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Sodium benzoate may reduce appetite in *Drosophila melanogaster* through serotonin upregulation

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Abstract: Sodium benzoate is a common artificial preservative in processed food, yet little is known about its long-term health effects. Since sodium benzoate could upregulate serotonin, we hypothesized that it may induce satiety and activate processes underlying caloric restriction that can lead to lifespan extension. In this study, the effects of sodium benzoate on tryptophan metabolism and its potential association with lifespan and stress tolerance in *Drosophila melanogaster* were investigated. We administered varying doses of sodium benzoate to male and female flies, monitoring their daily food consumption, serotonin levels, kynurenine/tryptophan (kyn/trp) ratio, Sirt1 levels, and survival under normal conditions. Additionally, separate groups of flies were exposed to stressors such as heat, ultraviolet A (UVA) radiation, and energy deprivation to assess the compound's effects on lifespan under diverse stress conditions. Our results demonstrated that fruit flies fed sodium benzoate exhibited reduced food consumption, decreased kyn/trp ratio, and increased serotonin. The expression of Sirt1, an indicator of the effect of caloric restriction, increased. Their lifespan was prolonged under normal and energy-deprived conditions but was unaffected under heat and UVA stress. Overall, our findings are consistent with our hypothesis that the upregulation of Sirt1 through sodium benzoate supplementation is associated with increased serotonin levels, which may explain delayed senescence and resilience under energy-deprived conditions.

Keywords: sodium benzoate, longevity, caloric restriction, serotonin, Sirt1, energy deprivation

INTRODUCTION

A study in France examining over 126,000 food products found that 54% contained at least one additive, highlighting the prevalence of additives like sodium benzoate in our diets [1]. While preservatives enhance product longevity and quality, emerging evidence suggests sodium benzoate might also have physiological effects linked to lifespan and metabolic regulation. Earlier studies have hinted that sodium benzoate may extend lifespan by reducing tumor prevalence in tumor-prone mice [2] and modulating appetite in various animals [3]. This appetite reduction is notable, as it parallels effects observed with caloric restriction.

One potential pathway through which sodium benzoate may affect lifespan and appetite is modulating tryptophan metabolism. In human studies, sodium benzoate has been shown to influence serotonin turnover, a neurotransmitter that helps regulate satiety and mood [4]. Sodium benzoate-treated cells exhibit a lower kynurenine-tryptophan (kyn/trp) ratio, enhancing serotonin production due to increased tryptophan availability [5]. This shift could link sodium benzoate to the mechanisms underlying caloric restriction, as serotonin reduces appetite and may activate pathways associated with energy balance and stress resistance. The kynurenine-tryptophan pathway, largely responsible for producing nicotinamide adenine dinucleotide (NAD^+), a vital molecule for cellular energy, is tightly regulated in organisms ranging from yeast to humans [6]. Lowering kynurenine levels or enhancing tryptophan availability, as sodium benzoate appears to do, may reduce oxidative stress and inflammation while promoting longevity [7].

Despite the intriguing potential, research on sodium benzoate's long-term effects on lifespan and metabolic pathways remains limited, particularly on tryptophan metabolism and caloric restriction. We previously hypothesized that sodium benzoate may upregulate serotonin production and activation of the NAD-dependent deacetylase sirtuin-1 (Sirt1), which promotes longevity and stress tolerance [8, 9]. In that study, we proposed a mechanistic link between sodium benzoate-induced serotonin upregulation and the response to caloric restriction, enhancing longevity and cognitive function.

Building on this premise, the current manuscript explores these effects using *D. melanogaster* as a model organism selected due to its well-established use in genetic, neurological, and behavioral research [9] along with its genetic and physiological parallels to humans. Its short lifespan allows for the rapid assessment of generational effects [9], and its conserved aging and kynurenine-tryptophan pathways make it an ideal system for examining sodium benzoate's multifaceted roles in caloric restriction and longevity [8]. We investigated sodium benzoate's impact on food consumption, appetite regulation, lifespan, tryptophan metabolites, Sirt1 expression, and resilience to stressors such as heat, UVA exposure, and energy deprivation. Our findings demonstrate that sodium benzoate reduced food intake, elevated serotonin levels, and increased Sirt1 expression, culminating in extended lifespan under normal and energy-deprived conditions. Interestingly, while sodium benzoate did not enhance resilience to heat or UVA-induced stress, its ability to mimic caloric restriction underscores its potential as a dietary intervention for aging and metabolic health.

MATERIALS AND METHODS

Ethics statement

This study does not involve experiments on vertebrates or human subjects.

Maintenance and husbandry of *Drosophila*

We cultivated wild-type Oregon-R *D. melanogaster* (Oregon stock, Bloomington Indiana University, USA) in a vial containing Formula 4-24® Instant *Drosophila* Medium (Carolina Biological Supply, NC, USA) at 25°C. To prepare the growth medium, a level measuring cup of Formula 4-24® and water were added to the *Drosophila* vial. We then waited for the medium to solidify. Finally, we filled the vial with 6 grains of yeast. To maintain the wild-type *D. melanogaster*, we moved the flies to fresh culture vials every 10 to 14 days to prevent crossbreeding of F1 offspring. The flies were age-synchronized by allowing ten male and female flies to breed per vial for 24 h. The flies were removed from the vial and eggs developed to eclosion. The flies were acclimatized for 5 days before treatment.

Storage and preparation of chemicals

Sodium benzoate (Kemrad Incorporated, Quezon City, Philippines) was stored at room temperature. In preparing the sodium benzoate, the compound was dissolved in water following the final volume of its desired concentration. The prepared solution was stored in the fridge at a temperature of 4°C before usage.

Sublethal assay

Thirty male and thirty female flies were collected 5 days post-eclosion. The flies were anesthetized with CO_2 and sorted by sex. Thirty flies from each sex were distributed into separate vials containing varying concentrations (0-20 mg/L) of sodium benzoate mixed in their food. The setups were maintained at 25°C, with live flies counted daily for 5 days and transferred to newly prepared culture tubes daily. The sublethal concentration was exhibited at least 90% survival after 5 days [10]. The mid and low concentrations were 2- and 4-fold lower, respectively, than the sublethal concentration.

Capillary feeder (CAFE) assay

In the capillary feeder assay, 30 male and 30 female flies were transferred into empty vials. The treatments were administered by mixing the different concentrations (0-2.5 mg/L) of sodium benzoate with 0.1 M sucrose in the capillary tubes [11]. The amount of food consumed by the group of flies was averaged daily to compute the food consumption rate. The consumption rate was computed every day for 8 days. This assay was conducted in 3 trials using a different set of flies.

Lifespan assay

Newly-eclosed male and female flies were collected on day 1 and allowed to acclimatize in standard culture vials at 25°C for 5 days. Flies were then treated with 2.5 mg/L, 1.25 mg/L, and 0.625 mg/L concentrations of sodium benzoate daily by adding these solutions to their food replacing distilled water (negative control). Each treatment consisted of 30 male and 30 female flies in separate vials. The number of live flies was recorded daily until all flies died. Live flies were transferred to newly prepared culture tubes daily, and this assay was repeated 3 times using a different set of flies.

Preparation of fly homogenates

We collected 90 male and 90 female flies 5 days after eclosion and supplemented their diet with varying concentrations (0, 0.625, 1.25, 2.5 mg/L) of sodium benzoate for 5 more days. These flies were homogenized using a Protein Extraction Kit (ABCAM, United Kingdom) in a Potter-Elvejhem glass homogenizer. The collected homogenates were centrifuged at 15,000×g for 10 min to collect the supernatants used for the subsequent assays.

Measurement of tryptophan, kynurenine, serotonin, and Sirt1

ELISA was used to quantify serotonin, tryptophan, kynurenine from the fly homogenates stored at -80°C, following the manufacturer's protocol (ImmuSmol, Inc., France); from the same pool of fly homogenates, we measured the level of Sirt1 following the ELISA kit protocol (Raybio, China). All reactions were measured at 450 nm. The standard curve of serotonin, tryptophan, kynurenine, and Sirt1 was analyzed by the 4PL regression model using a semi-log scale for plotting, using GainData® (Arigobiolaboratories, Taiwan). This assay was conducted in triplicate.

Heat stress assay

In this assay, we replicated the lifespan assay protocol with one modification: we exposed the flies to a daily 60-min incubation at 37°C [12]. The survival rate was measured daily until all flies had died. This assay was repeated 3 times using a different set of flies.

UVA stress assay

The same protocols as the lifespan assay were used, but the flies were also exposed to UVA light at 400 nm for 60 min daily [13]. The survival rate of the flies was measured daily until all flies had died. This assay was repeated 3 times using a different set of flies.

Energy deprivation assay

In this assay, we utilized two cohorts of flies. Adhering to the established protocols of the lifespan assay, the initial group of male and female flies received food supplemented with sodium benzoate for 3 days, after which they underwent a period of food and sodium benzoate deprivation, being supplied with distilled water instead [14]. The survival rates of these flies were recorded at 6-h intervals. The 2nd cohort was also provided with food containing sodium benzoate for 3 days, however, during the energy deprivation phase, sodium benzoate was incorporated into their water source. The survival rates of these flies were monitored at 6-h intervals. This assay was conducted in 3 trials using a different set of flies.

Statistical analysis

The data for mean lifespan, average food consumption, amount of serotonin, tryptophan, kynurenine, and Sirt1 were calculated as the average of 3 trials and presented as the mean±SD. Jamovi ver. 2.6.13 (Sydney, Australia) was used for statistical analysis, with significance set at α=0.05. For parametric data, the analysis of variance (ANOVA) was used, and for non-parametric data, the Kruskal-Wallis test was used. Post-hoc analysis using Tukey's test for parametric data and Dunn's test for non-parametric data. The lifespan assay was analyzed using the Kaplan-Meier test to determine if the mean lifespan is significant, followed by the Bonferroni test for post-hoc analysis.

RESULTS

Sublethal assay

The sublethal concentration of sodium benzoate for male flies was established at 2.5 mg/L, with a 100% survival rate observed after 5 days of exposure, comparable

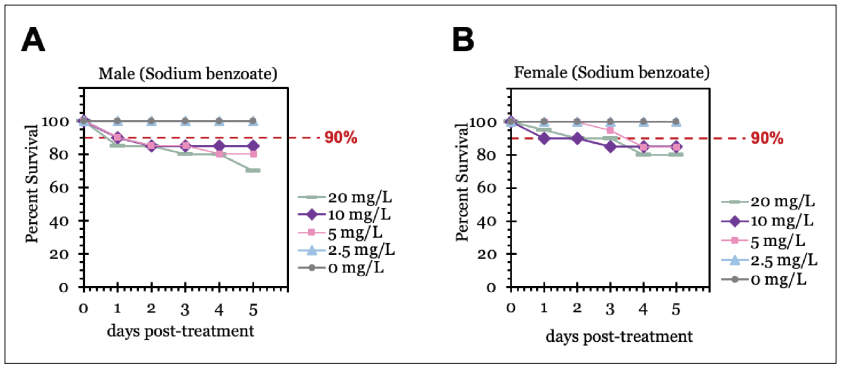


Fig. 1. Effect of sodium benzoate sublethal concentration in *D. melanogaster*. Thirty (**A**) male and 30 (**B**) female flies were given varying concentrations of sodium benzoate (0, 2.5, 5, 10, 20 mg/L) in their diet for 5 days. The survival rate was recorded daily. The sublethal concentration was determined as the highest amount of sodium benzoate with at least 90% survival after 5 days of treatment.

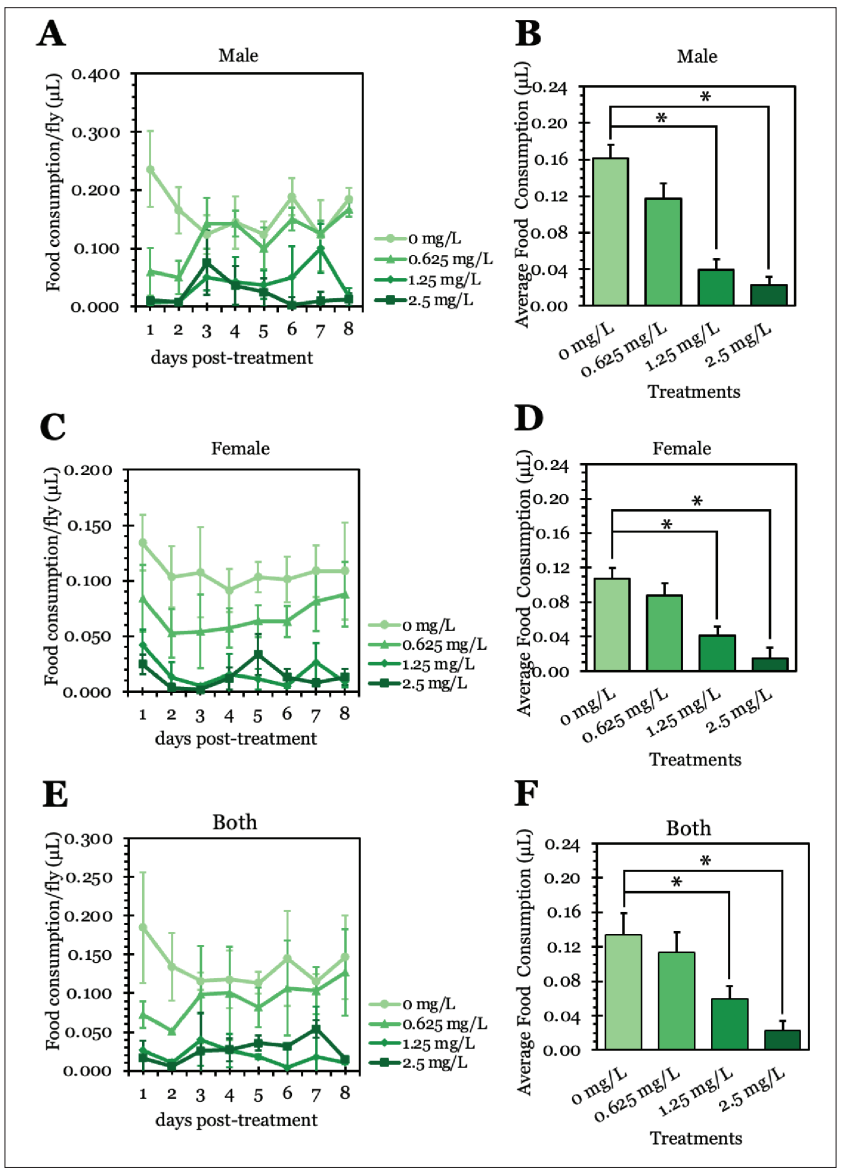


Fig. 2. Influence of varying concentrations of sodium benzoate on the food consumption patterns of *D. melanogaster*. A cohort comprising 30 male and 30 female *D. melanogaster* was provided *ad libitum* access to different concentrations of sodium benzoate (0, 0.625, 1.25, 2.5 mg/L) and subsequently evaluated for their daily food intake. The (**A**) daily food consumption, (**B**) average food consumption of male flies, (**C**) daily food consumption, and (**D**) average food consumption of female flies, were recorded. The results for (**E**) daily food consumption and (**F**) average food consumption of male and female flies was combined. This experiment was replicated across three independent trials. The asterisk (*) denotes instances of statistical significance, defined as P<0.05.

to the untreated control group, as shown in Fig. 1A. In contrast, higher concentrations of sodium benzoate (5, 10, and 20 mg/L) resulted in survival rates lower than 90%, indicating potential chronic toxicity at these levels. For female flies the sublethal concentration was also determined to be 2.5 mg/L, as shown in Fig. 1B. Exposure to higher concentrations led to survival rates falling below 90% after 5 days, suggesting a similar threshold for chronic toxicity as observed in male flies.

Effects of sodium benzoate on food consumption

Introducing sodium benzoate into the dietary regimen of *D. melanogaster* revealed a reduction in the food consumption of the flies (Fig. 2). Figs. 2A, C, and E illustrate the daily food consumption trends of male, female, and combined sex *D. melanogaster*, respectively. Male flies exposed to 1.25 and 2.5 mg/L of sodium benzoate exhibited a marked decrease ranging from 75.7% to 85.98% (P=0.0007 and P<0.0001, respectively) than in matching untreated counterparts (Fig. 2B). Likewise, female flies treated with 1.25 and 2.5 mg/L of sodium benzoate demonstrated a substantial decline in food consumption by 75.9% to 87.1% (P=0.0003, P<0.0001; Fig. 2D). When combined, the feeding activity of the fruit flies exposed to 1.25 and 2.5 mg/L of sodium benzoate

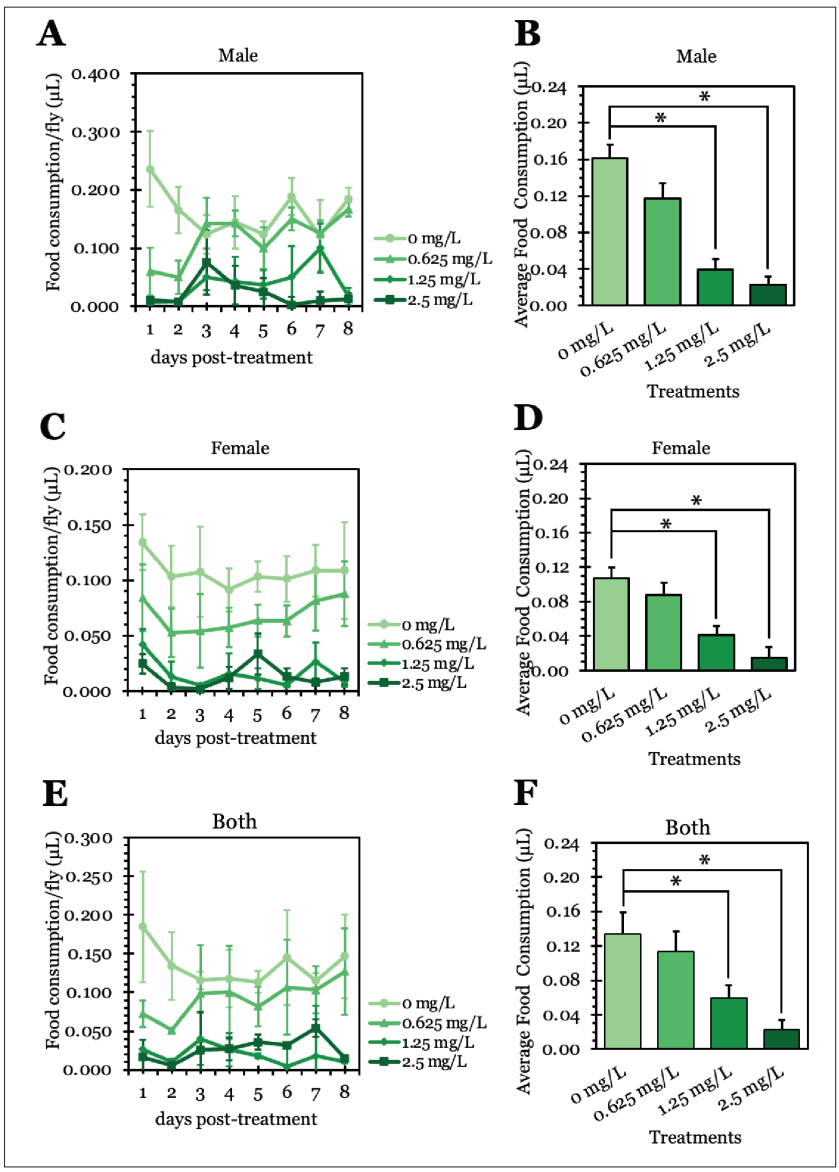


Fig. 3. Impact of various sodium benzoate concentrations on the lifespan of *D. melanogaster* under baseline conditions. A cohort of 30 male and 30 female *D. melanogaster* were provided with differing concentrations of sodium benzoate (0, 0.625, 1.25, 2.5 mg/L) *ad libitum* and assessed for lifespan. The (**A**) daily survival rates and (**B**) mean lifespan of male flies; (**C**) daily survival rates and (**D**) mean lifespan of female flies and combined (**E**) daily survival rates and (**F**) the mean lifespan of male and female flies. This experimental protocol was executed across 3 distinct trials. The asterisk (*) signifies statistical significance at P<0.05.

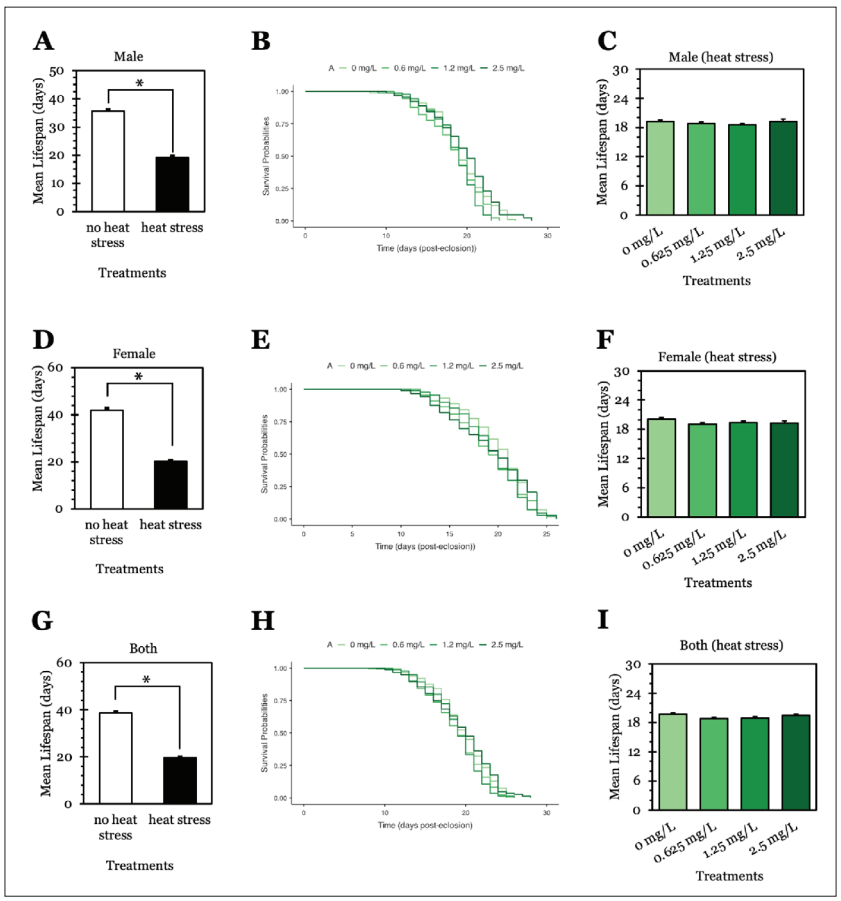
showed a 64.9-84.5% reduction (P<0.0001; Fig. 2F).

Effects of sodium benzoate on lifespan under normal conditions

The introduction of sodium benzoate into the dietary regimen of *D. melanogaster* yielded a notable extension in lifespan (Fig. 3). The survival trends among male, female, and combined sex *D. melanogaster* are shown in Figures 3A, C, and E, respectively. As shown in Fig. 3B, male *D. melanogaster* subjected to concentrations of 2.5 mg/L of sodium benzoate exhibited a significant lifespan extension of 5.87% (P=0.0037), compared to untreated counterparts. Female *D. melanogaster* administered 2.5 mg/L of sodium benzoate demonstrated a significant prolongation in lifespan by 7.26% (P=0.0054; Fig. 3D). When combined, 2.5 mg/L prolongs the lifespan of fruit flies by 6.68% (P=0.0001; Fig. 3F).

Effects of sodium benzoate on the levels of tryptophan metabolites and Sirt1

To explore whether sodium benzoate activates caloric restriction processes through tryptophan metabolism in *D. melanogaster*, we examined its impact on tryptophan metabolite and Sirt1 levels. Male flies treated with 1.25 and 2.5 mg/L of sodium benzoate exhibited significant 8.3- to 16-fold (P=0.0102; P=0.0047) reductions in the kynurenine/tryptophan (kyn/trp) ratio (Fig. 4A). These concentrations corresponded to substantial 3.6- and 6.4-fold (P=0.0375 and P=0.0047, respectively) increases in serotonin concentration compared to the untreated group (Fig. 4B). Sirt1 levels mirrored this trend, with male flies



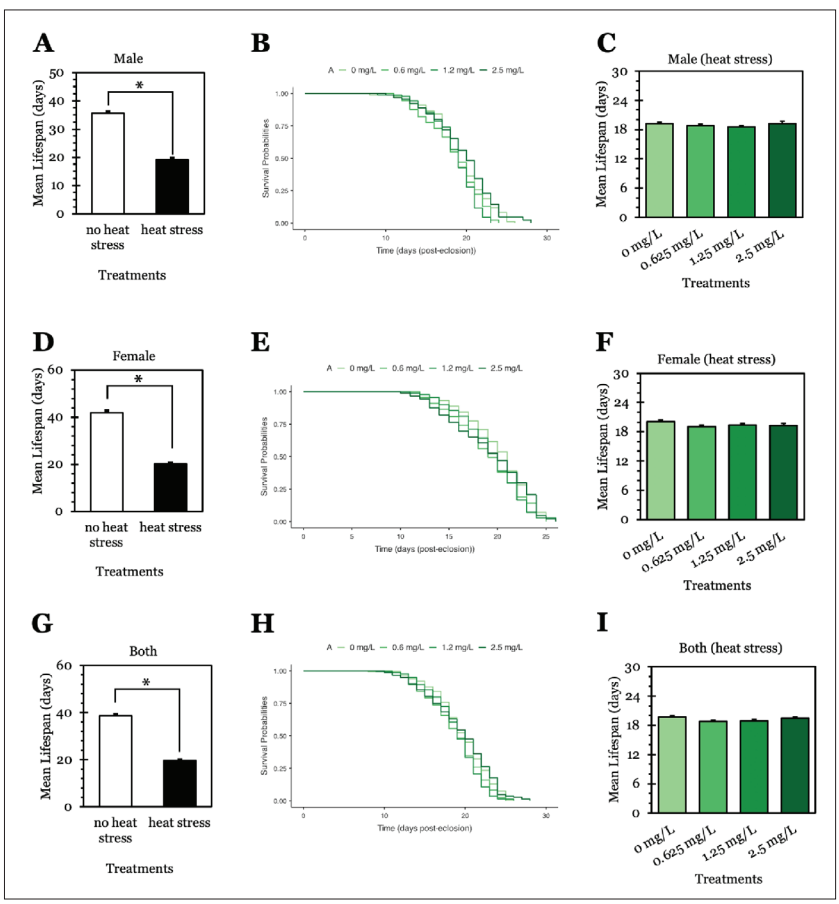


Fig. 4. The influence of sodium benzoate on tryptophan metabolites and Sirt1 levels in *D. melanogaster*. A cohort comprising 30 male and 30 female *D. melanogaster* was provided *ad libitum* access to different concentrations of sodium benzoate (0, 0.625, 1.25, 2.5 mg/L) evaluated for their effect on the (**A**) kynurenine/tryptophan (kyn/trp) ratio, (**B**) serotonin (5-HT) concentration, and (**C**) Sirt1 level in male flies; the (**D**) kynurenine/tryptophan ratio, (**E**) serotonin (5-HT) level, and (**F**) Sirt1 level in female flies. The results of the (**G**) kynurenine/tryptophan ratio, (**H**) serotonin (5-HT) concentration, and (**I**) the Sirt1 level for male and female flies. This experiment was replicated across three independent trials. The asterisk (*) denotes instances of statistical significance, defined as $P<0.05$.

treated with 1.25 and 2.5 mg/L of sodium benzoate showing significant 3.4- and 3.8-fold increases, respectively ($P=0.0004$ and $P=0.0001$; (Fig. 4C).

In female flies administered with 0.625, 1.25, and 2.5 mg/L of sodium benzoate, there was a significant 12.5-14.2-fold reduction ($P=0.0145$, $P=0.0006$, and $P=0.0004$) in the kyn/trp ratio, respectively (Fig. 4D) These same concentrations resulted in 2.35-fold ($P=0.0475$), 5.5-fold ($P=0.0276$), and 7.4-fold ($P=0.0193$) increases in serotonin concentration compared to the untreated group, respectively, (Fig. 4E). This pattern was reflected in

Fig. 5. Influence of sodium benzoate on the lifespan of *D. melanogaster* under heat stress conditions. A group of 30 male and 30 female *D. melanogaster*, administered with varying concentrations of sodium benzoate (0, 0.625, 1.25, 2.5 mg/L) *ad libitum*, underwent daily exposure to 37°C for 60 min. Male flies' (**A**) Mean lifespan after exposure to heat stress vs. unexposed flies, (**B**) daily survival rates and (**C**) mean lifespan when fed with sodium benzoate; female flies' (**D**) Mean lifespan when exposed to heat stress vs. unexposed flies; (**E**) daily survival rates and (**F**) mean lifespan when fed with sodium benzoate following each heat stress episode. Combined male and female flies (**G**) mean lifespan after exposure to heat stress vs. unexposed flies, (**H**) daily survival rates and (**I**) mean lifespan when fed sodium benzoate. This experimental design was replicated across three independent trials. The asterisk (*) denotes statistical significance at $P<0.05$.

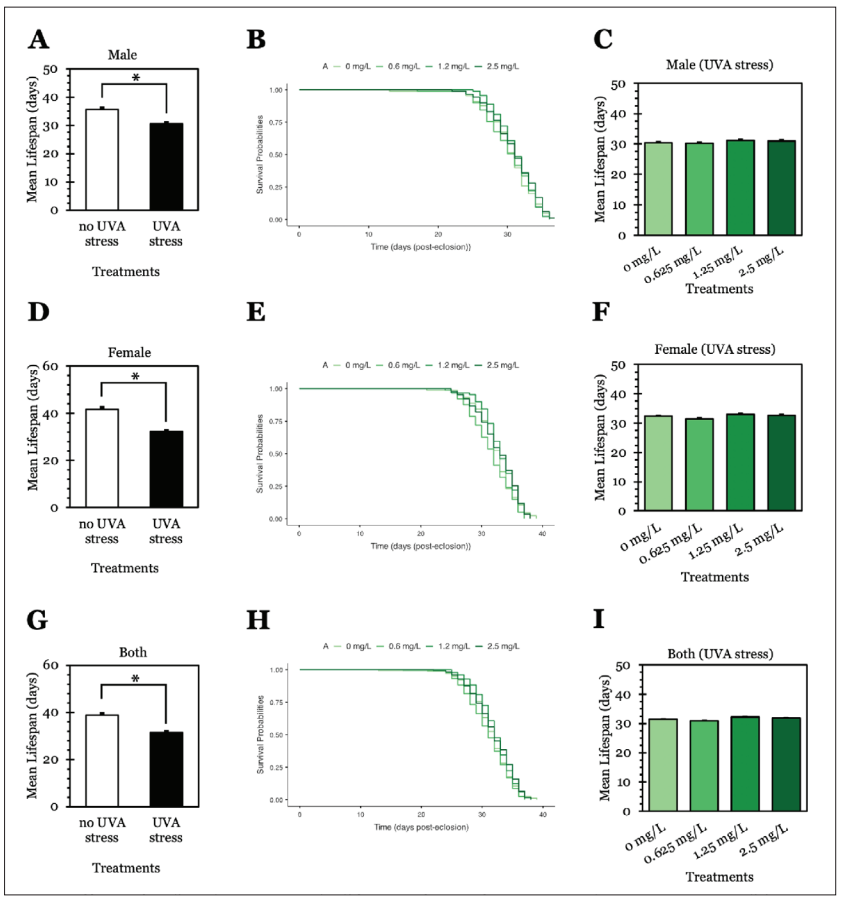


Fig. 6. Effects of sodium benzoate on the lifespan of *D. melanogaster* under UVA stress conditions. A group of 30 male and 30 female *D. melanogaster* was administered with varying concentrations of sodium benzoate (0, 0.625, 1.25, 2.5 mg/L) *ad libitum*, and subjected to daily UVA light (400 nm) exposure for 60 min. Male flies' (**A**) Mean lifespan of exposed to heat stress vs. unexposed flies, (**B**) daily survival rates, and (**C**) mean lifespan when fed sodium benzoate; female flies (**D**) Mean lifespan after exposure to heat stress vs. unexposed flies, (**E**) daily survival rates and (**F**) mean lifespan when fed sodium benzoate following each heat stress episode. Male and female flies (**G**) Mean lifespan after exposure to heat stress vs. unexposed flies, (**H**) daily survival rates, and (**I**) mean lifespan when fed sodium benzoate. This experimental design was replicated across three independent trials. The asterisk (*) denotes statistical significance at P<0.05.

Sirt1 levels, with female flies administered with 0.625, 1.25, and 2.5 mg/L of sodium benzoate displaying significant 3.4-fold (P=0.0086), 5.0-fold (P=0.0034), and 5.2-fold (P=0.0024) increases, respectively (Fig. 4F).

When we combined male and female flies, the kyn/trp ratio, serotonin, and Sirt1 mirrored the results observed in the male population (Fig. 4G, H, and I). The kyn/trp ratio was reduced 12.1 and 14.5-fold (P=0.0001 and P=0.0001, respectively) in flies fed with 1.25, and 2.5 mg/L sodium benzoate (Fig. 4G). This reduction was accompanied by 4.5- and 6.9-fold (P=0.0093 and P=0.0018, respectively) increases in serotonin levels, and 4.2- and 4.9-fold (P=0.0033 and P=0.0016, respectively) increases in Sirt1 level (Fig. 4H and I).

Effects of sodium benzoate on lifespan under heat stress

A separate cohort of male and female *D. melanogaster* was subjected to a regimen of heat stress. Analogous to the preceding assay, we investigated the impact of varying concentrations of sodium benzoate on their post-exposure lifespan, after daily exposure to 37°C for 60 min (Fig. 5). Notably, heat stress induced a significant reduction in the lifespan of male, female, and combined flies by 46.02% (P=0.0001), 51.95% (P=0.0001), and 49.26% (P=0.0001), respectively (Fig. 5A, D, and G). Intriguingly, the daily survival rates of male and female flies receiving sodium benzoate were comparable with those of the untreated group (Fig. 5B, C, E, and F). The mean lifespan of combined male and female flies administered sodium benzoate was unaffected (5H and I, respectively).

Effects of sodium benzoate on lifespan under UVA stress

Using a different set of male and female *D. melanogaster* subjected to varying concentrations of sodium benzoate, we exposed them to daily UVA stress sessions lasting 60 min each (Fig. 6). UVA stress shortened the lifespan of male and female flies by 14.57% (P=0.0001) and 22.59% (P=0.0001), respectively (Fig. 6A and D). The daily survival rates and mean lifespan of male flies administered sodium benzoate mirrored those of the untreated group (Fig. 6B and C). The daily survival rate and mean lifespan of female flies receiving sodium benzoate were unaltered (Fig. 6E and F). In the combined male and

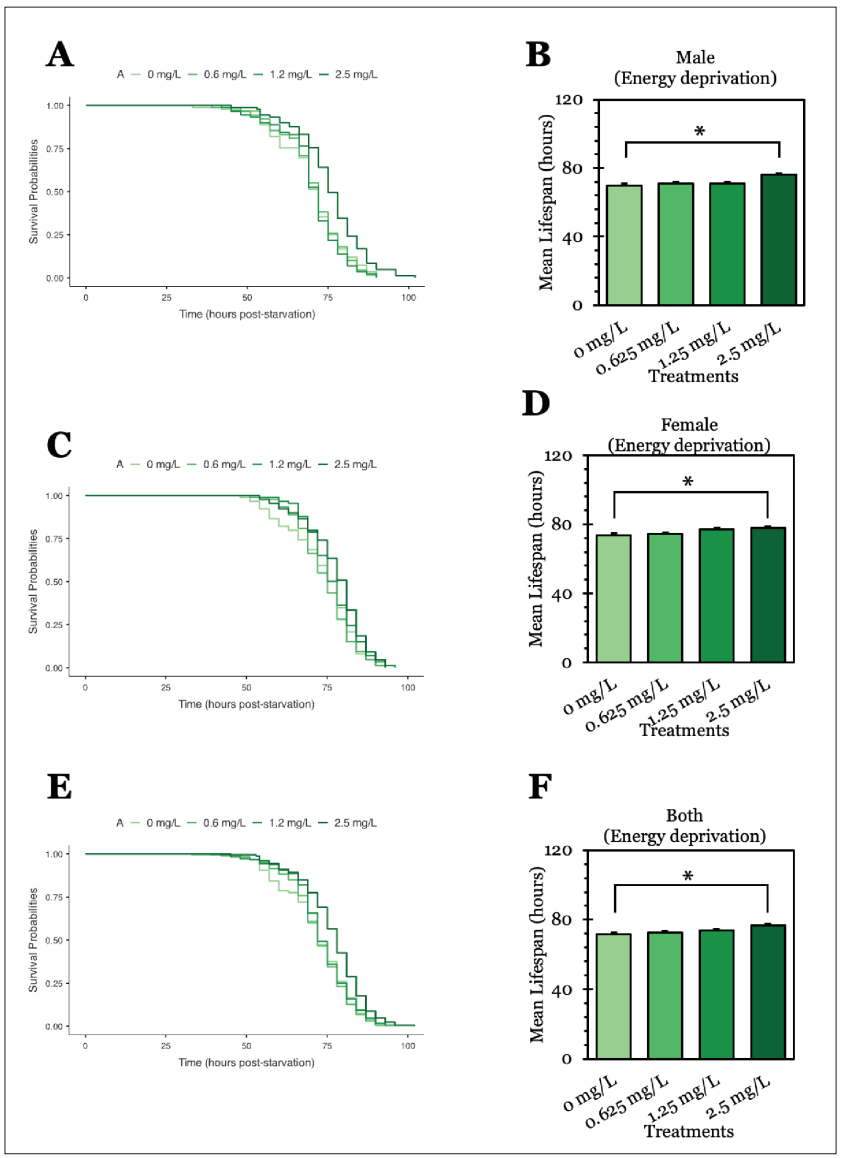


Fig. 7. Effects of acute sodium benzoate on the lifespan of *D. melanogaster* under energy deprivation. Two cohorts, each comprising 30 male and 30 female *D. melanogaster*, were exposed to varying concentrations of sodium benzoate (0, 0.625, 1.25, 2.5 mg/L) for 3 days or *ad libitum* intake. Following exposure, they were subjected to energy deprivation by withholding their caloric source. The (**A**) daily survival rates, (**B**) mean lifespan of male flies, (**C**) daily survival rates, and (**D**) mean lifespan of female flies treated exclusively with sodium benzoate for 3 days. The (**E**) Daily survival rates and (**F**) mean lifespan of male and female flies. This experimental design was replicated across 3 independent trials. The asterisk (*) denotes statistical significance at $P<0.05$.

female population, UVA stress reduced their lifespan by 18.96% (P=0.0001; Fig. 6G). Sodium benzoate did not exhibit any effect on the lifespan of the combined male and female fly population (Fig. 6H and I).

Effects of sodium benzoate on lifespan under energy deprivation

In the energy deprivation assay, two distinct cohorts of male and female *D. melanogaster* were used. The initial group received sodium benzoate as part of their diet for 3 days, followed by a switch to water exclusively during the energy deprivation phase. Male flies administered 2.5 mg/L of sodium benzoate for 3 days displayed a remarkable extension in lifespan by 8.68% (P=0.0420) following energy deprivation (Fig. 7A and B). Female flies given 2.5 mg/L for 3 days and subsequently subjected to energy deprivation exhibited lifespan extensions by 5.61% (P=0.0317; Fig. 7C and D). When the male and female fly populations were combined, their average lifespan was 7.1% (P=0.0001) longer in flies treated with 2.5 mg/L sodium benzoate than in untreated flies (Fig. 7E and F).

In the second cohort, male and female flies were likewise administered sodium benzoate in their diet for 3 days, and sodium benzoate was continued to be mixed in their water source even during the energy deprivation period. The daily survival rates and mean lifespan of male, female, and combined flies in this cohort are shown in Fig. 8A-F. Intriguingly, in contrast to the first batch, flies provided sodium benzoate did not have an altered lifespan.

DISCUSSION

The investigation into the effects of sodium benzoate on lifespan under various conditions, as documented in this study, presents an avenue for exploring its potential use as a longevity-promoting compound. The

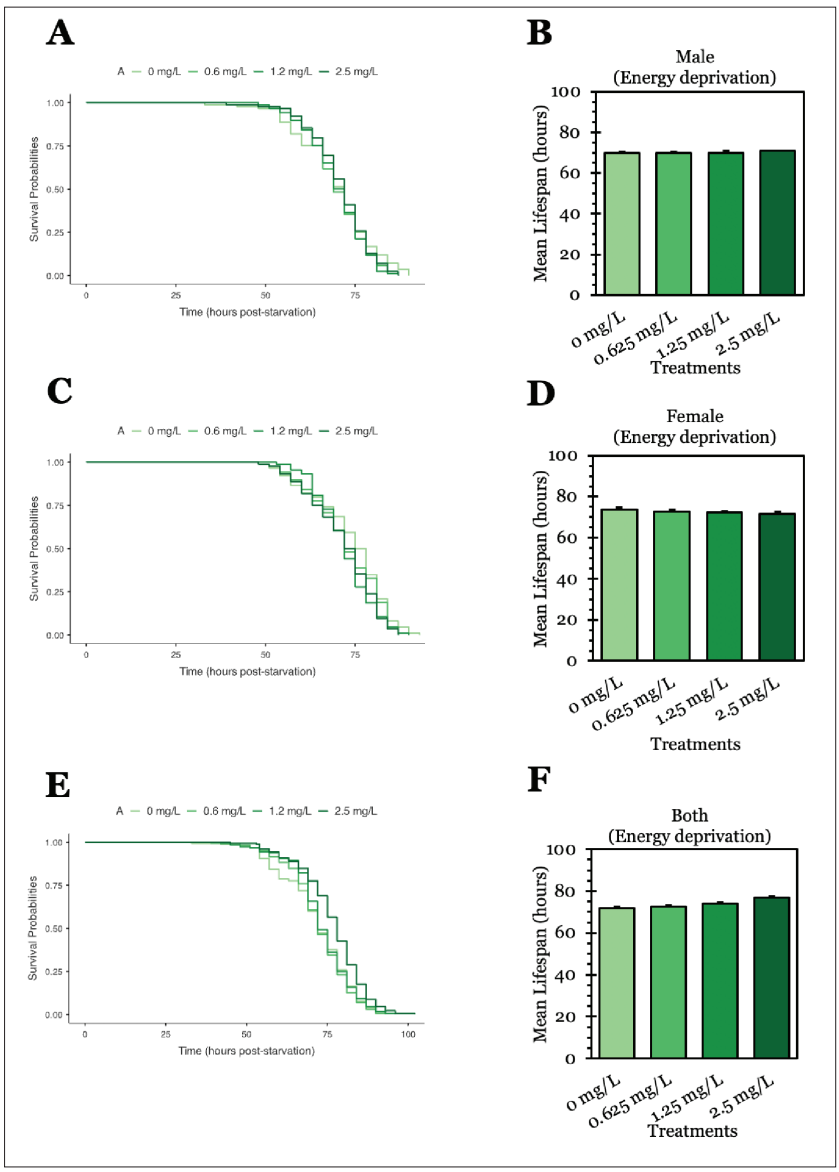


Fig. 8. Effects of chronic sodium benzoate on the lifespan of *D. melanogaster* under energy deprivation. Two groups, each comprising 30 male and 30 female *D. melanogaster*, were exposed to varying concentrations of sodium benzoate (0, 0.625, 1.25, 2.5 mg/L) for 3 days or *ad libitum* intake. Following exposure, flies were subjected to energy deprivation by withholding their caloric source but were provided with continuous intake of sodium benzoate. The (**A**) Daily survival rates and (**B**) mean lifespan of male flies, (**C**) daily survival rates, and (**D**) mean lifespan of female flies treated exclusively with sodium benzoate for 3 days. The (**E**) Daily survival rates and (**F**) mean lifespan of male and female flies. This experimental design was replicated across 3 independent trials. The asterisk (*) denotes statistical significance at $P<0.05$.

significant decrease in food consumption observed in *D. melanogaster* following supplementation with sodium benzoate underscores its potential impact on feeding behavior and metabolism. Evidence from studies on humans, rats, and cows has consistently shown that sodium benzoate reduces food intake [2-4,15]. This documented decline in food intake has prompted an investigation into the underlying mechanisms driving this behavior. Sodium benzoate may directly influence taste perception or alter neuroendocrine signaling pathways involved in appetite regulation. Humans find it challenging to distinguish the taste between plain water and water containing sodium benzoate, suggesting that most individuals could detect it at concentrations of around 0.04% (4 mg/L), whereas lower concentrations likely resulted in an aftertaste or flavor contrast effects [16]. Although no studies have specifically focused on *Drosophila*, it is conceivable that at the maximum concentration (2.5 mg/L) used in this study, the flies may have detected it, but whether they were repelled by it remains inconclusive. Regarding the effect of sodium benzoate on neuroendocrine signaling, its mechanism remains elusive. Studies have reported that sodium benzoate does not affect leptin release in murine adipocytes *in vitro* [17] and insulin levels in humans [19]. Studies on other appetite-regulating hormones, such as ghrelin and peptide YY (pYY), have not yet been conducted. However, one paper suggests sodium benzoate may reduce appetite due to increased serotonin levels [8]. The upregulation of serotonin may trigger processes of caloric restriction which in turn promote longevity [8].

The lifespan extension observed in *D. melanogaster* supplemented with 2.5 mg/L sodium benzoate does not align with what has been reported recently. To ensure our findings could be compared with existing research, we standardized all measurements to a percentage scale representing

the usage of sodium benzoate. Previous studies on *D. melanogaster* utilized concentrations ranging from 0.0005-0.5% of sodium benzoate, which resulted in fly lifespan reduction ranging from 8% to 30% [19-21]. Interestingly, there is a variation in the reported lifespan reductions across these studies. For instance, while a concentration of 0.2% sodium benzoate led to a lifespan reduction of 6-8%, a concentration of 0.002% resulted in a 30% reduction [19-20].

Our investigation yielded intriguing results. At the highest concentration tested, 0.00025% (equivalent to 2.5 mg/L), we observed about a 6.68% lifespan extension. Furthermore, a concentration of 0.0000625% of sodium benzoate did not alter the lifespan of *D. melanogaster*, suggesting that the differences, when compared to previous research, might be due to several factors, including the dosage used, the experimental conditions, and the genetic makeup of the flies. However, the most probable reason is that the impact of a substance like sodium benzoate on lifespan might vary depending on its concentration. Our study employed a lower concentration (0.00025%) than many previous studies, potentially explaining the disparities in outcomes.

This observed lifespan extension prompts an investigation into its underlying mechanisms. Notably, sodium benzoate is recognized for its antioxidant and antimicrobial properties, which can modulate gut microbiota by altering pH levels [20]. However, contrary to expectations, previous research has reported that changes in gut microbiota induced by sodium benzoate did not extend the lifespan of flies [20]. Alternatively, we explored a different hypothesis inspired by recent literature suggesting that sodium benzoate may induce the processes of caloric restriction by upregulating serotonin [8]. This activation of caloric restriction pathways, in turn, may lead to increased levels of Sirt1, potentially explaining the observed lifespan extension [8].

Our investigation into the potential role of sodium benzoate in activating caloric restriction pathways in *D. melanogaster* revealed intriguing findings regarding its impact on tryptophan metabolites and Sirt1 levels. The significant reduction in the kynurenine/tryptophan (kyn/trp) ratio observed in both male and female flies treated with sodium benzoate suggests a potential upregulation of serotonin, as lower kyn/trp ratios are indicative of reduced tryptophan catabolism to kynurenine. This kyn/trp ratio reduction was accompanied by an increase in serotonin concentration observed in both male and female flies treated with sodium benzoate supports that tryptophan was converted more to serotonin. Serotonin has been implicated in feeding behavior regulation, particularly in inducing satiety [8]. Moreover, the upregulation of Sirt1, an indicator of caloric restriction, suggests that serotonin may contribute to the increase in Sirt1, a key mediator of the metabolic effects of caloric restriction known to be upregulated under conditions of reduced nutrient availability [22].

Given the upregulation of Sirt1 in flies supplemented with sodium benzoate under normal conditions, we investigated whether this elevation could protect them under stress. Examining the impact of sodium benzoate under heat and UVA stress conditions provided intriguing insights. Unlike untreated flies, which experienced a significant decrease in lifespan under these stressors, those supplemented with sodium benzoate did not mitigate detrimental effects on lifespan. This suggests that the mechanisms responsible for sodium benzoate's lifespan-extending effects may not intersect with those underlying heat and UVA-induced damages. Despite sodium benzoate's inherent antioxidant properties, these findings highlight its limitations in rescuing *D. melanogaster* from thermal and UVA stress.

Since we did not measure the levels of tryptophan metabolites and Sirt1 in the presence of stress, we hypothesize that tryptophan catabolism may shift towards the kynurenine pathway under stress, despite sodium benzoate treatment. This, in turn, could downregulate the conversion of tryptophan to serotonin, thereby reducing its contribution to Sirt1 expression. Studies have shown that during stress conditions, kynurenine is upregulated which supports this hypothesis [23].

Short-term supplementation of sodium benzoate resulted in a longer lifespan under energy-deprived conditions compared to long-term supplementation. This effect may be related to the activation of Sirt1, as short-term exposure to sodium benzoate may induce transient activation of Sirt1, promoting adaptive metabolic responses to energy deprivation. However, long-term supplementation may lead to adaptation or

desensitization of Sirt1-related pathways, diminishing its protective effects under energy-deprived conditions. Sirt1 and serotonin mediate lifespan extension under energy deprivation [24-25]. Serotonin, known for its role in regulating feeding behavior and energy homeostasis, may contribute to the metabolic adaptation induced by sodium benzoate, as Sirt1, a key regulator of cellular metabolism and stress response, may promote protective mechanisms against energy deprivation-induced damage [24-27].

CONCLUSIONS

The study on dietary supplementation of sodium benzoate in *Drosophila melanogaster* provides valuable insights into its effects on caloric restriction and longevity. Sodium benzoate administration led to a reduction in food intake and an extension of lifespan, accompanied by elevated serotonin levels, which may account for appetite suppression, and an increase in Sirt1, a marker for caloric restriction activation. Despite these metabolic benefits, sodium benzoate did not enhance the resilience to heat or UVA stress. However, under conditions of acute energy deprivation, it significantly prolonged lifespan, while chronic supplementation under the same conditions showed no effect, suggesting that the benefits of sodium benzoate may be more pronounced with short-term usage. Sodium benzoate reduces food intake through serotonin upregulation. However, we acknowledge some limitations of the study, which did not establish a causal link between serotonin upregulation and increased Sirt1 levels. Further research targeting the serotonin pathway is needed to confirm this connection.

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Data availability: The dataset underlying the reported findings is available here: https://www.serbiosoc.org.rs/NewUploads/Uploads/Nas%20and%20Medina_Data%20Set.pdf

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