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Health status and lipidomic alteration of *Caenorhabditis elegans* under 1(3)-oleoyl-2-palmitoyl-3(1)-linoleoylglycerol and 1,3-dioleoyl-2-palmitoylglycerol exposure

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ABSTRACT: 1(3)-Oleoyl-2-palmitoyl-3(1)-linoleoylglycerol (OPL) and 1,3-dioleoyl-2-palmitoylglycerol (OPO) are the most abundant sn-2 palmitoyl lipids in human milk, whose intake in early life might influence the short- and long-term health of human. However, only OPO is widely approved for use in infant formula, scientific evidence regarding OPL's suitability and optimal dosage remains limited. Reliable model organisms are therefore needed to explore the effects of these structured lipids intervention in early life on the lipid metabolism and associated health outcomes. Using *Caenorhabditis elegans* (*C. elegans*), we investigated how dietary supplementation with OPL and/or OPO during L1 to L4 affected lipid composition, content, metabolism, and morphology at L4, and how these changes influenced growth, lifespan, movement, and stress resistance. Specifically, mixed OPL and OPO supplementation (10 mg/L) significantly increased ($P < 0.001$) the body length of *C. elegans*, with no effect on body width. While appropriate concentrations of OPO significantly extended lifespan, high concentrations of OPL reduced it, indicating that variations in structured lipid composition may differentially impact lipid metabolism and longevity. Locomotion was more enhanced in the OPO and OPL<OPO groups compared to OPL and OPL>OPO, suggesting that OPO more effectively improved mobility. Furthermore, mixed supplementation with OPO and OPL (10 mg/L) enhanced survival under heat, ultraviolet (UV), and oxidative stress, likely due to increased levels of PE, MUFA, PUFA, EPA, and DAG, along with alterations in lipid droplets. This study provides novel insights into the composition, function, and potential applications of structured lipids relevant to infant nutrition, and highlights the possible role of OPL in supporting infant nutrition.

Keywords: *Caenorhabditis elegans*, 1(3)-oleoyl-2-palmitoyl-3(1)-linoleoylglycerol, 1,3-dioleoyl-2-palmitoylglycerol, lipidomics, multi-structured illumination microscopy

1. Introduction

Early life nutrition is critical for short- and long-term health. Human milk is the best nutrition for infant with the content of lipid as the largest except water and carbohydrate. Sn-2 palmitoyl lipids in human milk

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can modulate the gut microbiota, regulate lipid metabolism, promote the absorption of calcium and fatty acids, reduce pro-inflammatory cytokine levels, and increase total bile acid concentrations [1, 2]. During digestion and absorption, the fatty acyl chains at the sn-1 and sn-3 positions of triacylglycerols (TAGs) are preferentially hydrolyzed and subsequently absorbed by small intestinal epithelial cells as free fatty acids. In contrast, saturated fatty acyl chains at the sn-2 position are absorbed as monoacylglycerol (MAG), avoiding the release of free palmitate with a melting point higher than body temperature. This mechanism prevents the formation of insoluble calcium soaps [3]. These hard calcium soaps can pass through the intestinal tract, potentially damaging the intestinal wall, disrupting the microflora, and being excreted in feces, ultimately leading to the loss of both fatty acids and calcium [1]. Sn-2 palmitoyl TAGs not only increase the abundance of *bifidobacteria*, but also support infant neurodevelopment by enhancing intestinal *bifidobacteria* levels, which in turn promote the development of fine motor skills [4, 5]. In summary, sn-2 palmitoyl TAGs can improve infant digestion, absorption, and overall comfort, thereby helping to narrows the nutritional gap between infant formula and human milk fat.

1(3)-oleoyl-2-palmitoyl-3(1)-linoleoylglycerol (OPL) and 1,3-dioleoyl-2-palmitoylglycerol (OPO) are the two most abundant sn-2 palmitoyl lipids in human milk. Currently, OPO is approved to be added to infant formula, mainly because approximately 70–90% of palmitoyl in human milk is esterized at sn-2 of TAGs, which emphasizes the significance of the position of palmitic acid rather than the importance of the sn-1, 3 position fatty acids. OPO and OPL both belong to sn-2 palmitoyl TAGs and have similar health benefits for infants based on the digestive characteristics of sn-2 palmitoyl TAGs. Some studies have proven that OPL is essential in Chinese human milk [6, 7]. The design of the current infant formula is almost entirely driven by the research findings of human milk in other countries. This also should be an essential guideline for lipid mimicking in Chinese infant formula. Human milk in most Chinese regions contains higher levels of linoleic acid and OPL-structured lipids, differing from Western human milk lipid compositions. However, only OPO is an approved nutritional fortifier, evidence on OPL’s suitability and optimal dosage remains insufficient.

Professor Deng Zeyuan's team from Nanchang University simulated infant gastrointestinal digestion and used 1,3-dilinoleoyl-2-palmitoylglycerol (LPL), OPL, and OPO structures with purities of 59.58, 50.04, and 55.79%, respectively, to explore their digestion differences in gastrointestinal tract [8]. The results indicated that structured TAGs rich in oleic acyl (OPO or OPO in high proportions) were more digestible than those rich in linoleic acyl (OPL, LPL, and OPL or LPL in high proportions). Caco-2 cells were treated with high-purity standards (>98% OPO, OPL, or LPL). The linoleic acid-rich structured TAGs were not only more easily absorbed than the oleic acid-rich structured TAGs but also more advantageous in the synthesis and transport of TAGs in Caco-2 cells [8]. Correspondingly, the protein expressions of fatty acid binding protein 1 (FABP1), peroxisome proliferator activated receptor alpha α (PPARα), and microsomal triglyceride transfer protein (MTP) in Caco-2 cells treated with linoleic acid-rich structured TAGs were significantly higher than those treated with oleic acid-rich structured TAGs [8]. In another study, human

hepatocyte (LO2 cells) were treated with TAGs in different configurations [9]. The lipid accumulation in LO2 cells was induced by OPL, whereas the expression of long-chain acyl-CoA synthetase 1 (ACSL1) protein was increased in the OPL group, promoting the conversion of long-chain fatty acids to TAGs, diacylglycerols (DAGs), and polar lipids (PLs). In another study, rat fed with human milk fat substitute containing 57.87% sn-2 palmitoyl TAGs and 1.4 times of OPL to OPO ratio obtained the lowest TAG accumulation in liver, synergistically improving the absorption of fatty acids and calcium, and increasing the levels of total bile acids [7]. Adding OPO to mice diet resulted in lower final weight, weight gain, and liver TAG, low-density lipoprotein-cholesterol, and the ratio of low-density lipoprotein-cholesterol to high-density lipoprotein-cholesterol compared to OPL supplement [10]. These studies provide preliminary evidence that structural TAGs (such as OPL, OPO, and LPL) have different effects on lipid metabolism. The concentrations of OPL and OPO in human milk vary by approximately 1–2% in different countries [11, 12]. Therefore, the proportion of structured lipids in human milk cannot be strictly mimicked using structured lipids with a purity of 50–80%. Currently, the high-purity OPL and OPO standards are primarily used in cell experiments to simulate structured lipid characteristics of different countries and assess their efficacy. Thus, more reliable model organisms and precise lipid ratios are needed for further investigation.

Caenorhabditis elegans (C. elegans), a multi-cellular eukaryote, is only 1.5 mm length with more than 70% of genes highly homologous to human genes. Its metabolic pathways of lipid biosynthesis are well-conserved and has a well-defined lipid metabolism network [13, 14]. *C. elegans* is in advantages of short life cycle, low food intake, simple cultivation, and independent survival [15]. Therefore, it is economically feasible and scientifically reasonable to investigate the effects of OPL and OPO on human health with high purity standards of up to 99% and *C. elegans*. Several studies have reported the effects of lipid nutrition on health efficacy and its mechanisms based on *C. elegans* models, including analysis of the effects of linoleic acid and conjugated linoleic acid on fat accumulation and activity [16], comparison of the effects of natural and synthetic medium/long-chain structured TAGs on lifespan [17], and elucidation of the benefits of membrane lipids in maintaining homeostasis [14]. Therefore, *C. elegans* is a reliable and promising in *vivo* model organism that provides significant advantages for investigating lipid nutrition.

OPL and OPO are the most abundant structural TAGs in human milk, and differences in their configurations affect lipid digestion, absorption, and metabolism in infants. However, their contents and ratios in infant formula remain scientifically unverified, particularly concerning Chinese human milk. Further research on the potential functionalities of different contents and ratios of OPL and OPO is still required, which will help guide the application of OPL and OPO in future Chinese infant formula. This study exposed juvenile *C. elegans* (L1–L4) to varying OPL and/or OPO concentrations to analyze changes during L4 and adulthood, assessing the impact of early nutritional intervention on short- and long-term health and lipid metabolism, which could provide scientific basis for regulation improvement and product innovation of infant formula and even for early-life nutrition guidance. Clarifying the specific nutritional effects of different contents and ratios of OPL and OPO is important strategies to ensure the rational

application of OPL in infant formula. It is also a fundamental guarantee to innovate infant formula based on Chinese human milk fat characteristic.

2. Materials and Methods

2.1 Chemicals and Reagents

Tryptone, yeast, NP40, and all reagents used for mass spectroscopy analysis were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Peptone was acquired from Becton Dickinson and Company (Franklin Lakes, NJ, USA). The standards used for lipidomics were obtained from Avanti Polar Lipids Inc. (Alabaster, AL, USA), Larodan AB (Sweden), and Shanghai ZZBIO Co., Ltd. (China), and were consistent with those used in previous studies [18]. The purity of OPL and OPO was usually between 50% and 80%. High-purity standard of OPL and OPO were selected to prepare a precise proportion of OPL and OPO mixture as structural lipid food sources of *C. elegans* in this study. The purity of both OPO and OPL is over 99%, and their mass numbers are 857.38 and 859.39, respectively. OPL and OPO belong to research grade lipids and were purchased from Larodan AB (Solna, Sweden).

2.2 Strains and Culture Conditions

The wild type *C. elegans* N2 and *E. coli* OP50 strains were provided by the Caenorhabditis Genetics Center (CGC) at the University of Minnesota (Minneapolis, MN, USA). *C. elegans* at the L1–L4 stages were placed in nematode growth media (NGM) with OPL- and/or OPO-containing *E. coli* OP50 as the food source. *C. elegans* were age-synchronized using a synchronization process prior to OPL and/or OPO diet experiments [19]. The concentrations of OPL and/or OPO coated onto the NGM are listed in Table 1, with one control group and eight experimental groups. The rationale for choosing the specific concentrations and mixing ratios of OPL and/or OPO depended on the reported literature and pre-experiments (Table S1-S2). *C. elegans* was subjected to further experiments after the intake of OPL and/or OPO to the L4 stage.

Table 1 Concentration of OPL and OPO in NGM

Group	Concentration (mg/L)	
	OPL	OPO
A: Control	/	/
B: Low OPL	4	/
C: Medium OPL	10	/
D: High OPL	40	/
E: Low OP	/	4
F: Medium OPO	/	10
G: High OPO	/	40
H: OPL > OPO	6.7	3.3
I: OPO > OPL	4	6

2.3 Physiological Parameters

2.3.1 Body Length and Width

Different concentrations of OPL and/or OPO along with *E. coli* OP50 were mixed and spread at the center of the NGM. Age-synchronized worms were maintained on NGM plates until the L4 stage. The length and width of the worms were observed and photographed using an SME-168 stereo microscope

(Motic, Xiamen, China). Approximately 50 worms from each group were captured, and the experiments were performed in triplicate.

2.3.2 Lifespan Assay

The lifespan assay was performed as previously described [19], with minor modifications. Briefly, synchronized worms were treated on NGM plates with *E. coli* OP50 and different concentrations of OPL and/or OPO from stages L1 to L4. Subsequently, approximately 200 worms were transferred to NGM plates containing only *E. coli* OP50. After transfer, movement and physical integrity were assessed, and abnormal worms were excluded. Subsequently, the plates were inverted and incubated in a biochemical incubator at 20 ℃, with the initial date recorded as day "0." Worms were transferred to fresh plates coated with OP50 at approximately 24 h intervals during the first 7 d of the lifespan experiment to allow the worms to be separated from their offspring. From day 8, the worms were transferred to new plates at 3-d intervals. The survival status of the worms was recorded at each transfer, including alive, dead, or vanished, until all the worms were dead. Worms that did not respond to the mechanical stimuli were scored as dead. Worms that were still alive but had abnormal survival behaviors, including hatching of live progeny inside the hermaphrodite, vulva protrusion, vulva eruption, paralysis or incoordination, and bacterial or fungal contamination, were removed from the analysis.

2.3.3 Body Bending and Head Swinging Tests

C. elegans were maintained with various concentrations of OPL and/or OPO from stages L1 to L4, and then body bending and head swinging tests were performed [19]. Briefly, the frequency of body bending and head swinging of worms was recorded under an SME-168 stereo microscope (Motic, Xiamen, China) after 1 min of free movement. Body bending frequency was determined as the number of body bends within 20 s. Single-body bending is defined as a shift of one wavelength in the direction of the worm relative to the long axis of the body. Worms were moved along the X-axis, observing changes in the posterior pharyngeal part along the Y-axis. The head swinging frequency was defined as the number of head swings within 1min [20]. A successful head swing was defined as the worm's head swinging back after swinging from one side to the other. Each group contained approximately 50 worms, and the experiments were performed in triplicate.

2.4 Stress Resistance Assay

2.4.1 High-Sugar Assay

High-sugar NGM was obtained by adding 10 mmol/L glucose during the preparation of NGM [19, 21]. The control and eight OPL and/or OPO supplementation groups are designated as Table 1. OPL and/or OPO supplementation and *E. coli* OP50 were evenly dispersed in the center of the high sugar NGM. The age-synchronized worms were maintained on high sugar NGM plates until L4 stages. The length and width of the worms were observed and photographed under SME-168 stereo microscope (Motic, Xiamen, China). Approximately fifty worms from each group were captured, and experiments were performed in triplicates.

2.4.2 Heat Stress Assay

Age-synchronized worms were maintained on control and experimental NGM plates (Table 1). The heat stress assay was conducted based on a previously published procedure with some modifications [19, 22]. When worms grew to L4 stage, different groups of worms were placed in an incubator at 37 ℃ for 2 h and then transferred to an incubator at 20 ℃ to recover. The survival status of the worms was observed after 24 h and 48 h of recovery. The experiments were conducted in triplicates and each group contained 50 worms.

2.4.3 Ultraviolet (UV) Stress Assay

Age-synchronized worms were maintained on control and experimental NGM plates (Table 1). When worms reached the L4 stage, they were exposed to UV light for 24 h and then transferred to an incubator at 20 ℃ for continued cultivation [23]. The survival status of the worms was observed once daily until all worms died.

2.4.4 Oxidative Stress Assay

The antioxidant activity of *C. elegans* fed OPL and/or OPL was assessed using a lifespan assay. Age-synchronized worms were placed on NGM plates with OPL and/or OPL at different concentrations (Table 1) until reaching L4. NGM surfaces were evenly coated with 30% H_2O_2 (30% H_2O_2: NGM = 1:1000, v/v) [24, 25]. The worms were then transferred to NGM plates. The survival of the worms was monitored at 20-min intervals until all worms were dead. Experiments were performed in triplicate, with approximately 30 worms per group, lifespan curves were drawn using Prism 10.

2.5 Lipidomics

2.5.1 Sample Collection

C. elegans was cultured up to the L4 stage according to the protocol described in Section 2.2. Subsequently, the NGM plates were rinsed using a 0.01 mol/L phosphate buffer saline (PBS) solution to collect worms. The collected worm suspensions were centrifuged at 3,000 r/min for 3 min at 4 ℃ before removing the supernatant. The centrifuged worms were then re-suspended in PBS and re-centrifuged under the same condition, this process was repeated three times. The collected worms were snap-frozen in liquid nitrogen for 15 min and then transferred to a −80 ℃ refrigerator for future use.

2.5.2 Lipid Extraction

The collected worms were weighed and diluted 20-fold with ultrapure water. Subsequently, the diluted worm solution was mixed with zirconia beads, ground in a grinder for 25 s, and then frozen at −80 ℃ for 10 min. This process was repeated three times to ensure that the worms were evenly mixed with the purified water. The procedure of lipid extraction was performed according to a previously described protocol description [26], with minor modification. Briefly, the standards, including TAG 16:0-18:1-18:1 (d5), phosphatidylcholine (PC) 14:1-17:0, phosphatidylethanolamine (PE) 14:1-17:0, phosphatidylinositol (PI) 14:1-17:0, phosphatidylserine (PS) 14:1-17:0 phosphatidylglycerol (PG) 14:1-17:0, sphingomyelin (SM) d18:1-17:0 and ceramides (Cer) d18:2-24:0, were dissolved in methanol–dichloromethane (1:1, v/v) containing 10 mmol/L ammonium acetate (Table S3). Next, 200 μL of worm dilution solution was mixed

with dichloromethane (900 μL), methanol (2 mL), and ultrapure water (200 μL), which was then agitated by shaking for 10 s. Subsequently, an additional 900 μL of dichloromethane and 200 μL of ultrapure water were added, followed by centrifugation at 3,300 × *g* for 15 min to separate the organic and aqueous phases. The organic phase was then mixed with 600 μL of dichloromethane, 2.2 mL of methanol, and 1 mL of ultrapure water. This mixture was then centrifuged at 3,000 × *g* for 10 min to obtain the organic phase. The aqueous phase was mixed with 1.8 mL dichloromethane and centrifuged at 3,300 × *g* for 15 min to obtain the remaining organic phase. The two fractions of the organic phase were dried under nitrogen gas flow and dissolved in 1 mL of methanol–dichloromethane (1:1, v/v) containing 10 mmol/L ammonium acetate. The extracted lipids were used directly for PLs and glycerolipids (GLs) analysis.

2.5.3 Lipid Detection

GLs and PLs were separated and detected using ultra- performance liquid chromatography/quadrupole-time-of-flight mass spectrometry (AB Sciex, 6600), as previously described [27]. The conditions for liquid chromatography and mass spectrometry were consistent with the published protocols [27]. GLs and sphingolipids were detected in positive ionization mode, whereas glycerol phospholipids were identified in negative ionization mode.

2.5.4 Qualitative and Quantitative Analysis

Qualitative and quantitative analyses of PLs were performed using Pearkview (version 2.2) and Multiquant (version 3.0.2) software. Glycerophospholipids were identified based on the adductive ion mass, head group fragments, and fatty acyl residues in negative ion mode. The mass of the parent ion and a characteristic fragment ion were used to detect sphingolipids, such as m/z 184 for SM and m/z 264 or 266 for Cer. Qualitative and quantitative analyses of GLs were performed using fragment ions and independent mass spectrometry data analysis (MS-DIAL) software. Positively charged $[M + NH_4]^+$ and $[DAG]^+$ (or $[M - RCOO + H]^+$) fragments were used to identify the TAG species. Regression equations were used to quantify the PLs and GLs concentrations, as shown in Table S4.

2.6 Multi-Structured Illumination Microscopy (Multi-SIM) Super-Resolution Microscopy

Age-synchronized worms were maintained on control, medium OPO, medium OPL, OPL>OPO, and OPO>OPL NGM plates from L1–L4, respectively. A maximum number of L4 worms were collected in 1.5 mL microcentrifuge tubes and washed with PBS containing 0.01% Triton X-100. The worms were allowed to settle under gravity for 10 min, and the supernatant was discarded. The worms were fixed with 150 μL of 50% isopropanol at 25 ℃ for 10 mins, then the supernatant was discarded, leaving 25 μL of 50% isopropanol and the worms. Subsequently, these worms were labeled with 125 μL of freshly prepared Liss Rhod PE (10 μg/mL, Merck) and Lipi-QA (10 μg/mL, Sunbloss) staining solution in a dark room for 4 h. After gravity settlement, 125 μL of dye working solution was removed, leaving 25 μL of solution and the worms. Subsequently, the samples were washed three times with 1 mL of M9 buffer. Next, the samples were fixed with a post-fixation solution (SunBloss, HXIE-2) for 10 min, and the post-fixed worms were washed

again three times with 1 mL of M9 buffer. The worms were evenly and flatly spread on glass coverslips (SunBloss, STGBD-035-1) for high-quality super-resolution SIM imaging.

TIRF-SIM/GI-SIM/Single, Slice-SIM/Stacked, and Slices-SIM/3D-SIM images of worms were acquired on a Multi-SIM imaging system (NanoInsights) with a 100X/1.49 NA oil objective (Nikon CFI SR HP Apo), semiconductor lasers (405 nm)/solid-state lasers (488 nm and 561 nm) and a CMOS (Complementary Metal-Oxide-Semiconductor) camera (Photometrics Kinetix). SIM image were reconstructed using SI-Recon 2.23.3 (NanoInsights) with a pixel size of 30.6 nm, channel-specific optical transfer functions, Wiener filter constants of 0.01 (2D) and 0.005 (3D), and background subtraction of negative intensities. The reconstructed SIM image was then denoised using total variation constraint. Pixel registration was corrected to be less than 1 pixel for all channels using 100 nm fluorescence beads.

2.7 Statistical Analysis

Lifespan analysis was conducted using the Kaplan–Meier method, and *P*-values were calculated using log-rank tests. When compared with the control group, $P < 0.05$ was marked with "*", $P < 0.01$ was marked with "**" and $P < 0.0001$ was marked with "****" in Fig. 2 and Fig. 3C–3H. The statistical methods employed included one-way ANOVA with Duncan's multiple tests for instances of equal variance, Tamhane's T2 test for cases of unequal variance, and a nonparametric test when the distribution was not normal. The results are presented either as the mean ± standard deviation or as a percentage. Statistical significance was set at a threshold of $P < 0.05$. The PLs and GLs were determined at a molecular resolution via multiple testing in MetaboAnalyst (https://www.metaboanalyst.ca/). Statistical significance was set at an AVOVA *P* -value (FDR) < 0.05.

3. Results and Discussion

3.1 Health Status of C. elegans Cultured with OPL and/or OPO

3.1.1 C. elegans Growth Ability Cultured with OPL and/or OPO

Body length and width are important parameters for evaluating the growth and developmental abilities of *C. elegans*. The changes in *C. elegans* body length and width are shown in Fig. 1. *C. elegans* showed a significant increase in body length in the medium-dose OPL groups and in the low-, medium-, and high-dose OPO groups compared with control group, which were marked different uppercase letters in Fig.1. Additionally, the body length of the worms was significantly longer in the low-dose OPO group than in the low-dose OPL group; however, there was no significant difference between the middle- and high- dose OPO and OPL groups. The increase in *C. elegans* body length was most significant ($P < 0.001$) with OPL and OPO mixed feeding compared with control group, which was significantly higher than that with the control and OPL or OPO alone feeding; however, the different proportions of OPL and OPO had no significant effect on the body length of *C. elegans*. These results indicate that even though either OPL or OPO could improve the body growth of worms, the mixed feeding of OPL and OPO had the better effect. Interestingly, mixed feeding of OPL and OPO had no significant effect on the body width of *C. elegans*. Among the

different treatments, only the medium-dose OPL and low- and medium- dose OPO groups exhibited greater body widths than the control group. Therefore, the effects of OPL and OPO exposure on worm body length were more obvious than those on body width. The changes in the body length of *C. elegans* were influenced by the feeding doses of OPO or OPL. When the added amounts of the structured lipids were 0, 4, and 8 mg/L, the body length of *C. elegans* increased with the amount of OPL or OPO added. Based on linear analysis, the R^2 values of OPL and OPO were 0.9426 and 0.8596, respectively, indicating that the concentrations of OPL and OPO could respectively explain 94.26% and 85.96% of the variation in body length. When the concentration of OPL or OPO increased to 40 mg/L, the body length of *C. elegans* began to decrease. This phenomenon indicates that the promoting effect on *C. elegans'* body length is restricted by high concentrations of OPL or OPO.

Feeding OPO alone has a better effect than OPL on body length, whereas OPL and OPO mixed feeding yields a more favorable result, significantly promoting the growth of body length in *C. elegans* without increasing body width.

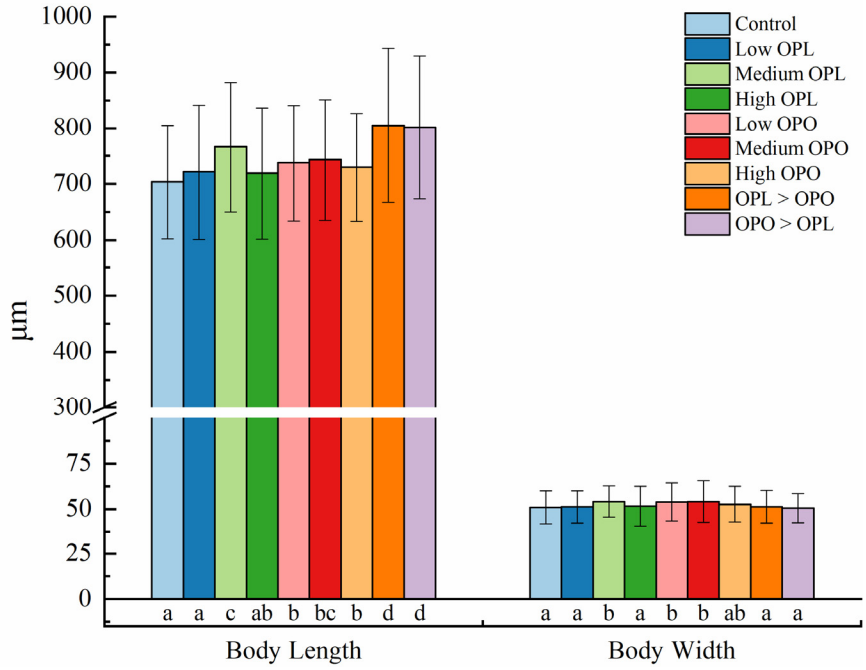

Fig. 1. Effects of OPO and/or OPL with different concentrations and proportions on C. elegans. body length and width. Different lowercase letters represent significant ($P < 0.05$) differences in body length and width.

3.1.2 Effect of OPL and/or OPO on C. elegans Lifespan

The lifespans of *C. elegans* grown on the control NGM and those supplemented with different concentrations of OPL and/or OPO were compared (Fig. 2). Feeding worms with high concentrations of OPL significantly reduced ($P < 0.05$) the lifespan of *C. elegans*, whereas low and medium concentrations of OPL had no effect on lifespan. However, different concentrations of OPO increased the lifespan of *C. elegans*, and a medium concentration of OPO had a significant effect ($P < 0.01$) on lifespan (Fig. 2 Control vs. OPO). Feeding with a different proportion of OPL and OPO did not affect the lifespan of *C. elegans*. Moreover, the median survival of *C. elegans* was 21 d in the control and OPL and OPO mixed feeding

groups. However, the median survival of *C. elegans* was 23 d in the medium- and high-concentration OPO groups. These results indicated that OPO is beneficial for prolonging the lifespan of *C. elegans*.

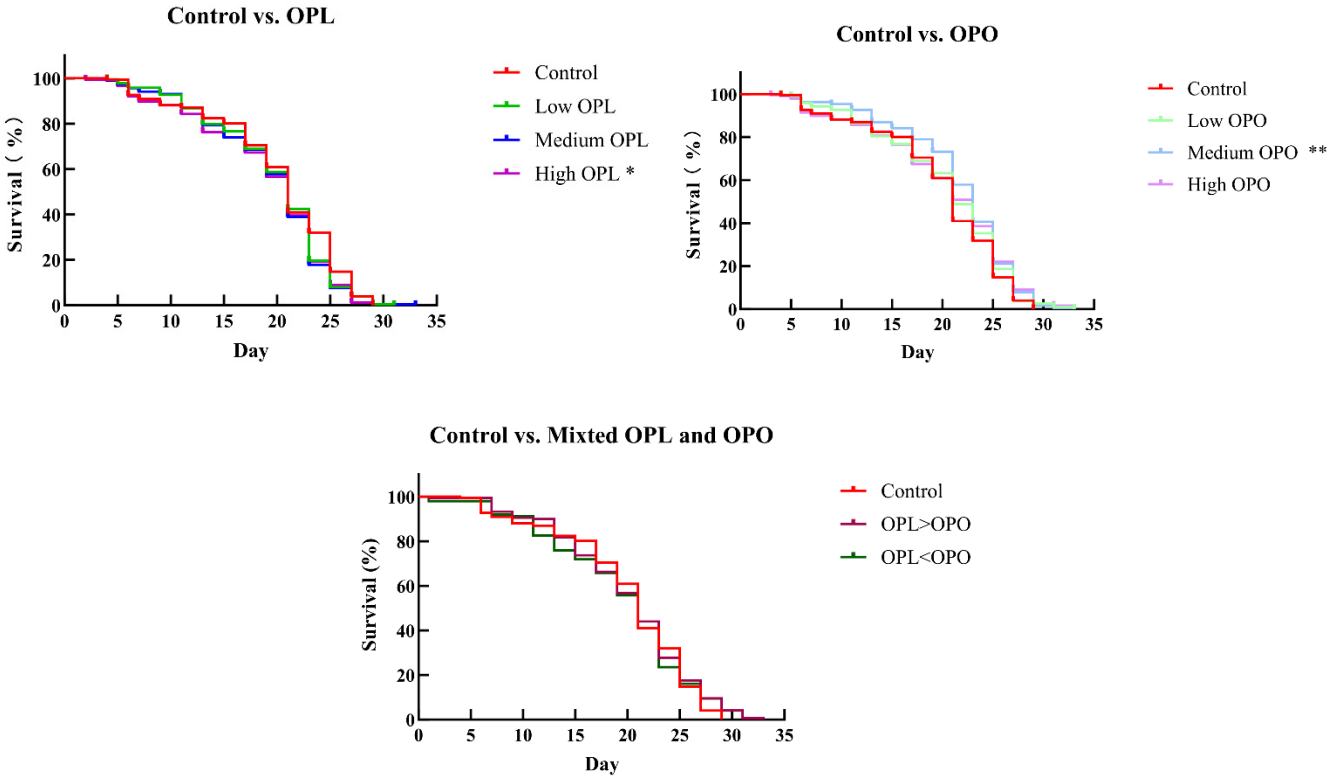

Fig. 2. Survival proportions of C. elegans grown on control NGM and NGM supplemented with different concentrations of OPL and/or OPO. The "*" represent $P < 0.05$ and "**" represent $P < 0.01$, when compared with the control group.

Dietary mono-unsaturated fatty acids (MUFAs) are linked to longevity in several species because they not only upregulate the number of lipid droplets and peroxisomes, which are critical for MUFA-induced longevity in *C. elegans*, but also modify the ratio of membrane lipids to ether lipids, a feature associated with reduced lipid oxidation [28, 29]. Moreover, polyunsaturated fatty acids (PUFAs) contain more double bonds than saturated fatty acids (SFAs) and MUFAs and are therefore more susceptible to peroxidation, which in turn leads to the propagation of reactive oxygen species and further damage to proteins and nucleic acids [30]. The longevity mutants of *C. elegans* were found to have increased MUFAs and decreased PUFAs [30]. Another study found that the lifespan of *C. elegans* cultured with large amounts of fish oil was shortened significantly, whereas an appropriate amount of fish oil extended their lifespan significantly [31]. These may explain why the lifespan of *C. elegans* was significantly inhibited by a high concentration of OPL, but promoted by a medium concentration of OPO, with no effect observed for the combination of OPL and OPO. Additionally, the maximum lifespan of *C. elegans* was higher in the experimental groups than that in the control group, demonstrating the potential of OPL and/or OPO to extend the maximum lifespan.

Overall, the effects of OPL and OPO on the lifespan of *C. elegans* are different. OPO is beneficial, whereas high concentrations of OPL have an inhibitory effect. The differential effects of OPL and OPO on *C. elegans* lifespan may be related to the differences in metabolites after the intake of OPL and OPO.

3.1.3 Effect of OPL and/or OPO on C. elegans Locomotory Behavior

The motility of *C. elegans* is an important indicator of its health statue. The body bends and head swings of *C. elegans* fed *E. coli* OP50 and *E. coli* OP50 supplemented with different concentrations of OPL and/or OPO are present in Table 2. Low and medium concentrations of OPL and OPO significantly increased body bending and head swinging frequencies, respectively. However, high concentrations of OPL or OPO significantly inhibited the motility of *C. elegans*. When worms were supplemented with the same concentrations of OPL or OPO, OPO supplementation had a more significant promotional effect on both body bending and head swinging. When worms were treated with a mixture of OPL and OPO, they moved more actively in the presence of a higher concentration of OPO than that of OPL. These results indicate that OPO structured lipids are more effective at promoting the movement of *C. elegans*. Among the different treatments, we found that at low concentration of OPO caused the most significant increase in body bending and heading swinging frequencies in worms, with a 57.23% increase in body bending and a 21.66% increase in heading swinging. Therefore, the low concentration is more suitable for the movement of *C. elegans*. This may be because low concentration of OPO not only enhances the physical condition of *C. elegans*, but also did not interfere with its movement due to the increase in body length. In another perspective, the mixed OPL and OPO treatments had a relatively positive impact on the growth and movement of *elegans*.

Table 2 Body bend and head swing frequencies of *C. elegans* fed *E. coli* OP50 and *E. coli* OP50 supplemented with different concentrations of OPL and/or OPO

	Body Bends	Head Swings
A: Control	6.57 ± 2.10^{C}	49.76 ± 6.63^{b}
B: Low OPL	8.47 ± 3.27^{EF}	56.39 ± 2.27^{d}
C: Medium OPL	7.85 ± 2.35^{D}	49.68 ± 2.96^{b}
D: High OPL	4.73 ± 2.23^{A}	44.11 ± 9.70^{a}
E: Low OPO	10.33 ± 3.58^{G}	60.54 ± 7.52^{e}
F: Medium OPO	8.42 ± 3.37^{EF}	52.13 ± 7.54^{c}
G: High OPO	5.76 ± 2.44^{B}	44.22 ± 7.70^{a}
H: OPL > OPO	7.22 ± 2.63^{DE}	49.03 ± 8.59^{b}
I: OPO > OPL	9.00 ± 2.72^{F}	56.61 ± 7.61^{d}

Note: Different uppercase letters indicate significant differences ($P < 0.05$) in body bending; different lowercase superscript letters indicate differences ($P < 0.05$) in head bending.

3.1.4 Effect of OPL and/or OPO on Stress Resistance

Fig. 3 illustrates the variations in body length and width induced by high sugar, survival rates at 24 h and 48 h following a 2-h treatment at 37 ℃, and the lifespan of *C. elegans* under UV and oxidative stress with different concentrations and ratios of OPL and OPO supplementation. Under high-sugar conditions, the body width of the worms increased significantly ($P < 0.05$, except for the OPL<OPO group), whereas the body length increased significantly only in the low OPL and low and high OPO groups (Fig. 3A). The changes trend in body length and width of worms with OPL and OPO supplementation were not consistent in high-sugar conditions or not, compared with the control group. In detail, the body width of *C. elegant* in the high-sugar environment containing OPL and/or OPO enhanced more significantly, whereas the body length of *C. elegant* in the environment only exposed to OPL and/or OPO changed more noticeably. This might be because the high-sugar environment provided excessive energy to *C. elegans,* thereby further

promoting their obesity under the expose of OPL and/or OPO, without playing a role in regulating metabolism or reducing fat accumulation. OPL and OPO supplementation improved the survival rate of *C. elegans* under heat stress at 37 ℃, and the effect was more significant at 48 h than at 24 h (Fig. 3B). OPL had a more significant effect than OPO at 48 h, but there was no significant difference between the mixed feeding groups (Fig. 3B). Proper supplementation with OPL and/or OPO extended the lifespan of *C. elegans* under UV and oxidation stress. Specifically, low OPL, medium OPO, and OPL>OPO significantly extended lifespan under UV stress (Fig. 3C-3E), while all treatments markedly prolonged lifespan under oxidative stress (Fig. 3F-3I).

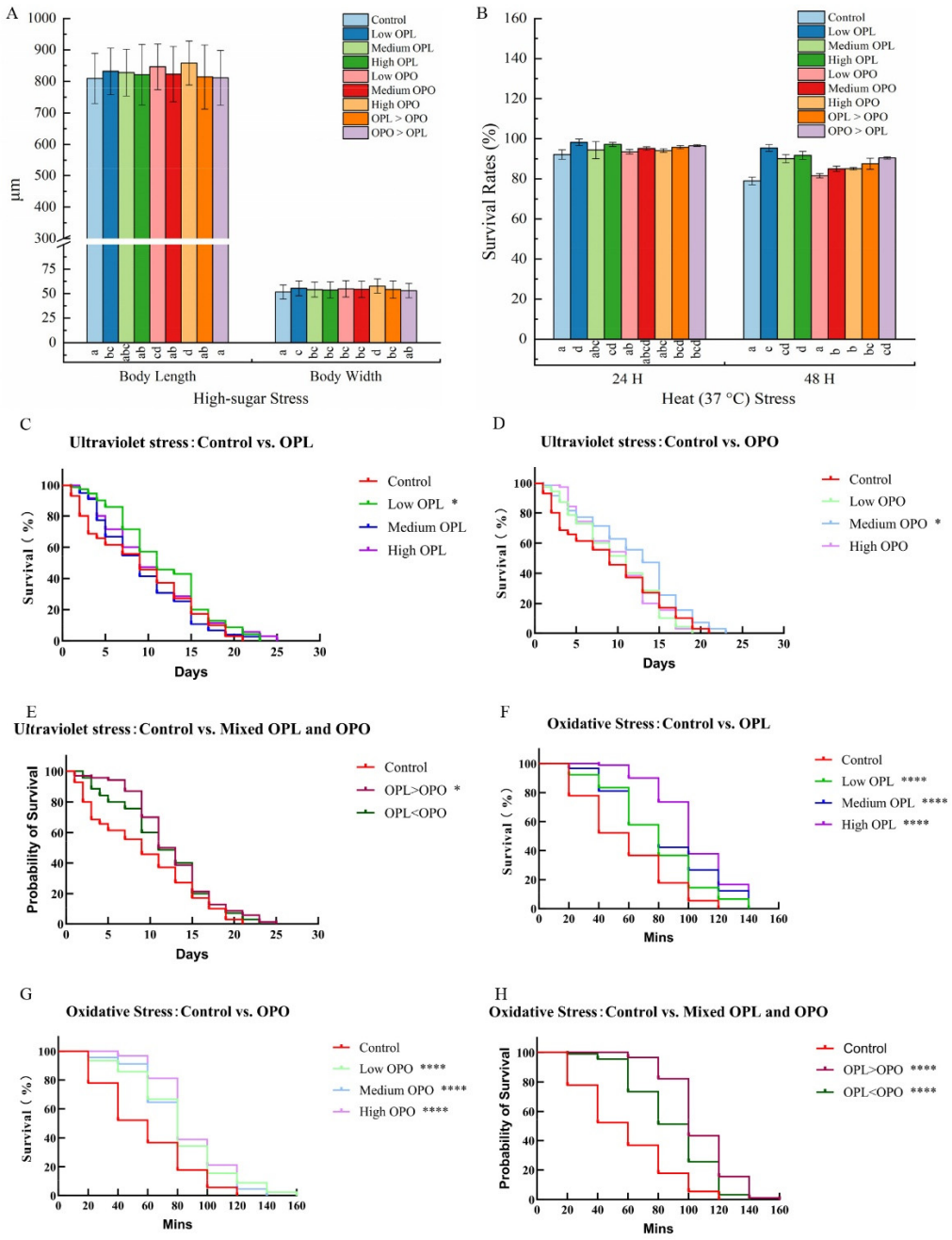

Fig. 3. Stress response of C. elegans under high-sugar, heat (37 ℃), UV and oxidation stress conditions, measured in body length and width (A), survival rates at 24 h and 48 h (B), and lifespan (C–H). Different lowercase letters represent significant ($P < 0.05$) differences in body length and width. The "*" represent $P < 0.05$ and "****" represent $P < 0.0001$, when compared with the control group.

C. elegans fed with a mixture of OPL and OPO exhibited more prominent performance. The OPL < OPO group had a stronger ability to survival (at 48 h), whereas the OPL > OPO group helped to inhibit ultraviolet radiation and oxidative damage (no statistical difference), thus extending lifespan. In conclusion, the preferential effects of different ratios of OPL and OPO were not consistent, but both enhanced the stress resistance of *C. elegans*.

3.2 Lipid Profiling of C. elegans Cultured with OPL and/or OPO

Lipids serve critical functions in *C. elegans*, acting as structural components of membranes, signaling molecules, and energy storage in TAGs [32]. Lipidomics enables comprehensive analysis of the tens of thousands of lipid species, which vary with physiological, pathological, and environmental conditions [33]. Changes in *C. elegans* food source affect lipid subclass composition and content as well as lipid molecule composition and content, altering physiological traits.

Overall, 186 lipid molecular species were detected in *C. elegans* from the control group. The number of lipid species increased in *C. elegans* fed different concentrations of OPL and/or OPO, and the number of PE, PS, and DAG species increased significantly ($P < 0.05$, Table S5). In addition, the number of molecular species was generally higher in the OPL < OPO group than in the control and other treatment groups (Table S5). The TAG species in *C. elegans* were abundant, representing approximately 42–50%, followed by PE, SM, DAG, PC, PG, PS, PI, Cer, LysoPG, MAG, and other lysophospholipids (Table S5). The quantitative results of the lipid subclasses in *C. elegans* showed that feeding OPL and/or OPO to *C. elegans* increased TAG and DAG levels in *C. elegans* (except for the low OPO group). Moreover, the concentration of TAG in *C. elegans* increased with the enhance feeding concentration of OPL or OPO in this study. The main PLs in *C. elegan*s were PC, PE, PS, and SM, followed by PI, PG, LPE, and LPC, whereas the LPI, LPG, LPS, and Cer contents were relatively low (Fig. S1), consistent with previous results [32, 34].

3.2.1 Change in Polar Lipids in C. elegans Cultured with OPL and/or OPO

PLs are important components of biological cell membranes and play crucial roles in various cellular functions. *C. elegans* obtains the majority of its membrane lipids by modifying the fatty acids present in its diet [14]. The metabolic pathways of membrane lipid biosynthesis are well-conserved across the animal kingdom, with glycerophospholipids produced by the cytidine diphosphate diacylglycerol (CDP-DAG) and Kennedy pathways and sphingolipids synthesized by different pathways in *C. elegans* [14].

Feeding *C. elegans* with different concentrations and ratios of OPL and/or OPO increased the concentration (from 65.07 mg/L to 125.27–291.30 mg/L) and proportion (from 25.95% to 31.55–48.32%) of PE in *C. elegans*, making it the most abundant PLs, surpassing PC (Fig. S1). PLs containing PUFAs in *C. elegans* play an important role in maintaining growth and adapting to environmental changes [34]. PE was the main carrier of phospholipid PUFAs; therefore, the increase in PE may contribute to the improvement of physiological function in body length and resistance to heat, UV, and oxidative stress. Furthermore, the concentrations of PS and PC in the experimental group also significantly increased, except for the low-dose group (Fig. S1). Dietary supplementation with PS increases reactive oxygen species and the expression of

stress-responsive genes, thereby improving anti-oxidative stress and exercise ability and prolonging the lifespan of *C. elegans* [24]. Additionally, desiccation stress depletes PC in cell membranes and enhances their interaction with trehalose to obtain a stronger hydration-induced gain in acyl chain free volume and a wider spread of structural relaxation rates of their lyotropic transitions and sub-headgroup H-bond interactions [14, 35]. Theoretically, the increase of PE, PC and PS in *C. elegans* would improve its growth, exercise and stress resistance. However, only the mixed feeding of OPL and OPO was both significant in body length, body bends, head swings, heat stress, and oxidative stress.

The changes in PL species in *C. elegans* fed *E. coli* OP50 containing OPL and/or OPO are shown in Fig. S2. Partial least squares discriminant analysis (PLS-DA) revealed distinct separation between control and experimental groups, with medium OPO and OPL<OPO groups differing from the others. PE and LPE were the main contributors to this variation, because PE and LPE accounted for 17 in the top 25 influencing factors based on the VIP score (Fig. S2B). The experimental group showed a general increase in the top 25 different PL molecules, particularly PE 36:5, PE 38:6, PE 36:4, PE 34:4, and PE 40:4. A heatmap (Fig. S2C) displays 73 significantly altered PL species, including PE 36:6, PE40:5, PE 38:6, PE 36:4, PE 38:5, PC 36:4, PI 38:6, PI 36:5, PC 36:6, PS 38:5, PC 36:5, LPE 20:4, and LPE 20:5, some of which were significantly higher in the experimental groups than in the control group. These PL molecules also contain PUFAs such as C20:5 (eicosapentaenoic acid, EPA), C20:4 (arachidonic acid, AA, eicosatrienoic acid, ETA), C18:3, or C18:2. PE containing EPA, such as PE 36:5, PE 38:6, PE 36:6, and PE 38:5, are precursors of eicosapentaenoyl ethanolamide that promote larval growth [36]. PUFAs supplementation preferentially diverts fat to somatic tissues over the germline in adult *C. elegans*, thereby increasing somatic resilience, improving stress responses, and improving survival [36, 37]. In response to this signal, PUFAs are released from the membrane by phospholipases and are further metabolized to form powerful short-range signaling molecules, collectively known as eicosane compounds. This might be linked to the fact that phospholipid PUFAs promote the growth of *C. elegans* and enable it to adapt to environmental changes, thereby prolonging its body length and enhancing its stress resistance [34].Therefore, the changes in the growth, lifespan, movement, and stress response abilities of *C. elegans* after OPL and/or OPO intervention may be related to the content and fatty acid saturation degree of PLs in its body.

The clustering correlation between the control and experimental *C. elegans* PL molecules showed that low, medium, and high concentrations of OPL and OPO were clustered, and the OPO and OPL mixed feeding groups were clustered. This indicates that the PLs in each group were mainly affected by the concentration of OPL and OPO, followed by their composition. In general, the significantly altered PL molecules in *C. elegans* were elevated in concentration, and the overall change in content was related to the supplementation dosage. Specifically, the content of LPE 18:0, PC 36:5, PC34:1, PC38:3, and LPE20:2 significantly increased in OPL>OPO and OPL<OPO groups, which was consistent with the significant increase in the body length of *C. elegans* in the same group. Whether these phospholipid molecules might be related to the rapid growth of the worms still needs to be explored. The content of LPE and PC molecular

species significantly increased in the OPL<OPO group. PC promotes lipid metabolism, emulsification, and excretion, thereby reducing lipid accumulation, which may be explain the reason why the body width of *C. elegans* in OPL<OPO group did not show significant changes under high sugar feeding. These results indicated that mixed feeding of OPL and OPO improved the physiological functions of *C. elegans*, which may be related to the variations in the compositions and contents of PLs, but the specific dose-response relationship and the underlying mechanism still require further exploration.

3.2.2 Change in GLs of C. elegans Cultured with OPL and/or OPO

Lipids are primarily stored in the form of TAGs in droplets in the gut granules, hypodermal cells, and germ line of *C. elegans* [38, 39]. The concentration of DAG increased significantly (Fig. S3B). At the lipid subclass level, the content of DAGs significantly increased from 100.32 mg/L in the control group to 169.07–309.47 mg/L in the experimental groups (Fig. 4). DAG not only controls many cellular processes mediated by neurotransmitters, growth factors, and hormones through the activation of protein kinase C, but also controls neurotransmission and certain transient receptor potential cation channels through the activation of the synaptic vesicle initiating protein UNC-13 [40]. After feeding with OPL and/or OPO, the significant increase in body length, stress ability, and frequency of body bending and head swinging may be related to the significant increase in DAG content and proportion. The correlation analysis revealed that the changes in DAG content in *C. elegans* were consistent with the trend of body length changes. The correlation coefficients between OPL and OPO groups were 80.07% and 60.69%, respectively, indicating a high degree of correlation between the two.

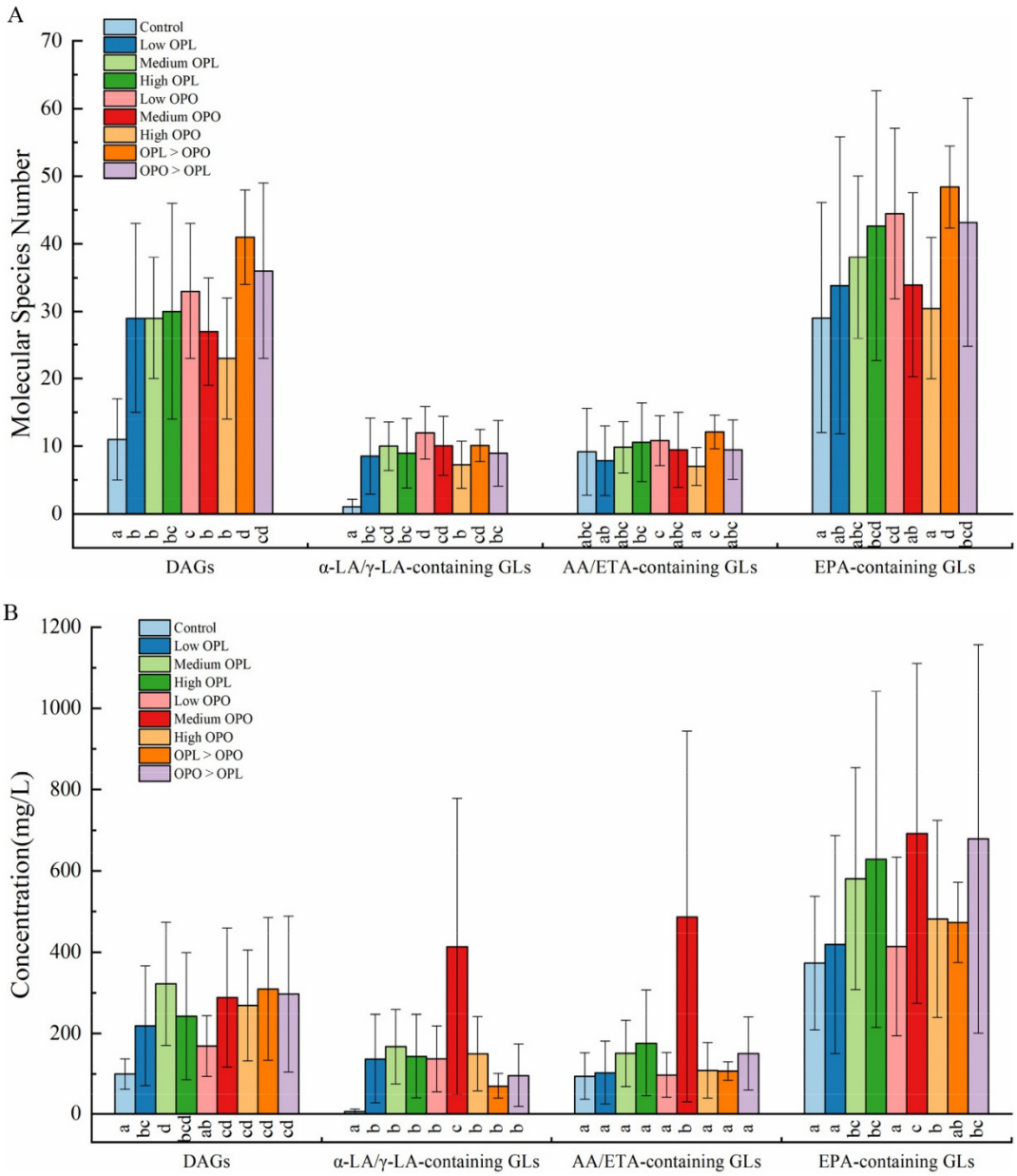

Fig. 4. Molecular species number (A) and concentration (B) of DAGs and fatty acid-containing TAGs in C. elegans fed *E. coli* OP50 and *E. coli* OP50 supplemented with different concentrations of OPL and/or OPO. α-LA, α-linolenic acid; γ-LA, γ-linolenic acid. Different lowercase letters represent significant ($P < 0.05$) differences in DAGs and certain fatty acid-containing GLs.

The molecular species and concentrations of GLs containing linolenic acid in *C. elegans* were significantly enhanced by treatment with OPO and/or OPL (Fig. 4). Unlike humans and most other animal species, *C. elegans* can use FAT-1 ω-3 desaturase to convert 18-carbon and 20-carbon ω-6 fatty acids into ω-3 fatty acids [30]. Moreover, FAT-3 ω-3 desaturase also can convert ω-6 linoleic acid into ω-6 γ-linolenic acid (GLA) [30]. Therefore, the enhanced molecular species and concentrations in GLs containing linolenic acid may be attributed to FAT-1 ω-3 and FAT-3 ω-3 desaturase activated by the increased oleic acid and linoleic acid levels in the body. Notably, α-linolenic acid (α-LA) treatment can activate the nhr-49/PPARα transcription factor, extending the lifespan of *C. elegans* [41]. Similarly, treating *C. elegans* with medium concentration of OPO resulted in a very significant increase in the contents of α-LA/γ-LA-containing GLs

(Fig. 4B). This was accompanied by a notable extension of lifespan, suggesting a potential correlation that requires further investigation using nhr-49/PPARα and FAT-1 mutants for verification. EPA-containing GL levels were higher in medium and high-concentration OPL or OPO groups, as well as in the OPL<OPO group, compared to the control and low concentration OPL and OPO groups. Omega-3 and omega-6 PUFAs, particularly EPA, contribute to growth, development, movement, neuronal function, reproduction, fitness, and cognition in *C. elegans* [36, 42]. Therefore, dietary supplementation with OPO and/or OPL should have a positive effect on GL composition in *C. elegans*.

Changes in GL species in *C. elegans* after being fed OPL and/or OPO are shown in Fig. S3. Similar to the PL analysis, the PLS-DA results showed significant differences between the control and experimental groups, indicating that the diet also changed the fatty acid composition and content of GLs in *C. elegans*. The top 25 differential GL molecules were found to be rich in odd-numbered carbon fatty acids C17:1 and C19:1 (or C17Δ and C19Δ), unsaturated fatty acids C18:1 and C18:2, and functional fatty acids C20:4 and 20:5 (Fig. S3B). This is consistent with the notion that the fatty acids of TAGs in *C. elegans* are mainly composed of dietary fatty acids and their derivatives obtained from *E. coli* and supplements [39]. The liquid chromatography-mass spectrometry cannot distinguish isomers in this study, thereby identifying C17Δ and C19Δ as C17:1 and C19:1, respectively. The abundant C17Δ and C19Δ identified in *C. elegans* are derived from *E. coli* OP50 that is rich in cyclopropane fatty acids (C17Δ and C19Δ) [32]. Similarly, C18:1 and C18:2 are derived from dietary OPL and/or OPO supplements and *E. coli* OP50 [39]. Moreover, *C. elegans* can resynthesize all essential fatty acids, including AA and EPA, using acetyl-CoA carboxylase, fatty acid synthase, and a series of fatty acid desaturases and elongators [30]. However, *C. elegans* lacks the specific elongase required to produce 22-carbon PUFAs [30]. This may explain why *C. elegans* is rich in AA and EPA but contains almost no 22-carbon fatty acids. The heatmap in Fig. S3C shows the 49 GL molecular species with significant differences based on ANOVA analysis. The content of GL molecules containing C17Δ, C19Δ, C18:1, and C18:2 generally increased, but those of TAG 20:5-20:5-20:5 and TAG 20:4-20:5-20:5 decreased significantly (Fig. S3B–S3C). This indicated that feeding OPO and/or OPL altered the concentration and composition of GLs in *C. elegans*. The diet rich in oleic acid and linoleic acid led to more GLs containing these acids in *C. elegans*, while simultaneously resulting in fewer TAGs with three functional fatty acids.

Lipidomics showed that *C. elegans* fed OPO and/or OPL had increased PE, PC, and PS as well as GLs containing LA and EPA; enrichment of these lipid molecules in worms may increase reactive oxygen species in their body, which actually increased resistance to oxidative stress through the process of stress-induced hormesis [24, 30]. This may also explain why high OPL concentrations shortened *C. elegans'* lifespan and medium OPO concentrations extended *C. elegans'* lifespan, whereas OPO and/or OPL treatment both prolonged the lifespan of *C. elegans* under oxidative stress. In conclusion, supplemented OPO and/or OPL to *C. elegans* changed the composition and content of GLs and PLs in *C. elegans*, and these changes were

related to *C. elegans* physiological function, but the specific regulatory mechanism and dose-effect relationship still need to be further explored.

3.3 Multi-SIM Profiling of C. elegans cultured with OPL and/or OPO

The merged images of *C. elegans* obtained through multi-SIM are shown in Fig. 5, in which the green or blue globules are GLs stained with Lipi-QA, and the diffuse red regions are PLs stained with Liss Rhod PE. GLs were mainly found in the intestinal tissue, hypodermal cells, and germ line in the form of dispersed lipid droplets, which is consistent with published research [38, 43]. GLs are surrounded by a phospholipid monolayer mainly composed of PC and PE; ACS-4, as a lipid droplet-associated protein, can bond to the surface of lipid droplets and revealed a ring pattern in live imaging, consistent with lipid droplet surface localization [43]. These results are not completely consistent with the imaging in this study. PLs were found throughout the *C. elegans* body, with red fluorescence dispersed across the body in this study (Fig. 5). As a living organism, the membrane structures of *C. elegans* throughout the body. The fluorescent probe Liss Rhod PE selected in this study can bind to all PLs components. Therefore, red fluorescence (PLs) was distributed throughout the body of *C. elegans*, and no typical spherical membrane structures were observed in imaging studies. However, the main components of membrane are still PE and PC, which is consistent with the results in 3.2. The 3D images more accurately depict the distribution of GLs and PLs in *C. elegans* (Fig. 5). Three-dimensional fluorescence imaging showed that as the imaging depth of the sample increased, the fluorescence became weaker; thus, the color of the lipid droplets gradually changed from green to blue (Fig. 5). This fluorescence reduction was primarily due to light absorption, scattering, fluorescence quenching, and optical system limitations.

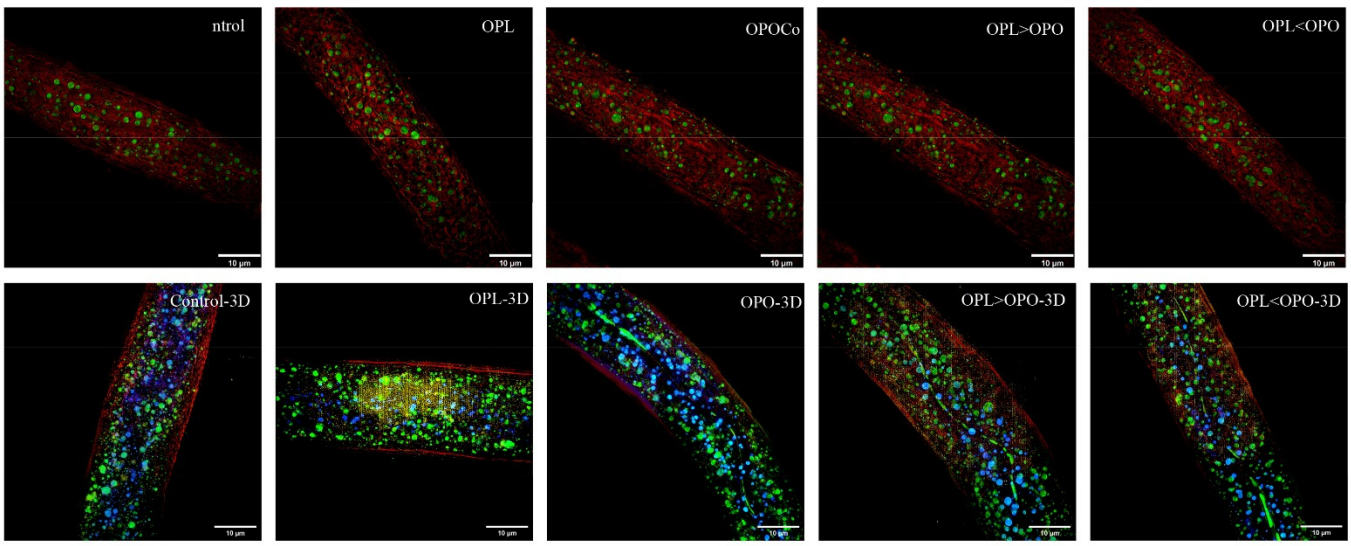

Fig. 5. Stacked-SIM (2D) and 3D-SIM images of C. elegans fed E. coli OP50 and E. coli OP50 supplemented with different concentrations of OPL and/or OPO. Stains: Liss Rhod PE for PLs (red) and Lipi-QA for GLs (green or blue).

Different concentrations and ratios of OPL and/or OPO alter the characteristics of lipid droplets in *C. elegans*. However, it is difficult to make a naked-eye assessment of the differences in lipid droplets among different groups. Therefore, the number, average size, and Feret diameter of lipid droplets were measured to evaluate the effects of different concentrations and ratios of OPO and/or OPL on lipid droplets in *C. elegans*

in the present study. Previous studies have shown that dietary supplementation of the cis-MUFA oleic acid upregulates lipid droplets, extends lifespan, and reduces lipid oxidation in middle-aged adults; moreover, increased numbers of lipid droplets in young or middle-aged individuals predicts their remaining lifespan [28]. These are consistent with the results of this study. Specifically, in terms of physiological characterization, medium concentration OPO prolonged the lifespan of *C. elegans* and obtained the maximum lifespan (shown in 3.12 Section). In terms of lipid droplets, the number of lipid droplets were higher in the medium concentration OPO groups than in the control, medium concentration OPL, OPL>OPO, and OPO>OPL groups as shown in Table 3. In all, OPO increased lipid droplet count, reduced lipid droplet size, and extended lifespan compared to OPL. The average size and Feret diameter of the OPO and OPO>OPL groups were smaller than those of the OPL and OPL>OPO groups, respectively. The smaller lipid droplets increase the specific surface area of lipid droplets, fluidity of adipose tissue, and energy supply rate of lipid metabolism [44], thereby improving the movement ability of *C. elegans*. This may explain why the OPO is more effective than OPL in promoting the movement of *C. elegans*. Compared with the 10 mg/L of OPL and/or OPO groups, the control group had the worst motility but not the largest lipid droplets, which may be related to the improved physical function of *C. elegans* due to OPO and/or OPL supplementation, such as providing energy, thereby enhancing its movement ability. Based on the imaging results, the lipid droplets appear to be nearly spherical. Therefore, the lipid droplet × average size³ and lipid droplet × Feret diameter³ values can be used to assess lipid concentrations in *C. elegans*. The values were significantly higher in the experimental groups than in controls, confirming increased GL content, consistent with the detection results in Section 3.2.

Different concentrations and ratios of OPL and/or OPO altered the number and size of lipid droplets in *C. elegans*, which can to some extent explain the changes in the lifespan and movement ability of worms. Besides, the multi-SIM imaging statistics are consistent with the quantitative results of GLs. In all, feeding *C. elegans* with OPL and/or OPO synergistically alters the physiological characterization as well as lipid composition, content, metabolism, and morphology of *C. elegans.*

Table 3 Lipid droplet of parameters of *C. elegans* fed *E. coli* and *E. coli* supplemented with different concentrations of OPL and/or OPO

Group	Count of lipid droplets	Average size (μm)	Feret diameter (μm)
Control	281	0.28 ± 0.58^{b}	0.58 ± 0.73^{b}
Medium OPL	335	0.28 ± 0.57^{b}	0.61 ± 0.70^{ab}
Medium OPO	417	0.25 ± 0.51^{b}	0.57 ± 0.63^{b}
OPL > OPO	267	0.45 ± 0.87^{a}	0.73 ± 0.87^{a}
OPL < OPO	304	0.29 ± 0.66^{b}	0.60 ± 0.79^{b}

Note: Different lowercase superscript letters indicate significant differences ($P < 0.05$) in average size and Ferret diameter.

4. Conclusion

This study investigated the effects of varying concentrations and ratios of OPO and/or OPL on short- and long- term health outcomes and lipid metabolism in *C. elegans*. Medium concentrations of OPO extended lifespan, whereas high concentrations of OPL reduced it. Mixed supplementation with OPL and

OPO had no significant effect on lifespan, a result likely influenced by fatty acid composition and lipid droplet number. The OPO > OPL group exhibited greater mobility compared to both the equal -concentration group and the control, likely due to higher OPO content and smaller lipid droplets. Although OPL or OPO promoted body growth in *C. elegans*, the mixed OPL and OPO feeding groups (total 10 mg/L) produced the most pronounced growth effect. Additionally, survival rates significantly improved in worms fed the mixed OPO and OPL diet following heat exposure at 37 ℃. While not all treatments showed statistically significant differences, supplementation with OPL and OPO enhanced resistance to ultraviolet and oxidative stress, particularly in the OPL > OPO group. Although the effects varied across physiological indicators, mixed OPL and OPO feeding generally improved physiological outcomes in *C. elegans*. Supplementation with mixed OPL and OPO altered lipid composition, content, and morphology, thereby affecting lipid metabolism and physiological traits. Enhancements in PE, PC, PS, MUFA, PUFA, EPA, and DAG were partially associated with improvements in movement, longevity, and stress resistance. These findings highlight the importance of considering the mixed application of OPL and OPO and their concentration and ratio in the design of infant formula. However, further genomic and proteomic studies are required to elucidate the specific mechanisms underlying OPO and OPL effects.

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Conflicts of interest

The authors declare no conflicts of interest in the submission and publication of this manuscript.

References

[1] T. Wei, A. Mueed, T. Luo, et al., 1,3-Dioleoyl-2-palmitoyl-glycerol and 1-oleoyl-2-palmitoyl-3-linoleoyl-glycerol: Structure-function relationship, triacylglycerols preparation, nutrition value, Food Chem. 443 (2024) 138560. https://doi.org/10.1016/j.foodchem.2024.138560

[2] T. Wei, D. Tan, S. Zhong, et al., 1-Oleate-2-palmitate-3-linoleate glycerol improves lipid metabolism and gut microbiota and decreases the level of pro-inflammatory cytokines, Food Funct. 14 (2023) 5949-5961. https://doi.org/10.1039/d3fo00723e

[3] Q. Liu, J.Y. Zhao, Y. Liu, et al., Advances in analysis, metabolism and mimicking of human milk lipids, Food Chem. 393 (2022) 133332. https://doi.org/10.1016/j.foodchem.2022.133332

[4] S. Wang, C. Zheng, D. Guo, et al., Dose-related effects of early-life intake of sn-2 palmitate, a specific positionally distributed human milk fatty acid, on the composition and metabolism of the intestinal microbiota, J. Dairy Sci. 106 (2023) 8272-8276. https://doi.org/10.3168/jds.2023-23361

[5] Q. X. Chen, Q. G. Xie, C. Q. Jiang, et al., Infant formula supplemented with 1,3-olein-2-palmitin regulated the immunity, gut microbiota, and metabolites of mice colonized by feces from healthy infants, J. Dairy Sci. 105 (2022) 6405-6421. https://doi.org/10.3168/jds.2021-21736

[6] W. Wei, Q. Z. Jin, X. G. Wang, Human milk fat substitutes: Past achievements and current trends, Progress in Lipid Research. 74 (2019) 69-86. https://doi.org/10.1016/j.plipres.2019.02.001

[7] L. Zhu, S. Z. Fang, W. W. Liu, The triacylglycerol structure and composition of a human milk fat substitute affect the absorption of fatty acids and calcium, lipid metabolism and bile acid metabolism in newly-weaned *Sprague-Dawley* rats, Food Funct. 14 (2023) 7574-7585. https://doi.org/10.1039/d2fo01800d

[8] N. Zhang, J. P. Zeng, Y. P Wu, et al., Human milk sn-2 palmitate triglyceride rich in linoleic acid had lower digestibility but higher absorptivity compared with the sn-2 palmitate triglyceride rich in oleic acid in vitro, J. Agric. Food Chem. 69(2020) 9137-9146. https://dx.doi.org/10.1021/acs.jafc.0c05116

[9] Y. Wu, N. Zhang, Z. Y. Deng, et al., Effects of the major structured triacylglycerols in human milk on lipid metabolism of hepatocyte cells *in vitro*, J. Agric. Food Chem. 69 (2020) 9147-9156. https://dx.doi.org/10.1021/acs.jafc.0c06976

[10] T. Wei, D. F. Tan, S. Y. Zhong, et al., Differences in absorption and metabolism between structured 1,3-oleate-2-palmitate glycerol and 1-oleate-2-palmitate-3-linoleate glycerol on C57BL/6J mice, J. Agric. Food Chem. 71 (2023) 19610-19621. https://doi.org/10.1021/acs.jafc.3c07234

[11] J. Y. Zhao, Q. Liu, Y. Liu, et al., Quantitative profiling of glycerides, glycerophosphatides and sphingolipids in Chinese human milk with ultra-performance liquid chromatography/quadrupole-time-of-flight mass spectrometry, Food Chem. 346 (2021) 128857. https://doi.org/10.1016/j.foodchem.2020.128857

[12] Q. Liu, W. Qiao, Y. Liu, et al., Effects of lipids from multiple sources on glyceride composition, concentration, and structure of infant formulas benchmarked to human milk, Heliyon 9 (2023) e21611. https://doi.org/10.1016/j.heliyon.2023.e2161

[13] N. H. Anh, Y. C. Yoon, Y. J. Min, et al., *Caenorhabditis elegans* deep lipidome profiling by using integrative mass spectrometry acquisitions reveals significantly altered lipid networks, J. Pharm Anal. 12 (2022) 743-754. https://doi.org/10.1016/j.jpha.2022.06.006

[14] M. Koyiloth, & S. N. Gummadi, Regulation and functions of membrane lipids: Insights from *Caenorhabditis elegans*, BBA Advances. 2 (2022) 100043. https://doi.org/10.1016/j.bbadva.2022.100043

[15] Y. Zhang, X. Zou, Y. Ding, et al., Comparative genomics and functional study of lipid metabolic genes in *Caenorhabditis elegans*, BMC Genomics. 14 (2013) 164-177. http://www.biomedcentral.com/1471-2164/14/164

[16] P. Shen, J. C. Kershaw, Y. Yue, et al., Effects of conjugated linoleic acid (CLA) on fat accumulation, activity, and proteomics analysis in *Caenorhabditis elegans*, Food Chem. 249 (2018) 193-201. https://doi.org/10.1016/j.foodchem.2018.01.017

[17] M. Chang, J. Yang, X. Guo, et al., Medium / long-chain structured triglycerides are superior to physical mixtures triglycerides in *Caenorhabditis elegans* lifespan through an AMPK modified pathway, Food Biosci. 39 (2021) 100815. https://doi.org/10.1016/j.fbio.2020.100815

[18] Q. Liu, Y. Liu, J. Zhao, et al., Impact of manufacturing processes on glycerolipid and polar lipid composition and ultrastructure in infant formula, Food Chem. 444 (2024) 138623. https://doi.org/10.1016/j.foodchem.2024.138623

[19] P. Li, Z. Wang, S. M. Lam, et al., Rebaudioside a enhances resistance to oxidative stress and extends lifespan and healthspan in *Caenorhabditis elegans*, Antioxidants. 10 (2021) 262. https://doi.org/10.3390/antiox10020262

[20] J. Y. Gu, Q. W. Li, J. Liu, et al., Ultrasonic–assisted extraction of polysaccharides from Auricularia auricula and effects of its acid hydrolysate on the biological function of *Caenorhabditis elegans*, Int J. Biol Macromol. 167 (2021) 423-433. https://doi.org/10.1016/j.ijbiomac.2020.11.160

[21] T. J. Schulz, K. Zarse, A. Voigt, et al., Glucose restriction extends *Caenorhabditis elegans* life span by inducing mitochondrial respiration and increasing oxidative stress, Cell Metabolism. 6 (2007) 2280-293. https://doi.org/10.1016/j.cmet.2007.08.011

[22] Z. Z. Yang, Y. T. Yu, H. R. Lin, et al., Lonicera japonica extends lifespan and healthspan in *Caenorhabditis elegans*, Free Radical Bio Med. 129 (2018) 310-322. https://doi.org/10.1016/j.freeradbiomed.2018.09.035

[23] S. J. Bai, Y. R. Yu, L. An, et al., Ellagic acid increases stress resistance via insulin/IGF-1 signaling pathway in *Caenorhabditis elegans*, Molecules. 27 (2022) 6168. https://doi.org/10.3390/molecules27196168

[24] B. K. Kim & S. K. Park, Phosphatidylserine modulates response to oxidative stress through hormesis and increases lifespan via DAF-16 in *Caenorhabditis elegans*, Biogerontology. 21 (2020) 231-244. https://doi.org/10.1007/s10522-020-09856-0

[25] N. D. Giraldo, J. D. Sánchez, A. López, et al., Functional assessment of a whey protein hydrolysate: *in vivo* responses in lipid regulation, oxidant defense, and neural protection in *Caenorhabditis elegans*, Biogerontology. 154 (2024) 105921. https://doi.org/10.1016/j.idairyj.2024.105921

[26] Y. Liu, W. Qiao, Y. Liu, et al., Quantification of phospholipids and glycerides in human milk using ultra-performance liquid chromatography with quadrupole-time-of-flight mass spectrometry, Front Chem. 10 (2023) 1101557. https://www.frontiersin.org/articles/10.3389/fchem.2022.1101557

[27] Q. Liu, Y. Liu, D. Yang, et al., Effects of excessive body fat on colostrum lipid patterns: overweight/obese vs. normal weight mothers, Food Sci. Hum Well. 13 (2024) 3708-3717. http://doi.org/10.26599/FSHW.2024.9250193

[28] K. Papsdorf, J. W. Miklas, A. Hosseini, et al., Lipid droplets and peroxisomes are co-regulated to drive lifespan extension in response to mono-unsaturated fatty acids, Nat Cell Biol. 25 (2023) 672-684. https://doi.org/10.1038/s41556-023-01136-6

[29] S. Han, E. A. Schroeder, C. G. Silva-García, et al., Mono-unsaturated fatty acids link H3K4me3 modifiers to *C. elegans* lifespan, Nature. 544 (2017) 185-190. https://doi.org/10.1038/nature21686

[30] J. Watts. Using *Caenorhabditis elegans* to uncover conserved functions of omega-3 and omega-6 fatty acids, J. Clin Med. 5 (2016) 19. https://www.mdpi.com/2077-0383/5/2/19/pdf?version=1454413472

[31] S. Sugawara, T. Honma, J. Ito, et al., Fish oil changes the lifespan of *Caenorhabditis elegans* via lipid peroxidation, J. Clin. Biochem. Nutr. 52 (20113) 139–145. https://doi.org/10.3164/jcbn.12-88

[32] M. Witting, & P. Schmitt-Kopplin. The *Caenorhabditis elegans* lipidome, Arch Biochem Biophys. 589 (2016) 27-37. http://dx.doi.org/10.1016/j.abb.2015.06.003

[33] B. T. Nguyen, N. T. H. Yen, N. K. T. Tung, et al., Lipid class-dependent alterations of *Caenorhabditis elegans* under harmane exposure, J. Pharmaceut Biomed. 231 (2023) 115401. https://doi.org/10.1016/j.jpba.2023.115401

[34] X. Sun, T. Zhang, P. Zhao, et al., 2D HILIC-ELSD/UPLC-Q-TOF-MS method for acquiring phospholipid profiles and the application in *Caenorhabditis elegans*, Eur J. Lipid Sci Tech. 142 (2021) 2100075. https://doi.org/10.1002/ejlt.202100075

[35] S. Abusharkh, C. Erkut, J. Oertel, et al., The role of phospholipid headgroup composition and trehalose in the desiccation tolerance of *Caenorhabditis elegans*, Langmuir. 30 (2014) 12897-12906. https://doi.org/10.1021/la502654j

[36] A. J. Connor, & J. L. Watts, Omega-3 and omega-6 fatty acid metabolism: modeling growth and disease using *Caenorhabditis elegans*, in R. R. Watson & and R. P. Victor (Eds.), Omega Fatty Acids in Brain and Neurological Health, E-Publishing Inc. pp. 107-116. https://doi.org/10.1016/B978-0-12-815238-6.00007-9

[37] S. M. Lam, Z. Wang, J. Li, et al., Sequestration of polyunsaturated fatty acids in membrane phospholipids of *Caenorhabditis elegans* dauer larva attenuates eicosanoid biosynthesis for prolonged survival, Redox Biol. 12 (2017) 967-977. http://dx.doi.org/10.1016/j.redox.2017.05.002

[38] I. C. Elle, L. C. B. Olsen, M. B. Mosbech, et al., *C. elegans*: A model for understanding lipid accumulation, Genetics. 207 (2008) 13-21. https://doi.org/10.4137/lpi.s1057

[39] J. L. Watts, & M. Ristow, Lipid and carbohydrate metabolism in *Caenorhabditis elegans*. *Genetics,* 207 (2017) 413-446. https://doi.org/10.1534/genetics.117.300106

[40] A. M. Jose, & M. R. Koelle, Domains, amino acid residues, and new isoforms of *Caenorhabditis elegans* Diacylglycerol Kinase 1 (DGK-1) important for terminating diacylglycerol signaling *in vivo*, J. Biol Chem. 280 (2005), 2730-2736. https://doi.org/10.1074/jbc.m409460200

[41] W. Qi, G. E. Gutierrez, X. Gao, et al., The ω-3 fatty acid α-linolenic acid extends *Caenorhabditis elegans* lifespan via NHR-49/PPARα and oxidation to oxylipins, Aging Cell. 16 (2017) 1125-1135. https://onlinelibrary.wiley.com/doi/pdfdirect/10.1111/acel.12651

[42] R. Menzel, X. Zhang, T. Pietrucik, et al., Omega-3 PUFA and the fitness and cognition of the nematode *Caenorhabditis elegans* under different environmental conditions, Comp Biochem Phys B. 270 (2024) 110925. https://doi.org/10.1016/j.cbpb.2023.110925

[43] T. L. Vrablik, V. A. Petyuk, E. M.Larson, et al., Lipidomic and proteomic analysis of *Caenorhabditis elegans* lipid droplets and identification of ACS-4 as a lipid droplet-associated protein, BBA-Mol Cell Biol L. 1851 (2015) 1337-1345. http://dx.doi.org/10.1016/j.bbalip.2015.06.004

[44] Y. L. Wang, C. X. Li, J. J. Zhang, et al., Polyunsaturated fatty acids promote the rapid fusion of lipid droplets in *Caenorhabditis elegans*, J. Biol. Chem.. 298 (2022) 102179. https://doi.org/10.1016/j.jbc.2022.102179