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Article

Sildenafil as a candidate geroprotective compound: Evidence from *Drosophila* lifespan extension

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SUMMARY

Sildenafil, a phosphodiesterase type five inhibitor originally developed to treat erectile dysfunction, has been reported to influence cellular metabolism, mitochondrial function, and vascular health, all of which are closely linked to aging. Based on these findings, we hypothesized that dietary sildenafil would exert anti-aging effects in the fruit fly, *Drosophila melanogaster*. To evaluate this, we examined its impact on lifespan, fecundity, body weight, and locomotor activity. Flies were fed food containing 20, 100, or 200 μM sildenafil. Low-dose treatment (20 μM) significantly extended lifespan in both sexes, while the intermediate dose (100 μM) increased lifespan only in males and the highest dose (200 μM) showed no effect. Sildenafil increased cumulative egg production over the seven-day experimental period in females. After three weeks, both male and female flies given sildenafil displayed increased body weight, suggesting that lifespan extension may be associated with altered energy balance rather than reduced metabolism. Males showed a temporary decrease in locomotor activity after one week, but there were no long-term differences in either sex. Overall, sildenafil produced dose-dependent and sex-specific effects on longevity and reproduction, supporting its potential as an anti-aging candidate.

INTRODUCTION

Aging is increasingly discussed as a potentially modifiable process that could be therapeutically targeted, although its classification as a disease remains debated. Current research therefore focuses on identifying interventions that modulate biological aging processes to extend healthspan and delay age-associated functional decline. Among these approaches, pharmacologic geroprotectors are agents that extend healthspan, the period of life spent in good health, and delay age-related decline (1). Metformin and rapamycin have been identified as potential geroprotectors in model organisms (2,3). These findings have accelerated interest in repurposing clinically approved drugs as candidate anti-aging interventions.

Drug repurposing involves identifying new therapeutic uses for existing drugs and offers strategic advantages by reducing time, costs, and risks associated with early-stage drug development due to well-characterized drug safety profiles. Sildenafil was originally developed as a therapeutic drug for erectile dysfunction and functions as a phosphodiesterase type five (PDE5) inhibitor (4). Sildenafil functions by increasing intracellular cyclic guanosine monophosphate (cGMP) concentrations, a key second messenger that mediates smooth muscle relaxation and vasodilation (5). Beyond its primary indication, sildenafil demonstrated therapeutic benefits in pulmonary hypertension and cardiovascular diseases (6). Recent studies further suggest that sildenafil exerts pleiotropic effects beyond vasodilation (4). Sildenafil activates AMP-activated protein kinase (AMPK), a key regulator of cellular energy balance, and suppresses the mechanistic target of rapamycin (mTOR), a central pathway involved in growth and aging (7). Modulation of these pathways may contribute to the maintenance of cellular homeostasis. Sildenafil also enhances mitochondrial function and antioxidant capacity while reducing oxidative stress and cellular injury (8). In addition, sildenafil may facilitate oxygen and nutrient delivery to tissues and mitigate stress-related neuronal damage by improving vascular tone (9). Together, these findings suggest that sildenafil may modulate healthy aging and longevity. However, direct experimental evidence for its effects on lifespan is limited.

Drosophila melanogaster is an ideal model organism for aging research due to the conservation of approximately 70% of human disease-related genes and the similarity of key signaling pathways and aging mechanisms (10). Their short lifespan of 60–70 days enables rapid and cost-effective assessment of age-related physiological changes, including reproductive output (10). Importantly, lifespan extension does not always coincide with preserved reproductive capacity, as many longevity interventions involve trade-offs between survival and reproduction (11). Therefore, evaluating both lifespan and fecundity allows the assessment of longevity and reproductive fitness as distinct outcomes, rather than providing a comprehensive measure of healthspan.

Although *Drosophila melanogaster* does not possess a direct ortholog of mammalian PDE5, it contains several phosphodiesterase enzymes with conserved catalytic domains and structural similarity to PDE family members, such as *dunce* (*dnc*) and *PDE11*-like genes (12). While the degree of sequence similarity varies, some of these enzymes exhibit structural features that allow interaction with PDE inhibitors. These enzymes regulate cyclic nucleotide signaling, including cAMP and cGMP pathways, and have been shown to respond to pharmacological inhibitors (13), supporting the use of *Drosophila* as a model system to investigate sildenafil's biological effects.

We hypothesized that dietary sildenafil would extend lifespan and preserve reproductive fitness in *Drosophila melanogaster*. To test this, we evaluated lifespan and fecundity. We selected three concentrations (20, 100, and 200 μM) to represent low, moderate, and high exposure levels, enabling assessment of dose-dependent effects. In

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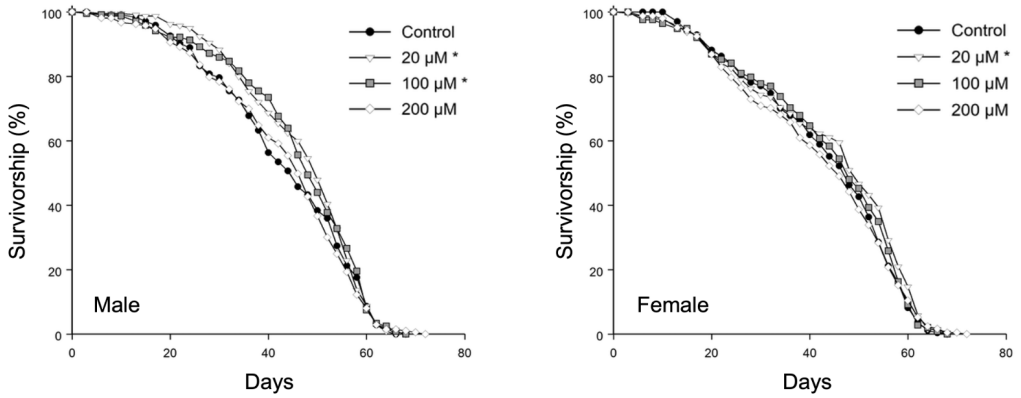

Figure 1: Sildenafil produces modest dose- and sex-dependent changes in survival in *Drosophila melanogaster*. Wild-type Canton-S flies were fed control or sildenafil-supplemented diets (20, 100, or 200 µM), and survival was analyzed by log-rank test. Low-dose sildenafil (20 µM) significantly extended lifespan in both sexes ($p < 0.05$), whereas the intermediate dose (100 µM) increased lifespan only in males ($p < 0.05$) and the highest dose (200 µM) showed no significant effect. Although statistically significant, the magnitude of the lifespan extension was modest, particularly in females. Each group consisted of three cages containing 100 flies per cage.

addition to lifespan and fecundity, body weight and locomotor activity were measured as complementary physiological and functional outcomes. Dietary sildenafil extended lifespan in a dose- and sex-dependent manner and increased cumulative egg production in females, without producing long-term changes in locomotor activity. Together, these findings suggest that sildenafil may promote longevity while preserving reproductive fitness and support its further investigation as a potential geroprotective intervention.

RESULTS

Sildenafil Extends Lifespan in a Dose- and Sex-dependent Manner

To assess the impact of sildenafil on longevity, we exposed wild-type flies to different concentrations of the drug (20, 100, or 200 µM) and monitored survival throughout the adult life course, which typically spans approximately 60–70 days in *D. melanogaster* under standard laboratory conditions. Control males and females had mean lifespans of 43.69 ± 0.90 and 43.71 ± 0.91 days (mean ± SEM), respectively. At the lowest concentration (20 µM), sildenafil significantly increased lifespan in both sexes, with treated males living 46.92 ± 0.79 days ($p = 0.0239$) and treated females living 44.67 ± 1.02 days ($p = 0.0374$) (**Figure 1**). At the intermediate concentration (100 µM), we observed a sex-specific pattern: sildenafil significantly increased lifespan in males to 46.44 ± 0.88 days ($p = 0.0267$), whereas females showed no significant difference from controls, with a mean lifespan of 44.27 ± 0.99 days ($p = 0.4276$) (**Figure 1**). At the highest concentration (200 µM), sildenafil did not significantly alter lifespan in either sex. Treated males had a mean lifespan of 43.47 ± 0.99 days ($p = 0.7264$), and treated females had a mean lifespan of 42.49 ± 1.01 days ($p = 0.4572$) (**Figure 1**). Because the 20 µM dose produced the most consistent increase in lifespan across both sexes, we selected this concentration for all subsequent experiments.

Sildenafil Increases Fecundity in Female Flies

To assess reproductive output, we measured fecundity by counting the number of eggs laid per female over a seven-day period under controlled mating conditions. Across the full experimental period, 20 µM sildenafil-treated females laid significantly more eggs than control females (73.5 ± 4.22 vs. 62.11 ± 5.13, mean ± SEM; $p = 0.0031$; **Figure 2A**). When egg production was examined on individual days, however, the difference between groups was statistically significant on only one day, while most day-by-day comparisons were not significant (**Figure 2B**). Both control and sildenafil-treated females showed changes in egg production over the seven-day period, with egg laying declining toward the end of the experiment. Thus, although sildenafil did not consistently increase egg production on each individual day, it significantly increased cumulative egg production across the seven-day experimental period (**Figure 2A**).

Long-term Exposure to Sildenafil Increases Body Weight

To determine whether low-dose sildenafil treatment (20 µM) might reduce metabolic activity, we measured body weight after early and prolonged exposure to sildenafil. After three weeks of treatment, both male and female flies receiving sildenafil showed significantly greater body weight than controls (males: control 0.83 ± 0.02 vs. sildenafil group 0.92 ± 0.02, $p = 0.006$; females: control 1.22 ± 0.02 vs. sildenafil group 1.28 ± 0.03, $p = 0.04$) (**Figure 3**). In contrast, body weight did not differ significantly between control and sildenafil-treated flies after one week (males: control 0.78 ± 0.02 vs. sildenafil group 0.83 ± 0.02, $p = 0.51$; females: control 1.05 ± 0.07 vs. sildenafil group 1.09 ± 0.01, $p = 0.49$) (**Figure 3**). These findings suggest that prolonged sildenafil treatment alters body weight, although body weight alone does not directly establish changes in metabolic activity.

Sildenafil Does Not Alter Long-term Locomotor Activity

To measure another indicator of health, we assayed locomotor activity. We examined locomotor performance in male and female flies treated with sildenafil (20 µM). Locomotor activity was evaluated using the rapid iterative negative geotaxis (RING) assay, which measures climbing ability as an indicator of neuromuscular function. After one week, sildenafil significantly reduced locomotor activity

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in males (average climbing height; control 6.67 ± 0.16 vs. sildenafil group 5.86 ± 0.23, $p = 0.008$), while females showed no significant change (control 4.93 ± 0.14 vs. sildenafil group 4.85 ± 0.12, $p = 0.63$) (**Figure 4**). After three weeks, we found no differences in either sex (males: control 3.39 ± 0.12 vs. sildenafil group 3.58 ± 0.1, $p = 0.23$; females: control 4.4 ± 0.23 vs. sildenafil group 4.64 ± 0.28, $p = 0.40$) (**Figure 4**).

DISCUSSION

In this study, we found that sildenafil showed dose-dependent and sex-specific effects on lifespan and reproductive ability in *D. melanogaster*. At low concentrations, sildenafil significantly enhanced survival in both sexes. The absence of beneficial effects on lifespan at higher concentrations may reflect potential toxicity or diminishing returns associated with overactivation of cGMP signaling pathways. The sex-specific differences observed at the intermediate dose could be explained by several factors, such as differences in hormonal regulation, reproductive investment, or sensitivity to mitochondrial function and oxidative stress between males and females. In addition, differences in feeding behavior between males and females could lead to unequal drug exposure despite identical dietary concentrations. Because females are known to consume more food than males, they likely received a higher effective dose of sildenafil despite being exposed to the same dietary concentration (14). As a result, the intermediate concentration may have exceeded the optimal range for females, diminishing its beneficial effects on lifespan. In contrast, males may have remained within the effective dose window at this concentration, leading to the observed male-specific lifespan extension. This suggests that sex differences in feeding behavior can shift the optimal therapeutic range between males and females and are an important consideration when testing drugs for therapeutic responses.

Although *Drosophila* does not possess a direct ortholog of mammalian PDE5, it contains several phosphodiesterase genes with conserved catalytic domains and structural similarity to PDE family members, including *dunce* (*dnc*) and PDE11-like genes such as *CG14227* and related loci (12). Some *Drosophila* PDEs, including PDE1-, PDE6-, and PDE11-like enzymes, have been reported to respond to vertebrate PDE inhibitors such as zaprinast and sildenafil, suggesting conservation of inhibitor sensitivity despite the absence of a direct PDE5 ortholog (13). Pharmacological inhibition of these enzymes can alter cyclic nucleotide signaling, including cGMP signaling, providing a plausible mechanism through which sildenafil could exert biological effects in *Drosophila*.

One interesting finding was the significant increase in cumulative fecundity among sildenafil-treated female flies over the seven-reday experimental period. Although egg production was not significantly higher on most individual days, the greater cumulative fecundity suggests that sildenafil may influence reproductive physiology. The mechanisms underlying this effect remain unclear, but changes in energy metabolism or cyclic nucleotide signaling could potentially contribute and warrant further investigation. In fact, some interventions that extend lifespan in *Drosophila* can also influence reproductive output, although the relationship between longevity and reproduction is complex and may

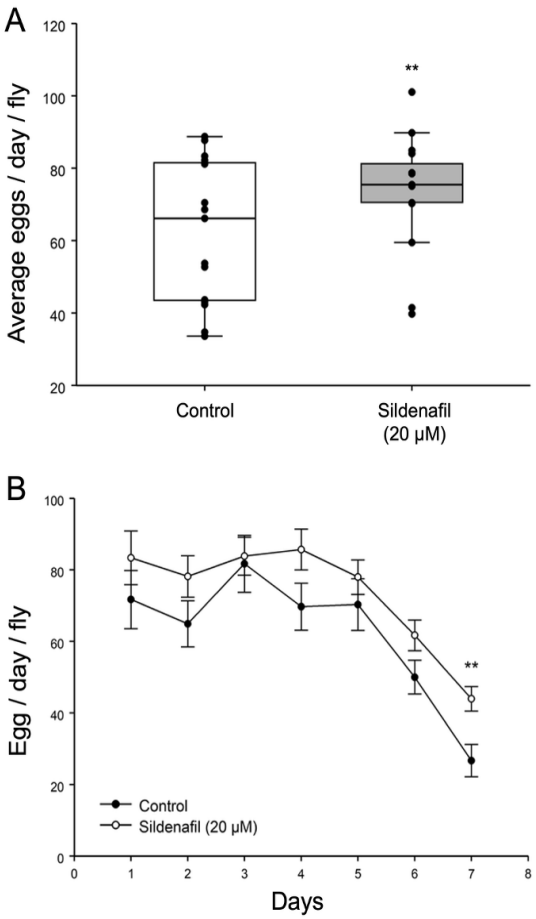

Figure 2: Sildenafil enhances cumulative egg production in female *Drosophila melanogaster*. Egg production was monitored daily for seven days and analyzed using Student's *t*-test. Across the seven-day experimental period, females treated with 20 µM sildenafil laid significantly more eggs than control females (73.5 ± 4.22 vs. 62.11 ± 5.13, mean ± SEM; $p = 0.0031$; **Figure 2A**). In contrast, day-by-day comparisons showed a statistically significant difference on only one day (day 7), while most individual-day comparisons were not significant (**Figure 2B**). Each treatment included 20 replicate vials (two males and one virgin female per vial), with egg counts recorded daily for seven days.

involve trade-offs (15). Therefore, the increase in cumulative egg production observed here should be interpreted as a reproductive outcome rather than as a comprehensive measure of healthspan. Sildenafil's known effects on animal reproduction are varied. Supplementing sows with sildenafil during gestation has shown the potential to increase the number of piglets born alive and improve litter birth weight (16).

In contrast, some studies in mice have shown that high doses of sildenafil can lead to embryo-fetal toxicity and a reduction in fetal growth and body weight (17). Research is ongoing to understand its potential uses and risks for reproductive health. Importantly, our observed enhancement of fecundity across the reproductive period raises the possibility that sildenafil may exert age-dependent effects on reproductive physiology. While increased egg production during early and peak reproductive stages suggests a beneficial role, it remains

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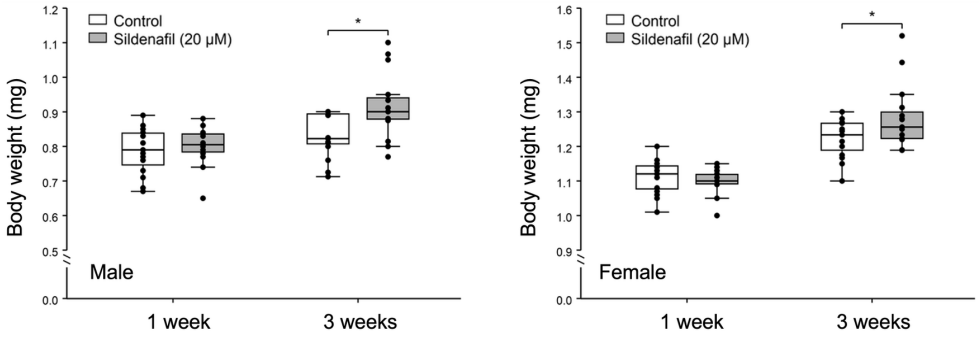

Figure 3: Sildenafil increases body weight after prolonged exposure in *Drosophila melanogaster*. Newly eclosed flies were maintained on control or 20 µM sildenafil-supplemented food and weighed at one and three weeks of treatment. Each group included 15 replicate vials (ten males and ten females per vial), and results are presented as mean body weight per fly ± SEM (standard error of the mean). Student's *t*-test was used for statistical analysis, with $p < 0.05$ considered statistically significant.

unclear whether such stimulation could have long-term trade-offs, particularly in later life stages. In many organisms, interventions that enhance early-life reproductive output can accelerate reproductive senescence or alter lifespan–fertility trade-offs (11). However, while sildenafil treatment increased fecundity in flies, the quality and long-term fitness of the offspring were not assessed in this study. It remains unclear whether the increased egg production translates into healthy progeny with normal development and reproductive potential. Future studies should therefore evaluate offspring viability, developmental outcomes, and reproductive capacity to better understand the broader implications of sildenafil treatment.

Two FDA-approved drugs currently being evaluated for their potential geroprotective effects through drug repositioning are metformin and rapamycin. Metformin is a widely used type two diabetes drug and has been reported to extend lifespan in *C. elegans* and rodents, likely via improved insulin sensitivity and AMPK activation (2). Rapamycin is an immunosuppressant used to prevent organ transplant rejection after surgery and significantly extends the lifespan of mice by inhibiting the mTOR pathway and promoting autophagy (3). These findings suggest that repositioning existing drugs as anti-aging interventions may be possible. Our findings support sildenafil as a candidate in this context for its potential geroprotective properties. Indeed, sildenafil is currently being investigated in mice within the framework of the National Institute on Aging's Intervention Testing Program (18).

Our study has several limitations that should be acknowledged and addressed in future work. First, only a limited range of concentrations and a single wild-type strain were tested, leaving open the possibility that genetic background and broader dose ranges could influence the observed responses. Second, feeding behavior and precise drug intake were not quantified, and tissue distribution was not measured, making it difficult to determine sex-specific pharmacokinetics. Third, direct comparative analyses with established geroprotectors such as metformin or rapamycin were not performed. Given that these compounds serve as well-characterized benchmarks in *Drosophila* aging research, such head-to-head comparisons would be valuable for positioning sildenafil within the broader geroprotector landscape. In addition, we did not examine whether sildenafil treatment at different stages of adult life produces distinct effects. Future studies comparing treatment initiated at defined ages could help determine whether the response to sildenafil

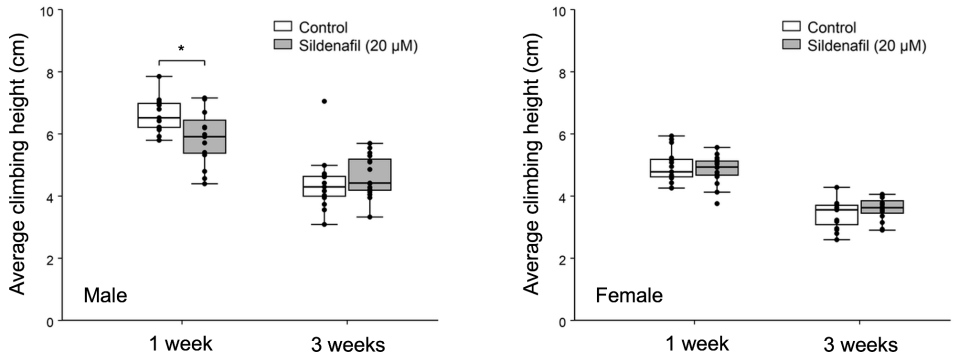

Figure 4: Sildenafil does not alter long-term locomotor activity in *Drosophila melanogaster*. Flies fed control or sildenafil-containing food were assessed for climbing ability after one and three weeks using the rapid iterative negative geotaxis (RING) assay. Each group included 20 replicate assays with four independent trials. The results are presented as mean climbing height ± SEM (standard error of the mean). Student's *t*-test was used for statistical analysis, with $p < 0.05$ considered statistically significant.

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is age dependent. Future work should therefore expand dose-response analyses across multiple genetic backgrounds, directly quantify feeding behavior and drug uptake, compare sildenafil head-to-head with established geroprotectors such as metformin and rapamycin, and evaluate the effects of treatment initiated at different stages of adult life.

Taken together, our results suggest that sildenafil may influence key aspects of aging, including longevity and fertility, in a dose- and sex-dependent manner. While these findings are limited to *Drosophila*, they provide experimental evidence for broader physiological roles of sildenafil that may warrant further investigation in higher organisms.

MATERIALS AND METHODS

Drosophila Stocks and Animal Husbandry

Wild-type Canton-S flies were originally obtained from the laboratory of Marc Tatar at Brown University and subsequently maintained in our laboratory. The flies were cultured and reared at 25°C and 65% humidity under a 12:12 hour light-dark cycle. To avoid larval crowding, approximately 150 eggs were placed in 250 ml fly bottles containing 25–30 ml of medium and allowed to develop until eclosion into adults. The standard medium for rearing fly larvae consisted of 5.2% cornmeal, 11% sugar, 2.5% Saf-instant yeast, 0.5% propionic acid, 0.04% methyl-4-hydroxybenzoate, and 0.8% agar. Sildenafil citrate (≥98% purity) was purchased from Sigma-Aldrich (catalog no. SML3033). Sildenafil was dissolved in dimethyl sulfoxide (DMSO) and added to the medium during food preparation at final concentrations of 20, 100, or 200 µM. Control food was supplemented with the same concentration of DMSO (vehicle) used to dissolve sildenafil.

Lifespan Assay

Longevity experiments were performed by placing male and female fruit flies in three cages per group, with 100 flies per cage (19). The food was replaced with fresh medium every two days, and the mortality rate was recorded by removing dead flies at the time of replacement.

Measurement of Fecundity

Male and female fruit flies were collected separately every three hours following the first eclosion. Female virginity was confirmed by the absence of progeny in the food after 24 hours. On day two, vials were set up with two males and one virgin female. Every subsequent 24 hours, the flies were transferred to new vials, and the number of eggs laid by each female was counted over a period of seven days. Twenty replicate vials were tested per treatment.

Measurement of Body Weight

Newly eclosed flies were collected after 48 hours. Ten male and ten female flies were lightly anesthetized with CO_2 and placed in vials (15 replicates per group) containing either control or sildenafil food. Food was changed every two days. On weeks one and three, the ten flies per vial were weighed using a precision microbalance.

Measurement of Climbing Ability

Males were fed either control or sildenafil containing foods seven days before assessing locomotor function through the rapid iterative negative geotaxis (RING) assay (20). Ten flies of the same sex were introduced into a climbing device and gently tapped down three times to trigger negative geotaxis. Their positions were recorded four seconds after the start of climbing, and the number of flies that surpassed a 4 cm mark was counted. Each experiment included 20 replicates and 4 independent trials per group, with statistical comparisons made using Student's *t*-test.

Statistical Analyses

Survival analyses were conducted using the standard survival model in JMP software, employing log-rank tests for survival data. Prior to analysis, fecundity, body weight, and locomotor activity data were assessed for normality using the Shapiro–Wilk test. Data satisfying the assumption of normality were analyzed using Student's t-tests. Statistical significance was denoted in figures by asterisks (*$p < 0.05$, ** $p < 0.01$) when compared to controls.

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