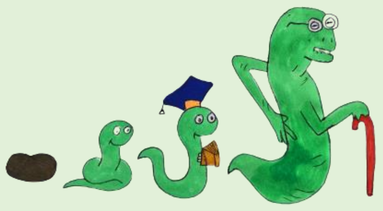

Effect of Caloric Restriction and Omega-3 EPA Supplementation on Sarcopenia in *C. elegans*

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Background

Sarcopenia, a progressive decrease of skeletal muscle mass and strength, is one of the primary changes associated with ageing and a major cause of physical frailty in the elderly. Despite extensive research, sarcopenia is still not well understood at the molecular level, although multiple factors have been found to influence the development of sarcopenia, including physical inactivity, and an unbalanced diet.

Using *C. elegans*, this study determines the effect of dietary interventions, like caloric restriction and dietary supplementation, on the rate of muscle loss. The specific *C. elegans* strain used (*unc54::GFP*) has its major myosin isoform (UNC-54) in body wall muscle fluorescently labelled, allowing UNC-54 myosin density to be measured throughout *C. elegans'* lifespan. Average lifespan, motility, and intestinal barrier function (IBF) were also determined.

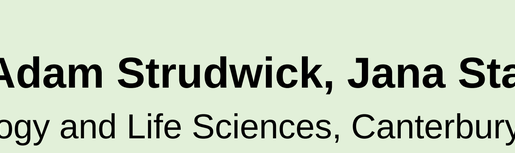


Figure 1: *C. elegans* is used as a model for sarcopenia: Left: one day old *C. elegans* nematode; Middle: 18 days old *C. elegans* nematode; Right: C. elegans with one of its main myosin muscle proteins (UNC54) labelled with GFP (UNC54::GFP).

Methods + Results

Lifespan Assay and media:

Worms were maintained on NGM plates with OP50 *E.coli* as standard food. Lifespan assays were done at 20°C on varying concentration of DR (figure 2) or EPA-supplemented plates (0.1mM and 0.3mM EPA) and transferred to new plates every other day. Peptone was removed from the DR media to prevent bacterial growth on the plates (5). FUDR was used to supress reproduction.

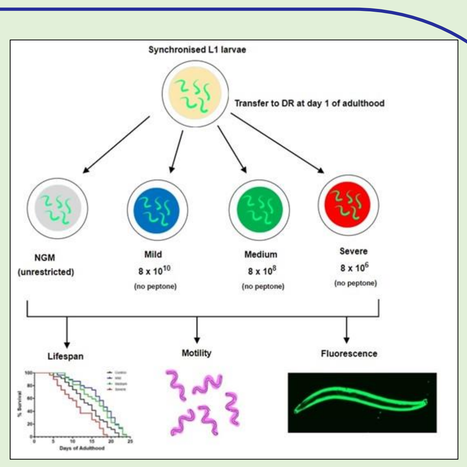


Figure 2: Set-up of CR assay using *C. elegans* expressing *UNC*54-GFP)

The EPA-rich algae oil was kindly provided by AlgaeCytes (Discovery Park, Sandwich).

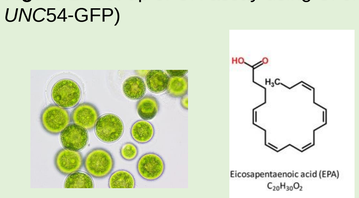


Figure 3. algae oil was used as a source of EPA

Motility Assay:

Motility was assessed daily by subjecting the worms to gentle stroking and their response to the stimulus was grouped into one of three motility classes (2).

Fluorescence Microscopy:

Fluorescence images were measured using a random sample of five worms from each population. Average pixel intensity of whole-body images was determined for quantification of GFP fluorescence using ImageJ software. All quantifications were calibrated using GFP-labelled beads as an internal standard and corrected for background fluorescence.

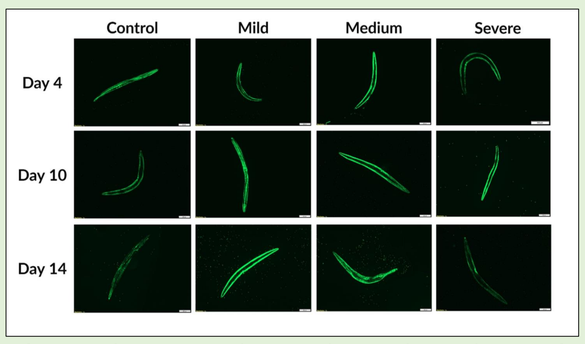


Figure 4: Fluorescence microscopy images show early onset of sarcopenia for worms under control and severe DR, compared to mild and medium CR.

Mass Spectrometry:

To quantify the uptake of EPA, synchronized L4 larvae were harvested after five days growth. FAMEs were generated after 1 hour incubation at 70 °C in 2.5% H_2SO4 in MeOH and extraction into a hexane/water (). The hexane layer was then transferred to a GC vial insert for GC-MS analysis using established methods ().

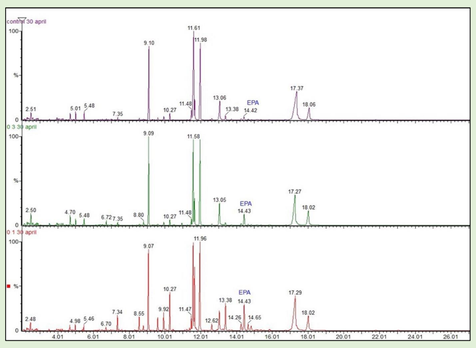


Figure 5: GC/MS of *C. elegans* supplemented with 0.1mM EPA (bottom) and 0.3mM EPA (middle) compared to control (top).

Intestinal Barrier Function (Smurf Assay):

Worms were raised with varying concentration (0.1 mM and 0.3 mM) of omega-3 Eicosapentaenoic acid (EPA) in comparison with control. 10 worms were removed from the experimental plates and suspended for 3 h in liquid cultures of OP50 *E.coli* mixed with Erigolaucine disodium salt. Worms were examined for the presence of blue dye in the body cavity and scored manually (Olympus IX83 microscope, 10x magnification).

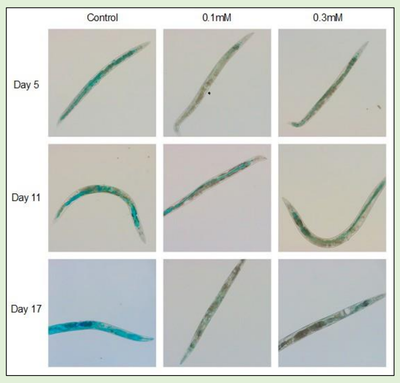


Figure 6: Microscopy images showing loss of intestinal integrity in nematodes grown under control and algae oil supplementation. During early adulthood, dye is restricted to the intestine but during middle age, dye is seen throughout the body cavity.

Results

CR + EPA supplementation increases lifespan of *C. elegans*

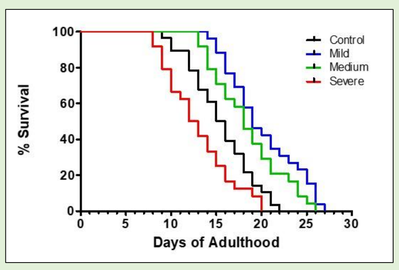


Figure 7. Effect of caloric restriction on life span in *C.elegans*. Mild and medium CR show a significant increase in mean lifespan compared to control ($p < 0.001$ and $p = 0.011$). Severe CR results in a reduced mean lifespan ($p = 0.026$). (Kaplan-Meier analysis)

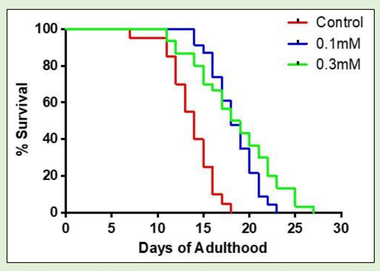


Figure 8. Effect of EPA-supplementation on life span of *C. elegans*. 0.1mM and 0.3mM EPA-supplementation increased the mean lifespan 34% and 38%, respectively, compared to control ($p < 0.0001$).

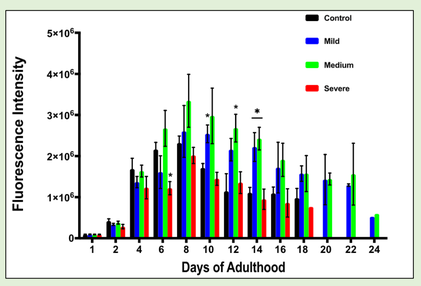
Age-related decline in motility is delayed with CR + EPA

Table 1: Age-related changes in motility. Average nr of days for 50% of initial population to move from fitness class 1 to class 2 (C_{1-2}) and for 50 % of the class 2 population to move to class 3 (C_{2-3}). Difference between two mid-points (ΔC_2) is average time spent in class 2.

	C_{1-2} (days)	C_{2-3} (days)	ΔC_2 (days)	Mean life span (days)
Control	6.9 ±0.2	12 ±3	5.1	15.5±0.7
Mild	8.6 ±0.1	15.4 ±0.3	6.8	19.9±0.8
Medium	8.5 ±0.4	14.3 ±0.4	5.8	18.3±0.8
Severe	6.9 ±0.1	10.0 ±0.5	3.1	13.0±0.8
0.1mM EPA	11.05 ±0.04	16.3±0.2	5.3	18.6±0.6
0.3mM EPA	11.15 ±0.03	17.0±0.1	5.9	19.4±0.6

Class I – Continuous smooth movement, fast movement when stimulated; Class II – Slow halting movement, smooth movement when stimulated; Class III – Small movement of head or tail, very slow movement when stimulated.

Age-related muscle loss is delayed under CR and EPA suppl



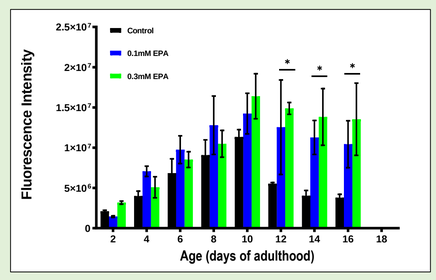


Figure 9: Average fluorescence intensity of whole-body images under different CR conditions (left) and EPA supplementation from algae oil (right). Error bars show SD. *P < 0.05 by 2-way ANOVA.

Age-related breakdown of intestinal barrier function is reduced for EPA-supplemented C. elegans

Figure 9: EPA-supplementation reduces age-related decline in intestinal barrier function. Representative images (10X) of control worms after soaking in dye for 3 hours on day 2 (left), day 8 (middle) and day 14 (right) of adulthood.

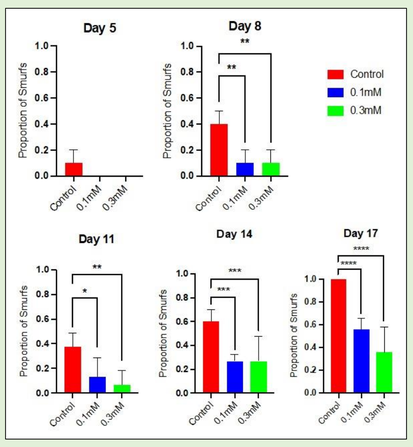


Figure 10: Age-related breakdown of intestinal barrier function is reduced in EPA from algae-oil supplemented worms. Proportion of worms showing loss of intestinal integrity as a function of age. Data are the mean ± SD of three independent assays (Two-way ANOVA, **P<0.05, **P<0.01, ***P<0.001, ***P<0.0001*).

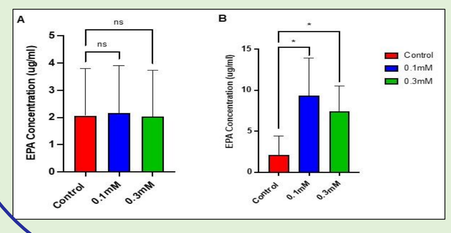


Figure 11: Uptake of supplemented EPA by E. coli OP50 (A) and C. elegans (B). OP50 and worms were harvested after 5 days, and the extracted FAMEs were analyzed with GC-MS. Data are the mean ± SD of three independent assays (One-way ANOVA, *P<0.05).

Conclusions

- Our data show that *C. elegans* exposed to mild and medium caloric restriction (CR) display an increase in lifespan and heath span, with a delayed onset of sarcopenia.
- Supplementation with sources of omega-3 EPA (eicosapentaenoic acid), has a similar effect, with improved lifespan and delayed sarcopenia.
- Age-related intestinal barrier function was also improved in EPA-supplemented worms, with GC-MS confirming the uptake of omega-3 EPA.
- These results show that dietary intervention can delay the onset of age-related diseases such as sarcopenia and intestinal barrier function in C. elegans.

Acknowledgements

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