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RESEARCH PAPER

# Transgenerational impact of maternal zinc deficiency on offspring metabolic outcomes in *Drosophila melanogaster*

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**Abstract**

Maternal zinc deficiency significantly influences fetal development and long-term health outcomes, yet its transgenerational effects remain poorly understood. This study aims to investigate the transgenerational effects of maternal zinc deficiency on metabolic outcomes in *Drosophila melanogaster*. Zinc deficiency was induced in Drosophila by incorporating TPEN (N,N,N',N'-tetrakis(2-pyridylmethyl)ethylenediamine) into their diet. Offspring (F1 to F3) were maintained on a standard diet for subsequent analyses. Various metabolic markers, including glucose, trehalose, glycogen, and triglyceride levels, were assessed, and gene expression analyses were conducted to examine the molecular responses across generations. Significant reductions in locomotor performance in female F1 flies and increased body weight in the F2 generation were observed. Maternal zinc deficiency exhibited gender- and generation-specific impacts on metabolic markers. Notably, an adaptive response in the F3 generation included increased catalase activity and total antioxidant capacity, along with decreased malondialdehyde levels. Gene expression analyses revealed upregulation of *DILP2* mRNA across generations and significant variations in *PEPCK, SOD1, CAT, EGR*, and *UPD2* mRNA levels, demonstrating intricate responses to maternal zinc deficiency. This study provides a holistic understanding of the consequences of maternal zinc deficiency, emphasizing the complex interplay between zinc status and metabolic outcomes across generations in Drosophila. These findings lay the foundation for future research elucidating the underlying molecular mechanisms, with potential implications for humans. The insights gained contribute to informing targeted interventions aimed at optimizing offspring health in the context of maternal zinc deficiency.
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*Keywords:* Maternal; Metabolism; Offspring; Transgenerational; Zinc deficiency; Drosophila.

## 1. Introduction

Maternal nutrition is a critical determinant of foetal development and long-term health outcomes, shaping the trajectory of offspring well-being across generations. As an essential micronutrient, zinc is involved in diverse physiological processes that are essential for optimal growth, neurodevelopment, and metabolic homeostasis [1]. The significance of maternal zinc sufficiency during pregnancy is well-established, with research primarily focusing on immediate outcomes such as birth weight and neurocognitive development [2–4]. However, a comprehensive exploration of the enduring consequences of maternal zinc deficiency across successive generations remains a significant gap in our understanding of developmental and health trajectories.

Zinc is an essential trace element integral to the function of numerous enzymes, transcription factors, and signalling pathways involved in cellular growth and differentiation. Its role as a cofactor in DNA synthesis, cell division, and gene expression underpins its importance during periods of rapid foetal growth and organ development [5,6]. Moreover, zinc is recognized for its involvement in neurodevelopment, influencing processes such as synaptogenesis and neurotransmitter function [7,8]. Consequently, deviations from optimal zinc levels during pregnancy may disrupt these intricate processes, leading to a spectrum of adverse outcomes that extend beyond the immediate perinatal period.

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The involvement of zinc in glucose metabolism is highlighted by its impact on insulin synthesis, secretion, and action. Zinc serves as an integral component of the insulin hexamer structure and is involved in the synthesis and processing of proinsulin [9]. Consequently, adequate zinc levels are crucial for maintaining insulin function and glucose homeostasis. Studies have demonstrated that zinc deficiency can impair insulin sensitivity and disrupt glucose regulation [10]. This disruption is often associated with decreased insulin receptor phosphorylation and compromised glucose transporter function. The interplay between zinc and insulin signalling pathways extends to the regulation of key enzymes involved in glucose metabolism, such as glycogen synthase and phosphoenolpyruvate carboxykinase (PEPCK). Zinc acts as an insulin-mimetic, promoting glycogen synthesis and inhibiting gluconeogenesis through its impact on these enzymes [11].

Oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) production and the antioxidant defence system, is closely linked to zinc status [12]. Zinc serves as a cofactor for several antioxidant enzymes, including superoxide dismutase (SOD) and metallothioneins [13]. These enzymes play a pivotal role in neutralizing ROS and preventing cellular damage. In conditions of zinc deficiency, a reduction in the activity of these antioxidant enzymes can occur, leading to increased oxidative stress. This oxidative stress, in turn, contributes to insulin resistance, further exacerbating disturbances in glucose metabolism [12].

Inflammation is closely linked to both zinc deficiency and oxidative stress. Zinc deficiency can potentiate inflammation by activating proinflammatory transcription factors, such as nuclear factor-kappa B (NF-$\kappa$B), and promoting the release of inflammatory cytokines [14,15]. Conversely, inflammation can induce zinc redistribution within the body, leading to decreased zinc availability in target tissues [16]. This bidirectional relationship contributes to a proinflammatory state that can impair insulin signalling and exacerbate insulin resistance.

Moreover, the anti-inflammatory properties of zinc are evident in its ability to inhibit the production of proinflammatory cytokines, such as tumour necrosis factor-alpha (TNF-$\alpha$) and interleukin-6 (IL-6) [17]. Zinc achieves this by modulating NF-$\kappa$B signalling and influencing immune cell function. The anti-inflammatory effects of zinc contribute to its potential role in mitigating insulin resistance and preserving glucose homeostasis.

Despite the wealth of knowledge on the acute effects of maternal zinc deficiency, our understanding of how these effects echo across generations is limited. This study aims to bridge this critical gap by examining the transgenerational impact of maternal zinc deficiency on the physiological parameters of Drosophila offspring. The *Drosophila melanogaster* model provides a valuable platform for investigating the multigenerational consequences of maternal nutrition due to its short generation time, ease of genetic manipulation, and conservation of essential biological pathways.

This study encompasses a holistic approach, delving into various facets of physiological well-being, including alterations in Drosophila body weight, locomotor performance, glucose metabolism, antioxidant defences, and inflammation. By assessing these diverse aspects, we aim to unravel the interconnected mechanisms through which maternal zinc status influences the health outcomes of successive generations.

## 2. Materials and methods

### 2.1. Drosophila melanogaster *(Fruit fly) husbandry*

The fruit flies used in this study were the $W^{1118}$ strain *Drosophila melanogaster* acquired from the fly laboratory of the Centre for Advanced Medical Research and Training (CAMRET) at Usmanu Danfodiyo University, Sokoto, Nigeria. They were cultured and maintained at an optimum temperature of 22-25°C, 50-60% relative humidity, had access to a standard cornmeal diet composed of corn flour, agar, yeast, methyl paraben, and distilled water, as well as a natural light-dark cycle. The parent flies (F0) were made zinc-deficient by incorporating a zinc-chelator, TPEN (N,N,N',N'-tetrakis(2-pyridylmethyl)ethylenediamine), at the concentration of 100 $\mu$M into their diet [18]. The diets were changed weekly to prevent contamination.

### 2.2. Experimental design

Gravid female flies aged 7-10 days were divided into two groups. Flies in the first group were transferred to a zinc-chelated diet for 24 hours to lay eggs. The second group of flies were transferred to a normal diet for egg-laying. The eggs from each group developed on their respective diets (zinc-chelated or normal). The resulting adult flies from the first and second groups were collected as the zinc-deficient and control F0 generation respectively (Fig. 1). To obtain subsequent generations (F1-F3) for the control group, virgin females (4 to 6 hours after eclosion) from the previous generation were mated with age-matched control males (seven to ten days post-eclosion). For the zinc deficient group; virgin females from the zinc-deficient F0 generation mated with control males produced the F1 generation, virgin females from the F1 generation mated with control males produced the F2 generation, and virgin females from the F2 generation mated with control males produced the F3 generation (Fig. 2). Importantly, all offspring from F1 to F3 generations were maintained on a normal diet for seven days before analysis of physical variables, biochemical variables, and gene expression.

### 2.3. Elemental analysis of fly body zinc level

Upon culturing the parent flies from embryo to adult stage on zinc-chelated diet, the total body zinc levels was assessed using Agilent Microwave Plasma Atomic Emission Spectrometer, MP-AES, (MY19479002, SC, USA). Ten adult female flies aged seven to ten days were collected in triplicate, anaesthetized by ice immobilization, and then rinsed with distilled water. The flies were digested in 1ml of 65% nitric acid ($HN0_3$) in a sterile microcentrifuge tube. The mixture was heated to 100°C for 10 minutes on a heating block. It was subsequently cooled, diluted to 5 mL with distilled water and analysed for total body zinc concentration against the calibration curves obtained between 0.00 and 6.00 ppm on the MPAES. More details on the MPAES can be found in the supplementary information.

### 2.4. Body weight measurement

Flies were anaesthetized by cooling on ice and weighed in groups of ten in triplicate per group (n=30). The weight was recorded in milligrams using a sensitive electronic weighing balance (Kern & Sohn Ltd Balingen, Germany).

### 2.5. Locomotor performance (negative geotaxis assay)

Flies were placed in an empty 50 mL measuring cylinder with a line 6 cm from the bottom. They were gently tapped to the bottom of the vial and the number of flies able to cross the line within 10 s were recorded. This was repeated thrice for each vial, and the percentage of live flies climbing above the line was averaged for a given group [19].

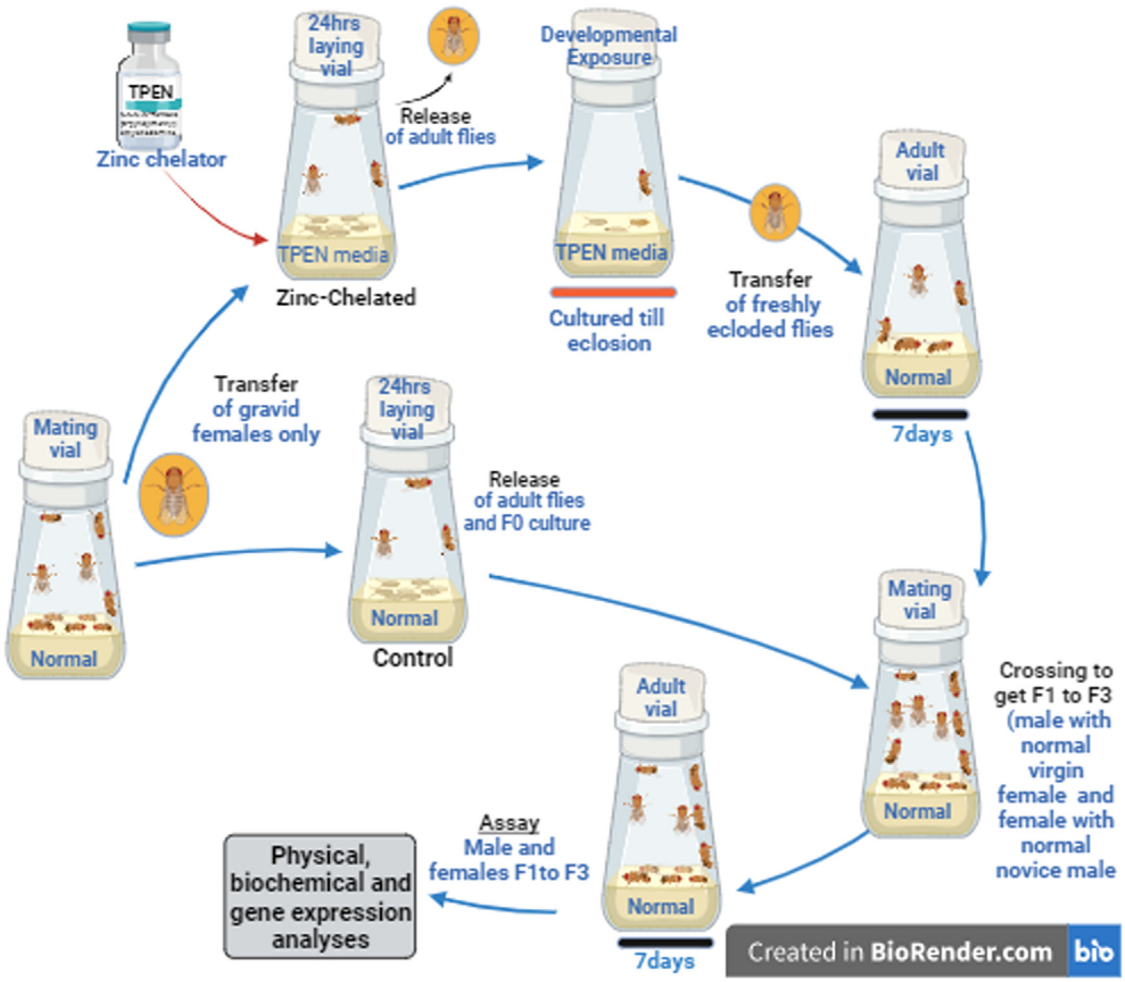



**Fig. 1.** Experimental design. Gravid female flies (7-10 days old) were divided into two groups: zinc-deficient (fed a zinc-chelated diet) and control (fed a normal diet) for egg laying (F0 generation). Eggs from each group developed on their respective diets. Virgin females (4-6 hours after eclosion) from each subsequent generation (F0 for control, zinc-deficient F0 for experimental) were mated with age-matched control males (7-10 days post-eclosion) to produce F1, F2, and F3 generations. All offspring (F1-F3) were raised on a normal diet for 7 days before analysis.

### 2.6. Biochemical analysis

#### 2.6.1. Sample preparation

A pool of ten flies in triplicate per group (n=30) were transferred to empty vials, and kept for 1 hour to eliminate their gut content. They were anaesthetized by cooling, rinsed in phosphate buffered saline (PBS) (pH 7.4 at 1:5 ratio (w/v)), homogenized, and then centrifuged at 3,000 × g for 6min in a refrigerated centrifuge (MX-301 Highspeed, Tomy Kogyo Co., Ltd., Tagara, Japan) at 4°C [20]. After centrifugation, the supernatant containing the haemolymph was used for the biochemical assays.

#### 2.6.2. Glucose assay

The haemolymph glucose level was determined using the Spinreact™ kit (Girona, Spain) according to the manufacturer's protocol. The absorbance of the samples and standard was read against the blank at 505 nm. The concentration was calculated using the following formula.

$$\text{Glucose concentration (mg/dL)} = \text{Absorbance of the sample/Absorbance of the standard} \times 100$$

#### 2.6.3. Trehalose assay

Trehalose level of haemolymph was quantified using the Anthrone colorimetric method (Solarbio Life Science, Beiging, China) according to the manufacturer's protocol. The absorbance of samples was read at 620 nm. The final concentration was calculated using the following formula.

$$\text{Trehalose (mg/g sample)} = \text{Concentrations from y-axis/Fresh weight of the sample}$$

#### 2.6.4. Glycogen assay

Glycogen level in the haemolymph was determined using the Anthrone method (Solarbio Life Science, Beiging, China) according to the manufacturer's protocol. The absorbance was detected at 620 nm. The final concentration was calculated using the following formula.

$$\text{Glycogen (mg/g fresh weight)} = (Cs \times V1) \times (A3 - A1) \div (A2 - A1) \div (W \times V1 \div V2) \div 1.11$$

Where 1.11= is a constant that glucose content converted to glycogen content; Cs=the concentration of the standard, 0.1

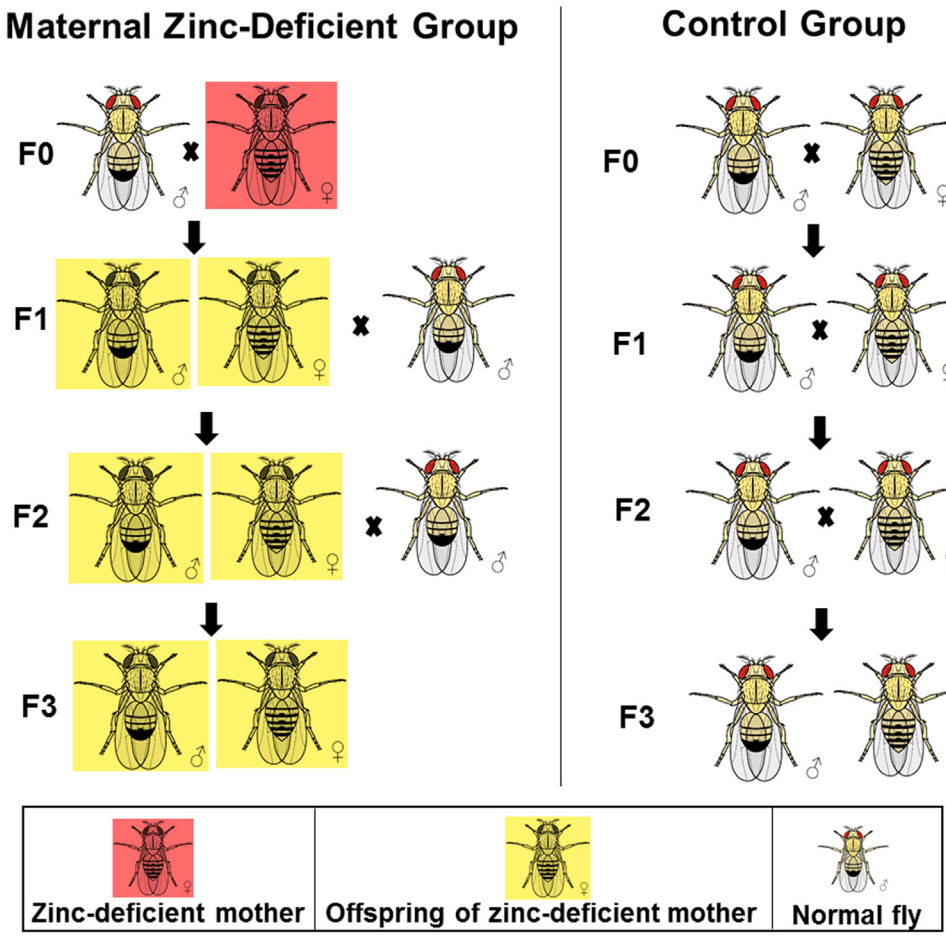



**Fig. 2.** The breeding schema for the fly crossing in both the zinc-deficient and control groups. In the zinc-deficient group, zinc-deficient F0 females were crossed with control males to produce F1 offspring. Then, F1 females were crossed with control males to obtain F2, and subsequently, F2 females were crossed with control males to obtain F3. Conversely, in the control group, normal diet-fed male and female F0 flies were initially crossed to generate F1 offspring. Then, both male and female F1 flies were crossed to produce F2, and finally, male and female F2 flies were crossed to generate F3.

mg/mL; V1=sample volume, 25 μL; V2=extraction volume, 1 mL; W=sample weight; A1=absorbance of blank; A2=absorbance of standard; A3=absorbance of sample.

*2.6.5. Triglyceride assay*

The triglyceride level in the haemolymph was quantified using a colorimetric kit (Spinreact, Girona, Spain) according to the manufacturer's protocol. The absorbance (A) of the samples and standard were read against the blank at 505 nm. The triglyceride concentration was calculated as follows.

$$\text{Triglycerides (mg/dl)} = \text{A(Sample)} - \text{A(Blank)/A(Standard)} - \text{A(Blank)} \times 100$$

*2.6.6. Catalase (CAT) assay*

CAT level in haemolymph was detected using a colorimetric assay kit (Solarbio Life Science, Beiging, China) according to the manufacturer's protocol. The principle is based on its action in decomposing $H_2O_2$ into $H_2O$ and $O_2$ and absorbance detected at 240 nm. The final concentration was calculated using the following formula.

$$\text{CAT activity (U/mL)} = \left(\Delta A \times \text{Extraction volume} \div (\varepsilon \times d) \times 10^9\right) \div \text{Sample volume} \div \text{Reaction time}.$$

Where ε=molar coefficient and d=light path.

*2.6.7. Total antioxidant capacity (TAOC) assay*

Total antioxidant capacity was determined in the haemolymph using a colorimetric assay kit (Solarbio Life Science, Beiging, China) according to the manufacturer's protocol. The kit is used to detect the total antioxidant levels of antioxidants and antioxidant enzymes in a given sample. In principle, $Fe^{3+}$-TPTZ is reduced to blue $Fe^{2+}$-TPTZ. This colour reaction reflects the total antioxidant capacity. The final concentration was obtained from the following formula.

$$\text{Total antioxidant capacity } (\mu\text{mol/mL}) = x \times \text{Vrv} \div \text{Vs} = 34 \times x$$

Where Vrv: total reaction volume, 1.02 mL; Vs: sample volume, 0.03 mL; x: concentration from the standard curve.

*2.6.8. Malondialdehyde (MDA) assay*

MDA level in the haemolymph was determined by lipid peroxidation assay colorimetric kit (Solarbio Life Science, Beiging, China) according to the manufacturer's protocol. The final concentration was calculated using the following formula.

$$\text{MDA (nmol/g)} = 5(6.45 \times (\Delta A532 - \Delta A600) - 1.29 \times \Delta A450) \div \text{sample weight}$$

### 2.7. Gene expression analysis

#### 2.7.1. Extraction of RNA

RNA was extracted from 45 flies aged seven to ten days taken from each group, using the nucleic acid isolation kit obtained from Hunan Runmei Gene Technology Co., Ltd, following the manufacturer's instructions. The extracted RNA purity readings were determined using the Bioevopeak Nucleic Acid Analyzer (SP-MUV2000F, Jinan, Shandong, China), and readings A260/230 and A260/280 of 1.8 to 2.2 were considered for this study.

#### 2.7.2. Primer design

The PrimerQuest software (https://www.idtdna.com/PrimerQuest/Home/Index) was used to design the primers for the genes of interest using sequences obtained from the GenBank Database of the National Centre for Biotechnology Information (Table 1). The mRNA expression of genes involved in some relevant metabolic pathways such as insulin signalling (*DILP2*- insulin-like peptide 2), gluconeogenesis (*PEPCK* – phosphoenolpyruvate carboxykinase), antioxidant defence (*SOD1*- Cu, Zn superoxide dismutase and *CAT*- catalase), and inflammation (*UPD2*- unpaired 2 and *EGR*- Tumour necrosis factor eiger) were assessed. *RPL32* was used as the housekeeping gene.

#### 2.7.3. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) analysis

To perform RT-qPCR, the TransScript Green One-Step qRT-PCR SuperMix (AQ211) kit from TransGen Biotech Co., Ltd (Beijing, China) was used in accordance with the manufacturer's instructions. The reaction mixture contains the RNA template (200ng/μl each), forward primer (0.4 μL of 10 μM), reverse primer (0.4 μL of 10 μM), SuperMix (10 μL), Enzyme mix (0.4 μL), and RNase-free water, resulting in a final volume of 20 μL. The mixture was then loaded onto a Rotor-Gene Q-5plex HRM platform thermal cycler (Qiagen, Hilden, Germany) and subjected to the following cycling conditions: 45°C for 5 minutes (reverse transcription), 94°C for 30 seconds (predenaturation), followed by 40 cycles of 94°C for 5 seconds (denaturation), corresponding annealing temperature (Table 1) for 15 seconds, and 72°C for 10 seconds (extension). The fold change was calculated using the comparative CT method with the formula $2^{-\Delta\Delta CT}$.

### 2.8. Data analysis

Statistical analysis was performed using GraphPad Prism 9.5.1.733 (GraphPad Software Inc, San Diego, CA). Student's t-test was used to analyse the data obtained from F0, while a two-way analysis of variance (ANOVA) was used to analyse the data obtained from F1 to F3, followed by Bonferroni's multiple comparison *post hoc* test. The data were presented as mean ± standard deviation. Statistical significance was determined at a $P<.05$.

## 3. Results

### 3.1. Effect of maternal zinc deficiency on body zinc level

Dietary zinc-chelation significantly ($P<.05$) reduced body zinc level in female parent (F0) compared to control. Interestingly, all offspring of zinc-deficient female flies also had significant ($P<.05$) reduction in body zinc level compared to control except the male offspring at F3, which was not significantly ($P>.05$) different from control (Table 2).

### 3.2. Effects of maternal zinc deficiency on body weight and locomotor performance

From F1 to F3, female offspring consistently demonstrated a significantly ($P<.05$) higher weight compared with the male. However, no significant differences ($P>.05$) were observed in the body weight of both the zinc-deficient parent and their offspring when compared to the control group, except for the F2 generation, which was significantly ($P<.05$) increased (Fig. 3A-D). In addition, no significant ($P>.05$) differences were observed in the locomotor performance of both the parent and their offspring, except for the female F1 flies, which showed a significant ($P<.05$) reduction compared to the control and their male counterpart (Fig. 3E-H).

### 3.3. Effects of maternal zinc deficiency on glucose and trehalose levels

In the zinc-deficient parent flies, a significant ($P<.05$) increase in glucose levels was observed compared to the control group. In the F1 and F3 generations, both male and female offspring from zinc-deficient mothers exhibited a significant ($P<.05$) reduction in glucose levels. However, in the F2 generation, there was no significant ($P>.05$) difference in glucose levels between male and

Table 1
List of primer sequences.

| S/N | Gene | Accession | Primer | Sequence | Annealing Temperature |
|---|---|---|---|---|---|
| 1 | *SOD1* | NM_057387 | Forward | CGGTCACACCATAGAAGATACC | 65 |
| | | | Reverse | CAGACAGCTTTAACCACCATTTC | |
| 2 | *CAT* | NM_080483 | Forward | TGGTCGTCTGTTCTCCTACT | 65 |
| | | | Reverse | CCGCTGGAAGTTCTCAATCT | |
| 3 | *DILP2* | NM_079288 | Forward | GTACTCAATTCCCTGGCTGAA | 55 |
| | | | Reverse | CGCAGAGCCTTCATATCACA | |
| 4 | *PEPCK* | NM_079060 | Forward | TCAATGGCGAATCCTGCTAC | 60 |
| | | | Reverse | CTTCACGTCCACCTTATCCTTC | |
| 5 | *UPD2* | NM_001370039 | Forward | TTGACCATAAACGCCTCCTATC | 60 |
| | | | Reverse | GTGAAAGTTGAGACGCTCCT | |
| 6 | *EGR* | NM_165735.4 | Forward | TGAGGCAACTTCCAAAGAGAG | 60 |
| | | | Reverse | CGGATCTGGCTGAAAGAAGAG | |
| 7 | *RPL32* | NM_170460.2 | Forward | GGATCGATTCCTGTGAGAGTTC | 60 |
| | | | Reverse | TGGGCAGTATCCATTGAGTTT | |

CAT, Catalase; dILP2, Drosophila Insulin like peptide-2; EGR, Eiger; PEPCK, Phosphoenolpyruvate carboxykinase; RPL-32- 60S, ribosomal protein large subnunit-32; SOD1. Superoxide dismutase 1; UPD2, Unpaired 2.

Table 2
Transgenerational zinc levels (F0-F3).

| Generation | Gender | Group | Body zinc level |
|---|---|---|---|
| F0 | Female | Control | 0.05000±0.00001 |
| | | Zinc-deficient | 0.03000±0.00033* |
| F1 | Male | Control | 0.05218±0.00185 |
| | | Zinc-deficient progeny | 0.04678±0.00006* |
| | Female | Control | 0.06285±0.00002 |
| | | Zinc-deficient progeny | 0.05503±0.00001* |
| F2 | Male | Control | 0.05218±0.00185 |
| | | Zinc-deficient progeny | 0.03631±0.00168* |
| | Female | Control | 0.06285±0.00002 |
| | | Zinc-deficient progeny | 0.02526±0.00865* |
| F3 | Male | Control | 0.05218±0.00185 |
| | | Zinc-deficient progeny | 0.05723±0.00009* |
| | Female | Control | 0.06285±0.00002 |
| | | Zinc-deficient progeny | 0.03370±0.00292* |

Samples were analysed on MPAES (microwave plasma atomic emission spectrometry), and values are expressed as mean ± SD (n = 30). F0 data were analysed using Student's t-test, while a two-way ANOVA was used to analyse the data from F1 to F3, followed by Bonferroni's multiple comparison post hoc test.
* Data with asterisks are significantly (P < .05) different from corresponding control.

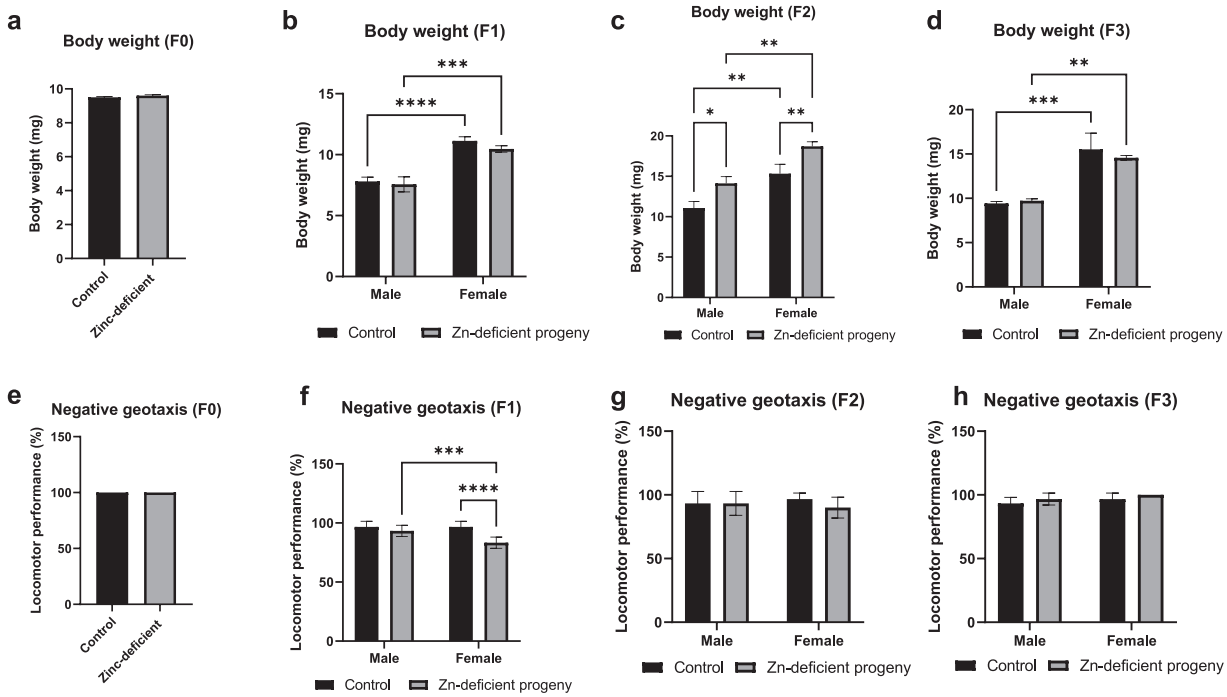



**Fig. 3.** Effects of maternal zinc deficiency on body weight (A-D) and locomotor performance (E-H). Bars represent mean±SD. F0 data were analysed using Student's t-test while F1-F3 data were analysed using two-way ANOVA followed by Bonferroni's *post hoc* test. Asterisks represent significant difference at varying *P*-value (*: .0332, **: .0021, ***: .0002, ****: <.0001). n=10.

female offspring and the control group (Fig. 4A-D). In addition, zinc deficiency resulted in a significant ($P<.05$) increase in trehalose levels in the female parent compared to the control. However, both male and female F1 generations exhibited a significant ($P<.05$) decrease in trehalose levels. The F2 and F3 generations showed a similar pattern with the zinc-deficient mothers, displaying a significant ($P<.05$) elevation in trehalose levels compared to the control. Notably, the trehalose levels in male F2 and F3 flies were significantly ($P<.05$) higher than in their female counterparts (Fig. 4E-H).

### *3.4. Effects of maternal zinc deficiency on glycogen and triglyceride levels*

In the F0 generation, zinc deficiency led to a significant ($P<.05$) decrease in glycogen levels compared to the control. In the F1 generation, male offspring exhibited significantly ($P<.05$) higher glycogen levels, while no significant ($P>.05$) difference was observed in the females compared to the control. However, the F2 and F3 generations demonstrated a similar pattern with the zinc-deficient mothers, showing a significant ($P<.05$) decrease in glyco-

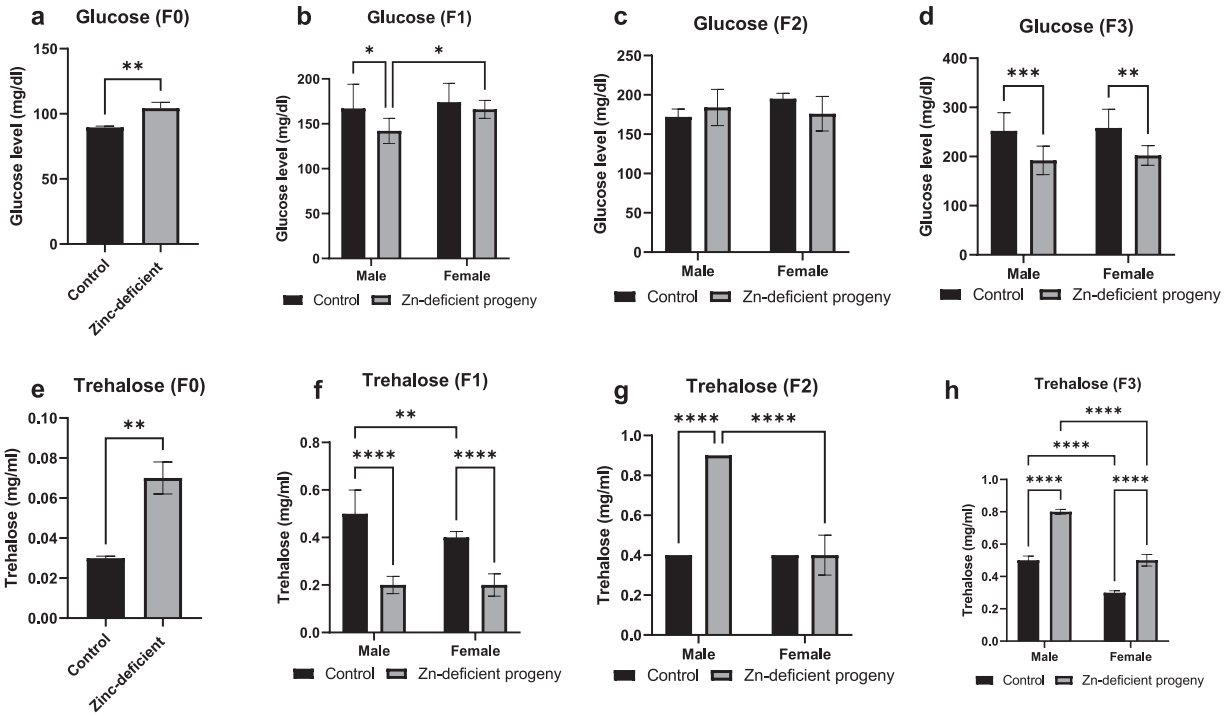



**Fig. 4.** Effects of maternal zinc deficiency on glucose (A-D) and trehalose (E-H) levels. Bars represent mean ± SD. F0 data were analysed using Student's t-test while F1-F3 data were analysed using two-way ANOVA followed by Bonferroni's *post hoc* test. Asterisks represent significant difference at varying *P*-value (*: .0332, **: .0021, ***: .0002, ****: <.0001). n=10.

gen level compared with the control (Fig. 5A-D). In addition, no significant ($P>.05$) difference in triglyceride levels was observed in the female parent compared to the control. However, in the F1 generation, both male and female offspring from zinc-deficient mothers exhibited a significant ($P<.05$) increase in triglyceride levels. In the F2 generation, a significant ($P<.05$) reduction was observed in the male triglyceride levels, while the females showed no significant ($P>.05$) difference compared to the control. In the F3 generation, both male and female offspring showed a significant ($P<.05$) decrease in triglyceride levels compared to the control (Fig. 5E-H).

### 3.5. Effects of maternal zinc deficiency on catalase activity, total anti-oxidant capacity and malondialdehyde levels

Zinc deficiency in the F0 resulted in a significant ($P<.05$) decrease in catalase activity compared to the control. However, a significant increase was observed in both the male and female F1 flies. Subsequently, no significant ($P>.05$) difference was observed in F2, whereas a significant ($P<.05$) reduction was observed in F3 compared to the control (Fig. 6A-D). Assessing the total antioxidant capacity (TAOC), a significant reduction ($P<.05$) in TAOC was observed in the F0 generation as well as in male and female F1 flies. In the F2 generation, no significant ($P>.05$) difference was observed in the male and female flies compared to the control. However, a significant ($P<.05$) increase in TAOC was noted in the male and female flies in the F3 generation compared to the control (Fig. 6E-H). Furthermore, zinc deficiency led to a significant ($P<0.05$) increase in MDA levels in the F0 generation, and this trend persisted in the subsequent F1 and F2 generations. However, there was a notable shift in the F3 generation, where a significant ($P<.05$) decrease in MDA levels was observed in both male and female flies (Fig. 6I-L).

### 3.6. Effects of maternal zinc deficiency on DILP2 and PEPCK mRNA

In the zinc-deficient female flies (F0), a significant ($P<.05$) increase in the fold change in *DILP2* mRNA was observed compared to the control. Interestingly, there was a consistent significant ($P<.05$) increase in *DILP2* mRNA in male and female offspring across F1 to F3 generations (Fig. 7A-D). On *PEPCK* mRNA, there was a significant ($P<.05$) increase in the fold change in the zinc-deficient female parent compared to the control. However, a significant ($P<.05$) reduction was observed in the male and female F1 flies. Sex-dependent effects emerged in the F2 generation, with a significant ($P<.05$) increase in males and a significant ($P<.05$) decrease in the female flies. Furthermore, *PEPCK* mRNA was significantly ($P<.05$) increased in both male and female F3 flies compared to the control (Fig. 7E-H). Notably, the fold change in *PEPCK* mRNA in the male zinc-deficient progeny ($P<.05$) was significantly higher than that of the female flies in F2 and F3.

### 3.7. Effects of maternal zinc deficiency on SOD1 and CAT mRNA

In the F0 generation, the fold change in *SOD1* mRNA was significantly ($P<.05$) reduced compared to the control. Across the generations of the offspring, there was a similar trend of significant ($P<.05$) decreases in *SOD1* mRNA, except in the male offspring in the F2 generation, which were significantly ($P<.05$) increased (Fig. 8A-D). In addition, there was a significant ($P<.05$) decrease in the fold change in *CAT* mRNA in the female parent compared to the control. This downward trend persisted in the F1 generation, where both male and female offspring displayed a significant ($P<.05$) reduction in *CAT* mRNA. Conversely, male and female offspring in the F2 and F3 generations showed a significant ($P<.05$) increase in the *CAT* mRNA compared to the control (Fig. 8E-H).

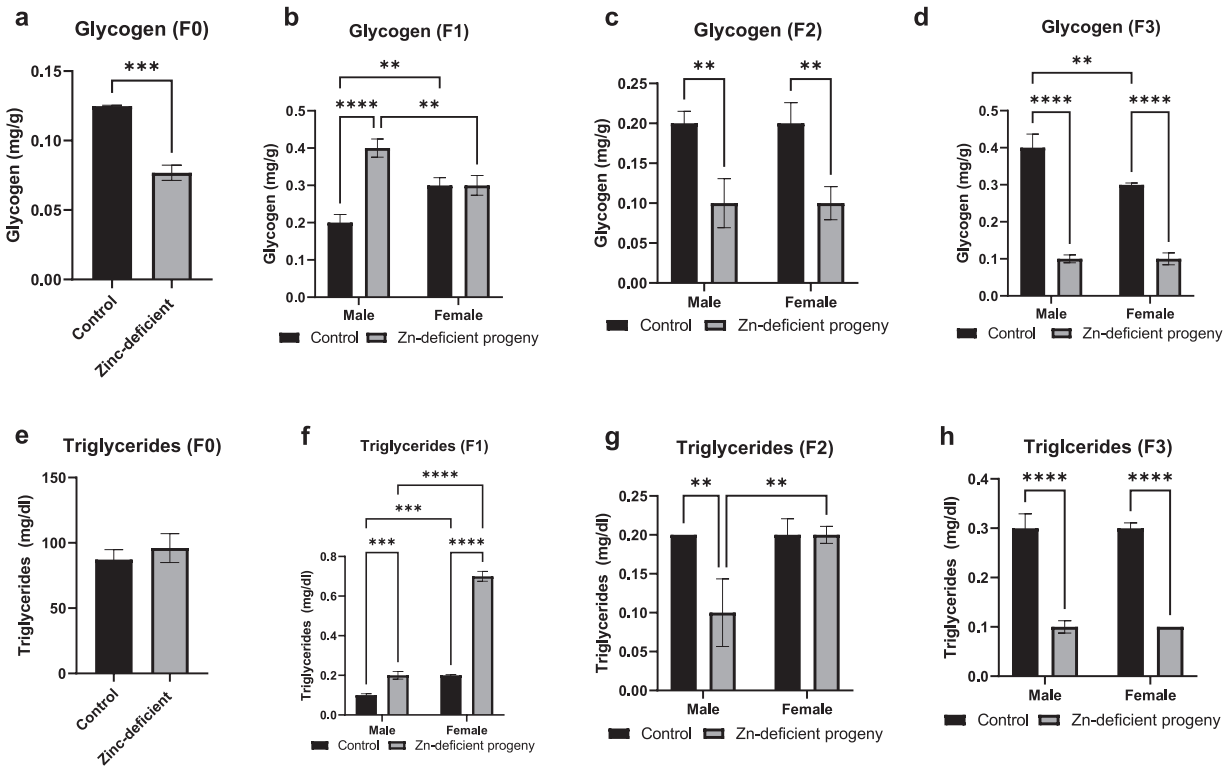



**Fig. 5.** Effects of maternal zinc deficiency on glycogen (A-D) and triglyceride (E-H) levels. Bars represent mean ± SD. F0 data were analysed using Student's t-test while F1-F3 data were analysed using two-way ANOVA followed by Bonferroni's *post hoc* test. Asterisks represent significant difference at varying *P*-value (*: .0332, **: .0021, ***: .0002, ****: <.0001). n=10.

### 3.8. *Effects of maternal zinc deficiency on* EGR *and* UPD2 *mRNA*

From the parent (F0), which was zinc-deficient, to the offspring (F1-F3), which were fed a normal diet, the expression of the *EGR* mRNA showed a significant ($P<.05$) increase compared to the control (Fig. 9A-D). In the addition, there was a significant ($P<.05$) reduction in the fold change of *UPD2* mRNA in the zinc-deficient female parent (F0) compared to the control. However, the male and female flies from F1 to F3 showed a significant ($P<.05$) increase in the *UPD2* mRNA compared to the control (Fig. 9E-H).

## 4. Discussion

Studies have shown that maternal zinc deficiency during pregnancy can impact foetal growth and development, ranging from lack of change in body weight and birth weight [21], reduced birth weight [22–24], to long term adult overweight [21,25] in offspring. In this study, the observed increase in body weight in the F2 generation may align with findings from other research that links maternal zinc status to the regulation of growth-related processes and predisposition to weight gain [25]. In the natural context of Drosophila, sexual dimorphism tends to favour females, as evidenced by their consistently larger body size compared to males throughout different developmental stages and across various measured parameters [26,27]. Thus, the observed gender-specific differences in body weight, with female offspring consistently weighing more, could have been influenced by genetic and associated factors.

The impact of maternal zinc deficiency on locomotor performance, especially the gender-specific reduction observed in female F1 flies, could be connected to the role of zinc in the development and maintenance of the nervous system. Zinc is known to play a crucial role in neurogenesis and synaptic function, and disruptions in these processes could contribute to alterations in motor behaviour [7,8]. This finding may be consistent with other studies that have explored the neurodevelopmental effects of maternal zinc deficiency causing decreased offspring fitness and physical activity [23,28,29] as well as learning and cognitive impairment [29,30].

Furthermore, the influence of maternal zinc deficiency on glucose levels aligns with existing literature on the role of zinc in insulin metabolism and glucose homeostasis [10,31]. The observed reduction in glucose levels in both male and female offspring in the F1 and F3 generations may suggest a transgenerational impact. Additionally, the increase in trehalose levels in the zinc-deficient F0 flies indicates a possible metabolic response. The significance of trehalose in energy metabolism and stress response has been established in studies [32,33]. The substantial change in trehalose levels from F1 to F3 in our study suggests the conversion of available glucose to trehalose, reflecting an adaptive strategy for robust energy metabolism. Considering earlier findings, although focused on F1, where male offspring of zinc-deficient mothers exhibited adverse metabolic responses [21,25,34], the gender-specific variations observed in the F2 and F3 generations in our study, with more pronounced effects in male flies, indicate a potential heightened susceptibility of males to the transgenerational adverse effects of maternal zinc deficiency.

Furthermore, in stored glycogen, the gender-specific response in the F1 generation, with increased glycogen levels in males but not females, may reflect sex-specific differences in metabolic adaptation. The observed increase in glycogen level at F1 in contrast to other generation shows that more of the glucose might have been converted to glycogen. This is also in consonance with the increased triglycerides at F1, indicating a potential link between

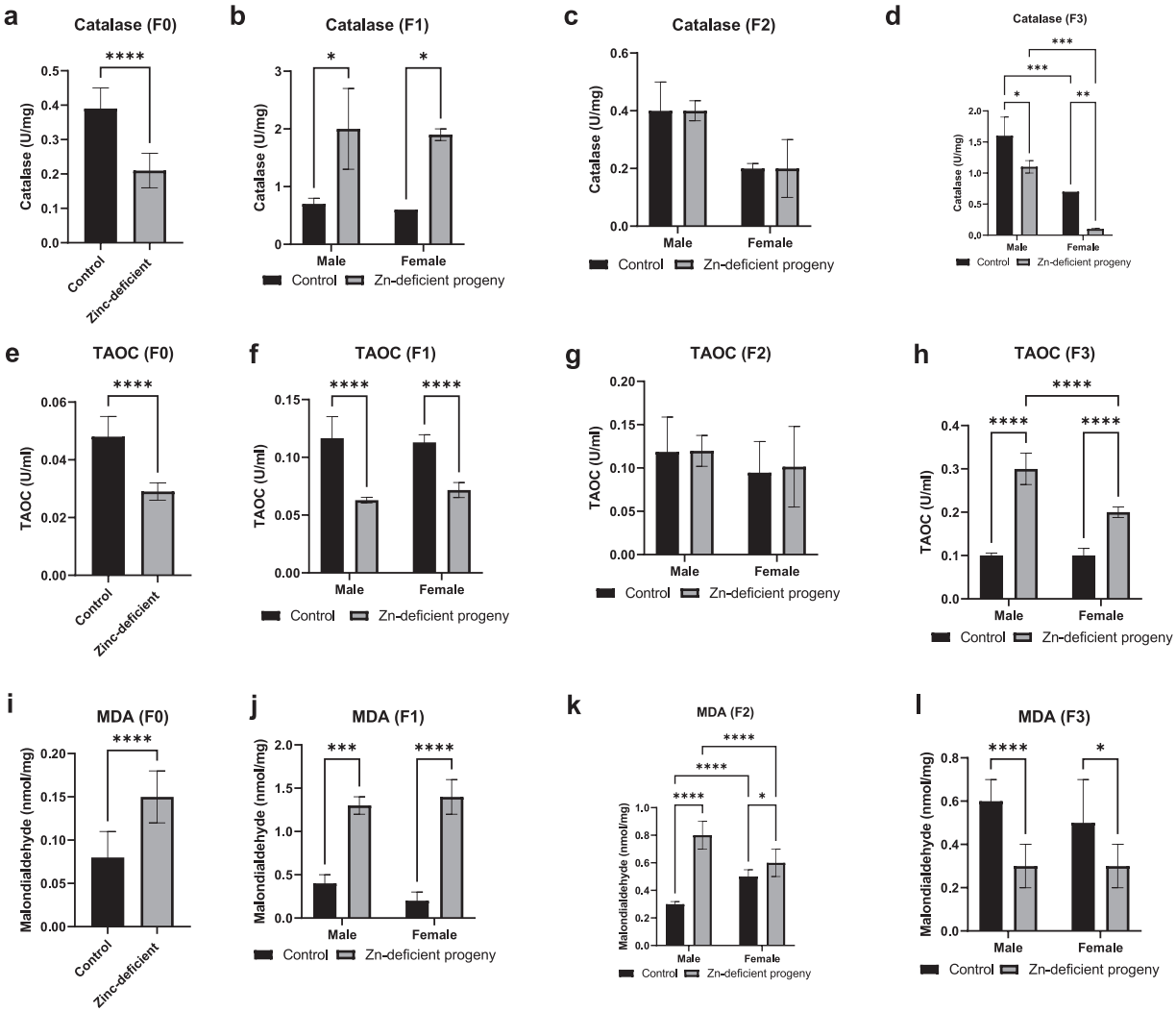



**Fig. 6.** Effects of maternal zinc deficiency on catalase activity (A-D), total anti-oxidant capacity (TAOC) (E-H) and malondialdehyde (MDA) levels (I-L). Bars represent mean ± SD. F0 data were analysed using Student's t-test while F1-F3 data were analysed using two-way ANOVA followed by Bonferroni's *post hoc* test. Asterisks represent significant difference at varying *P*-value (*: .0332, **: .0021, ***: .0002, ****: <.0001). n=10.

maternal zinc deficiency and altered lipid metabolism. However, F2, and F3 showed reduced glycogen and triglycerides probably due to more conversion of these substrates to glucose and in-turn trehalose, and a probable transgenerational inheritance. Similar patterns in the F2 and F3 generations suggest a potential transgenerational effect of maternal zinc deficiency on glycogen regulation.

While parent flies (F0) exhibited increased levels of glucose and trehalose, suggesting storage disturbances, subsequent generations showed a decrease in glucose levels, potentially indicative of increased utilization. Trehalose levels, however, displayed variability; F1 offspring exhibited lower trehalose levels, aligning with the decrease in glucose. Interestingly, F2 and F3 generations showed elevated trehalose levels, suggesting a possible compensatory mechanism. Additionally, changes in triglyceride levels indicate alterations in fat metabolism. We hypothesize that these metabolic shifts reflect the offspring's adaptive responses to maternal zinc deficiency. Further investigation is warranted to elucidate the precise mechanisms underlying these changes, particularly considering the observed sex-based differences in trehalose levels among F2 and F3 generations.

In addition, the observed significant increase in *DILP2* mRNA in zinc-deficient female flies (F0) and its persistence across F1 to F3 generations suggest a molecular basis for linking zinc deficiency to alterations in the insulin signalling pathways. Previous research, although focused on direct effect, has shown that zinc plays a crucial role in insulin regulation, and disruptions in this pathway can lead to metabolic consequences [12]. The sustained elevation in *DILP2* mRNA suggests an alteration in the insulin action making the system feel the need to produce more insulin, a potential link between maternal zinc status and insulin-related pathways in offspring across multiple generations. Transgenerational disease risk holds significant importance in the realm of metabolic diseases, offering insights into the intricate interplay among genetics, epigenetics, and environmental factors in disease progression [35,36]. Zinc, an essential mineral for various biological processes such as gene expression and enzyme activity, plays a critical role in the functioning of epigenetic enzymes involved in DNA and histone modifications. Animal studies have demonstrated the impact of zinc deficiency on these epigenetic modifications. Specifically, maternal zinc deficiency during pregnancy has been shown to induce changes in DNA methylation and histone modifications in offspring

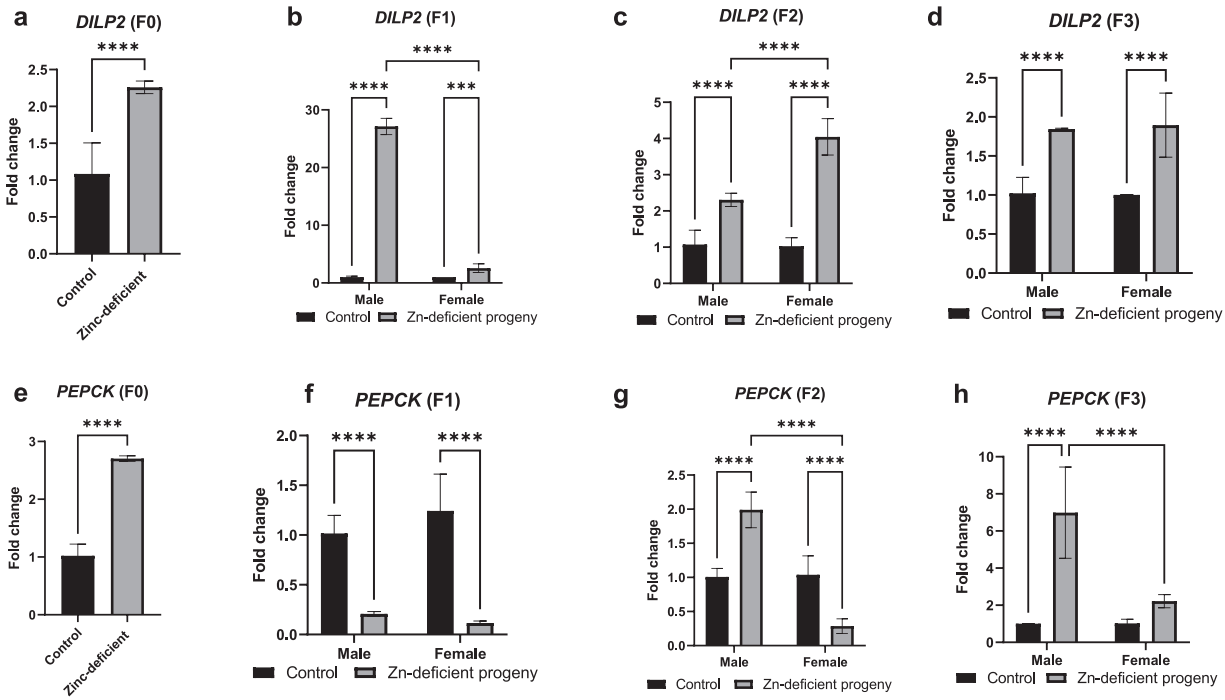



**Fig. 7.** Effects of maternal zinc deficiency on the expression of *DILP2* (A-D) and *PEPCK* (E-H). Bars represent mean ± SD. F0 data were analysed using Student's t-test while F1-F3 data were analysed using two-way ANOVA followed by Bonferroni's *post hoc* test. Asterisks represent significant difference at varying *P*-value (*: .0332, **: .0021, ***: .0002, ****: <.0001). n=10.

[37]. These alterations, induced by maternal zinc deficiency, can exert long-lasting effects on offspring health. Thus, understanding the role of zinc in epigenetic modifications is crucial for deciphering the mechanisms underlying the effects of zinc deficiency on offspring health. Further research is needed to better understand the specific epigenetic mechanisms at play and their potential implications.

Zinc regulates glucose metabolism and insulin signalling by exerting insulin-mimetic actions that promote glycogen synthesis and inhibit gluconeogenesis [11]. The significant increase in *PEPCK* mRNA in the zinc-deficient female parent flies affirm the role for zinc in gluconeogenesis regulation. However, the observed reductions in the F1 generation aligns with a homeostatic response and subsequent sex-dependent effects in the F2 generation highlight the complexity of the relationship between maternal zinc deficiency and *PEPCK* expression. The consistent elevation in *PEPCK* mRNA in the F3 generation suggests a programming effect that persists across generations and manifests in F3. In addition, a significant increase in *PEPCK* observed in male offspring at F2 and F3 suggests that the enzyme, PEPCK could trigger gluconeogenesis in the male offspring contributing to the increased trehalose level. Thus, adding to the fact that the males may be more prone to the glucose metabolism-related consequences of parental zinc deficiency.

As zinc plays a crucial role in antioxidant defence and managing oxidative stress, the observed decrease in catalase activity and total antioxidant capacity in the zinc deficient parent flies aligns with findings from studies linking zinc deficiency to impaired antioxidant enzymes functions [12]. Consistent with the catalase activities, the observed decrease in expression of *CAT* and *SOD1* mRNA expression in the F0 generation aligns with the association between zinc deficiency and impaired antioxidant defences. Zinc is a cofactor for SOD, an important antioxidant enzyme, while catalase is a key enzyme involved in the breakdown of hydrogen peroxide [38]. Their reduced expression suggests an impaired ability to neutralize reactive oxygen species. However, in our study, the increase in catalase activity in F1, the increase in the expression of *CAT* mRNA in F2 and F3, the absence of significant differences in TAOC in the F2, and an increase in the F3 generation suggests a dynamic and potentially adaptive response to maternal zinc deficiency. Also, the observed increase in *SOD1* mRNA particularly in the male offspring in indicates sex-specific responses necessary to be considered while studying the impact of maternal zinc deficiency. This variation across generations could be attributed to intricate interactions between antioxidant systems and compensatory mechanisms to counterbalance oxidative stress.

Moreover, the increase in MDA levels in the F0 is consistent with studies demonstrating that zinc deficiency can lead to elevated lipid peroxidation [12,39,40]. Interestingly, F1 and F2 generations fed normal diet also exhibited a significant elevation, a probable transgenerational inheritance. The decrease in MDA levels in both male and female offspring in the F3 generation presents a shift that could be due to the increased TAOC. Probably, the compensatory mechanisms or adaptations have occurred over successive generations to mitigate oxidative damage.

Exploring the pro-inflammatory status of the flies, we assessed the expression of *EGR* and *UPD2*. The Drosophila TNF-eiger (EGR) system is similar to the TNF-alpha system found in mammals, including humans. It is a signalling pathway that has been evolutionarily conserved across different species. In Drosophila, the EGR is involved in various biological processes, including immune responses, tissue development, and cell death [41]. The activation of the EGR system in Drosophila leads to the activation of downstream signalling pathways, such as the JNK (c-Jun N-terminal kinase) pathway and the NF-$\kappa$B (Nuclear Factor kappa B) pathway [42]. The metabolic functions of EGR extend to the regulation of glucose metabolism, lipid metabolism, and energy balance, with potential implications for various metabolic disorders such as di-

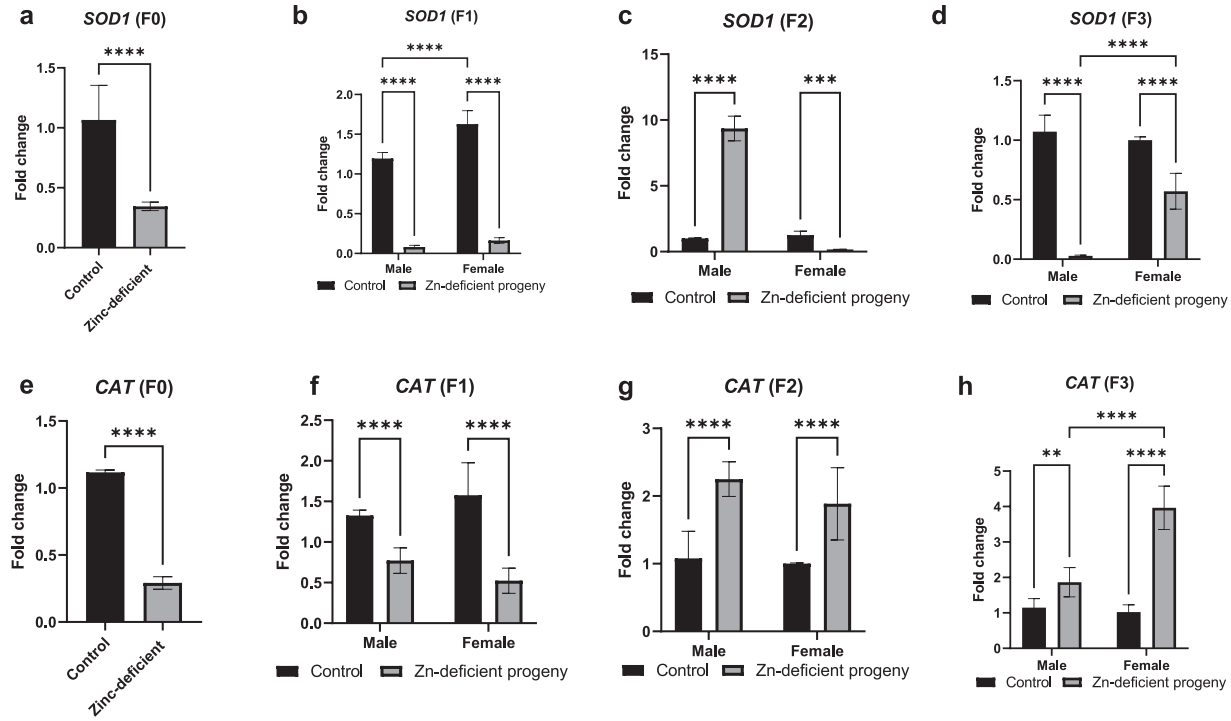



**Fig. 8.** Effects of maternal zinc deficiency on the expression of *SOD1* (A-D) and *CAT* (E-H). Bars represent mean ± SD. F0 data were analysed using Student's t-test while F1-F3 data were analysed using two-way ANOVA followed by Bonferroni's *post hoc* test. Asterisks represent significant difference at varying *P*-value (*: .0332, **: .0021, ***: .0002, ****: <.0001). n=10.

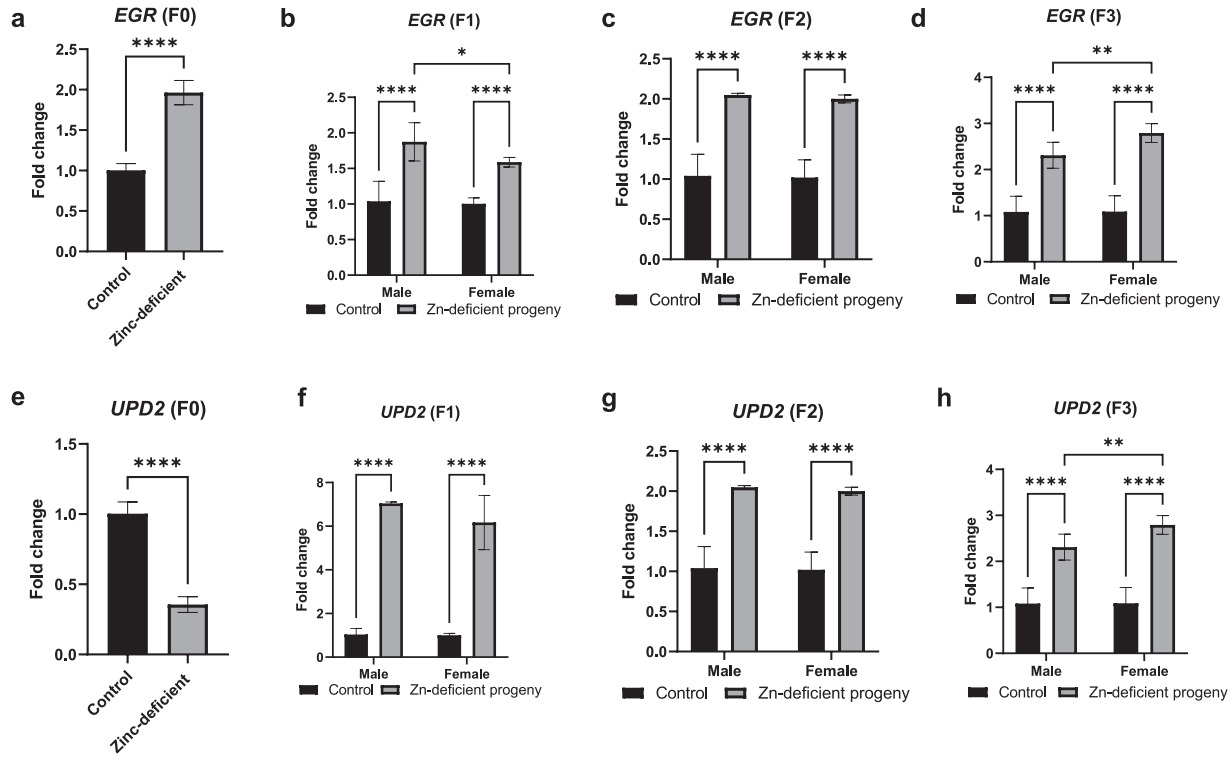



**Fig. 9.** Effects of maternal zinc deficiency on the expression of *EGR* (A-D) and *UPD2* (E-H). Bars represent mean ± SD. F0 data were analysed using Student's t-test while F1-F3 data were analysed using two-way ANOVA followed by Bonferroni's *post hoc* test. Asterisks represent significant difference at varying *P*-value (*: .0332, **: .0021, ***: .0002, ****: <.0001). n=10.

abetes and obesity [42,43]. Notably, in HL-60 cells under zinc deficiency, an increase in the proinflammatory cytokine TNF-alpha was observed [44]. This aligns with the observed elevation in *EGR* mRNA expression in the zinc-deficient parent (F0) flies. Interestingly, our study revealed a similar trend across the offspring (F1-F3), suggesting a transgenerational proinflammatory effect of maternal zinc deficiency. This effect may, in part, contribute to the observed energy dysmetabolism.

Similarly, Drosophila UPD2, activated in response to dietary factors, is recognized as a pro-inflammatory cytokine with a pivotal role in inflammation. Its activation triggers the JAK/STAT signalling pathway [45]. Moreover, UPD2 has been identified as a functional homologue of leptin, contributing to the regulation of energy metabolism within the organism [45]. Furthermore, the induction of proinflammatory cytokines, including UPD2, in damaged tissues is associated with JNK and Hippo signalling pathways [46]. The JAK-STAT pathway in Drosophila has been linked to intestinal homeostasis during stress or injury, highlighting its involvement in inflammatory responses [47]. In our study, the diminished expression of *UPD2* in the zinc-deficient parent suggests its role as an energy regulator, potentially contributing to the observed dysregulation in energy metabolism. However, the subsequent increase in *UPD2* mRNA observed in male and female offspring across generations may points to its proinflammatory role in the offspring. This emphasizes the complexity of gene regulation in response to maternal zinc status.

Importantly, the gender-specific effects of maternal zinc deficiency on offspring could stem from several mechanisms. Changes in gene expression triggered by epigenetic modifications or disruptions in sex hormone balance due to the role of zinc in their function are potential factors. Additionally, genes might respond differently to zinc levels based on the offspring's sex, and differences in the gut microbiome between male and female offspring could further influence how they handle zinc deficiency [48]. These factors could all contribute to the observed variations in how male and female offspring are affected by maternal zinc deficiency across generations.

## 5. Conclusions

Our study elucidates the transgenerational consequences of maternal zinc deficiency on various physiological aspects in Drosophila offspring. From altered body weight and impaired locomotor performance to disruptions in glucose metabolism and antioxidant defence mechanisms, our findings underscore the nuanced impact of maternal zinc status across multiple generations. Notably, the observed sex-specific responses and persistent molecular alterations in insulin signalling pathways and inflammatory markers suggest a complex interplay between maternal zinc deficiency and long-term offspring health. These results emphasize the importance of considering both gender-specific effects and molecular mechanisms including epigenetics in understanding the broader implications of maternal zinc deficiency, providing valuable insights for future research aimed at mitigating such adverse outcomes in human health.

## Author contributions

Mustapha Umar Imam contributed to the study conception and design. Material preparation, data collection, and analysis were performed by all authors. The first draft of the manuscript was written by Kamaldeen Olalekan Sanusi and all authors contributed to the review and editing of the manuscript. All authors read and approved the final manuscript.

## Ethics approval

No ethical approval is required.

## Declaration of competing interest

The authors have no relevant financial or non-financial interests to disclose.

## CRediT authorship contribution statement

**Kamaldeen Olalekan Sanusi:** Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Murtala Bello Abubakar:** Writing – review & editing, Supervision, Resources, Project administration, Data curation. **Kasimu Ghandi Ibrahim:** Resources, Project administration, Data curation, Supervision, Writing – review & editing. **Mustapha Umar Imam:** Supervision, Writing – review & editing, Conceptualization, Data curation, Funding acquisition, Project administration, Resources.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jnutbio.2024.109669.

## References

[1] Skalny A V, Aschner M, Tinkov AA. Zinc. Adv Food Nutr Res 2021;96:251–310. doi:10.1016/bs.afnr.2021.01.003.
[2] Ota E, Mori R, Middleton P, Tobe-Gai R, Mahomed K, Miyazaki C, et al. Zinc supplementation for improving pregnancy and infant outcome. Cochrane Database Syst Rev 2015;2015:CD000230. doi:10.1002/14651858.CD000230.pub5.
[3] Tamura T, Goldenberg RL, Ramey SL, Nelson KG, Chapman VR. Effect of zinc supplementation of pregnant women on the mental and psychomotor development of their children at 5 y of age123. Am J Clin Nutr 2003;77:1512–16. doi:10.1093/ajcn/77.6.1512.
[4] Boskabadi H, Maamouri G, Akhondian J, Ashrafzadeh F, Boskabadi A, Faramarzi R, et al. Comparison of birth weights of neonates of mothers receiving vs. not receiving zinc supplement at pregnancy. BMC Pregnancy Childbirth 2021;21:187. doi:10.1186/s12884-021-03598-8.
[5] Kambe T, Tsuji T, Hashimoto A, Itsumura N. The physiological, biochemical, and molecular roles of zinc transporters in zinc homeostasis and metabolism. Physiol Rev 2015;95:749–84. doi:10.1152/physrev.00035.2014.
[6] Hawrysz Z, Woźniacka A. Zinc: an undervalued microelement in research and treatment. Postep Dermatologii I Alergol 2023;40:208–14. doi:10.5114/ada.2023.127639.
[7] Choi S, Hong DK, Choi BY, Suh SW. Zinc in the Brain: Friend or Foe? Int J Mol Sci 2020;21:8941. doi:10.3390/ijms21238941.
[8] Grabrucker AM, Knight MJ, Proepper C, Bockmann J, Joubert M, Rowan M, et al. Concerted action of zinc and ProSAP/Shank in synaptogenesis and synapse maturation. EMBO J 2011;30:569–81. doi:10.1038/emboj.2010.336.
[9] Weiss M, Steiner DF, Philipson LH. Insulin biosynthesis, secretion, structure, and structure-activity relationships. In: Feingold KR, Anawalt B, Blackman MR, Boyce A, Chrousos G, Corpas E, et al., editors. Endotext [Internet], South Dartmouth (MA): 2014.
[10] Fukunaka A, Fujitani Y. Role of zinc homeostasis in the pathogenesis of diabetes and obesity 2018. https://doi.org/10.3390/ijms19020476.
[11] Tamura Y. The role of zinc homeostasis in the prevention of diabetes mellitus and cardiovascular diseases. J Atheroscler Thromb 2021;28:1109–22. doi:10.5551/jat.RV17057.
[12] Olechnowicz J, Tinkov A, Skalny A, Suliburska J. Zinc status is associated with inflammation, oxidative stress, lipid, and glucose metabolism. J Physiol Sci 2018;68:19–31. doi:10.1007/s12576-017-0571-7.

[13] Jarosz M, Olbert M, Wyszogrodzka G, Młyniec K, Librowski T. Antioxidant and anti-inflammatory effects of zinc. Zinc-dependent NF-κB signaling. Inflammopharmacology 2017;25:11–24. doi:10.1007/s10787-017-0309-4.
[14] Briassoulis G, Briassoulis P, Ilia S, Miliaraki M, Briassouli E. The anti-oxidative, anti-inflammatory, anti-apoptotic, and anti-necroptotic role of zinc in COVID-19 and sepsis. Antioxidants 2023;12:1942. doi:10.3390/antiox12111942.
[15] Gammoh NZ, Rink L. Zinc in infection and inflammation. Nutrients 2017;9:624. doi:10.3390/nu9060624.
[16] Roohani N, Hurrell R, Kelishadi R, Schulin R. Zinc and its importance for human health: an integrative review. J Res Med Sci 2013;18:144–57.
[17] Foster M, Samman S. Zinc and regulation of inflammatory cytokines: implications for cardiometabolic disease. Nutrients 2012;4:676–94. doi:10.3390/nu4070676.
[18] Hu Q, Duncan FE, Nowakowski AB, Antipova OA, Woodruff TK, O'Halloran TV, et al. Zinc dynamics during drosophila oocyte maturation and egg activation. Science 2020;23:101275. doi:10.1016/j.isci.2020.101275.
[19] Saka SO, Salisu YY, Sahabi HM, Sanusi KO, Ibrahim KG, Abubakar MB, et al. Nutrigenomic effects of white rice and brown rice on the pathogenesis of metabolic disorders in a fruit fly model. Molecules 2023;28:1–23. doi:10.3390/molecules28020532.
[20] Strilbytska O, Semaniuk U, Bubalo V, Storey KB, Lushchak O. Dietary choice reshapes metabolism in drosophila by affecting consumption of macronutrients. Biomolecules 2022;12:1201. doi:10.3390/biom12091201.
[21] Jou M-Y, Lönnerdal B, Philipps AF. Maternal zinc restriction affects postnatal growth and glucose homeostasis in rat offspring differently depending upon adequacy of their nutrient intake. Pediatr Res 2012;71:228–34. doi:10.1038/pr.2011.44.
[22] Padmavathi IJN, Kishore YD, Venu L, Ganeshan M, Harishankar N, Giridharan N V, et al. Prenatal and perinatal zinc restriction: effects on body composition, glucose tolerance and insulin response in rat offspring. Exp Physiol 2009;94:761–9. doi:10.1113/expphysiol.2008.045856.
[23] Bruno RS, Song Y, Leonard SW, Mustacich DJ, Taylor AW, Traber MG, et al. Dietary zinc restriction in rats alters antioxidant status and increases plasma F2 isoprostanes. J Nutr Biochem 2007;18:509–18. doi:10.1016/j.jnutbio.2006.09.001.
[24] Yu X, Jin L, Zhang X, Yu X. Effects of maternal mild zinc deficiency and zinc supplementation in offspring on spatial memory and hippocampal neuronal ultrastructural changes. Nutrition 2013;29:457–61. doi:10.1016/j.nut.2012.09.002.
[25] Chang E, Hafner H, Varghese M, Griffin C, Clemente J, Islam M, et al. Programming effects of maternal and gestational obesity on offspring metabolism and metabolic inflammation. Sci Rep 2019;9:16027. doi:10.1038/s41598-019-52583-x.
[26] Mathews KW, Cavegn M, Zwicky M. Sexual dimorphism of body size is controlled by dosage of the X-chromosomal gene Myc and by the sex-determining gene Tra in drosophila. Genetics 2017;205:1215–28. doi:10.1534/genetics.116.192260.
[27] Millington JW, Rideout EJ. Sex differences in Drosophila development and physiology. Curr Opin Physiol 2018;6:46–56. doi:10.1016/j.cophys.2018.04.002.
[28] Liu X, Adamo AM, Oteiza PI. Marginal zinc deficiency during gestation and lactation in rats affects oligodendrogenesis, motor performance, and behavior in the offspring. J Nutr 2023;153:2778–96. doi:10.1016/j.tjnut.2023.08.029.
[29] Beaver LM, Nkrumah-Elie YM, Truong L, Barton CL, Knecht AL, Gonnerman GD, et al. Adverse effects of parental zinc deficiency on metal homeostasis and embryonic development in a zebrafish model. J Nutr Biochem 2017;43:78–87. doi:10.1016/j.jnutbio.2017.02.006.
[30] Jiang Y-G, Wang Y, Zhang H, Wang Z-Y, Liu Y-Q. Effects of early-life zinc deficiency on learning and memory in offspring and the changes in DNA methylation patterns. Nutr Neurosci 2022;25:1001–10. doi:10.1080/1028415X.2020.1831259.
[31] Norouzi S, Adulcikas J, Sohal SS, Myers S. Zinc stimulates glucose oxidation and glycemic control by modulating the insulin signaling pathway in human and mouse skeletal muscle cell lines. PLoS One 2018;13:e0191727.
[32] Matsushita R, Nishimura T. Trehalose metabolism confers developmental robustness and stability in Drosophila by regulating glucose homeostasis. Commun Biol 2020;3:170. doi:10.1038/s42003-020-0889-1.
[33] Yasugi T, Yamada T, Nishimura T. Adaptation to dietary conditions by trehalose metabolism in Drosophila. Sci Rep 2017;7:1619. doi:10.1038/s41598-017-01754-9.
[34] Tomat AL, Juriol LV, Gobetto MN, Veiras LC, Mendes Garrido Abregú F, Zilberman J, et al. Morphological and functional effects on cardiac tissue induced by moderate zinc deficiency during prenatal and postnatal life in male and female rats. Am J Physiol Circ Physiol 2013;305:H1574–83. doi:10.1152/ajpheart.00578.2013.
[35] Zhu H, Ding G, Liu X, Huang H. Developmental origins of diabetes mellitus: environmental epigenomics and emerging patterns. J Diabetes 2023;15:569–82. doi:10.1111/1753-0407.13403.
[36] King SE, Skinner MK. Epigenetic transgenerational inheritance of obesity susceptibility. Trends Endocrinol Metab 2020;31:478–94. doi:10.1016/j.tem.2020.02.009.
[37] Sanusi KO, Uthman YA, Ooi DJ, Ismail M, Imam MU. Lifestyle and preventive medical epigenetics. In: Medical Epigenetics. Academic Press; 2021. p. 33–50.
[38] Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid. Alexandria J Med 2018;54:287–93. doi:10.1016/j.ajme.2017.09.001.
[39] Marreiro D do N, Cruz KJC, Morais JBS, Beserra JB, Severo JS, de Oliveira ARS. Zinc and oxidative stress: current mechanisms. Antioxidants (Basel, Switzerland) 2017;6:24. doi:10.3390/antiox6020024.
[40] Choi S, Liu X, Pan Z. Zinc deficiency and cellular oxidative stress: prognostic implications in cardiovascular diseases review-article. Acta Pharmacol Sin 2018;39:1120–32. doi:10.1038/aps.2018.25.
[41] Colombani J, Andersen DS. Drosophila TNF/TNFRs: At the crossroad between metabolism, immunity, and tissue homeostasis. FEBS Lett 2023;597:2416–32. doi:10.1002/1873-3468.14716.
[42] Karpac J, Hull-Thompson J, Falleur M, Jasper H. JNK signaling in insulin-producing cells is required for adaptive responses to stress in Drosophila. Aging Cell 2009;8:288–95. doi:10.1111/j.1474-9726.2009.00476.x.
[43] Sethi JK, Hotamisligil GS. Metabolic messengers: tumour necrosis factor. Nat Metab 2021;3:1302–12. doi:10.1038/s42255-021-00470-z.
[44] Wessels I, Haase H, Engelhardt G, Rink L, Uciechowski P. Zinc deficiency induces production of the proinflammatory cytokines IL-1$\beta$ and TNF$\alpha$ in promyeloid cells via epigenetic and redox-dependent mechanisms. J Nutr Biochem 2013;24:289–97. doi:10.1016/j.jnutbio.2012.06.007.
[45] Rajan A, Perrimon N. Drosophila cytokine unpaired 2 regulates physiological homeostasis by remotely controlling insulin secretion 2012;2:123–37. https://doi.org/10.1016/j.cell.2012.08.019.
[46] Panayidou S, Apidianakis Y. Regenerative inflammation: lessons from drosophila intestinal epithelium in health and disease. Pathogens 2013;2:209–31. doi:10.3390/pathogens2020209.
[47] Yang S, Li X, Xiu M, Dai Y, Wan S, Shi Y, et al. Flos puerariae ameliorates the intestinal inflammation of Drosophila via modulating the Nrf2/Keap1, JAK-STAT and Wnt signaling. Front Pharmacol 2022;13:893758.
[48] Morimoto J, Simpson SJ, Ponton F. Direct and trans-generational effects of male and female gut microbiota in Drosophila melanogaster. Biol Lett 2017;13:20160966. doi:10.1098/rsbl.2016.0966.