ABSTRACT

Metabolic disorders affect much of the population, and it is widely believed that they have a genetic basis as well as being influenced by dietary factors. Recent studies have revealed an important role for the *neil*1 gene in the prevention of metabolic diseases. *neil*1 is a DNA Glycosylase that catalyzes the repair of DNA and mtDNA. We aimed to understand how a deletion in the *neil*1 gene in a mouse model would affect susceptibility to high fat and high carbohydrate diets. We also sought to establish a role for pro and antioxidants in modulating disease risk. To accomplish this, Wild Type (WT) and *neil*1 Knockout (KO) mice were placed on either high fat or high carbohydrate diets. In a cohort of animals, the high fat diet was supplemented by the pro-oxidant $KBrO_3$, or the antioxidant N Acetyl Cysteine (NAC).

Under the oxidative stress of a high fat diet, the KO mice gained more weight than the WT mice. A similar difference was observed for the high carbohydrate treatment. The pro-oxidant $KBrO_3$ seemed to mitigate the weight gain phenotype that was characteristic of a high fat diet, whereas the antioxidant NAC had no effect in both kinds of mice.

At this point, the conclusion can be drawn that *neil*1-KO animals are more prone to the weight gain (and attendant effects of metabolic syndrome) induced by high fat and high carbohydrate diets.

METHODS

High Fat-treated Mice

1. 10 month old male WT (5), HET (4) and KO (5), mice were bred on-site.
2. All mice were fed diet containing 60% fat for the period of 17 weeks. Food was replaced every 2 weeks. Mice were housed in Thoren racks which provided them water.
3. Food consumption and weight of mice were measured weekly to track changes.
4. As mice died, they were dissected to look for phenotypes of cancer, cysts, etc.
5. At end of period, mice were sacrificed and dissected to observe phenotypic differences.

Potassium Bromate and NAC-treated Mice (3,4)

1. Procured 24 6-month old male mice, WT (12) and KO (12) bred on site.
2. Divided equally into 3 treatments: H_2O, $KBrO_3$ (pro-oxidant), NAC (antioxidant), NAC treatment is 2mg/L, $KBrO_3$ is 1g/L administered via drinking water.
3. Food was replaced every 2 weeks, solution is replaced every week (biweekly for KBrO3 due to more rapid degradation).
4. Food consumption, solution consumption and weight of all mice were tracked.

Sucrose-treated Mice

1. Procured 13 5-month old male mice, WT from Jackson Labs(6), KO bred on site (7)
2. Divided into 2 treatments: H2O (2 mice from both WT and KO) and Sucrose (4 WT and 5 KO mice). Sucrose solution was 30% weight/volume administered through drinking water. Mice were maintained on a regular chow diet.
3. Food was replaced every 2 weeks, solution was replaced every week.
4. Food consumption, solution consumption and weight of all mice were tracked.

LITERATURE CITED

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Figure 1: BW vs. Time for NEIL1-positive, NEIL1-deficient, and Heterozygote mice

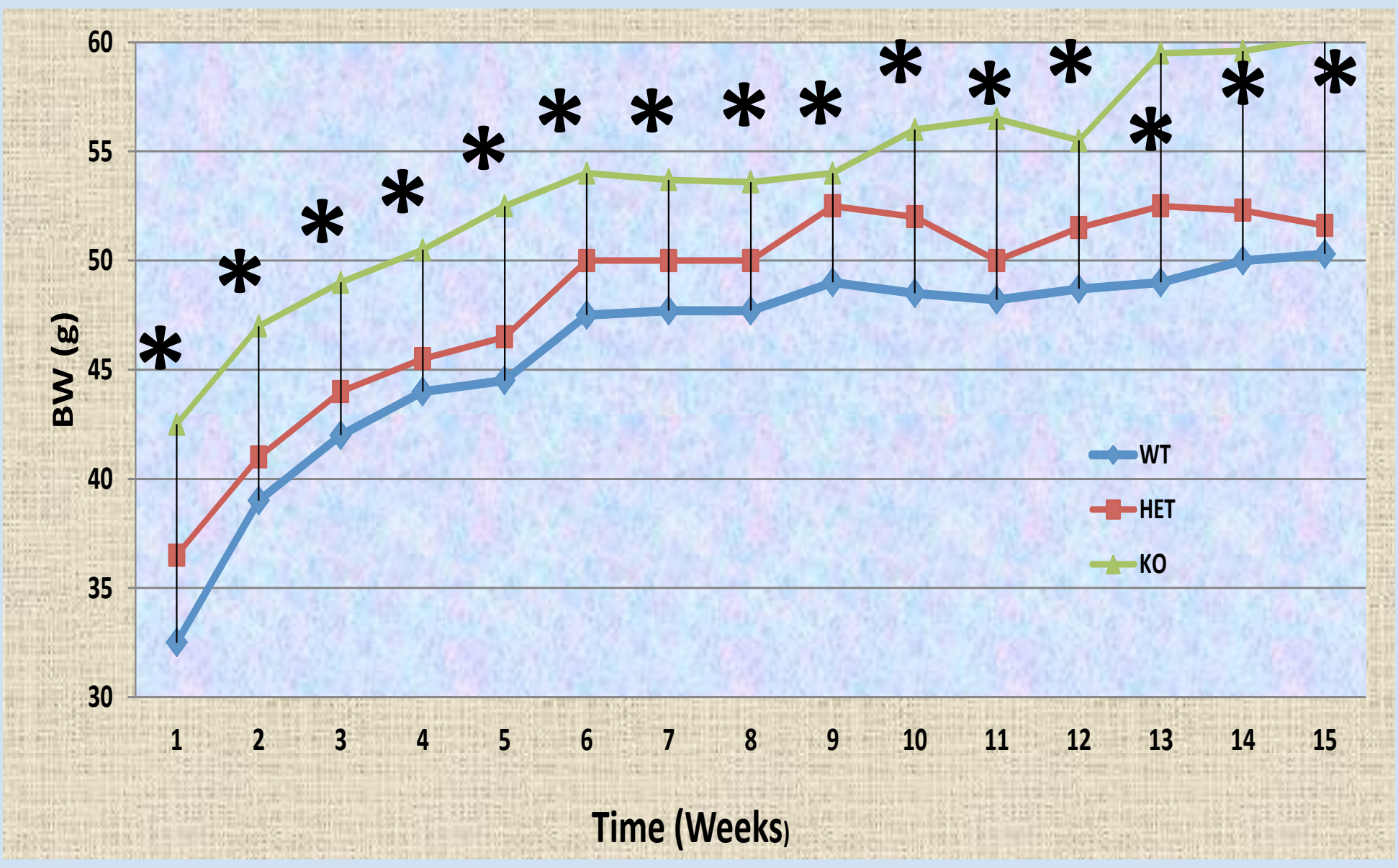

Figure 1: Male mice with varying genotypes for the *neil*1 gene were given a diet containing 60% fat. There was a significant difference between the weights of the KO and WT mice, and an intermediate phenotype was observed for the heterozygotes. *, $p<0.05$ vs. WT

Figure 2: Change in Body Weight vs. Time for NEIL1-positive and NEIL1-deficient mice given $KBrO_3$ (pro-oxidant), NAC (anti-oxidant) or Water (control)

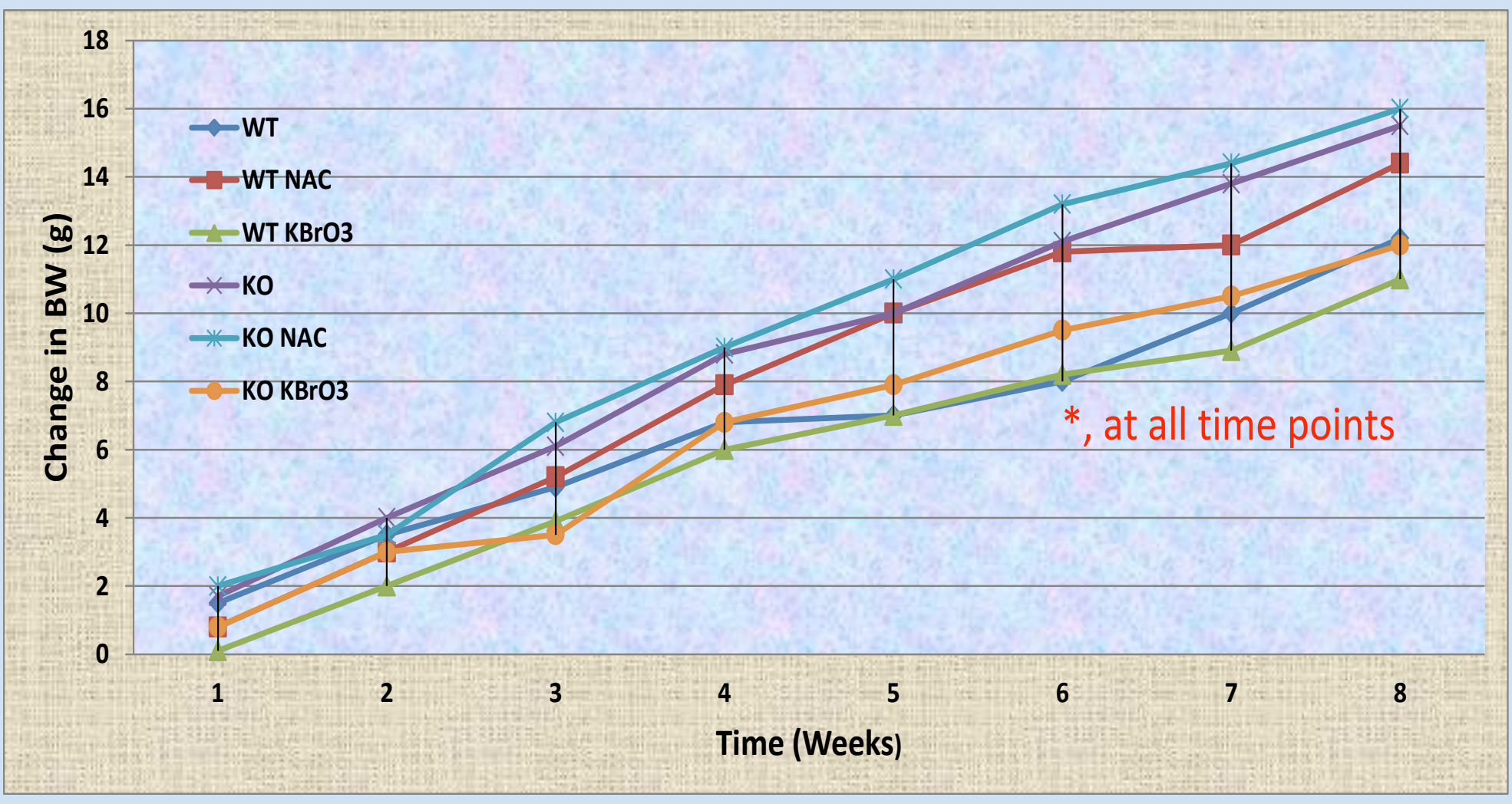

Figure 2: Male WT and KO mice were placed on a HFD and provided either drinking water alone (H_2O control) or water supplemented with $KBrO_3$ (pro-oxidant) or NAC (anti-oxidant). No significant differences were observed between the weight gains of H_2O and NAC mice of either genotype. However, $KBrO_3$ treated KO mice gained significantly less weight than H_2O treated controlled mice. While it is not clear precisely why $KBrO_3$ prevents weight gain, this suggests that the effects of $KBrO_3$ are magnified in KO animals. *, $p<0.05$ vs. KO

Table 1: Change in Body Weight for NEIL1-positive and NEIL1-deficient mice given $KBrO_3$ (pro-oxidant), NAC (anti-oxidant) or Water (control)

Genotype and Treatment	Change in Weight (grams)
WT H_2O	11.667
WT NAC	13.378
WT $KBrO_3$	10.435
KO H_2O	14.475
KO NAC	15.23
KO $KBrO_3$	11.465
Ttest: WT H_2O vs. $KBrO_3$	0.643961309
Ttest: WT H_2O vs. NAC	0.510432658
Ttest: KO H_2O vs. $KBrO_3$	0.001189632
Ttest: KO H_2O vs. NAC	0.579437558

Table 1: Changes in body weight were tracked over a period of 9 weeks for each of WT and KO mice on H_2O, $KBrO_3$ and NAC. The $KBrO_3$ treated mice displayed a lower weight gain than the H_2O and NAC-treated mice. No significant differences were observed between WT and KO mice in similar treatments.

Figure 3: Lean and Fat % of Body Mass Differences among NEIL1-positive and NEIL1-deficient mice on control and sucrose treatments

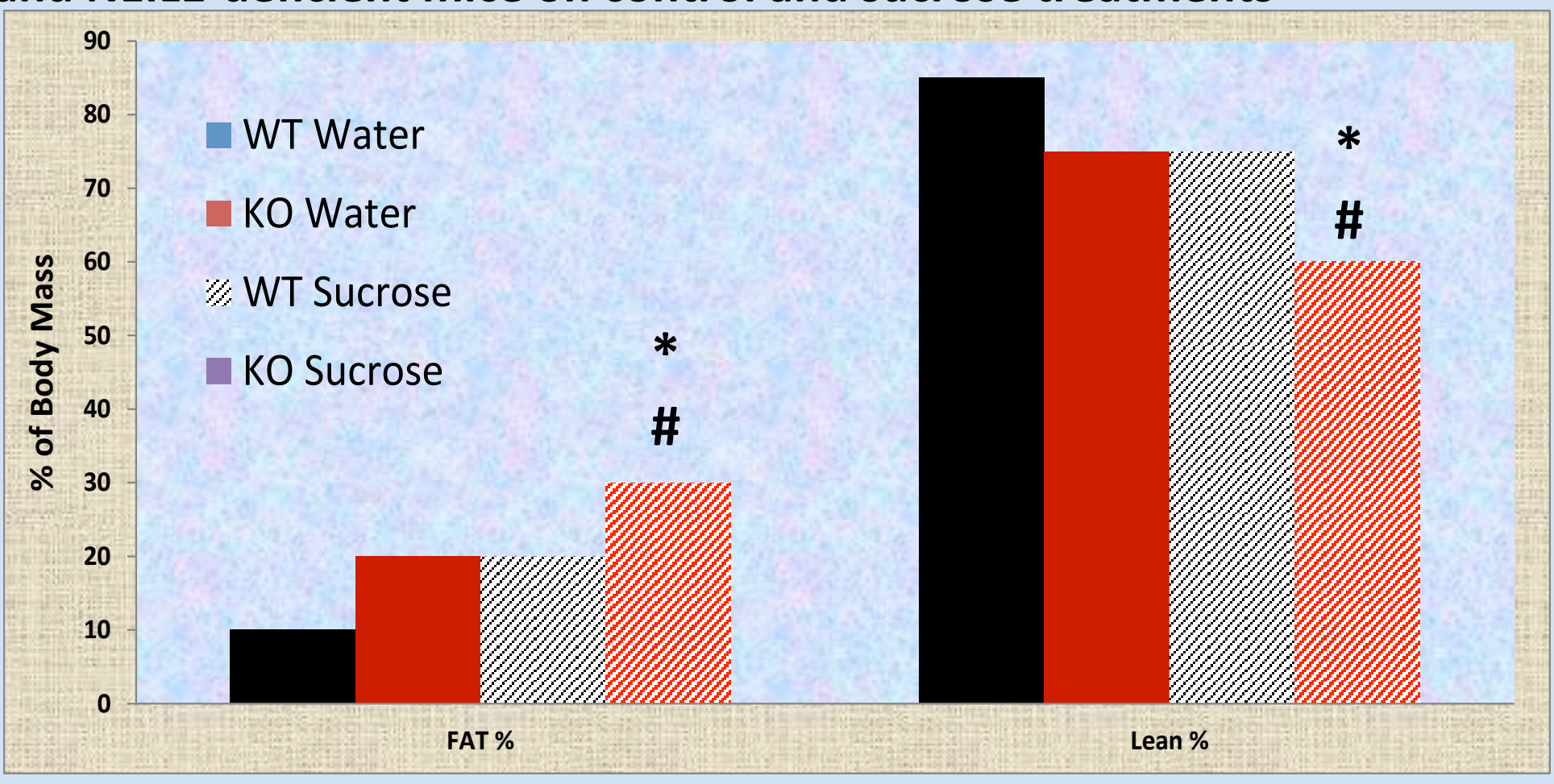

Figure 3: Male mice, both WT and KO for the *neil*1 gene, were given either water (control treatment) or a 30% sucrose solution (experimental treatment). NMR was done to calculate the percent of body mass that was fat and lean. There was no significant difference between the percentages of WT mice that were given sucrose or water, but there was between the KO mice. These data compel the conclusion that a NEIL1-deficiency aggravates the effects of a high-carbohydrate diet.

* Denotes $p<0.05$ by t-test between KO Sucrose and WT Sucrose

\# Denotes $p<0.05$ by t-test between KO Sucrose and KO Water

Table 2: Body Weight Differences among NEIL1-positive and NEIL1-deficient mice on control and sucrose treatments

Genotype and Treatments	Body Weight
WT Control	28.45
WT Sucrose-Fed	32.38
KO Control	28.10
KO Sucrose-Fed	31.67
Ttest: WT	0.11
Ttest: KO	0.031

Table 2: Male mice, both WT and KO for the *neil*1 gene, were given either water (control treatment) or a 30% sucrose solution (experimental treatment). The differences in body weight between the WT treatments were not significant. But they were for the KO treatment, compelling the conclusion that the KO genotype aggravates the effects of a high-carbohydrate diet.

DISCUSSION

High Fat-treated Mice

- KO mice were significantly heavier and fatter that WT and HET mice
- HET mice were not significantly heavier that WT mice
- Almost all (5 out of 6) KO mice died unexpectedly – autopsy revealed liver tumors
  - Possibility that phenotype of cancer in NEIL1 KO mice is aggravated by diet

Potassium Bromate and NAC-treated mice

- $KBrO_3$ mice gain significantly less weight than NAC and H_2O mice, as observed earlier in another model of DNA glycosylase deficiency (Ogg1-KO mice)
- NAC has no significant effect on weight gain relative to H_2O
- Further research needed on why $KBrO_3$ attenuates weight gain
- Further research needed on effects of oxidative stress specific to NEIL1 KO mice

Sucrose-treated mice

- High carb diet results in significantly higher weight gain without increased caloric consumption in KO, but not in WT mice
- MRI reveals significantly higher fat gain in KO mice on sucrose supplementation, relative to WT mice