

## Effects of Tea Tree Oil on Metabolic Rate and Reduction of Reactive Oxygen Species in *Mus musculus*

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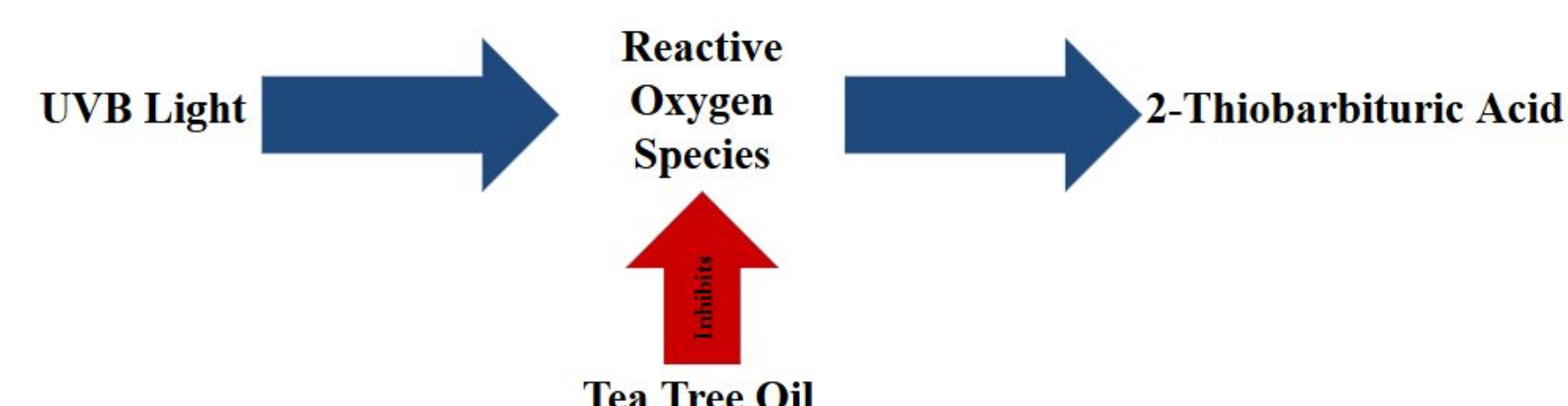


### Introduction:

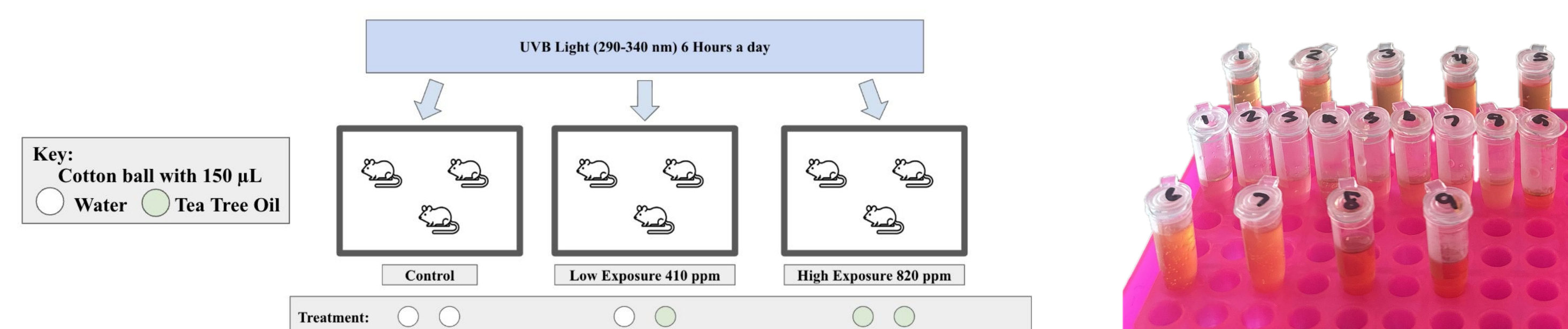
- Previous studies have shown anti-inflammatory, anti-fungal, anti-bacterial, and healing properties from exposure to *Melaleuca* oil or Tea Tree Oil (TTO) (Mohamed & Alotaibi 2023).
- T-4-ol is a phenolic compound in TTO that has been identified as the anti-inflammatory agent (Brand, et al, 2001).
- One proposed anti-inflammatory mechanism of TTO is associated with decreasing the production of reactive oxygen species (ROS) (Golab & Skwarlo-Sonta 2007).
- The production of reactive oxygen species creates 2-Thiobarbituric Acid which can be measured using a spectrophotometric ELISA kit that provides absorbance values for each sample (Figure 1).
- Upregulation of ROS can lead to damaging of proteins, lipids, carbohydrates, and nucleotides (Jakubczyk K, et al, 2020). The upregulation of ROS might also cause an increase in metabolic rates due to an increase in oxidative stress.
  - Exposure to UVB light has been demonstrated to induce the production of ROS in mice (De Jager, et al, 2017).
- Research Question:** Does the concentration of TTO exposure correlate to the downregulation of reactive oxygen species? Does TTO exposure correlate to changes in metabolic rate?
- Hypothesis:** If the concentration of TTO correlates with decrease of ROS production, then the group exposed to high concentrations of TTO will display a larger downregulation of ROS production when compared to low concentrations. Additionally, if the concentration of TTO correlates with a decrease of ROS production, then the groups with higher exposure will have lower metabolic rates and lower energy needs when compared to low concentrations because they are experiencing less oxidative stress.

### Methods and Materials:

- Purity of TTO was verified using chromatography paper.
- Experimental Design (Figure 2).
  - Two cotton balls were placed on opposite sides of each container for 3 hours of exposure per day.
  - Control: 150  $\mu$ L of water on each of the 2 cotton balls.
  - Low Concentration (410 ppm) (Golab & Skwarlo-Sonta, 2007): 150  $\mu$ L of TTO on one cotton ball and 150  $\mu$ L of water on the other.
  - High concentration (820 ppm) (Golab & Skwarlo-Sonta 2007): 150  $\mu$ L of TTO on both cotton balls.
- UVB Light (290-340 nm)
  - 6 hours of exposure daily for all treatments.
- Urine collection
  - Approximately 400  $\mu$ L of urine was collected for each mouse.
  - Samples were collected weekly for two weeks.
- Respiratory data was collected weekly for four weeks with a field metabolic system to assess general metabolic changes.
- Mass of mice were recorded weekly.
- The presence of lipid peroxides were quantified in urine with TBARS Assay kit from Eagle Biosciences (Figures 3 & 4).
  - Each absorbance value from the TBARS Assay kit was then used to calculate the concentration of the 2-Thiobarbituric Acid (Figure 4)
- T-Tests assuming unequal variances were performed to measure significant differences between the control and experimental groups.



**Figure 1.** Upregulation of ROS production will result in increased amounts of 2-TBA. TTO has been shown to inhibit ROS production in vivo (Red Arrow), leading to decreased amounts of 2-TBA. Therefore, the high-exposure experimental group should display a larger downregulation of 2-TBA when compared to low exposure.

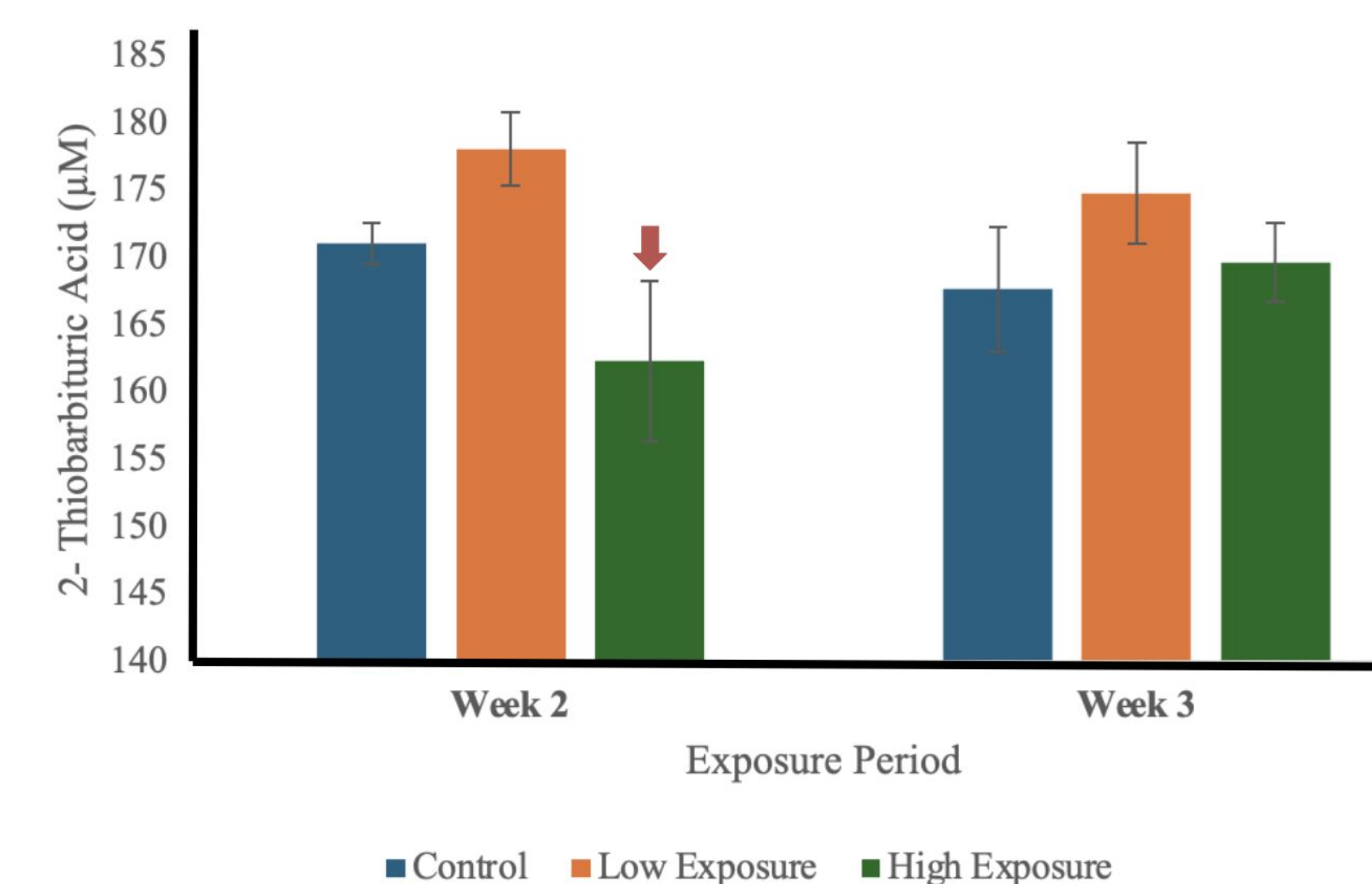


**Figure 2.** Experimental design with corresponding treatment of water or TTO on two cotton balls. 150  $\mu$ L of TTO is equivalent to 410 ppm of exposure (low), 2 cotton balls with 150  $\mu$ L is equivalent to 820 ppm of exposure (high). Control and experimental groups were exposed to UVB light six hours per day.

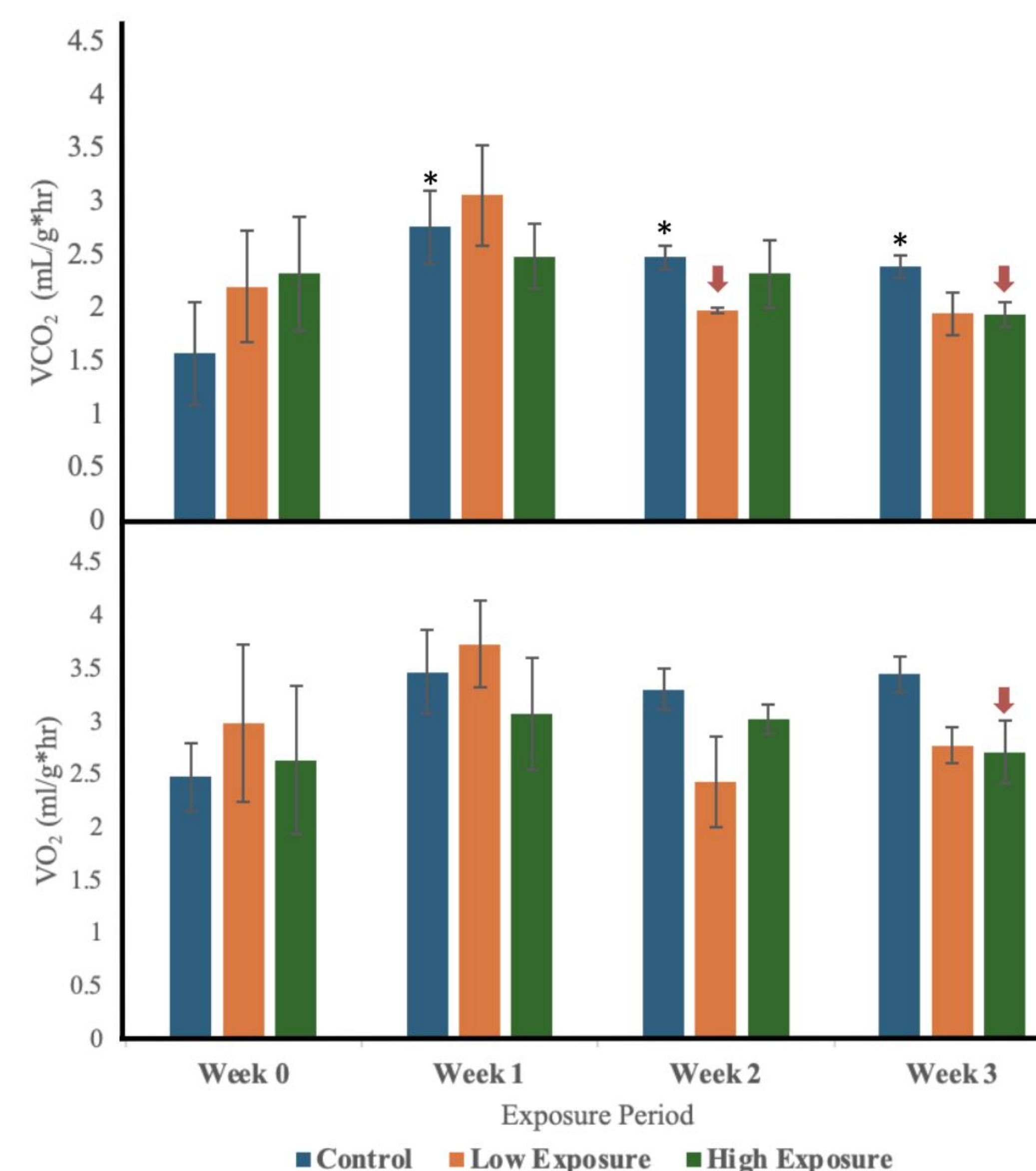


**Figure 3.** The TBARS Assay kit with urine samples from week two. In each sample, the color orange can be seen as a product of the 2-TBA reacting with ROS.

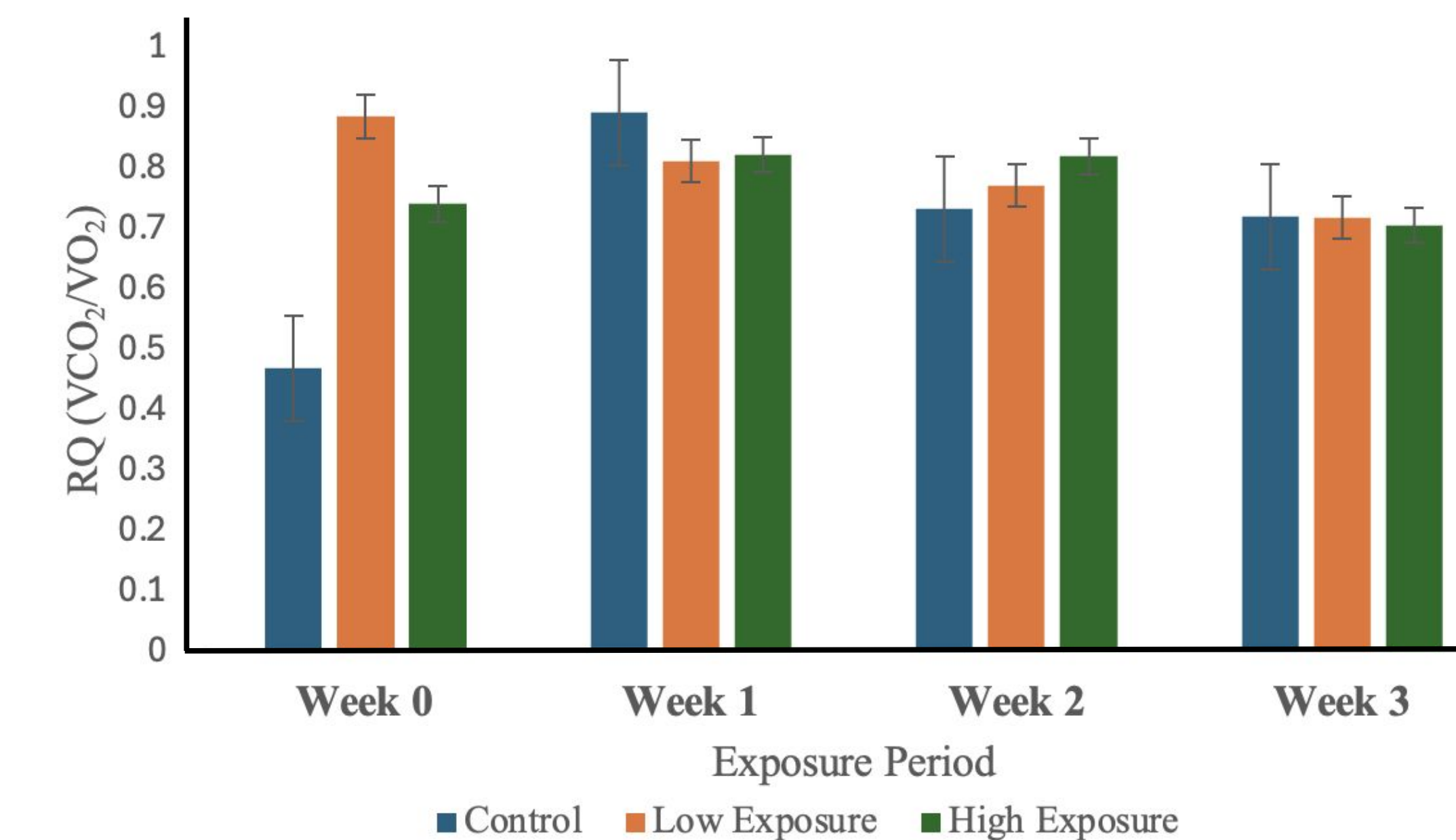
### Results:



**Figure 4.** Means  $\pm$  standard error of lipid peroxide 2-TBA concentration in control (0 ppm), low (410 ppm), and high (820 ppm) exposure to TTO in mouse urine. \* = Data with significant difference ( $p < 0.05$ ) compared to weekly control.



**Figure 5.** Means  $\pm$  standard error of  $VCO_2$  and  $VO_2$  respiratory values for control (0 ppm), low (410 ppm), and high (820 ppm) groups over four weeks. \* = Data with significant difference ( $p < 0.05$ ) compared to baseline.  $\downarrow$  = Data with significant difference ( $p < 0.05$ ) compared to weekly control.



**Figure 6.** Mean RQ values  $\pm$  standard error over four weeks. No significant differences ( $p < 0.05$ ) between groups was present.

### Discussion & Future Research Questions:

- The only treatment group with a significant 2-TBA difference compared to the control group ( $p < 0.05$ ) was the high exposure week 2 group ( $p = 0.0425$ ) (Figure 4).
- Significant differences in  $VCO_2$  averages ( $p < 0.05$ ) were observed between the week 0 and the controls for the weeks 1, 2, and 3. ( $p = 0.0243, 0.025, 0.0303$ ) (Figure 5).
  - The difference in average  $VCO_2$  between week 0 and each control for weeks one, two, and three support the hypothesis that UVB increases oxidative stress. The mice from the control groups of weeks with UVB light have higher metabolic rates and energy needs when exposed to oxidative stress inducing conditions.
- $VCO_2$  averages from the low exposure group week 2 ( $p = 0.0058$ ) and high exposure group week 3 ( $p = 0.0223$ ) were also statistically lower ( $p < 0.05$ ) when compared to the controls (Figure 5).
  - The lower  $VCO_2$  values could suggest there is an optimal exposure to TTO.
  - Further research with a larger concentration gradient and exposure time could supply more concrete results.
- $VO_2$  averages from the high exposure week 3 group were significantly lower than the control week 3 group with a p-value ( $p < 0.05$ ) at  $p = 0.02598315$  (Figure 5).
  - Long-term exposure to TTO for more than three weeks could lower the metabolic rates by reducing ROS production even more. Further downregulation of ROS production could lead to a gradual decrease in metabolic rates since individuals would be experiencing less oxidative stress. Therefore, future research could extend the timeline.
- RQ averages showed no significant differences ( $p < 0.05$ ) between groups (Figure 6).
  - This indicates that similar metabolic substrates were used by mice throughout experiment

### References:

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