

# **Long Term High-dose Aspartame Consumption Increased Global DNA Methylation In Various Rat Tissues**

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# What is Epigenetics?

- The genome of a eukaryotic organism is the same in all its cells.

## Epigenetics;

- Alteration of gene expression without changes in the underlying DNA sequence.
- Changes in the structure of DNA or the proteins that bind to it.
- Can be influenced by environmental exposures, lifestyle factors, and other external stimuli.

# Epigenetic Mechanisms

- **DNA Methylation**

- addition of a methyl group to a DNA molecule
- The 5-methylcytosine (5-mc) is the most common modification in eukaryotic genome.
- High levels of methylation at a specific site typically leads to decreased gene expression

- Histone Modification

- Chromatin Remodeling

- Non-coding RNA Regulation

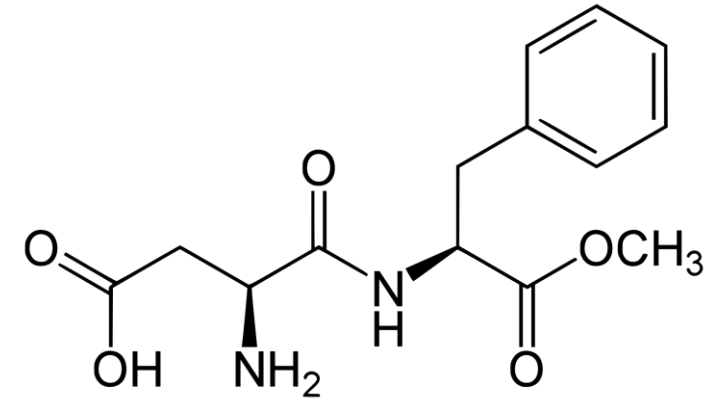
2. Li, Y., *Modern epigenetics methods in biological research. Methods*, 2021. 187: p. 104-113.

9. Schmitz, R.J., Z.A. Lewis, and M.G. Goll, *DNA Methylation: Shared and Divergent Features across Eukaryotes. Trends Genet*, 2019. 35(11): p. 818-827.

10. Dhar, G.A., et al., *DNA methylation and regulation of gene expression: Guardian of our health. Nucleus (Calcutta)*, 2021. 64(3): p. 259-270.

# Aspartame

- Low-calorie artificial sweetener (200 times sweeter than regular sugar).
- Approved for use in food products in more than 90 countries.
- One of the most rigorously tested food ingredients.
- There is still no consensus regarding its safety.



7. Kirkland, D. and D. Gatehouse, "Aspartame: A review of genotoxicity data". Food Chem Toxicol, 2015. 84: p. 161-8.

8. Borghoff, S.J., et al., Updated systematic assessment of human, animal and mechanistic evidence demonstrates lack of human carcinogenicity with consumption of aspartame. Food Chem Toxicol, 2023. 172: p. 113549.

# Materials and Methods

## 1

- 15 Sprague Dawley rats
  - Control (n=5)
  - Low-Dose = 50mg aspartame daily (n=5)\*
  - High-Dose = 250mg aspartame daily (n=5)\*\*
- Observed for 10 weeks
- Euthanasia



\*: FDA approved dose

\*\* : Dose at which excessive aspartame consumption was modeled and adjusted for animal metabolic rate.

FDA, F. (1981). Food additives permitted for direct addition to food for human consumption; aspartame. Food and Drug Administration. Federal Register, 46FR38285.

Nau H. 1986 "Species differences in pharmacokinetics and drug teratogenesis" Environ Health Perspect. 1986 Dec; 70: 113–129.

# Materials and Methods

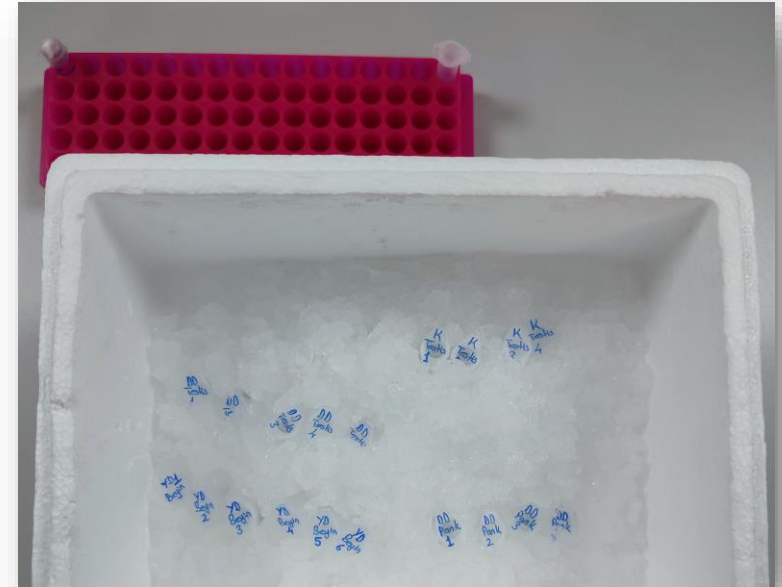
## 2

### 1. Collecting Tissues

- Cerebral cortex, pancreas, liver, kidney and testicular tissues were collected & then homogenized by bead-beating method.

2. DNA Extraction

3. Assessing the Global Methylation



# Materials and Methods

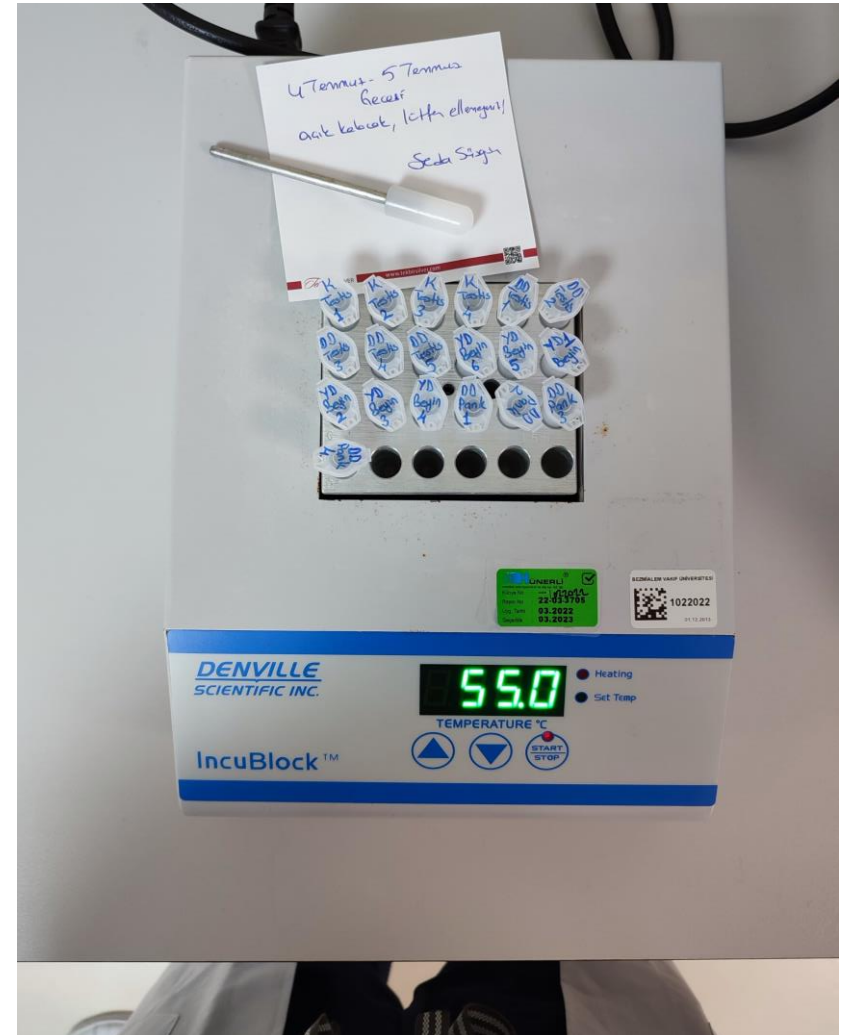
## 3

1. Collecting Tissues

2. DNA Extraction

- DNAs were extracted using *Quick-DNA™ Microprep Plus Kit* (Zymo Research, CA, USA).

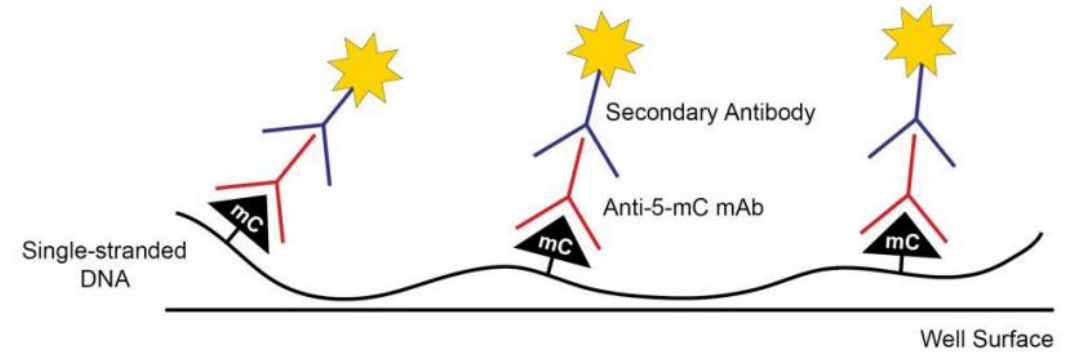
3. Assessing the Global Methylation



# Materials and Methods

## 4

1. Collecting Tissues
2. DNA Extraction
3. Assessing the Global Methylation
  - Percentage of global 5-mC in DNA samples were measured by *5-mC DNA ELISA Kit* (Zymo Research).



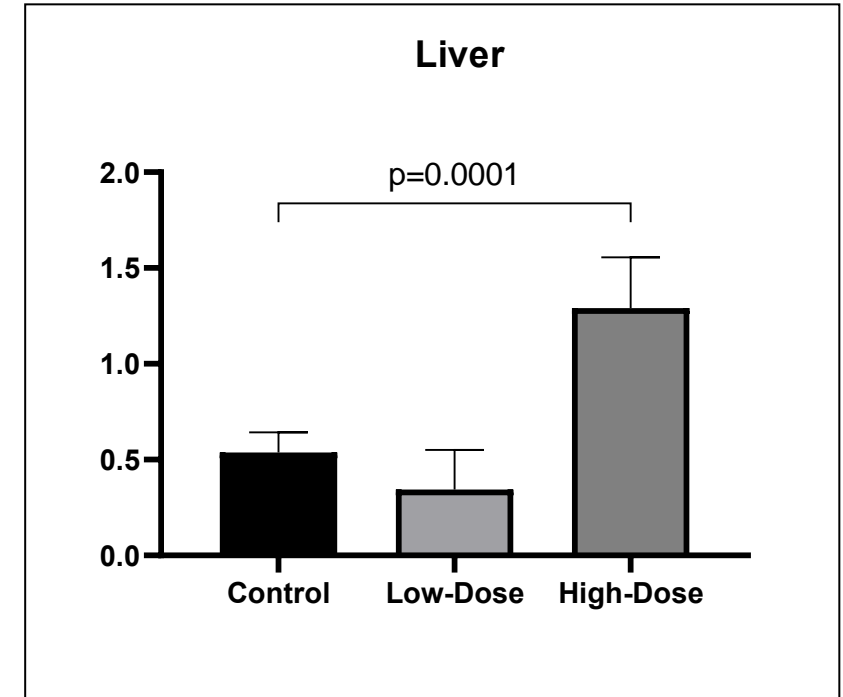
**The 5-mC DNA ELISA Kit utilizes the indirect ELISA technique in its workflow.** Denatured, single-stranded DNA samples are coated on the well surfaces in **5-mC Coating Buffer**. **Anti-5-Methylcytosine** monoclonal antibody (Anti-5-mC mAb) and the HRP-conjugated **Secondary Antibody** are prepared in **5-mC ELISA Buffer** and added to the wells. Detection of 5-mC occurs after addition of the **HRP Developer**.





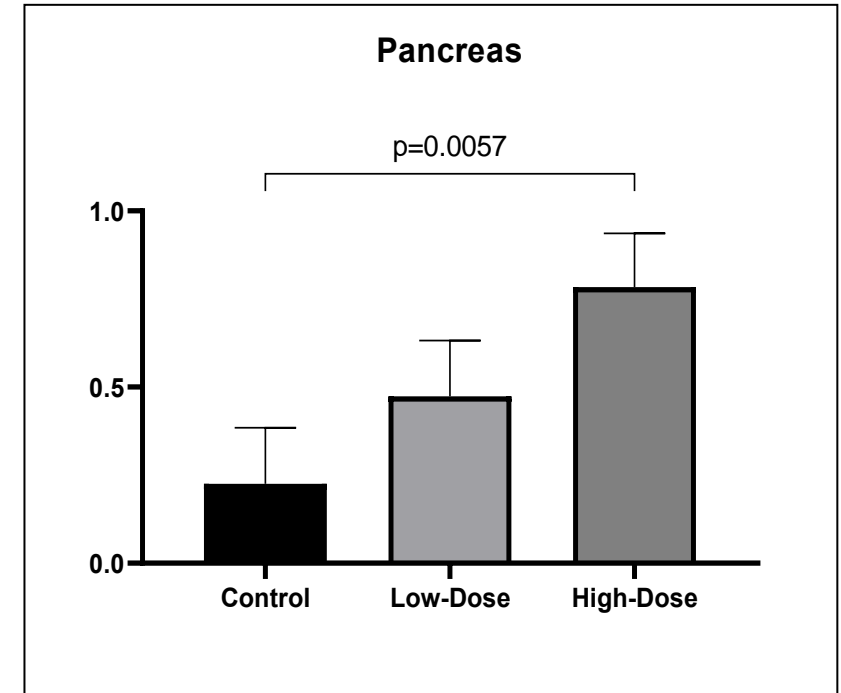
# Results - *Liver*

- A statistically significant **increase** was observed between the control group and the high-dose group ( $p=0.0005$ ).
- A statistically significant **increase** was observed between the low-dose group and the high-dose group ( $p=0.0001$ ).



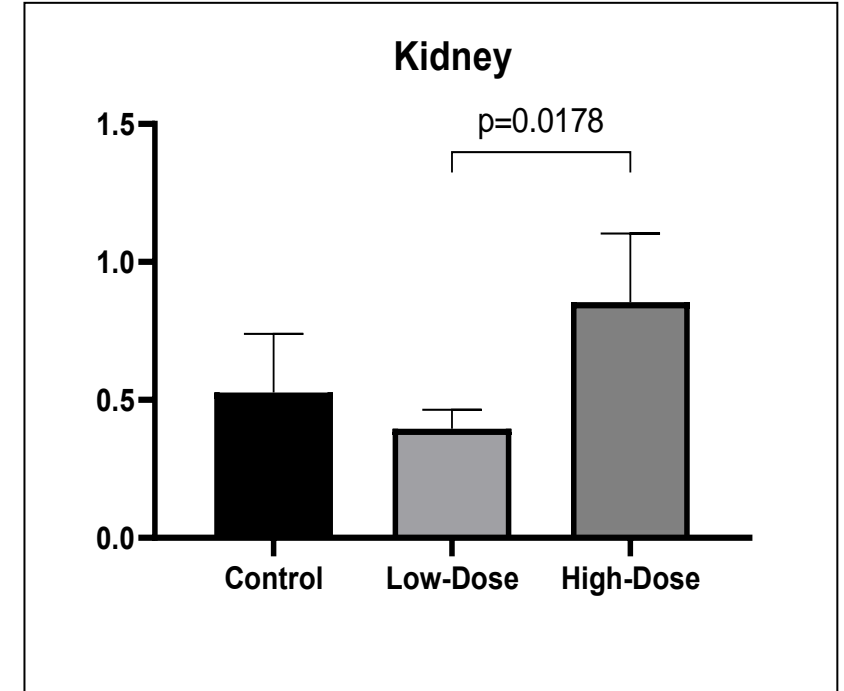
# Results - *Pancreas*

- A statistically significant **increase** was observed between the control group and the high-dose group ( $p=0.005$ ).



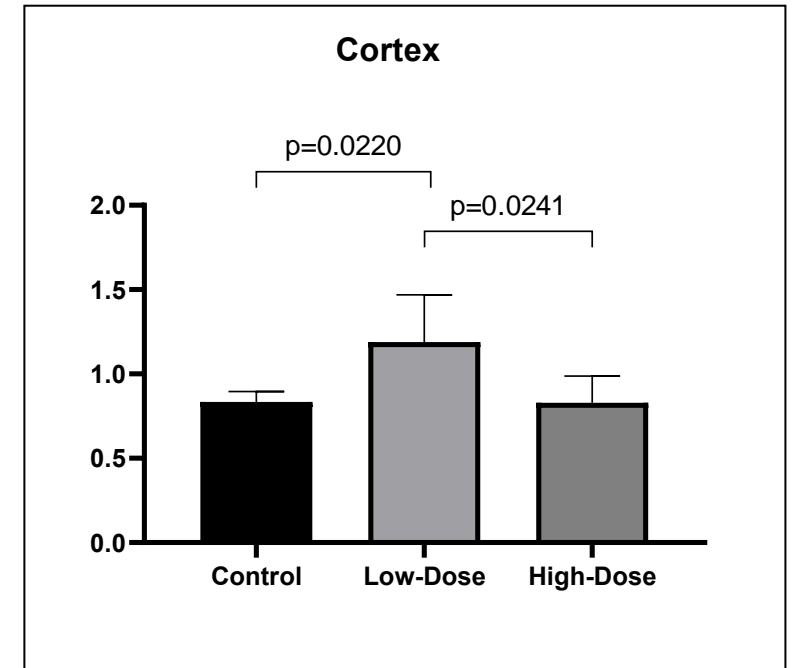
# Results - *Kidney*

- A statistically significant **increase** was observed between the low-dose group and the high-dose group ( $p=0.017$ ).



# Results – *Cerebral Cortex*

- A statistically significant **increase** was observed between the control group and the low-dose group ( $p=0.022$ ).
- A statistically significant **decrease** was observed between the low-dose group and the high-dose group ( $p=0.024$ ).



# Conclusion

- Our study found that dietary aspartame consumption leads to DNA hypermethylation in the **pancreas** and **liver**.
- DNA hypermethylation can alter gene expression and impact cellular function, potentially increasing the risk of developing metabolic disorders, cardiovascular diseases and cancer.
- More research is needed to fully understand the long-term effects of aspartame consumption on epigenetic regulation and overall health.