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Diet-Dependent Epigenetic Silencing of Transposable Elements

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Diet-dependent Epigenetic Silencing of Transposable Elements

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by

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Table of Contents

Abstract

Rapidly changing dietary conditions are of concern as to how they influence our genome function and overall health. Transposable elements (TEs) are genomic parasites that replicate and move throughout the genome, causing disruptions that have been associated with aging, various diseases, and cancers. To mitigate damage, cells deposit repressive epigenetic marks at TEs to reduce their movement. Interestingly, it has been found that dietary metabolites can act as substrates, cofactors, or inhibitors for the enzymes that deposit epigenetic marks. Accordingly, I hypothesize that altered diets, and thus metabolite profiles, change the silencing of TEs. By rearing flies under different dietary conditions, I discovered that a low-calorie diet led to concentration-dependent reduction or enhancement of TE silencing. The high sugar diet led to the enhancement of TE silencing, while the turmeric and ketogenic diets led to opposite effects. We also tested whether such diet-dependent epigenetic silencing of TEs can be inherited transgenerationally or developmentally. We found that developmental reversibility had diet and sex-specific impacts on TE silencing. Surprisingly, TE silencing was passed down across generations and there was a mixed parental dependency. Such observations suggest that transgenerational inheritance and dietary impacts through development are both important to diet-induced changes in TE silencing. We propose that dietary conditions could impact histone methylation processes, inducing changes to TE silencing, but we plan to further investigate the mechanistic impacts of diet on TE silencing. I am hopeful that my findings will inform the scientific community of the impact of a healthy lifestyle to prevent health complications caused by epigenetic changes.

Introduction

Dietary Changes Have Impacts on the Genome

The modern diet has changed drastically from that of our ancestors, evolving alongside the industrial, cultural, and lifestyle changes of the United States (Rogers et. al 2022). The prevalence of processed foods, added fats, and sweeteners have increased by over 100% in the past century, correlating with a similar increase in cardiovascular disease, hypertension, diabetes, and obesity (Lee et al. 2020). As a result, there has also been an emergence of fad diets as a "quick and easy" approach to weight loss. Fad diets have characteristic features that focus on one type of food group or on its elimination, promise rapid weight loss, and unfortunately, are unlikely to be maintained over a long period of time. A notable fad diet is the ketogenic diet, in which followers obey a strict ratio of one part carbohydrates and proteins combined with four parts of fat (Tahreem et al. 2022). The rapid change in dietary consumption and the subsequent emergence of fad diets have led researchers to investigate the impacts of diet and nutrition on overall human health.

It is widely understood that diet and nutrition can impact health through physiological mechanisms (Ravera et al. 2016, Luchsinger et. al 2004). Interestingly, recent studies have discovered that diet can also impact health through epigenetic mechanisms (Dai et al. 2020). Epigenetics is the phenomenon of altering the genome without directly changing the DNA sequence. Genetic information is stored in a chromatin structure that consists of DNA and histone proteins, which package and organize the DNA (Peterson et al. 2004). Protruding histone tails can be post-translationally modified, often through methylation and acetylation, and these epigenetic modifications at specific locations modulate gene expression. Methylation at different positions of histone tails often results in different impacts on gene expression. For example, a

prevalent mark is H3K9me2/3, a mark of constitutive heterochromatin, which silences genes (Nakayama et al. 2001). Likewise, acetylation at the same histone tail position prevents methylation at the H3K9 mark, leading to a reduction in gene silencing (Santa-Rosa 2002). Such modifications are induced by histone-modification enzymes. The degree of histone methylation is determined by the activities of histone methyltransferases, which add methyl groups, and histone demethylases, which remove them (Allis & Jenuwein 2016). In addition, acetyl groups are added via histone acetyltransferases (Marmorstein & Zhou 2014) and erased by histone deacetylases (Seto & Yoshida 2014). Interestingly, dietary metabolites can act as substrates, cofactors, or inhibitors to histone modification enzymes. For example, eggs, fish, and meat contain dietary methionine, and the metabolism of methionine produces S-adenosylmethionine (SAM) (Dai et al. 2020, Serefidou et al. 2019, Mentch et al. 2015). SAM provides methyl groups to histone methyltransferases and contributes to the regulation of histone methylation (Serefidou et al. 2019). Thus, diet has the ability to influence epigenetic modifications that modulate gene expression. Understanding the impacts of our diet on gene regulation provides insight into how diet may have the ability to influence genome functions.

Transposable elements are epigenetically regulated

Transposable elements (TEs) are genomic parasites that move throughout the genome. There are two main classes of TEs. The first are DNA TEs which use a “cut and paste” mechanism, inserting and excising themselves throughout the genome. The second are retrotransposons, which are much more prevalent and move in a “copy and paste” mechanism, duplicating and reinserting themselves using an RNA intermediate (Finnegan 1992).

TE movement can induce detrimental mutations or alter gene expression by inserting into protein-coding or regulatory regions (Cordaux & Batzer 2009), potentially disrupting the function of important processes. Expectedly, TEs have been associated with various neurological disorders, cancers, and aging (Gorbunova et al. 2021, Burns 2017, Ravel-Godreuil et al. 2021). Despite their harmful consequences, TEs manage to make up nearly 50% of the human genome (Wells & Feschotte 2020, Nurk et al. 2022). In order to mitigate damage caused by TE movement, eukaryotic hosts deposit repressive epigenetic marks, such as H3K9me2/3 and DNA methylation, to silence TEs and their movement. These chromatin modifications reduce the expression of genes required for transposition, limiting the movement of TEs, and these repressive modifications are mediated by histone methyltransferases (Slotkin & Martienssen 2007). H3K9 is a mark of constitutive heterochromatin, as H3K9me2/3 repressive marks are enriched in heterochromatic regions of the genome, contributing to its distinct condensed form (Nakayama et al. 2001). However, euchromatic TEs can assemble heterochromatin formation by recruiting H3K9me2/3 marks, creating islands of heterochromatin in the euchromatic region (Sentmanat & Elgin 2012). Little information is known about how dietary conditions impact H3K9me2/3 repressive marks in the euchromatic region. However, because previous studies have demonstrated that dietary metabolites can influence the epigenetic regulation of genes via histone modification enzymes, we hypothesize that diet can influence the epigenetic modifications of TEs, changing levels of TE silencing.

Transgenerational Effects of TE-induced Epigenetic Regulation

Dietary changes have only begun to occur within the last few centuries, so we have limited knowledge about how these changes will impact the genome and human health in the

long term. It is known that parental health, particularly through diet, is strongly correlated with the health and well-being of their offspring (Ahmad et al. 20221). An altered metabolism during pregnancy can cause negative health impacts, but the mechanisms are not well understood (Sferruzi-Perri et al. 2020). An area of interest as a possible explanation is the phenomenon of epigenetic inheritance. Epigenetic inheritance is the transmission of epigenetic modifications from one generation to the next (Yeshurun 2017). As previously mentioned, histone modifications, and other means of epigenetic modifications, could be highly sensitive to environmental factors, including diet, and studies have shown that some of these changes can be passed down across generations (Fang et al. 2014). For instance, a paternal high sugar diet is shown to cause changes to H3K9me3 marks in the next generation (Öst et al 2014). Previous studies investigated H3K9me3 found in the pericentromeric heteromatin regions, but not the marks enriched at TEs. Nonetheless, we hypothesize that diet-mediated changes in the epigenetic regulation of TEs may also be heritable.

Drosophila melanogaster **as a Model Organism**

Drosophila melanogaster is one of the most commonly used genetic model organisms for investigating genetic and biological questions. Since its first use over one hundred years ago, the *Drosophila* genome has been robustly studied and genetic tools such as transgenesis, RNAi, and CRISPR Cas-9 have been developed to perform genetic analyses (Jennings 2011, Gratz et al. 2015). These studies have revealed that several genes and biochemical processes are conserved between *Drosophila* and mammals. For example, nearly 75% of genes linked to human diseases have a match in the *Drosophila* genome (Jennings 2011), making it an effective organism for understanding processes that impact humans in a more time-sensitive and ethical way. Likewise,

its short life cycle allows it to be an optimal model organism for multi-generational studies (Jennings 2011). Although TEs make up a large portion of the human genome, they are largely unable to be studied as mutation accumulation has made a majority of them inactive and unable to move. TEs are prevalent and diverse in the *Drosophila* genome, with at least 30% of them being active, making them an effective model organism for the study of TEs (McCullers & Steiniger 2017).

Development of *mCherry* Reporter Assay

We developed a reporter assay to quantify levels of TE-mediated silencing/expression. The genome deposits repressive epigenetic marks at the TE to reduce movement and expression, but those marks can spread from TEs to nearby genes (Lee 2015, Lee & Karpen 2017, Choi & Lee 2020). Leveraging this observation, our lab developed a reporter assay that placed the fluorescent protein *mCherry* next to the TE 1360. The *mCherry* and 1360 were transgenically introduced into a specific, controlled genomic location in the euchromatic region of the 2L chromosome. The 1360 TE is one of the most abundant DNA TEs in the *Drosophila* genome (Reiss et al. 2003) and has been shown to support the assembly of repressive epigenetic marks (Sentmanat & Elgin 2012). The altered *mCherry* fluorescent intensity levels inform the extent of TE-mediated silencing on the neighboring reporter gene. The reporter assay was helpful because it provided an efficient and cost-effective way to quantify changes in the epigenetic silencing of TEs, serving as a practical alternative to molecular experimentation such as RNA-seq or CHIP-seq, which are commonly used in studies investigating repressive marks (Yang et al. 2023).

Research Goals

We hypothesize that dietary conditions impact chromatin-modifying enzymes, which would influence the epigenetic regulation of TEs. This modulation of epigenetic regulation would result in either an increase or decrease in the epigenetic silencing of TEs. We also hypothesize that these diet-induced epigenetic changes can be heritable as histone modifications can be inherited. In addition, we hypothesize that epigenetic changes can be developmentally reversible and histone modifications can be modified during development. Previous research has been done on the impact of dietary conditions on histone-modifying enzymes and epigenetic regulation of H3K9me2/3 in the heterochromatic region. However, our study will investigate how diet may impact the H3K9me2/3 silencing of TEs in the euchromatic region. This novel information is important as these TEs make up a substantial part of the human genome and can have detrimental impacts when epigenetic regulation is altered. Two lines of *Drosophila melanogaster,* one containing the 1360 TE and one without a TE, were exposed to various dietary conditions. This allowed us to directly compare diet-induced epigenetic changes in the presence and absence of a TE. To determine if these diet-induced changes can be developmentally reversible or heritable, a series of tests were developed that alter dietary conditions at different developmental and generational stages. *mCherry* fluorescence intensity levels were measured under a fluorescent microscope to infer the strength of TE silencing. This study will provide a new perspective on how diet can impact genome regulation, as well as the health and well-being of our future generations. This knowledge can be used to better inform nutritional impacts and interventions used to maintain human health and prevent disease.

Materials and Methods

Drosophila Strains and Overall Design

We used previously developed *Drosophila* lines: pAct5C –29616-1-M2 with noTE and pAct5C-31841-1-M3, with the 1360 TE. All *Drosophila* crosses were stored at 25 C, 12 hr light/12 hr dark cycles. The two strains of *Drosophila melanogaster* were placed onto control or altered diets (see Drosophila Crosses), and the ocular fluorescence intensity of each 3-5 day-old F1 individual was measured (see Imaging).

Food Protocol

Ketogenic Diet

Fly diets were based on a standard Nutri-Fly Bloomington Formulation diet to serve as a control. 4.8 mL propionic acid per liter of food was added to act as an antifungal agent. To emulate the ketogenic diet, we added (R)-(−)-3-Hydroxybutyric acid sodium salt (298360-1G) in 2 mM and 4 mM concentrations to the control diet. The ketogenic salt is the isolated form of the main ketone body that the liver produces under the ketogenic diet (Chriett et al. 2019). Equal portions were added to *Drosophila* vials and allowed to cool for one day.

Low Calorie Diet

The caloric restriction diet used 50% and 33% of the Bloomington Formulation and was supplemented with 12g and 24g of Agar per liter of food respectively. 4.8 mL propionic acid per liter of food was added to act as an antifungal agent. Equal portions were added to *Drosophila* vials and allowed to cool for one day.

High Sugar Diet

We supplemented the Nutri-Fly Bloomington Formulation diet with a 10% and 25% addition of granulated white cane sugar. 4.8 mL propionic acid per liter of food was added to act as an antifungal agent. Equal portions were added to *Drosophila* vials and allowed to cool for one day.

Turmeric Diet

The Nutri-Fly Bloomington Formulation diet was supplemented with a 75mM and 150mM concentration of curcumin powder supplied by Sigma-Aldrich (C1386-50G). 4.8 mL propionic acid per liter of food was added to act as an antifungal agent. Equal portions were added to *Drosophila* vials and allowed to cool for one day.

Drosophila **Crosses**

Overall dietary impact on TE silencing

To investigate whether diet has an effect on the epigenetic silencing of transposable elements, ten fresh animals of each sex (the GP generation) were placed onto experimental food and allowed mating to occur for two days. Ten male and female offspring (the P0 generation) were placed onto experimental food. Mating occurred for two days and the subsequent generation was allowed to develop on tested food (the F1 generation). At 1-3 days old, the F1 generation was collected and placed onto the experimental food for two days. The 3-5 day old F1 individuals were imaged under a fluorescent microscope to determine ocular fluorescent intensity.

Reversibility of diet-induced changes to TE silencing

This test investigated whether dietary impacts could be reversed during adult life. This test was identical to the previous test, but at 1-3 days old, the F1 generation was collected and placed onto

control food for two days. The 3-5 day old F1 individuals were imaged under a fluorescent microscope to determine ocular fluorescent intensity.

Heritability of diet-induced changes to TE silencing

We investigated if the dietary impacts of the epigenetic silencing of TEs could be passed down to the next generation. This test was identical to the first test, with the grandparents (GP generation) and their offspring (P0 generation) being exposed to the dietary condition. However, ten males and ten females of the parental generation were placed on control food, and the F1 generation developed on the control food. At 1-3 days old, the F1 generation was collected and placed onto control food for two days. The 3-5 day old F1 individuals were imaged under a fluorescent microscope to determine ocular fluorescent intensity.

Dietary changes to TE silencing during development

This test investigated if dietary impacts could be induced during development. This test was the opposite dietary set-up as the previous test. The grandparents (GP generation) and their offspring (P0 generation) were placed onto the control food. The P0 generation was then crossed onto the experimental food, where their offspring (the F1 generation) developed and emerged. At 1-3 days old, the F1 generation was collected and placed onto experimental food for two days. The 3-5 day old F1 individuals were imaged under a fluorescent microscope to determine ocular fluorescent intensity.

Imaging

Drosophila were placed on their side under the Zeiss SteREO Discovery V20 fluorescence microscope at a consistent magnification and exposure. The *Drosophila* eye was centered in frame and an image was captured. The average fluorescence intensity was measured from a 500-unit ring around the center of the eye.

Results

mCherry reporter lines for estimating the strength of TE silencing

mCherry intensity was significantly reduced in the line containing the 1360+ TE (median = 1442 (no TE) and 880 (with 1360 TE), Fig. 1), which is consistent with TE-mediated silencing spreading to the nearby *mCherry* gene and reducing expression. This demonstrated that the 1360 TE had increased silencing as compared to when no TE was present. Such an observation demonstrates the reporter assay's effectiveness in quantifying TE-mediated silencing using fluorescent intensity.

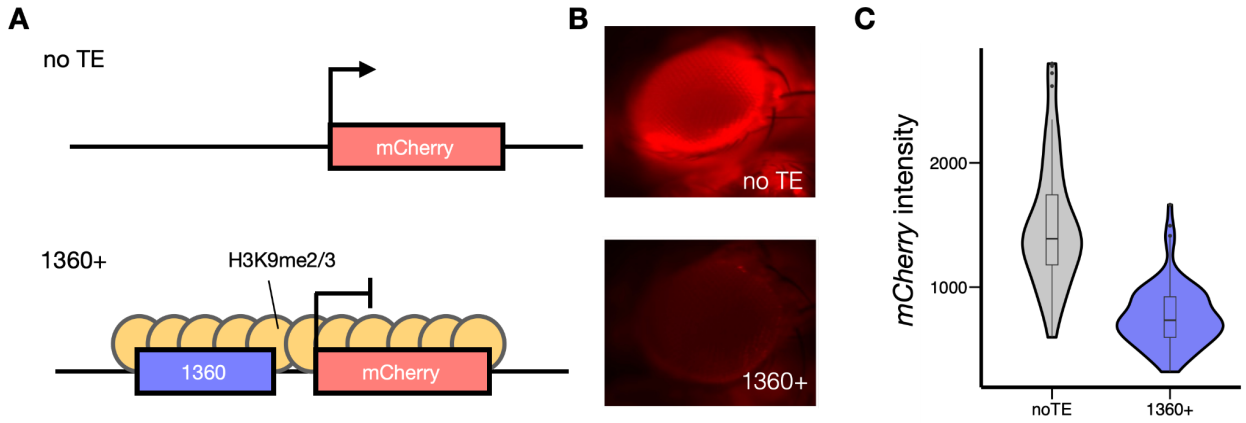

Figure 1. Reporter system effectively quantifies TE epigenetic silencing.

(A) Depiction of *mCherry* reporter system. no TE contains *mCherry* fluorescent protein without 1360 TE. 1360+ contains TE placed next to *mCherry* with hypothesized H3K9me2/3 enrichment.

(B) mCherry enrichment in 3-5 day old *Drosophila* eyes with TE and without TE.

(C) Measured fluorescence intensity in 3-5 day old *Drosophila* eyes with TE and without TE.

Dietary conditions influence *mCherry* intensity in the absence of TEs and affect sexes differently

I tested four dietary conditions: low calorie, high sugar, ketogenic, and turmeric.

Because we identified that some dietary regimes can influence *mCherry* intensity even in the absence of TEs (Fig. 2A), we normalized all *mCherry* intensities against the observation without the TE to highlight TE mediated changes. Another factor that influences *mCherry* intensity that is independent of the TE effect is sex. For all tested diets, males showed significantly lower *mCherry* intensity levels than females and were impacted by diet differently (Fig. 2B and see below). Accordingly, we analyzed males and females separately to mitigate sex-based differences. We developed a series of tests that placed flies on the dietary condition at different generations and developmental stages. In the first test, we placed parents and offspring onto dietary conditions to determine the dietary impacts on TE silencing. The second test investigated if diet-mediated TE silencing could be developmentally reversible. The third determined if changes in TE silencing could be passed between generations. Finally, we aimed to investigate if changes to TE silencing could be induced during development (details in Materials & Methods).

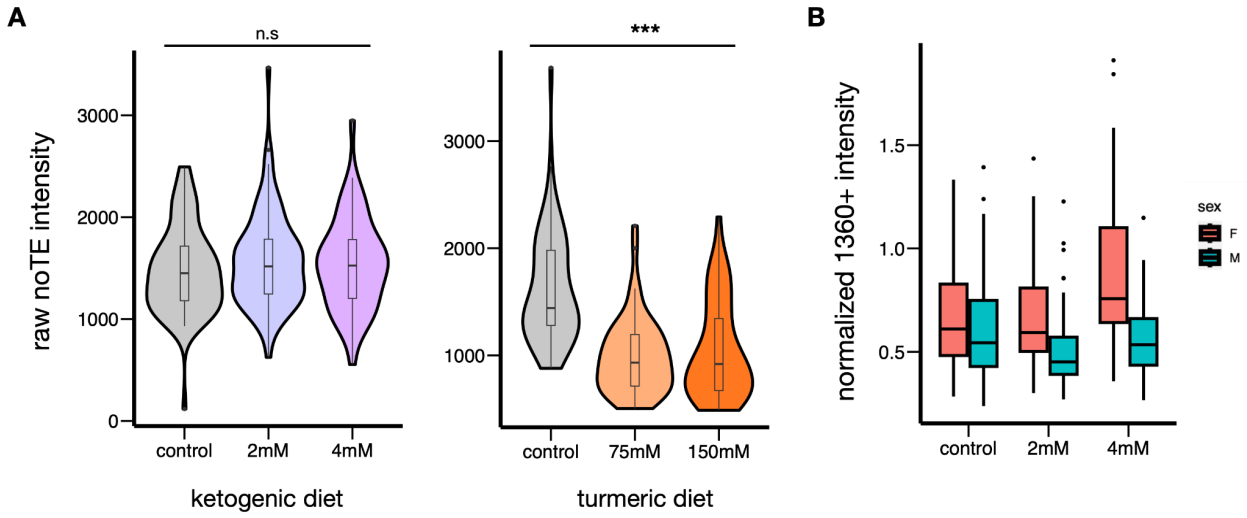

Figure 2. Data is normalized to highlight TE-specific changes and sexes are analyzed separately to mitigate sex-based differences.

(A) Raw *mCherry* intensity when noTE was present. The ketogenic diet did not change intensity, while the high sugar diet significantly changed *mCherry* intensity when noTE was present.

(B) Normalized intensity when the TE was present. Males showed significantly lower intensity levels than females. Female *mCherry* intensity increased with the ketogenic diet, while male intensity decreased.

High sugar diet enhances TE-silencing

First, we determined if the high sugar diet impacted TE silencing. When all three generations were exposed to a 5% increased sugar diet, the F1 generation showed an insignificant decrease in *mCherry* intensity (*Wilcoxon test*, p = 0.1612). Meanwhile, individuals on the 10% (*Wilcoxon test,* p = 3.37e-09) and 25% sugar increase diets (*Wilcoxon test*, p = 2.52e-06) showed significant decreases in *mCherry* intensity, suggesting an enhancement in TE silencing (Fig. 3A). This indicates that changes in TE silencing may be concentration-dependent as higher increases in sugar lead to more drastic changes in *mCherry* intensity. When investigating if dietary impacts could be reversible during development, we placed F1 adults onto control food for two days and found *mCherry* intensity insignificantly decreased from a 5% sugar increase (*Wilcoxon test*, p = 0.6825). Females exposed to the 10% sugar diet (*Wilcoxon test*, p = 0.04393) showed a significant increase in *mCherry* intensity while effects of the 25% sugar diet led to a significant decrease in intensity (*Wilcoxon test*, p = 1.569e-09) (Fig. 3B). Despite being moved off of the high sugar diet, changes to *Drosophila mCherry* intensity remain, suggesting that the effect of diet-mediated changes to TE silencing are not reversed within two days. The elevation in *mCherry* intensity in F1 adults on the 10% sugar diet could suggest concentration-dependent changes to *mCherry* intensity and accordingly TE silencing. Interestingly, when testing the dependence on the parental diet, both the 10% sugar diet (*Wilcoxon test,* p = 0.6019) and 25% sugar supplement (*Wilcoxon test*, p = 0.1431) led to an

insignificant increase in *mCherry* intensity (Fig. 3C). Overall, our observations suggest that, under the high sugar diet, changes in TE silencing did not occur within one generation, suggesting an increased parental dependency in the high sugar-mediated changes of TE silencing.

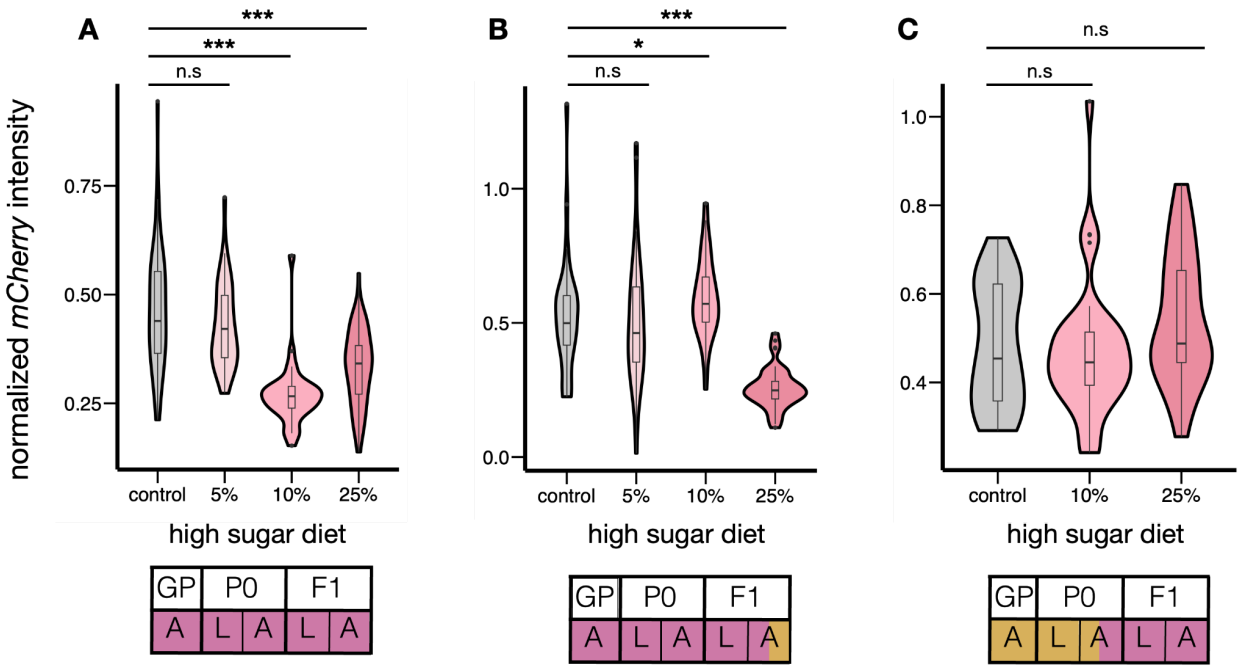

Figure 3. High sugar diet enhances TE-silencing that cannot be reversed or induced within one generation. Normalized *mCherry* intensity of female with TE. Control = control diet, 5% = 5% sugar increase, 10% = 10% sugar increase, 25% = 25% sugar increase. Violin plots show median (horizontal line), first and third quartile (lower and upper bounds), minimum and maximum values (whiskers), and data set density. Design of experimental test depicted in schematic below graph. Pink: experimental diet, yellow: control diet, A: adult individuals, L: larvae. *p<0.05, **p<0.01, ***p<0.001.

(A) *mCherry* intensity levels after exposure to the high sugar diet.

(B) Fluorescent levels in 1360 line after turmeric diet reversal.

(C) Intensity levels induced by dietary change at the start of development.

The ketogenic diet induces a reduction of TE silencing

First, we determined if the ketogenic diet had an impact on TE silencing. When all three generations of *Drosophila* were reared on a 2mM concentration of ketone supplement, females in the F1 generation showed an insignificant decrease in *mCherry* intensity (*Wilcoxon test*, p = 0.6904). The 4mM concentration led to a significant increase in *mCherry* intensity of *Drosophila*, suggesting a decrease in TE silencing (*Wilcoxon test,* p = 0.00029) (Fig. 4A). We next tested if the ketogenic diet could be developmentally reversible. At a 2mM concentration, *mCherry* intensity increased significantly, demonstrating a reduction in TE silencing (*Wilcoxon test,* p = 9.609e-06). When reared on a 4mM concentration of ketone bodies and moved to control food, there was a significant decrease in *mCherry* intensity, indicating an increase in TE silencing (*Wilcoxon test*, p = 0.03901) (Fig. 4B). This suggests that diet-induced changes to TE silencing cannot be reversed during development as changes to intensity remain after the diet has been reversed. When determining transgenerational inheritance, the F1 generation showed an insignificant decrease in *mCherry* intensity when parents were reared on 2mM of ketone supplement (*Wilcoxon test*, p = 0.2098). When parents were exposed to a 4mM keto concentration, the F1 generation also showed an insignificant decrease in *mCherry* intensity (*Wilcoxon test*, p = 0.9864) (Fig. 4C). Changes in *mCherry* intensity did not persist when F1 females were not exposed to the ketogenic food, suggesting that the changes in TE silencing from the ketogenic diet were not passed down to the next generation. When we tested if diet-induced changes could occur during development, we found that rearing F1 individuals on 2mM (*Wilcoxon test,* p = 0.52) and 4mM concentrations (*Wilcoxon test*, p = 0.84) led to insignificant changes in *mCherry* intensity (Fig. 4D). In summary, we found that, under the

ketogenic diet, changes to TE silencing did not occur during one generation, which could suggest that parental influence is necessary to produce keto diet-induced changes to TE silencing.

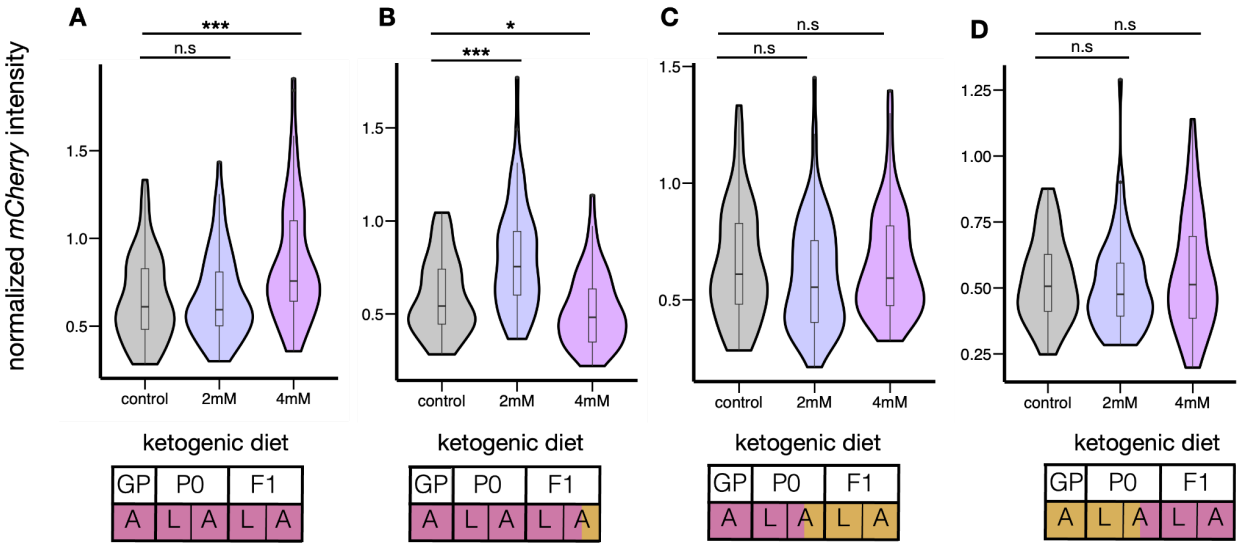

Figure 4. Ketogenic diet induces reduction of TE silencing that cannot be reversed, passed down, or induced within one generation. Normalized *mCherry* intensity of female with TE. Control = control diet, 2mM = 2mM of ketogenic salt, 4mM = 4mM of ketogenic salt. Violin plots show median (horizontal line), first and third quartile (lower and upper bounds), minimum and maximum values (whiskers), and data set density. Design of experimental test depicted in schematic below graph. Pink: experimental diet, yellow: control diet, A: adult individuals, L: larvae. *p<0.05, **p<0.01, ***p<0.001.

(A) *mCherry* intensity levels after exposure to the ketogenic diet.

(B) Fluorescent levels in 1360 line after ketogenic diet reversal.

(C) *mCherry* levels after parental exposure to the ketogenic diet.

(D) Intensity levels induced by dietary change at the start of development.

The turmeric diet leads to a reduction of TE silencing

First, we determined if the turmeric diet had an impact on TE silencing. When three generations were exposed to a diet with 75mM curcumin concentration (*Wilcoxon test*, p =

0.004452) as well as the 150 mM concentration (*Wilcoxon test,* p = 0.001722), *mCherry* intensity significantly increased in F1 individuals (Fig. 5A), a finding consistent with the turmeric diet leading to a reduction in TE silencing. When investigating the developmental reversibility of diet, we found that *mCherry* intensity insignificantly decreased from both diets with 75 mM concentration (*Wilcoxon test,* p = 0.1184) and 150 mM concentration of curcumin (*Wilcoxon test*, p = 0.5185) (Fig. 5B). The elevation in *mCherry* intensity did not persist after individuals were placed onto control food, suggesting that removal from the diet reversed changes on TE silencing. Interestingly, these results were not consistent with other diets, which showed that diet-induced changes to TE silencing were not reversible. Finally, we tested if changes in TE silencing could be passed down to the next generation. *mCherry* intensity insignificantly increased when parents were reared on a 75 mM turmeric diet (*Wilcoxon test*, p = 0.0645), but significantly increased from the 150 mM diet (*Wilcoxon test*, p = 2.717e-05) (Fig. 5C). Overall, we found that the turmeric diet consistently led to increased *mCherry* intensity levels, demonstrating reduced TE silencing in females. Our results could also suggest that the ability for inheritance is concentration dependent as a higher concentration allowed for changes in TE silencing to persist across generations.

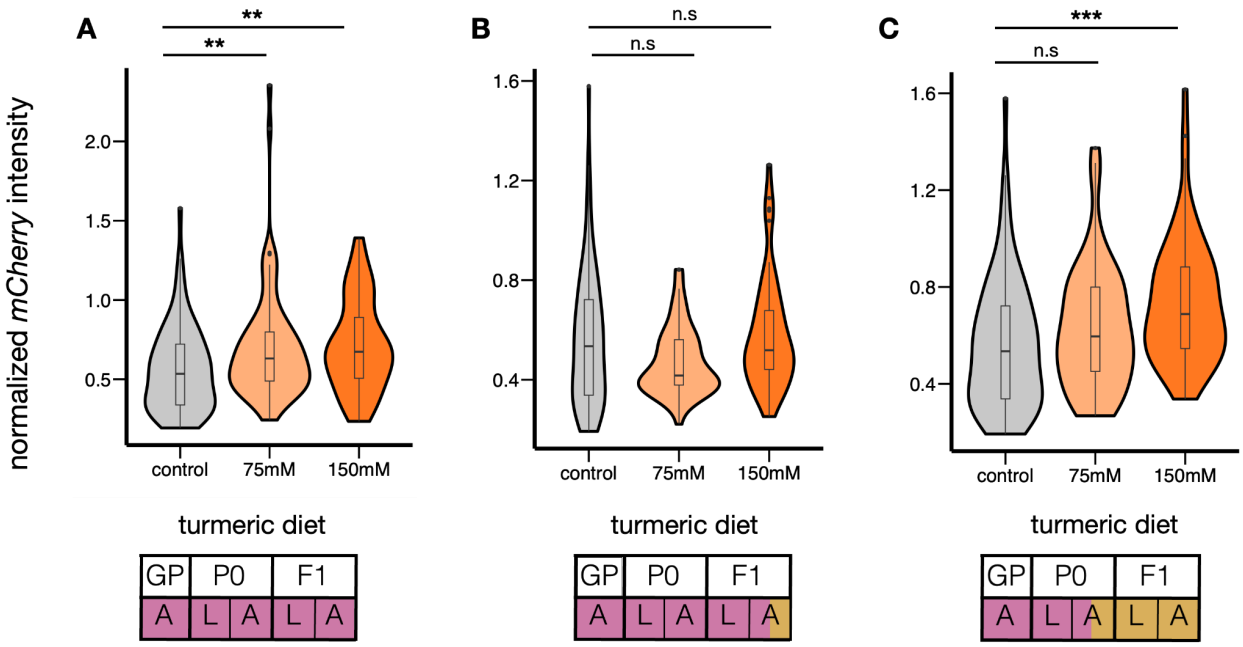

Figure 5. Turmeric diet leads to reduction of TE silencing that cannot be reversed but can be induced within one generation. Normalized *mCherry* intensity of female with TE. Control = control diet, 75mM = 75mM of curcumin, 150mM = 150mM of curcumin. Violin plots show median (horizontal line), first and third quartile (lower and upper bounds), minimum and maximum values (whiskers), and data set density. Design of experimental test depicted in schematic below graph. Pink: experimental diet, yellow: control diet, A: adult individuals, L: larvae. *p<0.05, **p<0.01, ***p<0.001.

(A) *mCherry* intensity levels after exposure to the turmeric diet.

(B) Fluorescent levels in 1360 line after turmeric diet reversal.

(C) *mCherry* levels after parental exposure to the turmeric diet.

(D) Intensity levels induced by dietary change at the start of development.

Low calorie diet influences TE silencing in a complex manner

First, we determined if the low calorie diet impacted TE silencing. Female *Drosophila* under 50% caloric diet showed a significant increase in *mCherry* intensity (*Wilcoxon test*, p

=0.01598), suggesting a reduction in TE silencing. However, a 33% calorie diet showed a significant decrease in *mCherry* intensity (*Wilcoxon test*, p = 1.75e-07), suggesting an increase in TE silencing (Fig. 6A). These results suggest that changes in TE silencing are impacted by the concentration of the dietary condition. When investigating if changes in TE silencing could be developmentally reversible, we found that the 50% caloric diet led F1 individuals to have an insignificant increase of *mCherry* intensity, demonstrating a reduction in TE silencing (*Wilcoxon test,* p = 0.9347). The 33% calorie diet led *Drosophila* to display a significant decrease in intensity, suggesting an increase in TE silencing (*Wilcoxon test,* p = 4.285e-05) (Fig. 6B). Altered TE silencing persisted after *Drosophila* were placed onto control food, which suggests that the changes in TE silencing were not reversed within two days. When testing if TE silencing could be transgenerationally inherited, the F1 generation displayed an insignificant increase in *mCherry* intensity when their parents were exposed to 50% calorie diet (*Wilcoxon test,* 0.3589) and the 33% calorie diet (*Wilcoxon test*, p = 0.99) (Fig. 6C). The changes do not persist in the F1 generation, suggesting the lack of transgenerational ability via the low-calorie diet. Finally, when investigating developmental impact of diet on TE silencing, offspring had a significant increase in *mCherry* intensity from the 50% calorie diet (*Wilcoxon test,* p = 0.03068) and a significant decrease from the 33% calorie diet (*Wilcoxon test,* p = 0.0009031) (Fig. 6D). Although aforementioned results suggest importance on the parental diet, this test indicates that changes in *mCherry* intensity and subsequently TE silencing can occur within one generation and independent of diets experienced by the parents. It is notable that our results consistently display that changes in the direction of TE silencing are dependent on the magnitude of calorie restriction: a 50% calorie diet leads to reduced TE silencing while a 33% calorie diet leads to enhanced silencing.

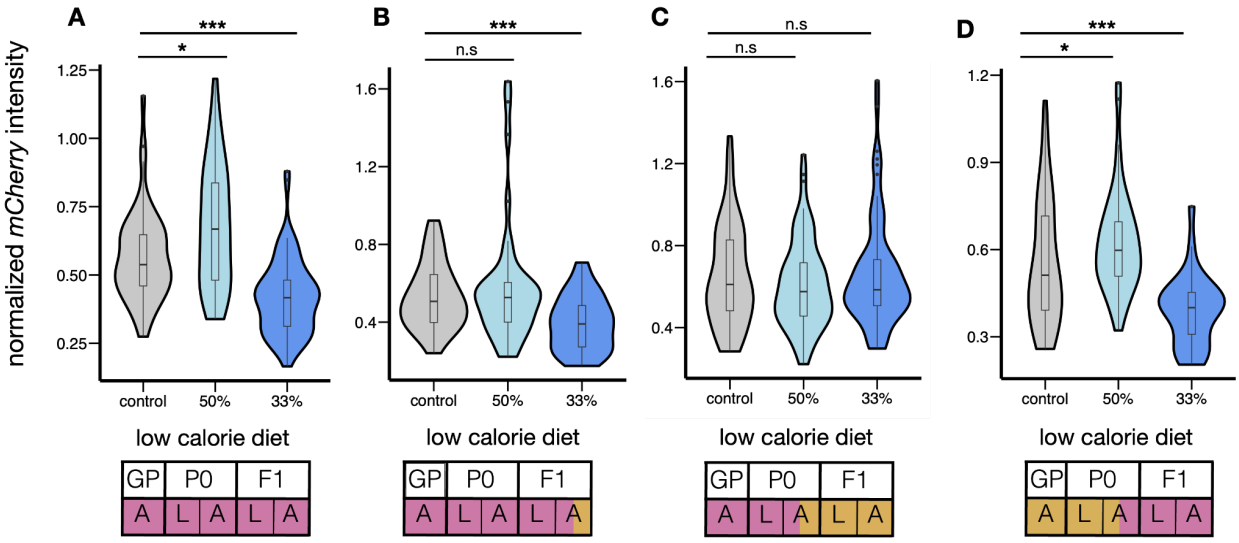

Figure 6. Low calorie diet induces concentration-dependent reduction or enhancement of TE silencing that cannot be reversed at higher concentrations, cannot be passed down, but can be induced at development. Normalized *mCherry* intensity of female with TE. Control = control diet, 50% = 50% calorie intake, 33% = 33% calorie intake. Violin plots show median (horizontal line), first and third quartile (lower and upper bounds), minimum and maximum values (whiskers), and data set density. Design of experimental test depicted in schematic below graph. Pink: experimental diet, yellow: control diet, A: adult individuals, L: larvae. *p<0.05, **p<0.01, ***p<0.001.

(A) *mCherry* intensity levels after exposure to the ketogenic diet.

(B) Fluorescent levels in 1360 line after ketogenic diet reversal.

(C) *mCherry* levels after parental exposure to the ketogenic diet.

(D) Intensity levels induced by dietary change at the start of development.

Dietary impacts on TE silencing show sex-specific differences

Even in the absence of dietary manipulation, males showed significantly lower levels of *mCherry* intensity, suggesting that they have higher levels of TE silencing than females (Fig. 2B). We also found that males and females were affected differently by the dietary conditions as

well as the developmental and generational tests. The low-calorie diet shows the most consistent results between males and females regarding directional and significant changes to TE silencing.

The high sugar diet shows sex-specific differences when investigating overall dietary impacts: females consistently have a decrease in *mCherry* intensity, while the intensity of males increases and decreases depending on concentration. *mCherry* intensity levels of females change significantly during exposure to 10% and 25% sugar increases (*Wilcoxon test,* $p = 0.04393$ (10%), $p = 1.569e-09$ (25%)), indicating that these concentrations of diet cannot be developmentally reversible. However, in males, only exposure to the 25% sugar increase shows a change in *mCherry* intensity (*Wilcoxon test,* $p = 0.0001046$), demonstrating that diet is not developmentally reversible in males at a higher concentration. This could suggest that lower concentrations of sugar are more reversible in males than females. When investigating parental dependence, females showed no significant changes to *mCherry* intensity, indicating that parental TE silencing more strongly influences females than males, where changes in *mCherry* intensity occurred within one generation for both magnitudes of increased sugar concentrations (*Wilcoxon test,* $p = 0.0461$ (10%), $p = 0.0232$ (25%)).

Our results found that a 2mM ketogenic supplement leads to an increase in *mCherry* intensity in females and a decrease in males. This suggests that the impacts of the ketogenic diet reduce silencing in females (*Wilcoxon test*, $p = 0.0003671$) but enhance TE silencing in males (*Wilcoxon test*, $p = 0.005561$). The impacts of the 4mM concentration diet were consistent between sexes, producing no change in intensity and TE silencing. Interestingly, males and females differed dramatically during the transgenerational tests. Females showed significant changes to *mCherry* intensity after being moved onto control food, while males did not. This suggests that the impacts of the ketogenic diet can be reversed in males, but not in females.

The turmeric diet consistently showed sex-specific differences: *mCherry* intensity was elevated in females exposed to a diet with 75mM and 150mM curcumin concentration (*Wilcoxon test,* $p = 0.004452$ (75 mM), $p = 0.001722$ (150 mM), but decreased in males (*Wilcoxon test*, $p = 0.00172$ (150 mM)). These results suggest that TE silencing was reduced in females but enhanced in males as a result of exposure to turmeric. Turmeric-mediated changes to *mCherry* intensity were reversed when females' diets were reverted. However, changes to intensity persisted in males (*Wilcoxon test*, $p = 0.0004497$ (75 mM), $p = 0.0009796$ (150 mM)), suggesting females are more susceptible to developmental reversal impacts on TE silencing. Changes in *mCherry* intensity were passed down in both females and males, with females showing decreases in intensity and males once again showing the opposite effect (*Wilcoxon test,* $p = 0.0001476$ (2 mM), $p = 0.0001027$ (150 mM). Nevertheless, such observation demonstrates that changes in TE silencing as a result of the turmeric diet can be passed down to both males and females.

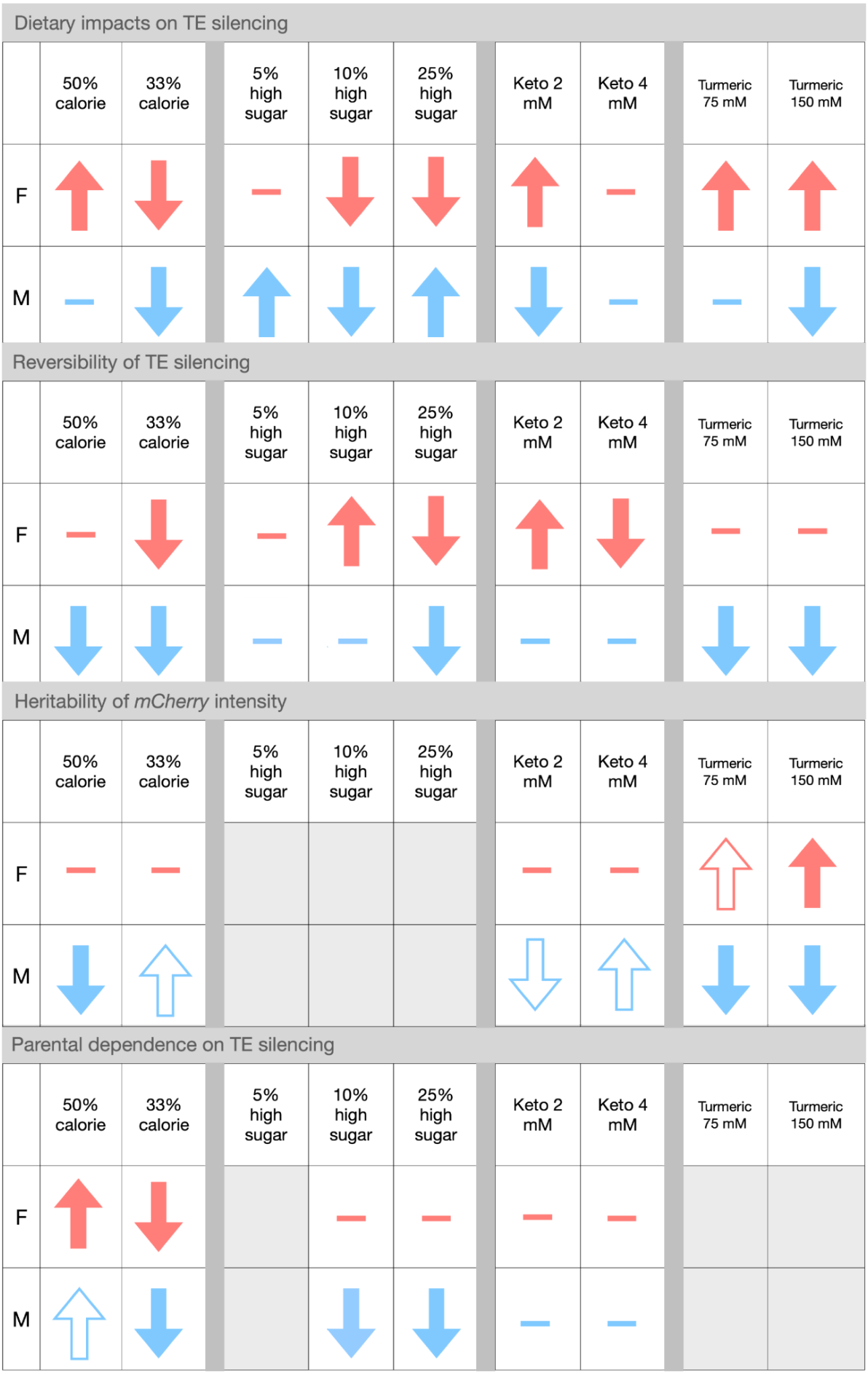
Dietary impacts on TE silencing
50% calorie
33% calorie
5% high sugar
10% high sugar
25% high sugar
Keto 2 mM
Keto 4 mM
Turmeric 75 mM
Turmeric 150 mM
F
M
Reversibility of TE silencing
50% calorie
33% calorie
5% high sugar
10% high sugar
25% high sugar
Keto 2 mM
Keto 4 mM
Turmeric 75 mM
Turmeric 150 mM
F
M
Heritability of mCherry intensity
50% calorie
33% calorie
5% high sugar
10% high sugar
25% high sugar
Keto 2 mM
Keto 4 mM
Turmeric 75 mM
Turmeric 150 mM
F
M
Parental dependence on TE silencing
50% calorie
33% calorie
5% high sugar
10% high sugar
25% high sugar
Keto 2 mM
Keto 4 mM
Turmeric 75 mM
Turmeric 150 mM
F
M

Table 1. Dietary impacts on TE silencing show sex-specific differences. Changes in *mCherry* intensity for males and females for dietary conditions and their generational tests. Upward arrow denotes increase in *mCherry* intensity and downward arrow denotes decrease in *mCherry* intensity. Solid color, $p < 0.05$, colored edge, $p < 0.1$ and > 0.05, "-", $p > 0.1$.

Discussion

A high sugar diet enhances TE silencing in females

This study investigated the impacts of diet on TE silencing. We hypothesized that diet will impact histone-modifying enzymes, which will modulate the strength of TE silencing. In support of our hypothesis, we observed that the high sugar diet increased TE silencing. Interestingly, our findings contradict previous mice studies that found that high glucose levels, which can result from a high sugar diet, were seen to decrease the activity of histone methyltransferases, contributing to reduced levels of H3K9me2/3 located at gene promoters (Sun et al. 2014). This previous study suggests the possibility that the high sugar diet could decrease the activity of histone methyltransferases, which could lead to reduced levels of H3K9me2/3 and TE silencing. In *Drosophila*, however, an ancestral high sugar diet has led to the increase of another repressive mark, H3K27me3 (Yang et al. 2023). If H3K27me3 is increased by a high sugar metabolism, there is a possibility that a high sugar diet could increase another type of repressive mark: H3K9me2/3. Our observation is consistent with a scenario where a high sugar diet enhances repressive marks, increasing TE silencing. The mechanisms by which the high sugar diet modulates histones are largely unknown. When sugar is being metabolized, it could generate many different metabolites, so it is difficult to predict how it will affect TE silencing. Further investigation addressing this knowledge gap would provide more information as to how sugar can impact the histone modification of species differently.

Ketogenic and turmeric diets induce a reduction of TE silencing in females

We found that the ketogenic diet reduced levels of TE silencing. Ketone bodies generated from the ketogenic metabolism act as histone deacetylase (HDAC) inhibitors (Dai et al. 2020).

Histone deacetylases remove acetyl groups, and this removal allows for methyl groups to be added. When the ketogenic diet inhibits the removal of acetyl groups, histone methylation is unable to occur (Kim & Bae 2011). Our observation is consistent with the scenario where the ketogenic diet prevents histone methylation and TE silencing as a reduction of silencing was seen in female *Drosophila* exposed to the ketogenic diet.

The turmeric diet also reduced levels of TE silencing. Likewise, curcumin, the active ingredient found in turmeric, acts as an effective HDAC inhibitor to limit the histone's ability to be methylated (Soflaei et al. 2018). Curcumin is also known to down regulate an enzyme required for the biosynthesis of SAM, the methyl donor for histone methyltransferases (Hu & Zhou 2020). The depletion of SAM has been associated with a decrease in the enrichment of H3K9me2/3 (Haws et al. 2020). This could suggest that curcumin's ability to inhibit methyl donors may reduce repressive marks, contributing to the reduction of TE silencing. These two mechanisms may work to inhibit methylation, which could contribute to our observed decrease in TE silencing caused by the turmeric diet.

Ketogenic and turmeric diets have sex-specific impacts on TE silencing

Both the ketogenic and turmeric diets showed consistent sex-specific differences in their ability to modulate TE silencing. Females exposed to the ketogenic and turmeric diets had a reduction in TE silencing, while the TE silencing of males was enhanced by these diets. Both diets are predicted to act as HDAC1 inhibitors. A previous study in mice found higher levels of HDAC1 in females exposed to arsenic than males (Tyler et al. 2015), suggesting that the impact of environmental and dietary conditions on histone modifications may differ between sexes. Interestingly, another study found that HDAC1 inhibitors affected males, but had no effect on

females (Tyler et al. 2018). While this study used a different type of HDAC1 inhibitor, it is intriguing to see that it impacts sexes differently. Mechanisms as to how sex contributes to the differing efficacy of HDAC1 inhibitors are unknown. Nevertheless, our findings and previous studies suggest that dietary conditions, particularly HDAC inhibitors, could affect the histone modifications of sexes differently.

The variation in sex chromosomes could provide another possible explanation for our observed sex-specific differences. The Y chromosome makes up a large portion of the male *Drosophila* genome and its presence strongly influences the enrichment of repressive marks across the genome, which has led to sex-based differential gene expression (Brown et al. 2020). If the epigenetic regulation of genes is influenced by the Y chromosome, then it is possible that the Y chromosome could also be affecting the epigenetic regulation of TEs. Diet may impact this sex-based regulation differently, contributing to our observed sex-specific differences in TE silencing.

Low calorie diet induces concentration-dependent reduction or enhancement of TE silencing in females

We found that the low-calorie diet increased TE silencing under higher levels of caloric restriction. Our results were consistent with a previous study that found that caloric restriction enhanced TE silencing in aging flies (Wood et al. 2016). Calorie restriction has been demonstrated to reverse the activity of KDM4D, a histone demethylase that removes methyl groups from H3K9 (Rapps et al. 2023). This could suggest a case where the reversal of demethylation enhances repressive marks, leading to the increased TE silencing we observed as a result of the low-calorie diet. Under less extreme caloric restriction, we found that TE silencing

levels were reduced. Previous studies have demonstrated that cells are able to use epigenetic alteration as a means to quickly adapt to changes in the epigenetic landscape, often overcompensating for changes to repressive marks (Wang et al. 2016). This may be an explanation for the decrease in TE silencing that occurs under 50% caloric restriction. The low calorie diet may be increasing levels of repressive marks. If this is occurring, the host organism may attempt to resist these changes, recruiting mechanisms to reduce epigenetic histone modifications, potentially overcompensating and leading to an overall reduction in TE silencing. However, under higher magnitudes of caloric restriction, epigenetic alterations may not be strong enough to reverse the effects of the diet, which generates an increase in TE silencing, which would be consistent with our results. Our observation of concentration-dependent effects could also be mediated by the possibility that different magnitudes of calorie restriction may have different mechanisms for altering TE silencing. One possibility is a threshold effect as a more strict calorie restriction elicits a different stress response. This could insinuate that dietary changes could be affecting TE expression in more indirect ways that are not mediated by histone modification enzymes. A future RNA-seq experiment may provide answers to explain the opposite effects observed because it can provide insight into the molecular mechanisms that are influencing TE silencing.

The turmeric diet is developmentally reversible unlike the low calorie or ketogenic diets

We observed that changes to TE silencing mediated by the low calorie diet were not developmentally reversible, while the high sugar, ketogenic, and turmeric diets showed sex-specific differences. Consistent with our results, previous studies have shown differing

responses to dietary reversal. One *Drosophila* study demonstrated that levels of repressive marks changed after the flies were removed from a low calorie diet, finding that epigenetic plasticity occurred within 72 hours (Jiang et al. 2013). In contrast, we found that the low calorie diet was not developmentally reversible after only two days of diet reversal, which suggests that the duration of reversal may be critical. Developmental reversibility could be dependent on the duration of reversal, which could demonstrate the importance of a healthy diet long term. The ketogenic diet is often used as a treatment for epilepsy, a disorder associated with DNA hypermethylation, another repressive mark that depends on methyl groups. The ketone bodies reduce DNA methylation in mice and have lasting effects for at least eight weeks after dietary reversal (Lusardi et al. 2015). Our results in reference to previous findings suggest that dietary reversibility is contingent on a variety of factors: the type of diet, the length of reversal, and sex. In addition, metabolism, which determines the availabilities of various nutrients that influence epigenetic modifications, can impact sexes differently as previous studies show sex-specific changes in mice metabolism when transitioned to a new diet during adulthood (Oraha et al. 2022), suggesting another possible mechanism by which diet may influence TE silencing.

Turmeric diet-induced changes to TE silencing are heritable unlike the low calorie and ketogenic diet

We found that changes in TE silencing via the turmeric diet were inherited for one generation, while changes from the low calorie and ketogenic diet were not. The heritability of TE silencing is surprising as germ cells go through epigenetic reprogramming during early development, a process that erases “epigenetic memory” by largely reducing epigenetic modifications in future functional germlines (Hajkova P. 2011). Previous studies have found that

environmental-induced changes in H3K9me2/3 formation can be passed down to the next generation (Seong et al. 2011). This study demonstrates that the environment can induce transgenerational changes to repressive marks, so it is plausible that dietary changes can exert similar impacts. Their findings are consistent with our observed results, suggesting a scenario where diet can change levels of repressive marks and that the effects can be transmitted between generations, altering TE silencing in individuals who had not been exposed to the dietary conditions. A previous study suggests that there may be a regulatory mechanism that maintains chromatin state during epigenetic reprogramming, which works to rescue environmental-induced changes to H3K9me2/3. They proposed that when environmental conditions alter this mechanism, it is unable to recover changes to repressive marks, causing the changes to be passed down to the next generation (Seong et al. 2011). If their hypothesis is true, the turmeric diet that we studied may have influenced regulatory mechanisms, allowing changes to TE silencing to be passed down, while the low calorie and ketogenic diets did not. This could also provide a possible explanation for the concentration-dependent inheritance ability. Our results showed that stronger magnitudes of dietary change showed larger and more consistent transgenerational effects.

The heritability of diet-mediated changes in TE silencing could be sex-specific

In the low calorie, ketogenic, and turmeric diets, diet-induced changes in TE silencing were more significantly transmitted to males than females. There is little information on how the sex of the offspring contributes to heritability, but these results could suggest that sexual dimorphism contributes to variation in epigenetic inheritance. This is another case demonstrating that sex can lead to different impacts on TE silencing and its transgenerational ability.

The ability of diet to induce changes during development differs between diets

We found that exposure to the low calorie and high sugar diet was sufficient to impact TE silencing in the larval stage of development. A previous study has identified the importance of timing required to initiate changes to histone modifications (Seong et al. 2011). Their study investigated heat shock, in which *Drosophila* were exposed to heat during early embryogenesis as well as exposure to an osmotic stress diet at the beginning of the larval stage. They identified that environmental changes at early embryogenesis are more likely to affect chromatin states than later in development (Seong et al. 2011). However, embryos cannot eat, so the earliest they are exposed to dietary conditions is the larval stage of development. Nevertheless, exposure to environmental impacts in the larval stage has the possibility to induce changes if pathways for histone modification are more strongly affected (Seong et al. 2011). If environmental factors can induce changes to chromatin during the larval stage, it is feasible that diet-induced changes to chromatin can occur during development. This possibility is consistent with our results, which demonstrate that being placed on low calorie and high sugar diets during the larval stage allowed for changes in TE silencing to occur within one generation. This could point out the possibility that the metabolic changes as a result of these diets impact histone modification processes very strongly, so they are able to alter chromatin states at later developmental stages. However, we found that the ketogenic diet did not cause changes to TE silencing after individuals were exposed during development, suggesting an increased parental dependency on TE silencing when exposed to the ketogenic diet. When taking into account the findings of Seong et al, our results could suggest that the ketogenic diet does not induce strong enough changes in histone modifications to induce changes in repressive marks during late development. This is intriguing as our results also show that the ketogenic diet did not have the ability to be heritable. This could

suggest that to have keto diet-induced changes to TE silencing, both the parental and offspring generations must be impacted and their effects could be added to produce a change.

Future Directions & Conclusion

Our study demonstrated that certain dietary regimes can impact the epigenetic silencing of TEs. We also found that many variables determine whether dietary impacts on TE silencing are developmentally reversible, heritable, and inducible during development. In the future, we will test the impacts of many more prevalent dietary conditions. We also plan to uncover the mechanisms by which diets impact TE silencing. The ketogenic diet and turmeric diets are proposed HDAC inhibitors, and my lab has found that TE-mediated silencing of *mCherry* intensity depends on the HDAC1 gene, suggesting the possibility that these diets' abilities as HDAC inhibitors could contribute to decreased silencing. We plan to test if the effects of the ketogenic and turmeric diets on TE silencing persist in HDAC1 mutants. We are in the process of performing transcriptome and epigenome experiments to confirm the results of the reporter assay as well as test the dietary effects genome-wide.

This study allowed for an understanding of how our diet can disrupt the regulation of this prevalent and detrimental component of the genome, and how these changes can impact future generations. I am hoping that these findings will inform the scientific community of the impact of a healthy lifestyle to prevent health complications caused by epigenetic changes.

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References

Ahmad, K., Kabir, E., Keramat, S. A., & Khanam, R. (2021). Maternal health and health-related behaviours and their associations with child health: Evidence from an Australian birth cohort. *PloS one*, *16*(9), e0257188. https://doi.org/10.1371/journal.pone.0257188

Allis, C. D., & Jenuwein, T. (2016). The molecular hallmarks of epigenetic control. *Nature Reviews Genetics*, *17*(8), 487–500. https://doi.org/10.1038/nrg.2016.59

Brown, E. J., Nguyen, A. H., & Bachtrog, D. (2020). The Drosophila Y Chromosome Affects Heterochromatin Integrity Genome-Wide. *Molecular biology and evolution*, *37*(10), 2808–2824. https://doi.org/10.1093/molbev/msaa082

Burns K. H. (2017). Transposable elements in cancer. *Nature reviews. Cancer*, *17*(7), 415–424. https://doi.org/10.1038/nrc.2017.35

Choi, J. Y., & Lee, Y. C. G. (2020). Double-edged sword: The evolutionary consequences of the epigenetic silencing of transposable elements. *PLoS genetics*, *16*(7), e1008872. https://doi.org/10.1371/journal.pgen.1008872

Cordaux R, Batzer MA. (2009). The impact of retrotransposons on human genome evolution. *Nat Rev Genet.*;10(10):691–703.

Dai, Z., Ramesh, V., & Locasale, J. W. (2020). The evolving metabolic landscape of chromatin biology and epigenetics. *Nature Reviews Genetics*, *21*(12), 737–753. https://doi.org/10.1038/s41576-020-0270-8

Fang, L., Wuptra, K., Chen, D., Li, H., Huang, S. K., Jin, C., & Yokoyama, K. K. (2014). Environmental-stress-induced Chromatin Regulation and its Heritability. *Journal of carcinogenesis & mutagenesis*, *5*(1), 22058. https://doi.org/10.4172/2157-2518.1000156

Finnegan D. J. (1992). Transposable elements. *Current opinion in genetics & development*, *2*(6), 861–867. https://doi.org/10.1016/s0959-437x(05)80108-x

Gorbunova, V., Seluanov, A., Mita, P., McKerrow, W., Fenyö, D., Boeke, J. D., Linker, S. B., Gage, F. H., Kreiling, J. A., Petrashen, A. P., Woodham, T. A., Taylor, J. R., Helfand, S. L., & Sedivy, J. M. (2021). The role of retrotransposable elements in ageing and age-associated diseases. *Nature*, *596*(7870), 43–53. https://doi.org/10.1038/s41586-021-03542-y

Gratz, S. J., Rubinstein, C. D., Harrison, M. M., Wildonger, J., & O'Connor-Giles, K. M. (2015). CRISPR-Cas9 Genome Editing in Drosophila. *Current protocols in molecular biology*, *111*, 31.2.1–31.2.20. https://doi.org/10.1002/0471142727.mb3102s111

Guillemette, B., Drogaris, P., Lin, H. H., Armstrong, H., Hiragami-Hamada, K., Imhof, A., Bonneil, E., Thibault, P., Verreault, A., & Festenstein, R. J. (2011). H3 lysine 4 is acetylated at active gene promoters and is regulated by H3 lysine 4 methylation. *PLoS genetics*, *7*(3), e1001354. https://doi.org/10.1371/journal.pgen.1001354

Hajkova P. (2011). Epigenetic reprogramming in the germline: towards the ground state of the epigenome. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, *366*(1575), 2266–2273. https://doi.org/10.1098/rstb.2011.0042

Haws, S. A., Yu, D., Ye, C., Wille, C. K., Nguyen, L. C., Krautkramer, K. A., Tomasiewicz, J. L., Yang, S. E., Miller, B. R., Liu, W. H., Igarashi, K., Sridharan, R., Tu, B. P., Cryns, V. L., Lamming, D. W., & Denu, J. M. (2020). Methyl-Metabolite Depletion Elicits Adaptive Responses to Support Heterochromatin Stability and Epigenetic Persistence. *Molecular cell*, *78*(2), 210–223.e8. https://doi.org/10.1016/j.molcel.2020.03.004

Hu, X., & Zhou, Y. (2020). Curcumin reduces methionine adenosyltransferase 2B expression by interrupting phosphorylation of p38 MAPK in hepatic stellate cells. *European journal of pharmacology*, *886*, 173424. https://doi.org/10.1016/j.ejphar.2020.173424

Jennings, Barbara. (2011). Drosophila – a versatile model in biology & medicine. *Materials Today.* 14. 190-195. 10.1016/S1369-7021(11)70113-4.

Jiang, N., Du, G., Tobias, E., Wood, J. G., Whitaker, R., Neretti, N., & Helfand, S. L. (2013). Dietary and genetic effects on age-related loss of gene silencing reveal epigenetic plasticity of chromatin repression during aging. *Aging*, *5*(11), 813–824. https://doi.org/10.18632/aging.100614

Keiser, A. A., & Wood, M. A. (2019). Examining the contribution of histone modification to sex differences in learning and memory. *Learning & memory (Cold Spring Harbor, N.Y.)*, *26*(9), 318–331. https://doi.org/10.1101/lm.048850.118

Kim, H. J., & Bae, S. C. (2011). Histone deacetylase inhibitors: molecular mechanisms of action and clinical trials as anti-cancer drugs. *American journal of translational research*, *3*(2), 166–179.

Lee, J. H., Duster, M., Roberts, T., & Devinsky, O. (2022). United States Dietary Trends Since 1800: Lack of Association Between Saturated Fatty Acid Consumption and Non-communicable Diseases. *Frontiers in Nutrition*, *8*, 748847. https://doi.org/10.3389/fnut.2021.748847

Lee, Y. C. G., & Karpen, G. H. (2017). Pervasive epigenetic effects of *Drosophila* euchromatic transposable elements impact their evolution. *eLife*, *6*, e25762. https://doi.org/10.7554/eLife.25762

Lee Y. C. (2015). The Role of piRNA-Mediated Epigenetic Silencing in the Population Dynamics of Transposable Elements in Drosophila melanogaster. *PLoS genetics*, *11*(6), e1005269. https://doi.org/10.1371/journal.pgen.1005269

Luchsinger, J. A., & Mayeux, R. (2004). Dietary factors and Alzheimer's disease. *The Lancet Neurology*, *3*(10), 579–587. https://doi.org/10.1016/S1474-4422(04)00878-6

Lusardi, T. A., Akula, K. K., Coffman, S. Q., Ruskin, D. N., Masino, S. A., & Boison, D. (2015). Ketogenic diet prevents epileptogenesis and disease progression in adult mice and rats. *Neuropharmacology*, *99*, 500–509. https://doi.org/10.1016/j.neuropharm.2015.08.007

Marmorstein, R., & Zhou, M. M. (2014). Writers and readers of histone acetylation: structure, mechanism, and inhibition. *Cold Spring Harbor perspectives in biology*, *6*(7), a018762. https://doi.org/10.1101/cshperspect.a018762

McCullers, T. J., & Steiniger, M. (2017). Transposable elements in *Drosophila*. *Mobile genetic elements*, *7*(3), 1–18. https://doi.org/10.1080/2159256X.2017.1318201

Mentch, S. J., Mehrmohamadi, M., Huang, L., Liu, X., Gupta, D., Mattocks, D., Gómez Padilla, P., Ables, G., Bamman, M. M., Thalacker-Mercer, A. E., Nichenametla, S. N., & Locasale, J. W. (2015). Histone Methylation Dynamics and Gene Regulation Occur through the Sensing of One-Carbon Metabolism. *Cell metabolism*, *22*(5), 861–873. https://doi.org/10.1016/j.cmet.2015.08.024

Nakayama , J., Rice, J. C., Strahl, B. D., Allis, C. D., & Grewal, S. I. (2001). Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. *Science (New York, N.Y.)*, *292*(5514), 110–113. https://doi.org/10.1126/science.1060118

Nurk, S., Koren, S., Rhie, A., Rautiainen, M., Bzikadze, A. V., Mikheenko, A., Vollger, M. R., Altemose, N., Uralsky, L., Gershman, A., Aganezov, S., Hoyt, S. J., Diekhans, M.,

Logsdon, G. A., Alonge, M., Antonarakis, S. E., Borchers, M., Bouffard, G. G., Brooks, S. Y., Caldas, G. V., … Phillippy, A. M. (2022). The complete sequence of a human genome. *Science (New York, N.Y.)*, *376*(6588), 44–53. https://doi.org/10.1126/science.abj6987

Oraha, J., Enriquez, R.F., Herzog, H. *et al.* (2022). Sex-specific changes in metabolism during the transition from chow to high-fat diet feeding are abolished in response to dieting in C57BL/6J mice. *Int J Obes* 46, 1749–1758. https://doi.org/10.1038/s41366-022-01174-4

Öst, A., Lempradl, A., Casas, E., Weigert, M., Tiko, T., Deniz, M., Pantano, L., Boenisch, U., Itskov, P. M., Stoeckius, M., Ruf, M., Rajewsky, N., Reuter, G., Iovino, N., Ribeiro, C., Alenius, M., Heyne, S., Vavouri, T., & Pospisilik, J. A. (2014). Paternal diet defines offspring chromatin state and intergenerational obesity. *Cell*, *159*(6), 1352–1364. https://doi.org/10.1016/j.cell.2014.11.005

Peterson, C. L., & Laniel, M.-A. (2004). Histones and histone modifications. *Current Biology*, *14*(14), R546–R551. https://doi.org/10.1016/j.cub.2004.07.007

Rapps, K., Kisliouk, T., Marco, A., Weller, A., & Meiri, N. (2023). Dieting reverses histone methylation and hypothalamic AgRP regulation in obese rats. *Frontiers in endocrinology*, *14*, 1121829. https://doi.org/10.3389/fendo.2023.1121829

Ravel-Godreuil, C., Znaidi, R., Bonnifet, T., Joshi, R. L., & Fuchs, J. (2021). Transposable elements as new players in neurodegenerative diseases. *FEBS letters*, *595*(22), 2733–2755. https://doi.org/10.1002/1873-3468.14205

Ravera, A., Carubelli, V., Sciatti, E., Bonadei, I., Gorga, E., Cani, D., Vizzardi, E., Metra, M., & Lombardi, C. (2016). Nutrition and Cardiovascular Disease: Finding the Perfect Recipe for Cardiovascular Health. *Nutrients*, *8*(6), 363. https://doi.org/10.3390/nu8060363

Reiss, D., Quesneville, H., Nouaud, D., Andrieu, O., & Anxolabehere, D. (2003). Hoppel, a P-like element without introns: a P-element ancestral structure or a retrotranscription derivative?. *Molecular biology and evolution*, *20*(6), 869–879. https://doi.org/10.1093/molbev/msg090

Rogers, M. A., & Amer, H. (2022). Food as we knew it: Food processing as an evolutionary discourse. *Trends in Food Science & Technology*, *128*, 68–74. https://doi.org/10.1016/j.tifs.2022.07.015

Sentmanat, M. F., & Elgin, S. C. (2012). Ectopic assembly of heterochromatin in Drosophila melanogaster triggered by transposable elements. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(35), 14104–14109. https://doi.org/10.1073/pnas.1207036109

Seong, K. H., Li, D., Shimizu, H., Nakamura, R., & Ishii, S. (2011). Inheritance of stress-induced, ATF-2-dependent epigenetic change. *Cell*, *145*(7), 1049–1061. https://doi.org/10.1016/j.cell.2011.05.029

Serefidou, M., Venkatasubramani, A. V., & Imhof, A. (2019). The Impact of One Carbon Metabolism on Histone Methylation. *Frontiers in genetics*, *10*, 764. https://doi.org/10.3389/fgene.2019.00764

Seto, E., & Yoshida, M. (2014). Erasers of histone acetylation: the histone deacetylase enzymes. *Cold Spring Harbor perspectives in biology*, *6*(4), a018713. https://doi.org/10.1101/cshperspect.a018713

Sferruzzi-Perri, A. N., Lopez-Tello, J., Napso, T., & Yong, H. E. J. (2020). Exploring the causes and consequences of maternal metabolic maladaptations during pregnancy: Lessons from animal models. *Placenta*, *98*, 43–51. https://doi.org/10.1016/j.placenta.2020.01.015

Slotkin, R. K., & Martienssen, R. (2007). Transposable elements and the epigenetic regulation of the genome. *Nature Reviews Genetics*, *8*(4), 272–285. https://doi.org/10.1038/nrg2072

Soflaei, S. S., Momtazi-Borojeni, A. A., Majeed, M., Derosa, G., Maffioli, P., & Sahebkar, A. (2018). Curcumin: A Natural Pan-HDAC Inhibitor in Cancer. *Current pharmaceutical design*, *24*(2), 123–129. https://doi.org/10.2174/1381612823666171114165051

Sun, G. D., Cui, W. P., Guo, Q. Y., & Miao, L. N. (2014). Histone lysine methylation in diabetic nephropathy. *Journal of diabetes research*, *2014*, 654148. https://doi.org/10.1155/2014/654148

Tahreem, A., Rakha, A., Rabail, R., Nazir, A., Socol, C. T., Maerescu, C. M., & Aadil, R. M. (2022). Fad Diets: Facts and Fiction. *Frontiers in Nutrition*, *9*, 960922. https://doi.org/10.3389/fnut.2022.960922

Tyler, C. R., Hafez, A. K., Solomon, E. R., & Allan, A. M. (2015). Developmental exposure to 50 parts-per-billion arsenic influences histone modifications and associated epigenetic machinery in a region- and sex-specific manner in the adult mouse brain. *Toxicology and applied pharmacology*, *288*(1), 40–51. https://doi.org/10.1016/j.taap.2015.07.013

Tyler, C. R. S., Smoake, J. J. W., Solomon, E. R., Villicana, E., Caldwell, K. K., & Allan, A. M. (2018). Sex-Dependent Effects of the Histone Deacetylase Inhibitor, Sodium Valproate, on Reversal Learning After Developmental Arsenic Exposure. *Frontiers in genetics*, *9*, 200. https://doi.org/10.3389/fgene.2018.00200

Wang, J., Jia, S. T., & Jia, S. (2016). New Insights into the Regulation of Heterochromatin. *Trends in genetics : TIG*, *32*(5), 284–294. https://doi.org/10.1016/j.tig.2016.02.005

Wells, J. N., & Feschotte, C. (2020). A Field Guide to Eukaryotic Transposable Elements. *Annual Review of Genetics*, *54*(1), 539–561.

Wood, J. G., Jones, B. C., Jiang, N., Chang, C., Hosier, S., Wickremesinghe, P., Garcia, M., Hartnett, D. A., Burhenn, L., Neretti, N., & Helfand, S. L. (2016). Chromatin-modifying genetic interventions suppress age-associated transposable element activation and extend life span in Drosophila. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(40), 11277–11282. https://doi.org/10.1073/pnas.1604621113

Yang, J., Tang, R., Chen, S., Chen, Y., Yuan, K., Huang, R., & Wang, L. (2023). Exposure to high-sugar diet induces transgenerational changes in sweet sensitivity and feeding behavior via H3K27me3 reprogramming. *eLife*, *12*, e85365. https://doi.org/10.7554/eLife.85365

Yeshurun, S., Short, A. K., Bredy, T. W., Pang, T. Y., & Hannan, A. J. (2017). Paternal environmental enrichment transgenerationally alters affective behavioral and neuroendocrine phenotypes. *Psychoneuroendocrinology*, *77*, 225–235. https://doi.org/10.1016/j.psyneuen.2016.11.013