Large-Scale Inference on Age and Diet Effects on Mouse Phenotypes

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Introduction

In this memo, we investigate how an anti-aging intervention—including genetic modifications and dietary restrictions—affects phenotypic changes in mice over time, aiming to understand how aging is associated with physiological changes and whether targeted treatments can mitigate, slow, or reverse these effects. Using a cross-sectional design, we utilize a set of measured diverse phenotypes at different ages, comparing treated and untreated groups to assess intervention impacts. Given the large-scale nature of the data (~200 phenotypes studied), we leveraged statistical techniques such as multiple hypothesis correction to ensure robust findings. This study focuses on the "Figure5_phenotypes" dataset, one of five datasets from the original study (source: https://www.nature.com/articles/s41467-022-34515-y), which contains 76 observations across four groups: young control, young restricted, old control, and old restricted. These groups allow us to examine how both age and diet influence phenotypic outcomes and whether dietary restriction modifies aging-related changes.

Methods

To investigate the impact of a calorie-restricted diet on phenotypic aging, mice were divided into young (3 months) and old (26 months) age groups and assigned to either a restricted diet or a regular diet. Phenotypic measurements, including heart rate, body mass, and metabolic markers, were collected for each mouse. This initial exploratory data analysis was performed using boxplots, which visualized differences in phenotype distributions across the four experimental groups (young-regular, young-restricted, old-regular, old-restricted). These visualizations provided insight into potential age and diet effects and whether their influence appeared independent or interactive.

For formal statistical analysis, we applied Ordinary Least Squares (OLS) regression to model each phenotype as a function of age, diet, and their interaction. This model allows us to assess both main effects and interactions. Given the large number of phenotypes analyzed (~200), we performed multiple hypothesis correction to control for family-wise error rates. The resulting regression outputs enable large-scale inference across phenotypes. To systematically assess the effects of age and dietary interventions across phenotypes, we visualized both raw Z-scores and standardized effects using a heatmap, allowing for an intuitive comparison of effect sizes across different phenotypes. Z-scores provide a measure of statistical significance by standardizing effect estimates relative to their standard errors, while standardized effects rescale these estimates based on the variability in age and diet, offering a more interpretable measure of effect size. To further summarize the prevalence of significant effects, we applied threshold-based filtering, counting the number of phenotypes exceeding predefined significance thresholds for both Z-scores and standardized effects. This approach quantifies the proportion of phenotypes significantly influenced by age, diet, and their interaction, helping to distinguish broad intervention patterns from noise in large-scale inference.

Results

The boxplots in Figure 1 illustrate the distributions of ST, Body_mass_NMR, and HR across the four experimental groups. ST is highest in the old-restricted group and lowest in the young-restricted group, while Body_mass_NMR peaks in the old-control group and is lowest in the young-restricted group. HR follows a different pattern, with the highest median in the young-control group and the lowest in the old-restricted group. Next, regression analysis on over 200 phenotypes examined the effects of age, diet, and their interaction in Figure 2. For HR, the model explained 51.6% of the variance ($R^2 = 0.516$). Diet had a significant negative effect ($p < 0.001$, $\beta = -1.4974$), while age alone was not significant ($p = 0.442$). The interaction term was significant ($p = 0.008$, $\beta = 1.2775$), suggesting that diet's effect on HR varied by age. Regression diagnostics confirmed model validity.

The Z-score heatmap in Figure 3 reveals a range of positive and negative deviations, while the standardized effects heatmap in Figure 4 shows a similar trend but with a more constrained range. Diet and age effects appear more pronounced than interaction effects. To quantify these effects, we applied significance thresholds to identify the proportion of phenotypes exceeding predefined cutoffs. With a Z-score threshold of 2, 51.6% of phenotypes were significant for age, 39.5% for diet, and 18.5% for interaction (Figure 5). Using a standardized effects threshold of 0.5, 33.1% of phenotypes were significant for age, 15.9% for diet, and none for interaction (Figure 6). Lowering the standardized effects threshold to 0.3 increased significance rates to 55.4% for age, 37.6% for diet, and 6.4% for interaction (Figure 7).

Interpretation

The results show that age and dietary restriction significantly impact phenotypic outcomes, with varying effects across traits.The boxplots indicate that aging causes consistent physiological changes, while dietary restriction can mitigate or amplify these effects depending on the phenotype. For example, in some cases, dietary restriction appears to slow age-related decline, while in others, it induces changes independent of aging. This suggests that the benefits of dietary restriction are not uniform but rather depend on the specific trait being measured. The varying median values further highlight that age and diet interact differently for different phenotypes, reinforcing the need to analyze their effects on a case-by-case basis.

The regression analysis and heatmaps clarify that age has a broad and consistent impact across phenotypes, whereas diet has a more selective but still substantial effect. Many phenotypes show significant changes with age and diet, but weaker interaction effects suggest dietary restriction affects traits without universally altering aging trajectories. Threshold-based analysis confirms this pattern, showing many phenotypes are significantly affected by age and diet, while fewer show strong interactions. Notably, the interaction term was not significant when using a standardized effects threshold of 0.5, indicating that its influence is generally weaker than the main effects of age and diet. However, lowering the threshold to 0.3 revealed interaction effects in a small subset of phenotypes, suggesting that while interactions exist, they are more subtle and require less stringent significance criteria to be detected. This further supports the idea that dietary restriction does not systematically counteract aging but may have nuanced effects in specific cases.

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Figures

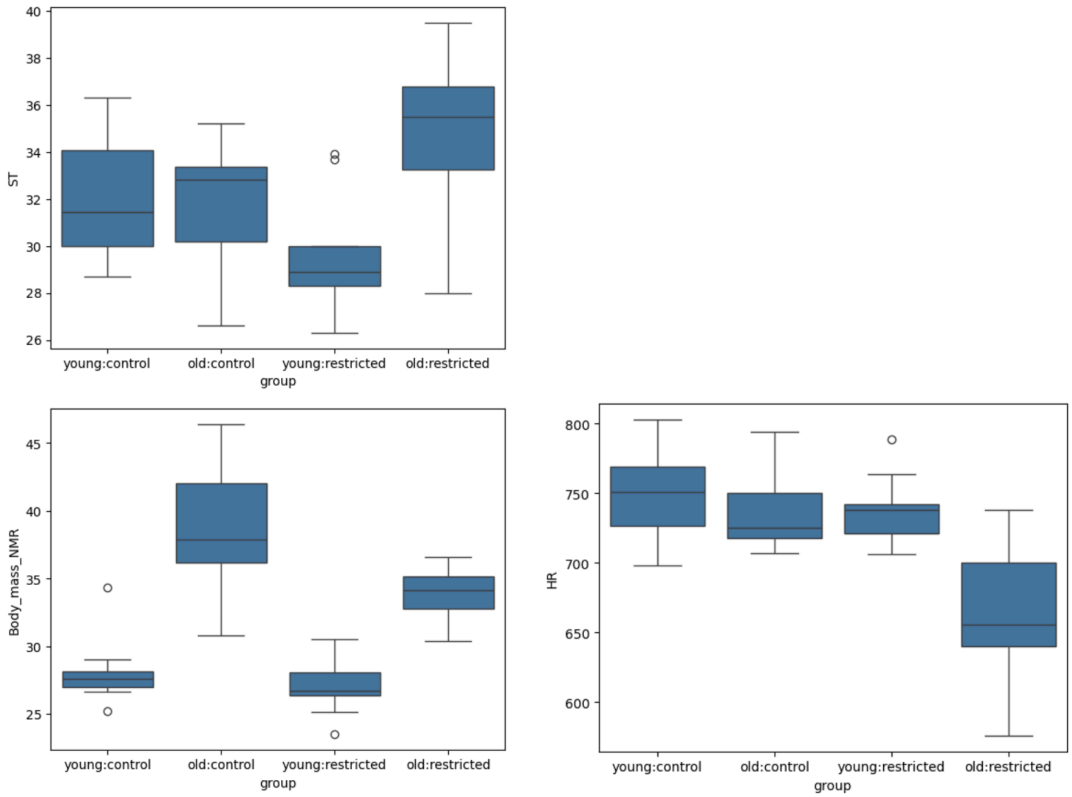

Figure 1: Boxplots of selected phenotypes grouped by age and diet intervention.

OLS Regression Results

Dep. Variable:	HR	**R-squared:**	0.516
Model:	OLS	**Adj. R-squared:**	0.477
Method:	Least Squares	**F-statistic:**	13.15
Date:	Wed, 19 Feb 2025	**Prob (F-statistic):**	5.37e-06
Time:	18:40:06	**Log-Likelihood:**	-42.791
No. Observations:	41	**AIC:**	93.58
Df Residuals:	37	**BIC:**	100.4
Df Model:	3		
Covariance Type:	nonrobust		

	coef	**std err**	**t**	**P>|t|**	**[0.025**	**0.975]**
Intercept	0.3361	0.218	1.541	0.132	-0.106	0.778
age[T.young]	0.2458	0.316	0.778	0.442	-0.395	0.886
diet[T.restricted]	-1.4974	0.308	-4.855	0.000	-2.122	-0.872
age[T.young]:diet[T.restricted]	1.2775	0.453	2.818	0.008	0.359	2.196

Omnibus:	0.506	**Durbin-Watson:**	2.028
Prob(Omnibus):	0.776	**Jarque-Bera (JB):**	0.118
Skew:	0.119	**Prob(JB):**	0.943
Kurtosis:	3.111	**Cond. No.**	6.58

Figure 2: OLS Regression Results

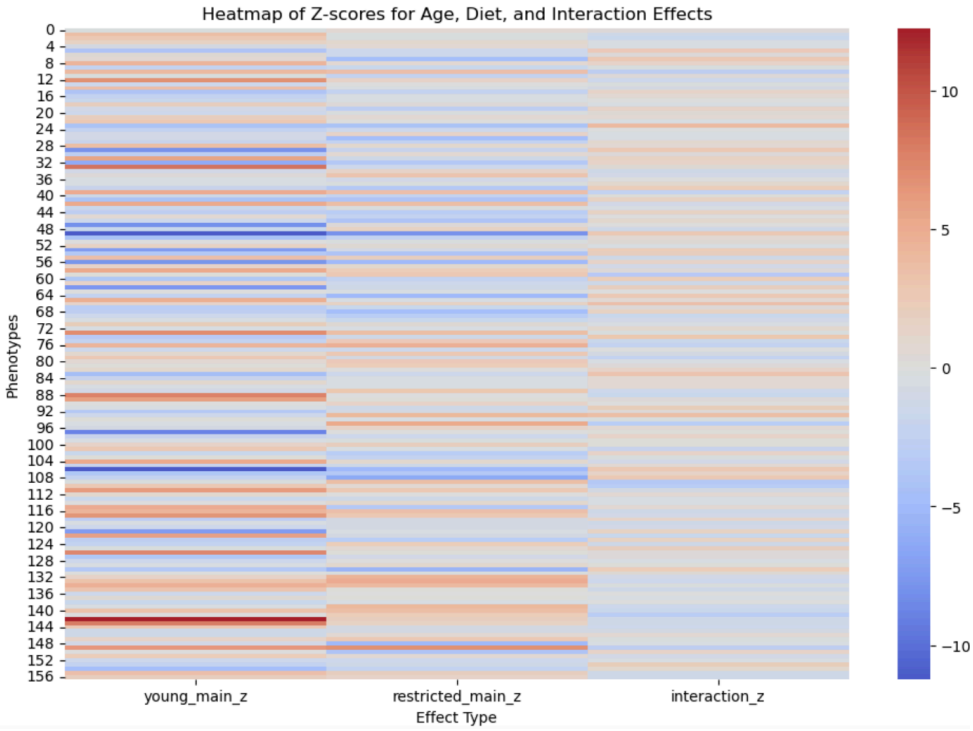

Figure 3: Heatmap of Z-scores for Age, Diet, and Interaction Effects

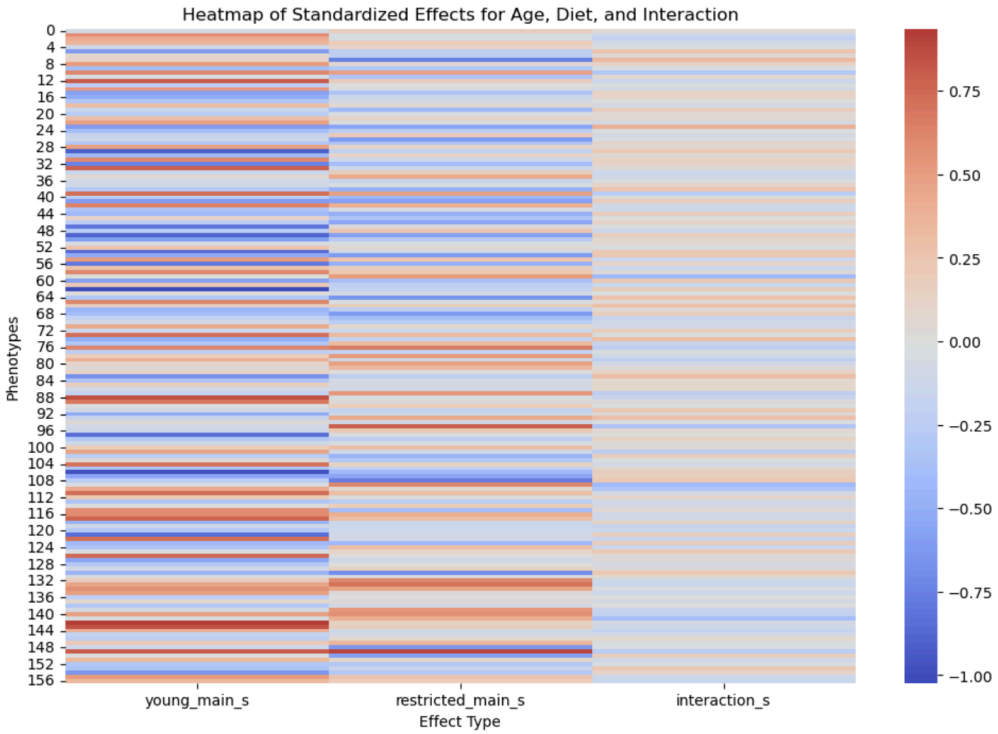

Figure 4: Heatmap of Standardized Effects for Age, Diet, and Interaction

	Effect Type	Significant Phenotypes	Percentage
0	Age Effect	81	51.592357
1	Diet Effect	62	39.490446
2	Interaction Effect	29	18.471338

Figure 5: Significant phenotypes based on Z-scores (threshold = 2).

	Effect Type	Significant Phenotypes	Percentage
0	Age Effect	52	33.121019
1	Diet Effect	25	15.923567
2	Interaction Effect	0	0.000000

Figure 6: Significant phenotypes based on standardized effects (threshold = 0.5).

	Effect Type	Significant Phenotypes	Percentage
0	Age Effect	87	55.414013
1	Diet Effect	59	37.579618
2	Interaction Effect	10	6.369427

Figure 7: Significant phenotypes based on standardized effects (threshold = 0.3).