results of this study are expected to provide a new understanding of the dynamics of eating behavior due to dietary intervention and its contribution to the prevention of metabolic disorders such as NAFLD. This is in line with the goal of Sustainable Development Goals (SDGs) point 3, which has a focus on implementing a Healthy and Prosperous Life, which emphasizes the importance of health and well-being for all ages.

B. METHODS

This study utilized secondary data on food intake levels in mice obtained from laboratory research conducted at the Faculty of Veterinary Medicine, Airlangga University, in 2021. The data reflects the effects of various treatments on food intake levels in mice, comprising seven treatment groups: normal diet, HFD + vehicle, HFD + CUR50, HFD + CUR100, HFD for 14 days followed by HFD-CUR50 from day 15 to 28, HFD for 14 days followed by HFD-CUR100 from day 15 to 28, and HFD + repairfeed. Observations were conducted over four time periods, from the first week to the fourth week. The analytical procedures used in this study are as follows.

1. Presenting data exploration with graphical visualizatio n for food intake levels at each measurement time point for each diet group. Although the specific instrument used to measure food intake was not detailed in the original study, it is assumed that food consumption was quantified by weighing the food before and after feeding sessions, which is a common procedure in dietary experiments.
2. Basic Assumption Analysis for Repeated Measures Design (RMD-ANOVA)
   a. Sphericity Test

      The assumption of sphericity is an important requirement stating that the variance of differences between pairs of time or treatment must be homogeneous. Mauchly's Test of Sphericity is used in testing for fulfillment of the assumption that variances between factors are equal (Blanca et al., 2023). To test this assumption, Mauchly's Test of Sphericity is used with the following hypotheses

      H_0 : The variances of the differences between pairs of factor groups are equal

      H_1 : The variances of the differences between pairs of factor groups are unequal

      The Mauchly Test of Sphericity statistic is as follows

$$W = \frac{|T|}{\frac{tr(T)}{p}.d} \tag{1}$$

When the assumption of is not fulfilled, then a correction step is first carried out, one of which uses the Greenhouse-Geisser or Huynh-Feldt correction to be applied in modifying the degrees of freedom, thereby preserving the validity and accuracy of the ANOVA results. The Greenhouse-Geisser correction is defined by the following equation (Haverkamp & Beauducel, 2017).

$$\varepsilon = \frac{(k-1) * \Sigma\left(s_j - s\right)^2}{\left(\Sigma\Sigma\left(s_j - s\right)^2 - 2\Sigma\left(s_j - s\right)^2 + ks^2\right)} \tag{2}$$

b. Normality Test
The Kolmogorov-Smirnov test is used to test the fulfillment of the assumption that the residual data is normally distributed comparing the theoretical and empirical cumulative frequencies (Cardoso et al., 2023). The test statistic for Kolmogorov-Smirnov is given by the following equation

$$D = {}^{sup}_{Y}|F_n(Y) - F(Y)| \tag{3}$$

3. Modeling Food Intake Levels with Repeated Measures Design (RMD-ANOVA)
Repeated Measures Design (RMD) is a method of repeated measurements on the same subjects to understand changes in response over time (Chaka & Njuho, 2022). The general model of Repeated Measurement Design (RMD) is given in the following equation.

$$y_{ijk} = \mu + \beta_i + \tau_j + \gamma_{ijk} + \varepsilon_{ijk} \tag{4}$$

a. Analyzing RMD to see if there are significant differences in food intake levels based on time (over 4 weeks) and diet group (7 different treatments)
b. Analyzing the main effect, namely the main effect of time, whether food intake levels change significantly over time.
c. Analyzing the main effect of the group, whether food intake levels differ overall between diet intervention groups.
d. Analyze whether there is an interaction between time and group, causing changes in food intake levels over 4 weeks to differ between groups.

4. Interpreting Analysis Results
a. If significant results are found from the interaction of time and diet group, then a second test, namely post-hoc analysis, is needed to determine at which time point the differences are in food intake levels occur.
b. Interpret the effects of time, type of dietary intervention, and their interaction in a biological context, particularly in understanding the effects of diet on the feeding behavior of Mus musculus.

5. Test the assumptions of the RMD model using a normal probability plot or residual plot to analyze whether the errors in the model are identically independent, normally distributed with a mean of 0 and variance σ^2 (Allik et al., 2016).
6. Determine and Interpret the Best Model
   Concluding the optimal model from RMD results that show significant factors influencing food intake levels. Additionally, providing further explanation to draw biological conclusions regarding the effects of dietary interventions on food intake during the observation period.

C. RESULT AND DISCUSSION

1. Data Exploration

The dataset is initially explored through graphical visualization, which illustrates the longitudinal food intake patterns of 56 Mus musculus under dietary interventions over a four week period, as shown in Figure 2.

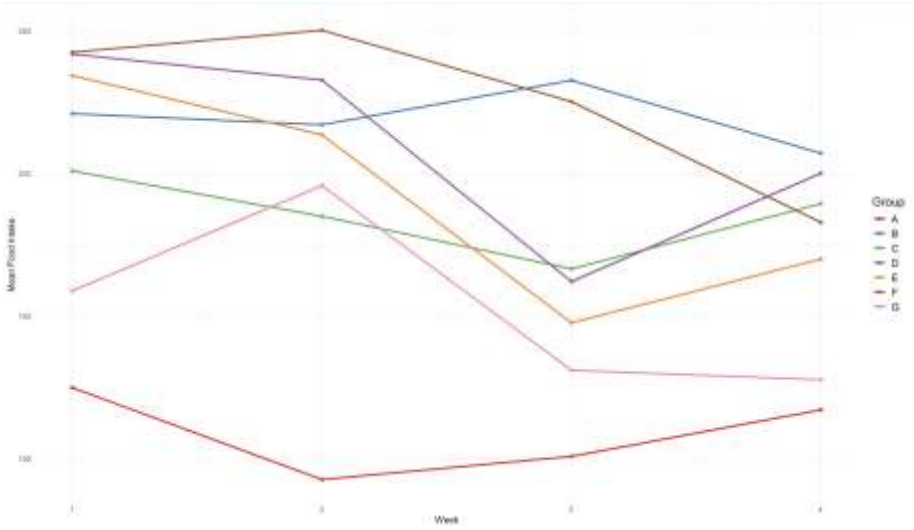

Figure 1. Mean Structure by Group

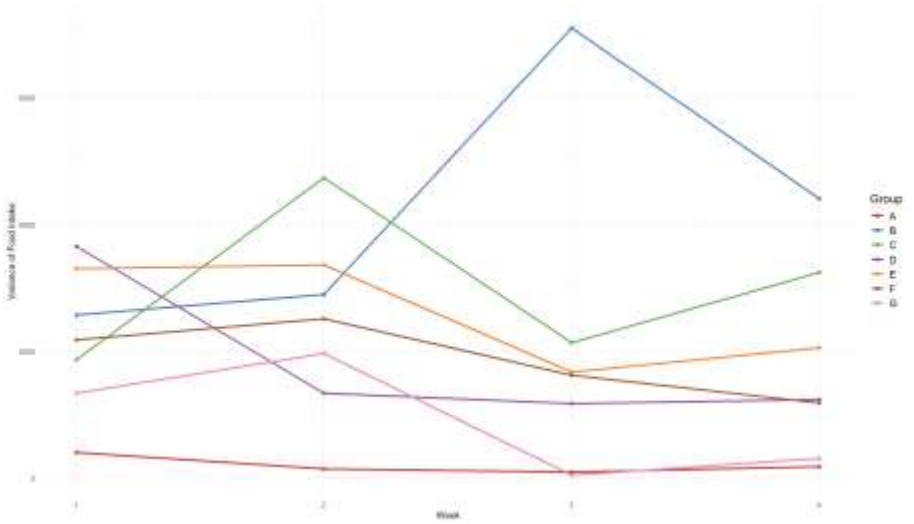

Figure 2. Variance Structure by Group

Figure 1 illustrates the mean food intake trends across the seven treatment groups over the four-week period, highlighting distinct differences in response patterns. While several groups exhibited a significant decline in intake by Week 3, others maintained stable or slightly increased consumption, suggesting that the type of dietary intervention significantly influences average food intake. Additionally, Figure 2 presents the variance structure among groups, revealing that Groups B (HFD + Vehicle) and C (HFD + CUR50) display higher and more fluctuating variability in food intake responses. This elevated variance indicates heterogeneous individual responses within these groups, potentially driven by biological or metabolic differences affecting curcumin treatment efficacy. In contrast, other groups exhibited more stable or decreasing variance patterns, reflecting more uniform food intake responses despite smaller overall consumption changes, as shown in Figure 3.

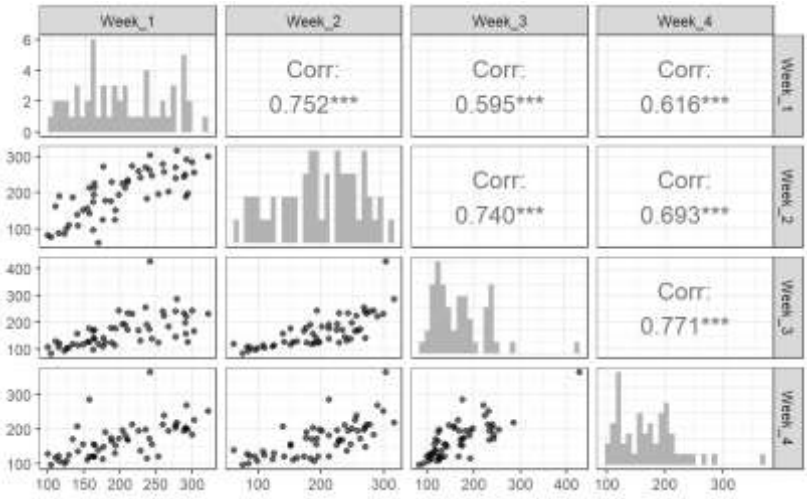

Figure 3. Scatterplot and Correlation within Weeks

Figure 3 present scatterplots and correlation coefficients of weekly food intake measurements in *Mus musculus* over a four-week period. The results reveal strong and statistically significant positive correlations between consecutive weeks, indicating consistent individual food intake patterns over time. Mice with higher intake in one week tended to maintain similar levels in subsequent weeks. The linear distribution of data points in the scatterplots further supports the presence of positive inter-week correlations.

2. Mauchly's Test of Sphericity

The assumption of sphericity must be made in ANOVA of repeated measures, in order to fulfill the requirement that the variances of the differences between repeated measures must be equal (Maurissen & Vidmar, 2017). This assumption ensures that the results of the analysis remain reliable and interpretable, as shown in Table 1.

Table 1. Mauchly's Test of Sphericity

Within Subjects Effect	Test Statistic *W*	Sig.
Weeks	0.69089	0.0056422

Based on the output of Mauchly's Test of Sphericity in Table 1 with a significance level of alpha = 0.05, it is decided that the null hypothesis is rejected if the p-value is less than 0.05. The test result shows a p-value of 0.0056, which is less than 0.05. Although the p-value is close to the cutoff, the decision is to reject the null hypothesis, so then the conclusion is that the variances of the differences between pairs of groups are not equal, so the assumption of sphericity is not fulfilled. Therefore, the Greenhouse-Geisser correction is needed, that is shown in Table 2.

Table 2. Greenhouse-Geisser Correction

Within Subjects Effect	Sig.
Weeks	0.80989

After applying the Greenhouse-Geisser correction, which adjusts the degrees of freedom to prevent Type I errors caused by violations of sphericity (Haverkamp & Beauducel, 2017). The Greenhouse-Geisser epsilon value was obtained at 0.80989. Generally, the sphericity index ranges from 0 to 1, and a value of $\varepsilon = 0.80989$ indicates that the violation of the sphericity assumption is not severe. Therefore, the analysis results remain valid and are more accurate due to the correction applied, despite the initial violation of the sphericity assumption.

3. Classical Assumption

a. Normality Test

In repeated measures ANOVA, verifying classical assumptions is crucial to ensure the validity of the analysis. Among these, the assumption that residuals are normally distributed is particularly important because violations can compromise the accuracy of test statistics and affect the interpretation of within-subject effects (Khatun, 2021), as shown in Table 3.

Table 3. Normality Test

Normality Test	Sig.
Kolmogorov-Smirnov	0.1924

Based on the output of the Table 3 with a significance level of 0.05, the critical region for rejecting the null hypothesis is when the p-value < α this figure is greater than 0.05, meaning that the conclusion is that the residual data is normally distributed.

b. Homogeneity Test

The assumption of homogeneity of variances ensures that the variability across time points or treatment conditions does not differ significantly (Zhou et al., 2023) To assess this, Levene's Test is commonly used to determine if the residual variances of all data are equal, as shown in Table 4.

Table 4. Levene's Test

Weeks	*p-value*
1	0.957
2	0.866
3	0.164
4	0.348

Based on the Levene's Test output in Table 4 with a significance level of 0.05, the null hypothesis is rejected if the p-value < α. This indicates that the residual variances across groups are equal, meaning the assumption of homogeneity is fulfilled.

4. Repeated Measures ANOVA

The algorithm analysis is applied to evaluate whether there are significant differences in repeated measurements of the same variable within the same subject unit. This method accounts for the correlation between measurements with the same subject over time but under different conditions (Janczyk & Pfister, 2023), as shown in Table 5.

Table 5. Repeated-Measures ANOVA Test Results

Observation	Sum Sq	df	Error SS	den df	F-value	p-value
Intercept	7644156	1	291687	49	1284,1268	$2{,}2e^{-16}$
Group	327318	6	291687	49	9,1643	$9{,}99e^{-7}$
Weeks	59400	3	120357	147	24,1833	$8{,}878e^{-13}$
Group**Weeks*	67994	18	120357	147	4,6136	$6{,}065e^{-8}$

a. Testing the mean effect of group
H_0: $\mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5 = \mu_6 = \mu_7 = 0$ (There is no differences were observed in the mean food intake across treatment groups)
H_1 : At least one $\mu_i \neq 0$ (There is differences were observed in the mean food intake across treatment groups)
Based on the test results in Table 5. the significance value for the treatment factor is 9.99 × 10^{-7}, which is less than 0.05. indicating a significant difference in the mean food intake among treatment groups in mice.

b. Testing the mean effect of group and time (interaction)
H_0:$(\alpha\beta)_{11} = (\alpha\beta)_{12} = \cdots (\alpha\beta)_{74} = 0$ (There is no difference in food intake due to the interaction between time and treatment)
H_1 : At least one ij where $(\alpha\beta)_{ij} \neq 0$ (There is difference in the mean food intake due to the interaction between time and treatment)
Based on the test results in Table 5. the significance value for the interaction between weeks (time) and treatment is 6.065 × 10^{-8}, This resulted in the decision to reject the null hypothesis, meaning that there was a significant difference in mean food intake due to the interaction between time and treatment in mice. This suggests that the effect of drug treatment on the food intake of rats is not constant from time 1 to time, but varies depending on the week of observation.

c. Testing the mean effect of time

$H_0 : \mu_1 = \mu_2 = \mu_3 = \mu_4 = 0$ (There is no difference in average food intake across observation times)

H_1 : At least one $\mu_i \neq 0$ (There is a difference in average food intake across observation times)

Based on the test results in Table 5. the significance value for the factor weeks (time) is less than 0.05. Therefore, the decision to reject the null hypothesis, indicating that there is a significant difference in the mean food intake across observation times in mice. Consequently, the analysis can be continued with post-hoc tests, such as Tukey's HSD, to see which time of day makes a difference in the test.

5. Tukey's Post-hoc Test

The test is used to determine whether the means of two treatments differ statistically (Agbangba et al., 2024). Also, it is is a follow-up procedure applied to identify which specific treatments show significant differences when is known to be at least 1 different. Based on the results of the repeated measures ANOVA, it was found that, in addition to the main effect of time, both the group effect and the interaction between time and group showed significant differences in food intake levels. However, the Tukey HSD post-hoc test was focused only on the time factor. This is because the time effect represents the longitudinal changes in food intake, which is the primary focus of the study in assessing the dynamics of change over time, as shown in Table 6.

Table 6. Tukey HSD Post-hoc Analysis of Time Effect

***Weeks* (I)**	***Weeks* (J)**	***Estimate***	***SE***	***p-value***
1	2	5,33	5,69	0,7853
	3	36,91	6,31	<0,0001
	4	32,87	6,42	<0,0001
2	3	31,58	4,53	<0,0001
	4	27,58	5,01	<0,0001
3	4	-4,05	4,03	0,7482

Table 6 shows significant differences in food intake among mice between week 1 and week 3, week 1 and week 4, as well as between week 2 and weeks 3 and 4. These differences are indicated by p-values less than 0.05, suggesting a substantial and consistent decrease in food intake after the second week, with the most pronounced change occurring between weeks 2 and 3. This pattern can be interpreted as strong evidence that week 3 represents a critical point in the longitudinal dynamics of food intake. These findings also support the conclusion that time is the primary factor influencing variations in food intake in mice On the other hand, no significant differences were found between weeks 1 and 2 or between weeks 3 and 4, as the p-values were greater than 0.05, indicating relative stability in food intake during these intervals.

Based on several findings obtained in this study, it can be concluded that different dietary treatments significantly affect food intake in mice (Mus musculus) during the four-week observation period. The amount of food consumed varied between dietary groups, meaning that each type of dietary intervention had a different impact on the eating behavior of mice. Additionally, the interaction between diet type and time was significant, indicating that the

effects of dietary interventions change over time. In other words, food intake patterns are not static but are influenced by the combination of diet type and duration of administration.

A new finding revealed in this study and not extensively described in previous research is the identification of the third week as a critical point in the dynamics of food consumption changes. Results from repeated measures ANOVA and Tukey's post-hoc tests showed that the sharpest decrease in food intake occurred after the second week and tended to stabilize after the third week. This indicates a significant physiological response phase to the high-fat diet, which has not been extensively studied from a weekly temporal perspective. Additionally, this study provides evidence that the group combining the HFD + curcumin 50 mg/kg (CUR50) and HFD + vehicle diets exhibited higher variability in food intake compared to other groups, reflecting the possibility of more heterogeneous biological responses due to these dietary components. This finding has also not been explicitly discussed in previous studies.

D. CONCLUSION AND SUGGESTIONS

Based on some of the previous descriptions found in this study, it can be concluded that different dietary treatments significantly affect food intake in Mus musculus over the four-week observation period. he amount of food consumed varied between the diet groups, meaning each diet affected the mice differently. Also, the interaction between diet type and time was significant, indicating that the impact of the diets changed over the weeks. This means that food intake did not stay the same throughout the study but varied depending on the week and the diet given. These results highlight that both the type of diet and how long it is given play important roles in influencing food intake in mice. For future research, it is recommended to conduct more detailed investigations focusing on the effects of diet types that showed the highest variability, incorporating additional factors such as sex, age, and physiological parameters of the mice to provide a more comprehensive understanding. Extending the observation period could also offer valuable insights into the long-term effects of dietary interventions.

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