Lysergic Acid Diethylamide extends lifespan in *Caenorhabditis elegans*

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Abstract

Aging is modulated by nutrient-sensing pathways that integrate metabolic and hormonal cues to regulate growth, stress resilience, and lifespan. Caloric restriction (CR), a well-established intervention, extends longevity in diverse species primarily through inhibition of the TOR signaling pathway. Lysergic acid diethylamide (LSD), a classic serotonergic psychedelic with emerging therapeutic applications, remains largely unexplored in the context of aging. Here, we show that LSD treatment significantly extends lifespan in *Caenorhabditis elegans* and reduces age-associated lipofuscin accumulation, indicative of delayed cellular aging. LSD reproduces several CR-like phenotypes, including decreased reproductive output and increased nuclear localization of the transcription factor PHA-4/FOXA, without affecting food intake. Moreover, LSD treatment reduces lipid stores and downregulates global protein synthesis, both hallmark signatures of TOR inhibition. These findings establish LSD as a modulator of evolutionarily conserved longevity pathways and suggest that psychedelic signaling can mimic a caloric restriction-like metabolic state, paving the way for the development of novel geroprotective strategies.

Introduction

Aging is a complex biological process characterized by the progressive accumulation of sequential biological alterations over time, which increases susceptibility to disease and mortality (Harman, 1993). As global population ages, diseases such as cardiovascular conditions, diabetes, Alzheimer's, and Parkinson's disease have become increasingly prevalent, posing significant health and socioeconomic challenges (Fang et al., 2017). Addressing these challenges requires innovative strategies to extend longevity, delay the aging process, and mitigate the risk of age-associated diseases.

Over the past decades, research has focused on identifying and optimizing interventions to slow aging and promote healthier and longer lives (Hine et al., 2015; Jones et al., 2023; Ma et al., 2020). Among these, dietary modifications and regular physical activity have emerged as critical approaches. Specifically, calorie restriction (CR), defined as a reduction of 30–60% of caloric intake without malnutrition, stands out as one of the most effective strategies for extending lifespan and enhancing healthspan (Mercken et al., 2012). CR has been shown to significantly lower the incidence of aging-related diseases such as diabetes, cancer, heart diseases, and neurodegenerative disorders(Colman et al., 2009). Furthermore, fasting, which involves periodic abstinence from food, shares many benefits with CR, enhancing cellular resilience and reducing the risks associated with aging and disease (Longo & Mattson, 2014).

The mechanisms underlying the benefits of CR and fasting involve the activation of key molecular pathways, including the insulin/IGF-I-like (IIS) pathway and the target of rapamycin (TOR) pathway. These signaling pathways play crucial roles in cellular protection against oxidative, metabolic, and proteotoxic stress, thereby enhancing longevity and delaying age-associated pathologies (Mattson, 2012). However, while the

potential of CR to promote healthy aging is well-documented, sustaining a strict CR regimen in humans is challenging due to social, psychological, and practical constraints. This has led to growing interest in identifying alternative approaches, such as compounds that mimic the effects of CR without requiring drastic dietary restrictions.

Recent research has helped understand the interplay between dietary interventions, serotonin signaling, and aging mechanisms. Serotonin, traditionally recognized for its role in mood regulation, has emerged as a key regulator of aging and longevity (Fidalgo et al., 2013). Studies have shown that serotonin influences lifespan in several organisms, including *Caenorhabditis elegans* (Murakami & Murakami, 2007). In murine model, serotonin signaling is intricately linked to pathways such as mTORC1, which mediates the cognitive and metabolic benefits associated with dietary restriction (Teng et al., 2019). Targeting serotonin signaling may represent a novel strategy to promote longevity.

In mammals, serotonergic signaling can be modulated by psychedelics, which have gained significant attention for their potential to promote neuroplasticity, enhance mental health, and influence biological pathways linked to longevity (de Vos et al., 2021). Compounds such as LSD (Lysergic acid diethylamide), psilocybin, and N,N-DMT, are known to interact with serotonin receptors, impacting neuroplasticity and emotional regulation (Nichols, 2016).

Our recent findings highlight the use of *C. elegans* as a model organism for investigating the effects of psychedelics on serotonergic signaling and behavior. In this previous study, we demonstrated that acute LSD exposure reduces locomotion speed in *C. elegans* by 52%, mediated by serotonin receptors SER-1 and SER-4, which are homologous to human serotonin receptors 5HT2 and 5HT1, respectively (Ornelas et al., 2024). Additionally, metabolic profiling revealed that LSD is rapidly absorbed and metabolized into compounds such as Nor-LSD and O-H-LSD, analogous to metabolic pathways in

humans (Silveira et al., 2023). While psychedelics have shown protective effects across various physiological systems, their role in modulating aging and promoting longevity remains an underexplored area of research (Inserra et al., 2023; Saeger & Olson, 2022).

Given these findings, *C. elegans* emerges as an ideal model for further exploration of the links between serotonin signaling, psychedelics, and aging mechanisms. This study aims to investigate the effects of LSD on longevity. We hypothesize that LSD promotes longevity in *Caenorhabditis elegans* by modulating conserved nutrient-sensing pathways, particularly through mechanisms resembling caloric restriction, thereby delaying physiological markers of aging.

Methods

Chemicals

Potassium phosphate monobasic (CAS 7778-77-0, Sigma Aldrich), Sodium phosphate dibasic (CAS 7558-79-4, Sigma Aldrich), Calcium chloride (CAS 10043-52-4, Merck), Magnesium sulfate (CAS 7487-88-9, Sigma Aldrich), Cholesterol (CAS 57-88-5, Sigma Aldrich), Streptomycin sulfate salt (CAS 3810-74-0, Sigma Aldrich), Agar bacteriological (Kasvi), Bacto peptone (Thermo Scientific), Potassium phosphate dibasic (CAS 7758-11-4, Thermo Scientific), LB Broth (Merck), Sodium chloride (CAS 7647-14-5, Merck), LSD (CAS 50-37-3, Lipomed), Oil Red O (CAS 1320-06-5, Sigma Aldrich), PBS (10X), pH 7.4 (Gibco), Triton™ X-100 (CAS 9036-19-5, Sigma Aldrich) and Click-iT™ Plus OPP Alexa Fluor™ 647 Protein Synthesis Assay Kit (Invitrogen).

Maintenance and cultivation of animals

The strains used in this study were obtained from the *Caenorhabditis* Genetic Center (CGC), University of Minnesota, USA. The strains N2, OH15876 (*pha-*

4::3xGAS::GFP::TEV::LoxP::3xFLAG), AQ866 (ser-4 KO), DA1814 (ser-1 KO) and DV3208 (*daf-15::mNG::2xHA*) were cultivated using standard techniques (Brenner, 1974). Briefly, the animals were maintained on solid medium in plates prepared with nematode growth medium (NGM) seeded with *Escherichia coli* OP50-1 as a food source and supplemented with 100 µg/L of streptomycin. The plates were kept at 20°C. To ensure adequate food supply, the animals were regularly transferred to fresh plates.

Synchronization

The animals were synchronized using the bleaching method according to Porta-de-la Riva *et al.* (Porta-de-la-Riva et al., 2012). In summary, plates containing young adult hermaphrodites and eggs were collected and washed three times with M9 buffer to remove bacterial residues. Then, the bleaching solution (1.65% [v/v] NaCl, 0.5 M NaOH) was added, and the mixture was agitated to ensure complete lysis of the animals while preserving the eggs. M9 was added to stop the reaction, and the eggs were washed, transferred to bacteria-free plates, and stored at 20°C for 24 hours. The next day, L1 larvae were transferred to fresh plates containing bacteria to allow the animals to develop.

Preparation for experiments

Except for the oviposition experiment, in long-term assays, to prevent egg hatching, 5-fluorouracil was added at a concentration of 80 µM to the medium, a compound that interferes with DNA synthesis and blocks cell division. Additionally, to prevent the degradation of the compounds by bacteria, *E. coli* was previously inactivated.

Longevity assessment

Synchronized animals at the young adult stage (day 0 of adulthood) were transferred to Petri plates containing treatment (1 µM LSD) or not. Approximately 60 animals were allocated to each experimental condition: Control or LSD 1 µM. After exposure periods

of 24, 48, 72, 96, or 120 hours, animals from both groups were transferred to treatment-free plates. Survival was assessed every two days, with animals considered dead if they showed no body or head movement upon mechanical stimulation. In parallel, a second longevity assay was conducted in which animals were continuously exposed to LSD throughout their lifespan, with survival counts performed at the same intervals and using the same criteria. The analyses were performed using the Kaplan-Meier survival curve. The results presented refer to the time at which 50% of the animals were dead.

Pharyngeal pumping

Synchronized animals at the young adult stage (day 0 of adulthood) were placed on plates containing treatment (LSD 1 μM) or not. Approximately 12 animals were transferred to a plate containing 12 wells (one animal per well). Each well was recorded when the animal reached 1 and 5 days of treatment for 10 seconds using a Leica Investa 3 (Leica, Wetzlar, Germany) stereomicroscope with an attached camera and a 55x magnification. The analysis of each video was performed by playing them in slow motion (0.25x) and counting the pharyngeal pumping rate.

Lipid quantification

Synchronized animals at the young adult stage (day 0 of adulthood) were placed on Petri plates containing treatment (LSD 1 μM) or not. Approximately 100 animals were transferred to each plate, and each plate represented a treatment time point: 1, 5 and 10 days. Once the treatment period ended, lipid quantification was performed according to Nicole Stuhr (Stuhr et al., 2022). In summary, the animals were washed, permeabilized with PBST, and fixed with 60% isopropanol. They were then incubated with Oil Red O 60% for staining. The animals were imaged using a Leica SP8 confocal microscope

(Leica, Wetzlar, Germany) with the same gain, offset, and pinhole aperture parameters for all experimental groups.

Oviposition assessment

Synchronized adult animals were placed on Petri plates containing treatment (LSD 1 μM) or not. At the end of the L4 larval stage, approximately six animals were transferred to plates, totaling six plates with one animal per plate. Every 24 hours, the eggs were counted using a Leica Investa 3, and the adult animals were transferred to new plates containing the treatment or not, until no animals laid eggs anymore.

Larval development

Synchronized animals at the L1 stage were placed on Petri plates containing treatment (LSD 1 μM) or not. Approximately 20 animals were transferred to each plate, and after 72 hours, the number of animals with or without a developed vulva was counted. The animals were imaged using a Leica SP8 confocal microscope.

Protein synthesis quantification

To quantify protein synthesis, we used the Click-iT™ Plus OPP Alexa Fluor™ 647 Protein Synthesis Assay Kit, adapted for the *C. elegans* model. After synchronization, animals were transferred to treatment plates and, at the end of the exposure period (1 or 10 days), were collected using M9 buffer, washed three times, and concentrated into a pellet. Click-iT® OPP was diluted to 20 μM in M9 supplemented with inactivated bacteria, added to the pellets, and the animals were incubated for 4 hours at 20 °C under agitation. Subsequently, fixation was performed with 4% PFA for 10 minutes at 4 °C, followed by permeabilization with PBST for 24 hours at 20 °C. After washing, animals were incubated with the Click-iT® Plus OPP reaction cocktail for 30 minutes at room

temperature, protected from light, and then washed with the rinse buffer. Quantification was performed using the GloMax (Promega, Winsconsin, EUA) plate reader .

Fluorescence analysis and quantification

Fluorescence from the *pha-4::GFP* and *daf-15::mNG* reporters was analyzed using the OH15876 and DV3208 strains, respectively, while lipofuscin autofluorescence was assessed in the N2 (wild-type) strain. Synchronized animals at the young adult stage (day 0 of adulthood) were placed on Petri plates containing either 1 μM LSD or not. Each plate corresponded to a specific time point: days 1, 5, or 10 of adulthood. At the end of each treatment period, animals were imaged using a Leica TCS-SP8 confocal microscope at 63x magnification for *pha-4::GFP* and *daf-15::mNG* fluorescence, and at 10x magnification for lipofuscin autofluorescence. Image analysis was performed using Image J software, and fluorescence levels were expressed as a percentage relative to the control group.

Statistical analysis

Lifespan assays were analyzed by comparing treated and untreated animals of the same strain using the log-rank (Mantel–Cox) test (See supplementary table 1). For all other experiments, statistical significance was assessed using the Student's *t*-test. All analyses were performed using GraphPad Prism version 8.0.

Results

LSD extends lifespan and reduces lipofuscin accumulation in aging *C. elegans*

Given that serotonergic signaling plays a crucial role in modulating longevity in *C. elegans* (Murakami & Murakami, 2007; Petrascheck et al., 2007), we first investigated

whether chronic exposure to LSD increases nematode longevity. We find that 1 μM LSD-treatment increases lifespan by 25% compared to controls (Figure 1A).

Although studying longevity provides insights into aging, an increase in lifespan does not necessarily imply an extension of the healthy period of life. To determine whether LSD also delays aging, we quantified lipofuscin, a fluorescent pigment that accumulates due to the incomplete degradation of cellular components, primarily because of autophagic and lysosomal activity. As its accumulation progressively increases with age, lipofuscin is considered a reliable marker of aging (Porta, 2002). We analyzed 10-day-old and 15-day-old worms, as these time points ensure sufficient lipofuscin accumulation to assess aging-related changes. LSD significantly reduced lipofuscin accumulation compared to controls suggesting that, in addition to its lifespan extension effect, LSD also delay the aging process (Day 10: CT 100% ± 5.0, LSD 80.7% ± 5.7, $p < 0.01$; Day 15: CT 100% ± 8.0, LSD 56.0% ± 7.0, $p < 0.001$) (Figure 1B and C).

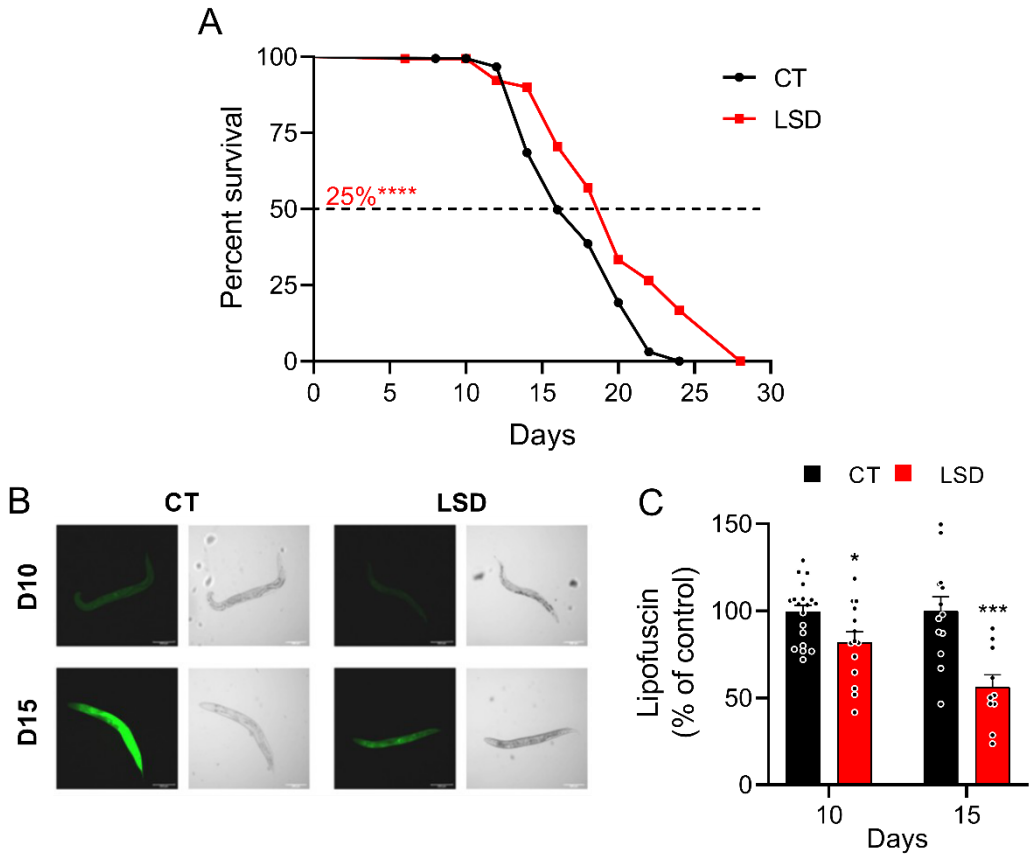

Figure 1 - LSD extends the lifespan of *C. elegans* and delays aging. **(A)** Percentage survival of untreated animals (black line) or animals treated with 1 µM LSD (red line) over time. Data presented as Kaplan-Meier survival curves, and group differences analyzed using the Log-rank test. n=3, with at least 60 animals per condition. **(B)** Representative images of lipofuscin (green) animals treated (right) and untreated (left). **(C)** Quantification of lipofuscin accumulation in untreated (black line) and treated with 1 µM LSD (red line) animals over time. Fluorescence intensity was measured in arbitrary units (A.U.) and is presented as mean ± standard error of the mean (SEM) from 3 independent biological replicates. n=3, with at least 5 animals per condition. Statistical analysis performed using Student's t-test. *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$ compared to the control.

LSD-induced longevity does not require continuous exposure

Next, we tested whether shorter, non-continuous exposures could also increase longevity. Five groups of animals received a 1 µM LSD treatment for 24, 48, 72, 96, or 120 hours, respectively (Figure 2). Our data show that all treatments can extend lifespan to a similar

extent (24h: 11%; 48h: 11%; 72h: 12%; 96h: 12%; 120h: 12%). It is important to note that the lifespan extension with short treatments was less than half that observed in continuously treated animals. For subsequent experiments, we used chronic treatment, as it resulted in a greater increase in longevity.

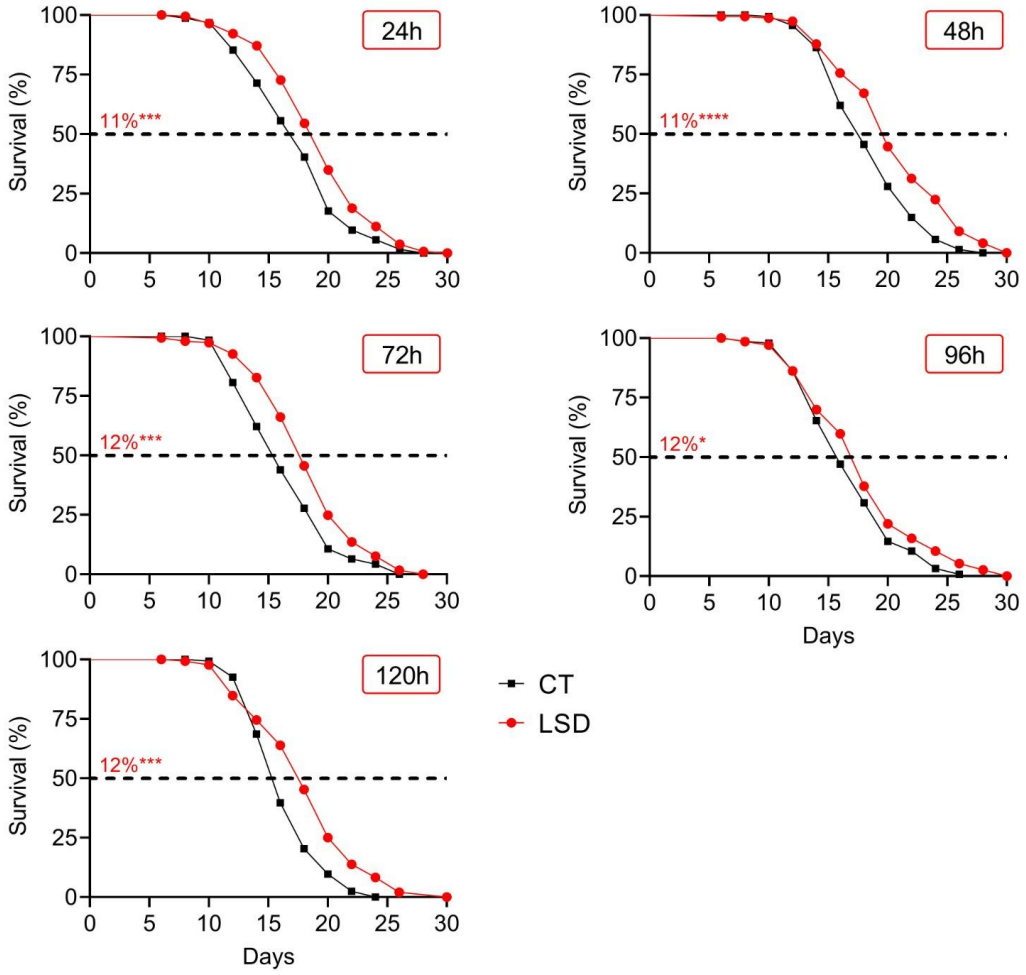

Figure 2 - LSD treatment extends longevity partially independent of exposure duration. Percentage of survival of animals treated with LSD 1 µM for 24, 48, 72, 96 or 120 hours (red line) or untreated (black line) over time. All data presented as Kaplan-Meier survival curves, and group differences analyzed using the Log-rank test. n=3, with at least 60 animals per condition. *p<0,05. ***p<0,001. ****p<0,0001.

LSD promotes longevity through SER-1 and SER-4-dependent mechanisms

We next asked whether serotonin receptors mediate the LSD-induced increase in lifespan. In our previous work, we identified SER-1 and SER-4 as key receptors involved in the behavioral changes triggered by LSD (Ornelas et al., 2024). Building on these findings, we assessed whether these receptors play a role in longevity by testing *ser-1* and *ser-4* mutant strains. In *ser-1* mutants, LSD resulted in an 11.6% decrease in lifespan, while no effect was observed in *ser-4* mutants (Figures 3A, 3B, and table 1). These results suggest that both receptors are necessary for LSD-mediated lifespan extension.

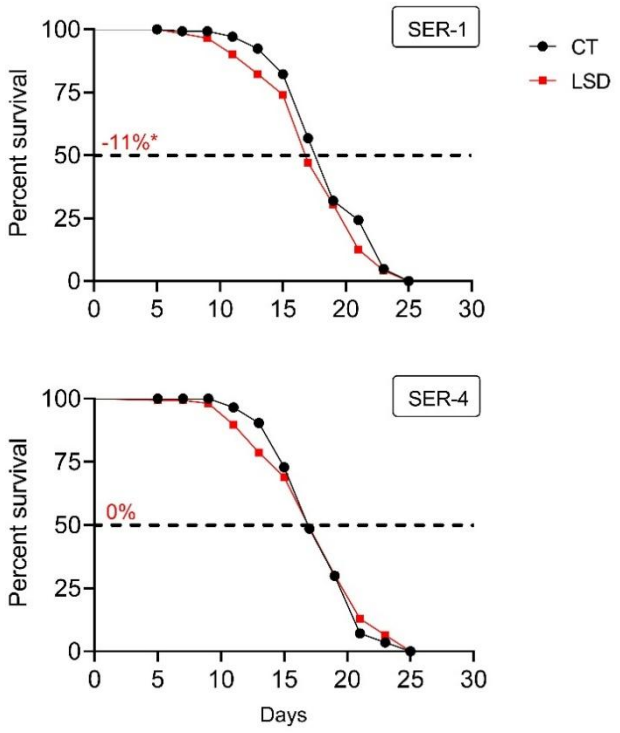

Figure 3 - LSD promotes longevity through SER-1 and SER-4-dependent mechanisms. (A) Percentage of survival of mutant animals (ser-1 KO) treated with LSD 1 µM (red line) or untreated (black line) over time. All data presented as Kaplan-Meier survival curves, and group differences analyzed using the Log-rank test. n=3, with at least 45 animals per condition. *p<0,05. **(B)** Percentage of survival of mutant animals (ser-4 KO) treated with LSD 1 µM (red line) or untreated (black line) over time. All data presented as Kaplan-Meier survival curves, and group differences analyzed using the Log-rank test. n=3, with at least 45 animals per condition.

LSD induces caloric restriction-like phenotype in *C. elegans*

In *C. elegans*, serotonergic signaling is not only involved in locomotor responses to food availability, such as the enhanced slowing response (Chase & Koelle, 2007) but also plays a broader role in nutrient-sensing and metabolic regulation. Since our previous work showed that LSD can elicit this response (Ornelas et al., 2024), we hypothesized that LSD-treated animals might display additional characteristic typical of caloric restriction (CR).

A well-established consequence of CR in *C. elegans* is reduced oviposition (Lakowski & Hekimi, 1998). We quantified egg-laying in LSD-treated animals compared to untreated controls and found that animals exposed to LSD exhibited a significant reduction in egg-laying ($p<0.01$; Figure 4A), suggesting a CR-like effect on reproductive output. CR is also known to reduce body size in *C. elegans*. We measured the length and width of treated and untreated animals on day 1, 5, and 10 and found that LSD treatment leads to a significant reduction in body length at all time points (Day 1: CT 0.79 mm ± 0.01, LSD 0.72 mm ± 0.01, $p<0.01$; Day 5: CT 1.04 mm ± 0.01, LSD 0.93 mm ± 0.02, $p<0.01$; Day 10: CT 1.12 mm ± 0.1, LSD 1.01 mm ± 0.03, $p<0.05$) (Figure 4B). In contrast, body width remained unchanged at day 1 and 5 and decreased at day 10, when a significant difference between groups was observed (Day 1: CT 47.4 µm ± 1.5, LSD 45.2 µm ± 1.5; Day 5: CT 74.4 µm ± 2.3, LSD 70.8 µm ± 3.0; Day 10: CT 69.8 µm ± 2.2, LSD 58.71 µm ± 2.8, $p<0.01$) (Figure 4C).

CR has lowers lipid content (Kuhla et al., 2014; Zheng et al., 2018). We next quantified total lipid accumulation using Oil Red O staining. Although we detected that LSD increased total lipid content at day 1 of adulthood, this trend was reversed at later time points (Figures 4D and 4E; Day 1: CT 100% ± 2.3, LSD 125.7% ± 6.8, $p<0.001$; Day 5: CT 164.4% ± 11.0, LSD 151.6% ± 8.1; Day 10: CT 115.1% ± 6.7, LSD 86.6% ± 6.4,

$p<0.01$). This is consistent with a CR-like metabolic shift. Together, our findings indicate that LSD induces physiological changes in *C. elegans* that closely resemble caloric restriction.

One of the key downstream markers of CR-induced signaling is the transcription factor PHA-4, the *C. elegans* ortholog of mammalian FOXA, which translocates to the nucleus under conditions of reduced TOR activity (Sheaffer et al., 2008). To investigate whether LSD could modulate PHA-4, we observed PHA-4's cellular localization. We observed an increase in PHA-4 nuclear translocation in LSD-treated animals after 1 and 5 days of treatment (Day 1: CT 52.0 ± 2.2, LSD 64.0 ± 5.1, $p<0.001$; Day 5: CT 61.0 ± 1.9, LSD 66.4 ± 1.4; Day 10: CT 53.7 ± 2.7, LSD 56.7 ± 2.8, $p<0.01$) (Figures 4F and 4G), indicating a potential inhibition of the TOR pathway which is associated with CR.

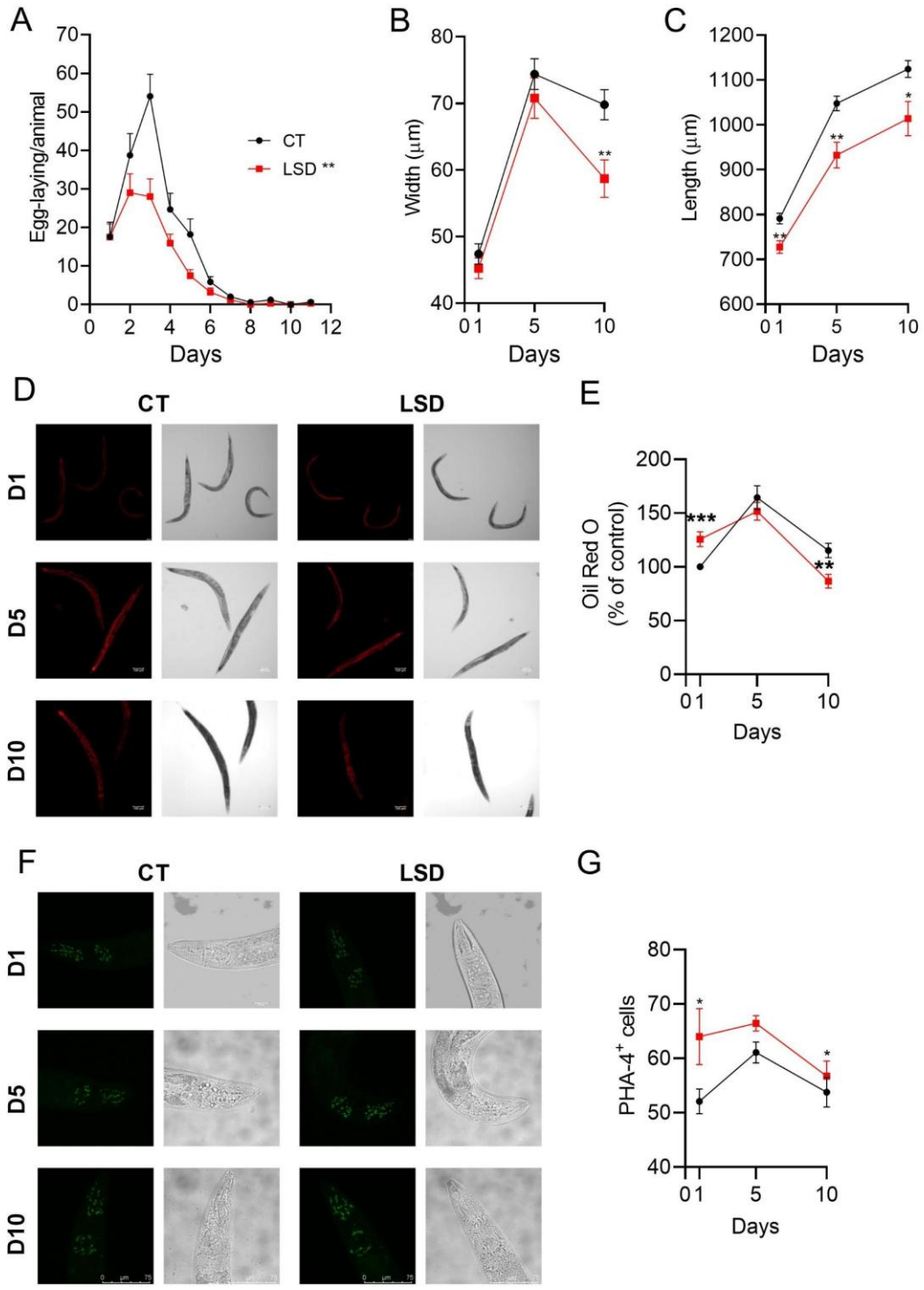

Figure 4 - LSD induces calorie restriction phenotypes. (A) Number of eggs laid during the reproductive period by treated animals (red bar) or untreated animals (black bar) per animal/day. Data presented as mean ± standard error of the mean (SEM) from 3 independent biological replicates. n=3. Statistical analysis performed using Student's t-test. **p < 0.01 compared to the control. **(B)** Measurement of body width of treated animals (red line) or untreated animals (black line) over time. Statistical analysis performed using Student's t-test. **p < 0.01 compared to the control. **(C)** Measurement

of body length of treated animals (red line) or untreated animals (black line) over time. Statistical analysis performed using Student's t-test. **$p < 0.01$ compared to the control. **(D)** Representative images of treated (right) and untreated (left) animals stained with Oil Red O (ORO) over time. **(E)** Quantification of total lipids in treated animals (red line) or untreated animals (black line) through ORO staining. Data presented as mean ± standard error of the mean (SEM). n=3. Statistical analysis performed using Student's t-test. ***$p < 0.001$; **$p < 0.01$ compared to the control. (**F)** Representative images of PHA-4::GFP animals, treated (right) and untreated (left), showing nuclei-positive cells for the transcription factor PHA-4 over time. **(G)** Quantification of nuclei-positive cells for the transcription factor PHA-4 in treated animals (red line) or untreated animals (black line) in the head region. Statistical analysis performed using Student's t-test. *$p < 0.05$ compared to the control.

LSD-treated *C. elegans* exhibit CR-like traits independently of a decrease in food consumption

In wild type animals, CR is triggered by reduced food intake. To assess whether LSD could induce alterations in food intake and thus promote the CR response in an indirect manner, we quantified the animals' pharyngeal pumping rate. We analyzed animals at day 1 and 5 and our results indicated that LSD did not alter pumping frequency (Day 1: CT 244.8 ± 11.9, LSD 246.8 ± 15.2; Day 5: CT 197.5 ± 16.1, LSD 204.2 ± 11.6) (Figure 5). This suggests phenotypes induced by LSD are not due to reduced food intake. This leads us to hypothesize that LSD activates CR-like signaling.

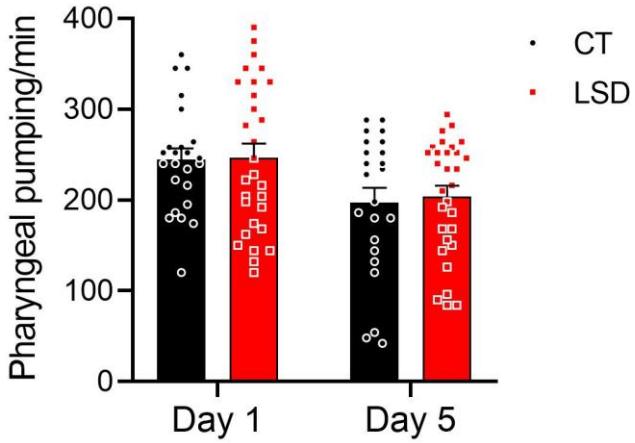

Figure 5 - LSD does not reduce food intake of *C. elegans*. Quantification of pharyngeal pumping in treated animals (red bar) and untreated animals (black bar) per animal/minute. Data presented as mean ± standard error of the mean (SEM). n=3. Statistical analysis performed using Student's t-test.

LSD modulates TOR signaling pathway in *C. elegans*

To further investigate the mechanisms through which LSD may mimic CR, we focused on the TOR pathway that acts upstream of PHA-4. Since TOR signaling plays a key role in development, we assessed vulval formation in young adult nematodes to determine whether LSD induces a delay in larval growth. For this, we treated animals from the L1 stage and monitored continued until fully vulval development in young adults. Our results indicate that the number of animals with fully developed vulva was significantly lower in the LSD-treated group (CT 77.9% ± 8.0, LSD 45.7% ± 10.2, $p<0.05$) (Figure 6A and 6B). This suggests that LSD may be inhibiting TOR modulation. To further explore this hypothesis, we measured protein synthesis in animals treated with LSD for either 1 or 10 days. After 24 hours of treatment, we observed a significant increase in protein synthesis (CT: 100%, LSD: 136.5 ± 12.2) (Figure 6C), consistent with previous reports showing that psychedelics enhance mTORC1 signaling (Ly et al., 2018a).However, after 10 days of treatment, protein synthesis was significantly reduced (CT: 100%, LSD: 62.9 ± 13.0),

suggesting that chronic LSD exposure leads to TORC1 pathway inhibition. These results support the idea that LSD exerts biphasic effects on TOR signaling, with early activation followed by long-term suppression. Next, we measured protein levels of DAF-15, an essential component of the TORC1 complex and homologous to the human Raptor protein. We observed that LSD treatment increased DAF-15 levels on day 1 and 5, followed by a decrease on day 10 (Day 1: CT 9.4 ± 1.6, LSD 14.0 ± 1.8, $p<0.05$; Day 5: CT 11.3 ± 1.3, LSD 16.0 ± 1.1, $p<0.05$; Day 10: CT 24.5 ± 3.1, LSD 14.7 ± 3.0, $p<0.01$) (Figure 6D and 6E). Consistent with our previous findings, chronic LSD treatment appears to inhibit TOR signaling.

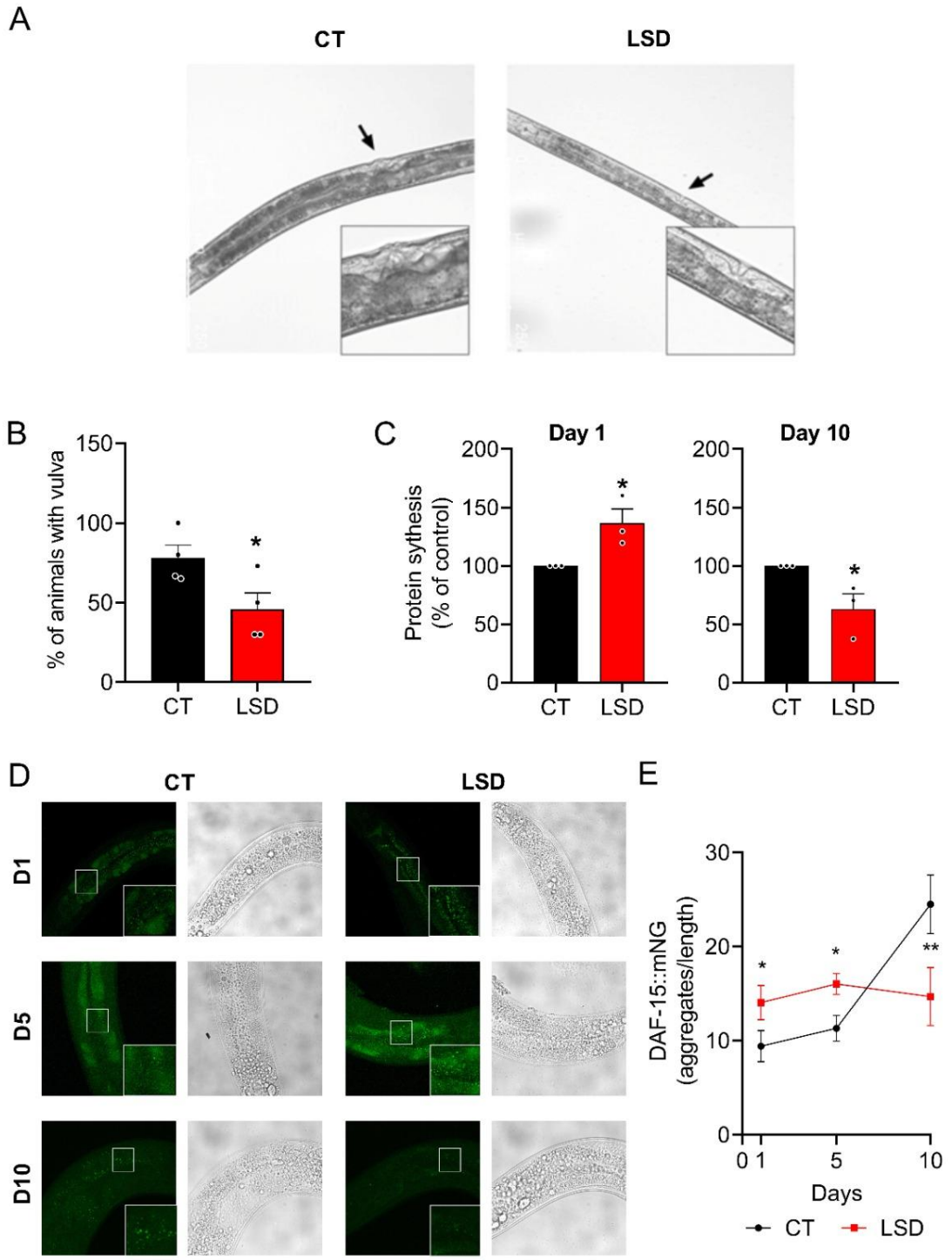

Figure 6 - LSD modulates the TOR pathway. (A) Representative image of the vulva (black arrow) in treated (left) and untreated (right) animals. **(B)** Percentage of treated (red bar) and untreated (black bar) animals with developed vulvas at the end of the L4 larval stage. Data presented as mean ± standard error of the mean (SEM). n=4. Statistical analysis performed using Student's t-test. *$p < 0.05$ compared to the control. **(C)** Quantification of protein synthesis in treated animals (red bar) and untreated animals (black bar) through Click-iT™ Plus OPP Alexa Fluor™ 647 Protein Synthesis Assay Kit.

Data presented as mean ± standard error of the mean (SEM). n=3. Statistical analysis performed using Student's t-test. *p < 0.05 compared to the control. **(D)** Representative images of DAF-15::mNG animals, treated (right) and untreated (left). **(E)** Quantification of daf-15 aggregates relative to the body length of treated animals (red line) and untreated animals (black line). Data presented as mean ± standard error of the mean (SEM). n=3. Statistical analysis performed using Student's t-test. **p < 0.01; *p < 0.05 compared to the control.

Discussion

In this study, we demonstrate that LSD increases longevity. This effect is accompanied by a reduction in lipofuscin accumulation, suggesting not only an extended lifespan but also healthier aging. When evaluating whether the longevity-enhancing effect of LSD requires chronic exposure, we found that shorter treatment durations (24, 48, 72, 96, and 120 hours) each produced a comparable effect, approximately half the lifespan extension observed with continuous, lifelong treatment. This suggests that LSD may have two distinct components, one dependent on chronic exposure and another independent, with each contributing equally to the overall lifespan extension.

In our previous study, we showed that the behavioral slowing effect of LSD in *C. elegans* was absent in animals lacking serotonin receptors SER-1 and SER-4, indicating that both are involved in mediating its neuromodulatory actions. Building on those findings, we asked whether these receptors also play a role in the longevity effects induced by LSD.

In *ser-4* mutants, LSD failed to extend lifespan, indicating that the pro-longevity effect of LSD is dependent on SER-4. In contrast, *ser-1* mutants, which exhibited a longer baseline lifespan than wild-type animals, a finding also reported in the literature (Murakami & Murakami, 2007), showed a lifespan reduction upon LSD treatment. This suggests that, in the absence of SER-1, LSD may either exert a deleterious effect or counteract the lifespan increase typically observed in *ser-1* mutants, as the average lifespan in this group

approximated that of wild-type controls. Altogether, these results indicate that LSD-induced longevity is not only receptor-specific but also dependent on the balance of serotonergic signaling.

The longevity effects of LSD exhibit parallels with those of serotonergic transporter inhibitors such as fluoxetine, paroxetine, and sertraline, which extend lifespan in *C. elegans* by modulating serotonin receptor pathways (Liang et al., 2024; C. Zhou et al., 2024; Y. Zhou et al., 2024). Notably, the serotonin antagonist mianserin has been proposed to mimic CR, a phenomenon wherein lifespan extension is achieved without reducing nutrient intake, by engaging serotonin receptor-mediated signaling (Petrascheck et al., 2007b). Similarly, LSD may share these effects, as our findings indicate that it modulates CR-like features, such as reduced egg-laying and animal size, without altering feeding behavior, as evidenced by the lack of pharyngeal pumping alteration. Supporting this hypothesis, we observed that LSD induced PHA-4 nuclear translocation, a key event in dietary restriction-mediated longevity (Panowski et al., 2007). Since PHA-4 is essential for CR-induced lifespan extension (PHA-4/Foxa mediates diet-restriction-induced longevity of C. elegans), its activation by LSD suggests that the psychedelic may promote longevity through nutrient-sensing mechanisms.

On the other hand, LSD has been shown to exhibit unique therapeutic properties compared to other CR mimetic agents. For example, rapamycin, which also modulates nutrient-sensing pathways via mTOR inhibition, is associated with adverse effects such as glucose intolerance and increased diabetes risk (Unnikrishnan et al., 2020). Psychedelics have been associated with reduced metabolic risks, such as protective effects on pancreatic β-cells, lower odds of diabetes and heart diseases (Gojani et al., 2024; Simonsson et al., 2021). These findings support the potential of LSD to mimic CR-

like benefits without the negative metabolic consequences observed with rapamycin and other compounds.

Furthermore, in the CR-like signaling, TOR pathway plays a pivotal role in growth, metabolism, and aging (Keith Blackwell et al., 2019). LSD's ability to delay larval development, decrease size, protein synthesis and DAF-15 (RAPTOR) protein levels suggests inhibition of TORC1, consistent with mechanisms observed in dietary restriction and rapamycin treatment (Bar et al., 2016; Duong et al., 2020; Keith Blackwell et al., 2019). Notably, the reduction in protein synthesis and lipid levels observed on day 10 of LSD treatment reinforces TOR inhibition hypothesis, which is known to shift energy metabolism away from storage toward cellular maintenance (Bar et al., 2016). This biphasic pattern, where lipid levels, protein synthesis and DAF-15 levels increase initially and then decline by day 10, may reflect temporal changes in TORC1 activity driven by receptor dynamics. Psychedelics like LSD and DMT activate 5-HT2A receptors, which have been shown to mediate mTOR activation during short-term treatments, such as those enhancing neuroplasticity in cortical neurons (Ly et al., 2018b; N. Costa et al., 2024). Proteomic analyses of overnight LSD-treated human cerebral organoids reveal upregulation of proteins involved in mTOR signaling and synaptic plasticity, further supporting this mechanism during acute exposure (N. Costa et al., 2024).

Chronic treatments might produce the opposite effect due to receptor internalization and downstream signaling adaptations. Studies on 5-HT6 receptor signaling demonstrate that receptor desensitization and decreased membrane expression attenuate mTORC1 activity over time, a phenomenon that may also apply to 5-HT2A receptors with prolonged LSD exposure.

In our study, the biphasic response observed suggests a temporal shift in TOR signaling. This aligns with the hypothesis that acute psychedelic-induced mTOR activation

transitions into an inhibitory state with prolonged exposure. Further experiments are needed to delineate the mechanisms driving this switch and its implications for longevity.

Conclusion

Our findings demonstrate that lysergic acid diethylamide (LSD) extends lifespan and delays age-associated physiological decline in *Caenorhabditis elegans*. These longevity-promoting effects are mediated, at least in part, through serotonergic signaling via the SER-1 and SER-4 receptors, and are associated with a range of phenotypes that closely mimic those induced by caloric restriction (CR). Notably, LSD treatment induces nuclear translocation of the PHA-4/FOXA transcription factor, reduces reproductive output and lipid accumulation, and ultimately suppresses TOR signaling, a central pathway in nutrient sensing and aging. LSD-treated animals exhibited these CR-like traits independently of reduced food intake, indicating that the compound modulates intrinsic metabolic signaling rather than acting through dietary restriction per se. Taken together, our results position LSD as a novel modulator of evolutionarily conserved aging pathways, and support the broader concept that psychedelics may have geroprotective potential beyond their neuropsychiatric effects. These findings open new avenues for the exploration of psychedelics as caloric restriction mimetics and warrant further investigation into their mechanisms and translational applications in higher organisms.

CRediT authorship contribution statement

Beatriz de S. Carrilho: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Aline Duarte:** Writing– review & editing, Methodology, Investigation. **Ariane Moura:** Methodology, Investigation. **Matheus Antonio V. de C. Ventura:** Methodology, Investigation. **Francisco Moll:** Investigation. **Hugo Aguilaniu:** Writing – review & editing, Supervision. **Ivan Domith:** Writing – original draft,

Validation, Investigation, Formal analysis, Supervision. **Stevens Rehen:** Writing – review & editing, Supervision, Resources, Conceptualization, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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