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Oat consumption reduced intestinal fat deposition and improved health span in *Caenorhabditis elegans* model

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Abstract

In addition to their fermentable dietary fiber and the soluble β -glucan fiber, oats have unique avenanthramides that have anti-inflammatory and antioxidant properties that reduce coronary heart disease in human clinical trials. We hypothesized that oat consumption will increase insulin sensitivity, reduce body fat, and improve health span in *Caenorhabditis elegans* through a mechanism involving the *daf-2* gene, which codes for the insulin/insulin-like growth factor-1–like receptor, and that hyperglycemia will attenuate these changes. *Caenorhabditis elegans* wild type (N2) and the null strains *sir-2.1*, *daf-16*, and *daf-16/daf-2* were fed *Escherichia coli* (OP50) and oat flakes (0.5%, 1.0%, or 3%) with and without 2% glucose. Oat feeding decreased intestinal fat deposition in N2, *daf-16*, or *daf-16/daf-2* strains ($P < .05$); and glucose did not affect intestinal fat

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Conflict of interest statement

This research was supported by a nonrestricted donation from PepsiCo Inc. Oats used in this study were a gift of PepsiCo Inc. Y. Chu is an employee of PepsiCo, Inc, which manufactures oatmeal products under the brand name Quaker Oats. The views expressed in this article are those of the author and do not necessarily reflect the opinion or policies of PepsiCo, Inc.

deposition response. The N2, *daf-16*, or *sir-2.1* mutant increased the pharyngeal pumping rate ($P < .05$), a surrogate marker of life span, following oat consumption. Oat consumption increased *ckr-1*, *gcy-8*, *cpt-1*, and *cpt-2* mRNA expression in both the N2 and the *sir-2.1* mutant, with significantly higher expression in *sir-2.1* than in N2 ($P < .01$). Additional glucose further increased expression 1.5-fold of the 4 genes in N2 ($P < .01$), decreased the expression of all except *cpt-1* in the *daf-16* mutant, and reduced mRNA expression of the 4 genes in the *daf-16/daf-2* mutant ($P < .01$). These data suggest that oat consumption reduced fat storage and increased *ckr-1*, *gcy-8*, *cpt-1*, or *cpt-2* through the *sir-2.1* genetic pathway. Oat consumption may be a beneficial dietary intervention for reducing fat accumulation, augmenting health span, and improving hyperglycemia-impaired lipid metabolism.

Keywords

Oats; *C elegans* model; Insulin sensitivity; Lipid metabolism; Health span

1. Introduction

Modern lifestyle-related chronic diseases, such as obesity, insulin resistance, and type 2 diabetes mellitus, are major health challenges of the 21st century [1]. Changes in dietary habits and lifestyle are among the primary causes for this undesirable development, which is already being seen in the younger population.

An inverse connection of longevity and fat mass is characteristic of humans above 25% \pm 5% body fat when accompanied by obesity-related disease [2]. Both obesity and aging decrease insulin sensitivity, impair the immune response, increase inflammation, weaken the gut-bloodstream barrier, and decrease physical mobility [2]. Promotion of dietary intervention, exercise, and lifestyle modification, including second-line obesity treatments, to sustain weight loss has been elusive [3,4]. Even though a direct relationship between calorie restriction and health span has been observed in animal models [5,6], controlling food intake in humans is difficult, mainly transient, and often unsustainable because it involves multiple factors including psychological factors.

Caenorhabditis elegans is well suited to obesity studies. Lipid oxidation pathways are present in the *C elegans* model, such as cluster differentiation transport protein, carnitine palmitoyltransferase-1, acetyl coenzyme A carboxylase, and acetyl CoA synthetase. Resistant starch and short-chain fatty acids reduce intestinal fat deposition (IFD) in *C elegans*, demonstrating that the model can be used to evaluate bioactive components from gut fermentation [7–9]. *Caenorhabditis elegans* is a small, free-living soil nematode, a multicellular eukaryotic organism, distributed widely around the world. *Caenorhabditis elegans* is the first animal to have its genome completely sequenced and conserves 65% of the genes associated with human disease [8]. *Caenorhabditis elegans* deposits fat for energy storage along its intestinal tract of its transparent body [10]. Thus, lipid-staining dyes such as Nile red can be visualized directly and quantitated photometrically in the intact *C elegans* [11]. Food intake and transport in *C elegans* are regulated by pharyngeal movements and relaxation in the terminal bulb of the pharynx. The pharyngeal pumping rate (PPR) declines with age because of sarcopenia, and PPR is a surrogate marker of aging [12].

Many dietary interventions with enriched nondigestible but fermentable carbohydrates may reinforce optimal nutrition, enhance physiological function, reduce obesity, improve metabolic dysfunction, limit disease [4], and produce anticancer effects in humans [13,14]. The mechanisms of these interventions have been reported to be due to increased mRNA expression of the GLP-1 precursor, proglucagon and peptide YY in rodents [15], improved glucose tolerance [16,17], increased phase I xenobiotic metabolizing enzymes that are a hallmark of long-lived mice [18], promotion of mitochondrial fatty acid oxidation, and increased energy expenditure in *Drosophila* or adult rodents at ages analogous to 56 to 65 years in humans [17,19,20]. Consequently, fermentable dietary fiber reduces body fat accumulation [19,21], plasma cholesterol and triglycerides [22], and insulin resistance [23] in rodents and humans.

In addition to resistant starch, oats (*Avena sativa*) contain the soluble fermentable dietary β -glucan fiber and the unique phytoalexins avenanthramides (avns). Avenanthramides provide safe, antioxidant, anti-inflammatory, and antiangiogenic properties [24–26]. Consuming oats introduces a hypocaloric intervention through caloric dilution [27,28]; reduces low-density lipoprotein cholesterol [29], body fat [30], and coronary heart disease risk factors in human clinical trials [31,32]; increases fasting peptide YY, GLP-1, postmeal satiety, and insulin sensitivity [33]; and elevates endothelium nitric oxide production [34,35]. Like resistant starch, β -glucans are fermentable dietary fibers resistant to digestion and high-temperature cooking. An association was detected between total fermentable phenolics, a reduction in triacylglycerols, and fewer aorta inflammatory lesions in mice [36].

Decreased *daf-2* signaling, the only homolog of the human insulin and insulin-like growth factor (IGF) receptor gene in *C elegans*, increases *C elegans*' life span in a DAF-16/FOXO-dependent manner [37–39], which is reversed by hyperglycemia [40]. Furthermore, doubling the *C elegans sir-2.1* gene number increases life span by 50% in a *daf-16*-dependent manner [41,42]. We hypothesized that oat consumption would reduce IFD, improve insulin resistance created by added glucose (2%) to the *C elegans* feeding media, improve hyperglycemia-impaired lipid metabolism, and increase health span through *daf-16* and/or *sir-2.1* pathway(s). A number of genes in *C elegans* have been proven to promote fat storage and mediate satiety, for example, guanylyl cyclases and the cholecystokinin (CCK) receptor. The objectives of this study were to quantify IFD by the fluorescence intensity of Nile red staining, to evaluate health span by counting PPR, and to determine the genetic pathway(s) using *daf-16*- and/or *sir-2.1*-deficient mutants.

2. Methods and materials

Caenorhabditis elegans strains and their standard laboratory food source *Escherichia coli* (OP50, uracil auxotroph) were obtained from the *Caenorhabditis* Genetics Center (University of Minnesota, Minneapolis, MN). The *C elegans* model does not require Institutional Animal Care and Use Committees regulation or approval [43].

2.1. Culture of *E coli* (OP50)

OP50 were cultured by the standard method described elsewhere [7]. Briefly, approximately 10 μ L of stock *E coli* solution was added to media and incubated at 37°C for 24 hours. The

OP50 were then subcultured in Petrifilm (3M Corporate, St Paul, MN) at 37°C for 24 hours, the colonies were confirmed to be at a density of 5×10^8 to 5×10^{11} cfu/mL, and the *C. elegans* were allowed to eat ad libitum.

2.2. Culture of *C. elegans*

Caenorhabditis elegans wild type (N2) and null mutants *sir-2.1(ok434)IV*, *daf-16(mgDf50)I*, and *daf-16(mgDf50)I;daf-2(m65)III* were used in this study (Table 1). They were grown on Nematode Growth Media agar plates (Ø35 mm) and stored in a low-temperature incubator (Revco Tech, Nashville, NC) at 20°C or 15°C (*daf-16(mgDf50)I;daf-2(m65)III*).

Caenorhabditis elegans were age synchronized. Mature gravid *C. elegans* were transferred individually onto the agar plates and treated with a NaOH (1 mol/L) and sodium hypochlorite solution (5.25%, 5:2 ratio) to dissolve the body and release viable eggs [7]. One day before the experiment, 200 µL of a feeding media containing E coli was added to the agar dish.

2.3. Diet composition

Oats (Quaker Oats instant flakes, PepsiCo Inc, Chicago, IL) (Table 2) were powdered using a centrifugal mill with a 0.75-mm sieve (ZM 200; Retsch, Haan, Germany), autoclaved at 121°C, and suspended in distilled deionized water (5% w/v).

Control *C. elegans* were fed with OP50 only. The experimental groups were fed OP50 supplemented with 0.5%, 1.0%, or 3.0% oats at the larval stage L2 (Table 3). Two percent glucose was added to an additional group of each strain with the oats. The dietary nutrient composition is listed in Table 4 [44]. *Caenorhabditis elegans* were transferred to fresh dishes every other day receiving treatment (50 µL).

2.4. Pharyngeal movement (PPR)

Caenorhabditis elegans were examined periodically using a stereomicroscope (SMZ1500; Nikon, Melville, NY) with transmitted light. The PPR was recorded manually by independent observers then were returned to the incubators [9,45].

2.5. Fluorescence microscopy

Lipophilic dye Nile red was used to stain for IFD [7]. S-basal solution was added to the dish to wash the *C. elegans*. The solution containing the *C. elegans* was centrifuged for 20 seconds at 805g, and this procedure was repeated twice. *Caenorhabditis elegans* were then fixed with 4% paraformaldehyde over 2 hours at 4°C and washed with phosphate-buffered saline for 5 minutes \times 3. Nile red (50 µL) was applied to the specimens for 10 minutes. Ten microliters of Fluoromount-G (Southern Biotechnology Associates, Birmingham, AL) was applied to a glass slide followed by 20 µL of the medium containing Nile red-stained *C. elegans*. A cover glass was mounted on the glass slide, and the slides were viewed with an epifluorescence microscope (Nikon Eclipse, Ti) equipped with a Texas red filter. Fluorescent micrographs were taken with a digital camera (Andor, DU-885k) and were analyzed using Nikon-Elements (version 3.22.11). Optical densities (arbitrary units, % of control) of Nile red-labeled IFD were determined for adult *C. elegans* (larvae stage 4).

2.6. Quantitative real-time reverse transcription polymerase chain reaction reagents

Trizol reagent (T9424), chloroform (C2432), and isopropanol (I9516) were purchased from Sigma-Aldrich (St Louis, MO). Taqman PCR core kit (N8080228), MuLV Reverse Transcriptase (N8080018), and Ribonuclease (RNase) Inhibitor (N8080119) were obtained from Life Technologies (Grand Island, NY). RNase free micro tubes and pipette tips were used in RNA isolation and quantitative real-time reverse transcription polymerase chain reaction (Q-RT-PCR).

2.7. RNA isolation

Total RNA was extracted using the Trizol reagent as described elsewhere [23]. *Caenorhabditis elegans* samples were homogenized in 1 mL Trizol reagent per 50 to 100 mg of tissue. Samples went through 5 freeze-thaw cycles in which they were frozen in liquid nitrogen and thawed in a 37°C water bath. The homogenized samples were vortexed at room temperature to allow the complete dissociation of nucleoprotein complexes. Two hundred microliters of cold pure chloroform was added, and the samples were vortexed for 15 seconds and incubated at room temperature for 5 minutes. The samples were centrifuged at 15,616g for 10 minutes at 4°C, and the top aqueous phase was transferred to a fresh tube. A 600- μ L cold isopropanol was used for the initial homogenization; and the samples were incubated at room temperature for 10 minutes, vortexed for 15 seconds, and centrifuged at 15,616g for 10 minutes at 4°C. The supernatant was removed, and the RNA pellet was washed once with 1 mL of cold 75% ethanol. The sample was vortexed and centrifuged at 6100g for 5 minutes at 4°C. The supernatant was removed completely, and the RNA pellet was briefly air-dried. The RNA pellet was resuspended in 0.1% diethyl pyrocarbonate-treated water and stored at -80°C. The RNA concentration was analyzed with a Nanodrop ND-1000 spectrophotometer (Wilmington, DE).

2.8. Quantitative RT-PCR

The mRNA levels of *ckr-1*, *gcy-8*, *cpt-1*, and *cpt-2* were determined using Taqman Q-RT-PCR. The following components were added in 1-step RT-PCR: 2.2 μ L H₂O, 1 μ L 10 \times Taqman buffer A, 2.2 μ L 25 mmol/L MgCl₂ solution, 0.3 μ L 10 mmol/L dATP, 0.3 μ L 10 mmol/L dCTP, 0.3 μ L 10 mmol/L dGTP, 0.3 μ L 10 mmol/L dUTP, 0.05 μ L 20 U/ μ L RNase inhibitor, 0.05 μ L 50 U/ μ L MuLV reverse transcriptase, 0.05 μ L 5 U/ μ L Ampliqaq gold, 0.25 μ L Taqman probe and primers, and 3 μ L 5 ng/ μ L RNA. The PCR was conducted in triplicate using a Taqman probe and primer set (Life Technologies, Grand Island, NY) for cholecystokinin receptor homolog (*ckr-1*, Ce02408606_m1), guanylyl cyclase-8 (*gcy-8*, Ce02456184_g1), carnitine palmitoyltransferase-1 (*cpt-1*, Ce02440434_m1), and carnitine palmitoyltransferase-2 (*cpt-2*, Ce02459919_g1). The mRNA signal was normalized over a eukaryotic 18S rRNA (Hs99999901-s1) internal control. The reaction was conducted using a 7900 HT Fast real-time PCR system (Life Technologies). Reverse transcription was added at 48°C for 30 minutes, and Ampliqaq gold activation (denaturation) was performed at 95°C for 10 minutes. Amplification of the DNA involved 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. Data were analyzed using the Sequence Detector Software (Life Technologies, Carlsbad, CA). The relative quantification of gene expression (2^{-Ct}) was calculated.

2.9. Statistical analyses

All data were presented as means \pm SEM. Student t tests and analysis of variance were used for IFD, and analysis of covariance was used to compare the slopes of PPR data (SAS 9.4, Cary, NC). Statistical significance was set at $P < .05$. Principle component analysis (PCA) was performed to cluster the factors of the oats results (SAS 9.4). Power analyses were performed to predict the sample size to achieve 80% power for PPR (minimum $n = 7$) and IFD ($n = 5$) with statistical significance as $.05$.

3. Results

3.1. Intestinal fat deposition

Oat feeding reduced IFD by 30% (0.5% and 1.0%) in the N2 strain ($P < .05$), and the addition of glucose to the oats increased IFD. Intestinal fat deposition decreased by 17% in response to 0.5% oats and 37% in response to 1.0% oats in the *daf-16(mgDf50)I* and increased with glucose treatment compared with the group that did not receive glucose ($P < .05$). The *daf-16(mgDf50)I;daf-2(m65)III* showed a similar dose-dependent reduction in IFD in response to oat feeding as well as in the presence of glucose ($P < .05$). Intestinal fat deposition was mildly lower following oat (0.5% and 1.0%) consumption in the *sir-2.1(ok434)IV* ($P > .05$) (Fig. 1).

3.2. Pharyngeal pumping rate

The PPRs declined in all groups as the *C elegans* aged. The oat treatment (0.5%, 1.0%, or 3%) increased the PPR in N2, *daf-16*, and *sir-2.1* mutants ($n = 24 C elegans/3$ dishes, $P < .001-.05$). The PPR in N2 was increased in the oat group (0.5%, 1.0%, or 3%) in the presence of glucose ($P < .005-.03$). This increase persisted in the presence of glucose at a low dose in *daf-16* or *daf-16/daf-2* mutant ($P < .05$) (Fig. 2).

3.3. Lipid metabolism gene expression

Oat feeding (0.5% and 1%) increased the mRNA expression of *ckr-1*, *gcy-8*, *cpt-1*, and *cpt-2* in the *C elegans* N2, and *sir-2.1*- and *daf-16*-deficient strains ($P < .01$). Additional glucose further increased these mRNA expressions in N2 and *sir-2.1*. In the *daf-16*-deficient strain, *ckr-1* and *gcy-8* were increased after oat feeding ($P < .01$). All 4 genes tested were elevated in response to glucose treatment alone, and oat consumption in the presence of glucose significantly reduced mRNA expression of the 4 genes ($P < .01$). Oat feeding with glucose reduced mRNA expression of all 4 genes in *daf-16/daf-2*-deficient strains ($P < .01$). Oats (0.5%) plus glucose increased *cpt-1* and *cpt-2* ($P < .01$, Fig. 3).

3.4. Principle component analysis

Principle component analyses revealed 2 theoretical strong factors that played important roles in the relationship of the components that were tested in this study (Fig. 4, Table 5). Inverse relationship between the IFD and the PPR was detected in N2, *daf-16/daf-2*, and *sir-2.1* mutants. The PPRs were strongly and positively related to the 2 factors in the 3 strains. By contrast, the IFDs were strongly and positively related to the factor 1 in the N2 and the *daf-16/daf-2* double mutant, which was negatively associated in the *sir-2.1* mutant.

The IFD or PPR seemed weakly related to the *ckr-1*, *cpt-1*, *cpt-2*, and *gcy-8*. The *ckr-1*, *cpt-1*, *cpt-2*, and *gcy-8* had strong positive relationships with factor 1 N2, *daf-16/daf-2*, and *sir-2.1* mutants. A loose but positive relationship of the 4 genes with the factor 2 was seen in the *daf-16* mutant, whereas the IFD was negatively related to the factor 1 and the PPR did not have any association with either the factors or the 4 genes.

4. Discussion

The effect of oat consumption paired with or without 2% glucose was evaluated in the *C elegans* model by observing changes in IFD determined by the fluorescent intensity of Nile red, alterations in health span indicated by PPR, and variations in selected mRNA expression indicated by PCR in this study. The IFD was reduced in N2, and the *daf-16*– and *daf-16/daf-2*–deficient mutants. Apart from natural age-related decline of the PPR in all groups, oat treatment sustained the PPR in N2 with or without glucose. Oat treatment also sustained the PPR in *daf-16* and *sir-2.1* mutants without glucose. Low-dose oat treatment (0.5%) increased the PPR in the presence of glucose. Oat consumption increased mRNA expressions of *ckr-1*, *gcy-8*, *cpt-1*, and *cpt-2* genes, which were greater in N2 than in the *sir-2.1* mutant; and additional glucose further increased the expression by 1.5-fold in both strains. The effects of oats on health span and IFD in N2 was mediated by *daf-2* and partially depended on *daf-16*, with and without the presence of hyperglycemia.

Hyperglycemia often coexists with hyperlipidemia. In *C elegans*, inactivation of more than 300 genes has been shown to reduce IFD; and inactivation of more than 100 genes, to increase fat storage [46]. Oat consumption significantly reduced IFD in the N2, and *daf-16*– and *daf-16/daf-2*–deficient mutants, whereas the presence of glucose increased IFD. However, oats reduced IFD even when there was insulin resistance, as seen in the *daf-16/daf-2*–deficient group and other groups with additional glucose. Glucose did not attenuate the oat-induced IFD reduction in the *daf-16/daf-2*–deficient mutant, suggesting an indirect improvement in insulin sensitivity through lipid metabolism activating other pathway(s), such as the serotonin pathway. In rodent diabetes, hyperglycemia is associated with an increase in peroxisome proliferator activator alpha (PPAR α) activity associated with insulin resistance [10]. In a *C elegans* model, deletion of the nuclear hormone receptor–49, a homologue of PPAR α , prevents hyperlipidemia and restores insulin sensitivity in a manner similar to PPAR α -null mice [47]. The reduced IFD following oat consumption in the *daf-16/daf-2*–deficient mutant suggests that oats may improve hyperglycemia-impaired lipid metabolism.

Reduced food intake, reduced lipid metabolism, and a reduction in metabolic turnover in N2 are associated with an extension of life span [41]. Unlike the N2, or *daf-16/daf-2*– and *daf-16*–deficient mutants, the IFD was only mildly reduced by the same amount of oat consumption in the *sir-2.1*–deficient mutant with increased mRNA expression of the 4 genes similar to N2, suggesting increased lipid metabolism. The ability of both the wild-type and mutant nematodes to sustain the PPR indicates that oat consumption improved health span independent of the *sir-2.1* pathway, which relates to the co-regulator/genes in the aging process in *C elegans* [48,49]. Our data are in agreement with the literature that shows that involvement of *sir-2.1* in lipid metabolism has multiple factors similar to life span [41], and

either a higher dose is required in the absence of the *sir-2.1* gene pathway or the *sir-2.1* pathway was not critically involved in the IFD reduction.

Hyperglycemia reduced the *C elegans* life span [50] and completely suppressed the long life span of *daf-2(-)* insulin/IGF-1 receptor mutants in *C elegans* [4]. The *daf-16* gene plays a central role in activating the downstream insulin signaling pathways and is the major target of the *daf-2* that negatively regulates *daf-16* [50,51]. In the present study, oat feeding increased PPR and decreased IFD, which were mediated by the *daf-16/daf-2* pathways. The benefit from oats' may be attributed to its being high in fibers, β -glucan, proteins, and unique avns.

Although activation of the CCK receptor does not change circulating triacylglycerol or adipose tissue, it increases glucose tolerance and insulin sensitivity and decreases liver triacylglycerol in mice [52,53]. Similarly, in humans, activation of CCK pathway does not appear to play a central role in long-term energy balance but signals postprandial satiety [53,54]. However, *ckr-1*, the analogous gene in the *C elegans* species, increased in response to oat consumption that decreased IFD in the presence of glucose and preserved PPR, suggesting that *ckr-1* has a potential role in the lipid metabolism. The *gcy-8* is exclusively expressed in AFD neurons and contributes to thermotaxic behavior [55]. Increased expression of *gcy-8* may indicate an increase in metabolic rate. In the *daf-16*-deficient mutant, the mRNA expression of *ckr-1* and *gcy-8* was increased in response to oat feeding. This effect was reversed in presence of glucose that initially elevated *cpt-1* and *cpt-2* followed by a reduction when oats were added.

An elevated *cpt-1* or *cpt-2* suggests an augmented lipid β -oxidation and turnover. Expression of all 4 genes was reduced in the *daf-16/daf-2*-deficient mutant when treated with oats, and *cpt-1* and *cpt-2* expression increased nearly 2-fold with additional glucose. The reduced *cpt-1* or *cpt-2* in *daf-16/daf-2*-deficient mutant suggests that the *C elegans* were either unable to use/metabolize the nutrients from the food as seen in *daf-16*-deficient mutant or were unable to adequately downregulate metabolic turnover as observed in the *daf-16/daf-2*-deficient mutant, thus reducing IFD. In the 3 strains, N2, *daf-16/daf-2*, and *sir-2.1* mutants, the factor 2 of the PCA was closely related to health span, which is inversely related to IFD; and the factor 1 had strong positive relationships with *cpt-2* and *gcy-8*. The PCA data showed an inverse relationship of the *daf-16* to the IFD, *cpt-1*, and *cpt-2*.

The exclusive and unique avns in oats are fermentable polyphenols that also resist digestion and provide health benefits. A high β -glucan concentration increases viscosity in the gastrointestinal tract, delays nutrient absorption, decreases the glycemic peak by 50%, lowers low-density lipoprotein cholesterol, reduces body fat in humans and rodents [56], counteracts obesity-related inflammation [25], and enhances immunity in *C elegans* by activating the dectin-1 receptor and DAF-2/insulin-like receptor pathways [57,58]. These avns, along with the β -glucan content of oats, make oats a unique food that may aid in lowering body fat and controlling body weight. Future studies will investigate possible synergistic effects of avns and β -glucan, as well as the role of the serotonin pathway in improving hyperglycemia-induced hyperlipidemia and increasing health span.

This study had limitations as well. *Caenorhabditis elegans* have two-thirds of genes that are related to human diseases. Thus, *C. elegans* is used as a stage for use in high-throughput screening to search for nutritional interventions for health before confirmatory studies in higher animal models and human clinical trials. Compensatory pathways that exist in higher animals may be not discovered by the present study using *C. elegans*. Active components of the oats extract will be further tested in followup studies using higher animal species.

In conclusion, our data indicate that oats have unique properties that induce an improvement in insulin sensitivity, reduce IFD, and extend health span in the *C. elegans* model organism, giving support to our study hypothesis. As part of a healthy diet, oats are an appropriate food to improve public health. Avenanthramides and β -glucan are functional food components of oats that can be incorporated into the daily diet in various ways. *Caenorhabditis elegans* is an attractive in vivo animal model for initial studies of nutrition interventions before confirmation in higher animal species.

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Abbreviations

Avns	avenanthramides
IFD	intestinal fat deposition
IGF	insulin-like growth factor
PPARα	peroxisome proliferator activator alpha
PPR	pharyngeal pumping rate
Q-RT-PCR	quantitative real-time reverse transcription polymerase chain reaction

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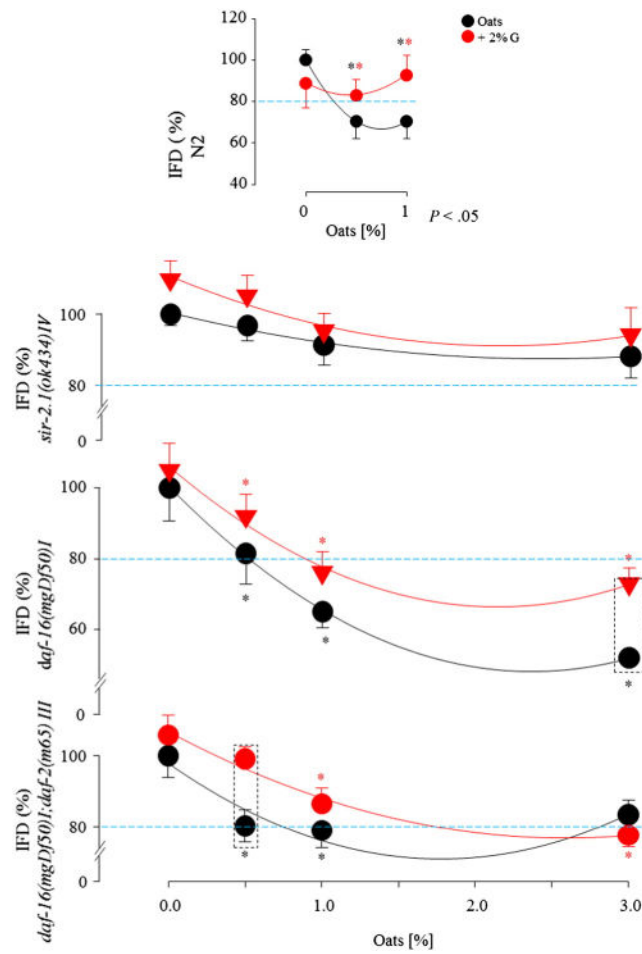


Fig. 1. Nile Red staining of the IFD in *C. elegans* after oat feeding in the absence or presence of 2% glucose.

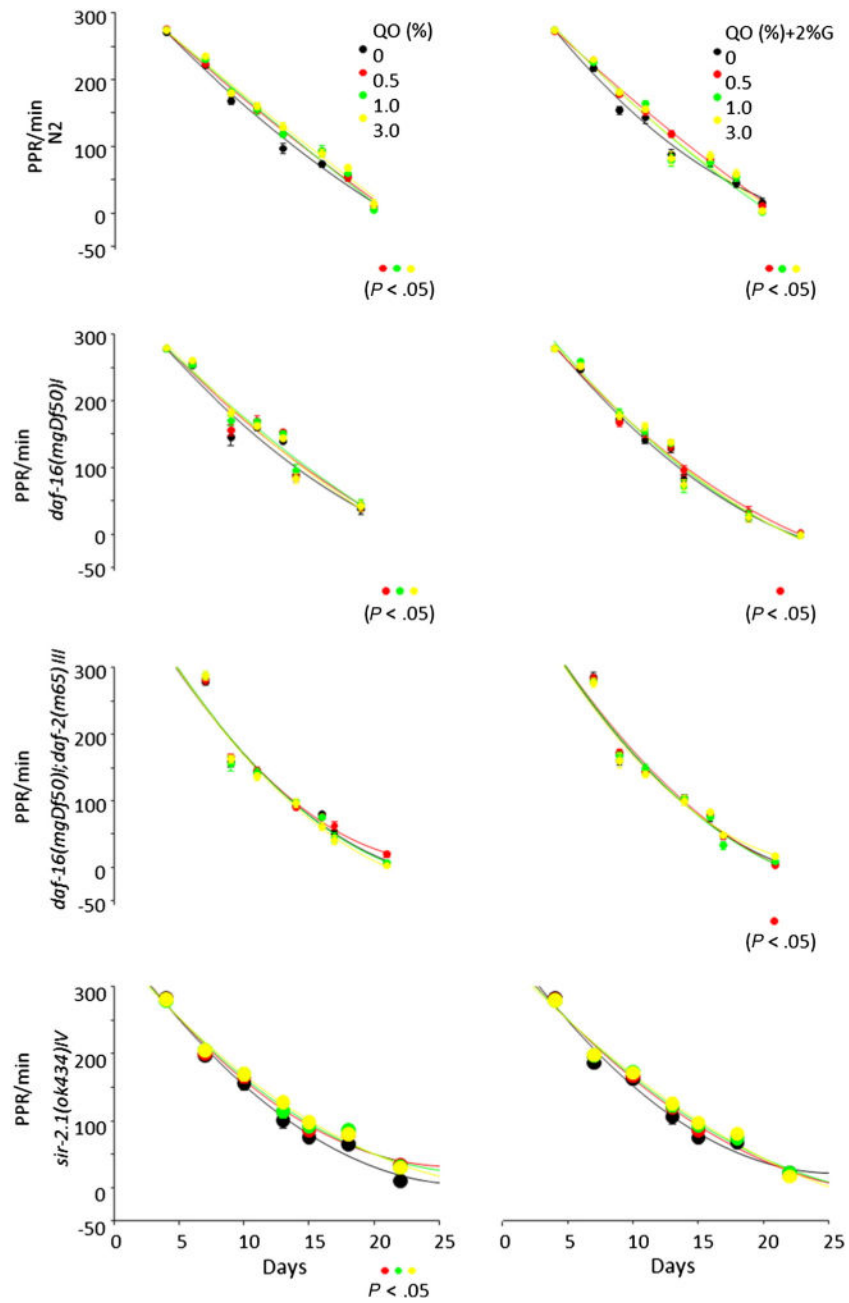


Fig. 2. Oat feeding affected PPR, a surrogate marker of life span in *C. elegans*.

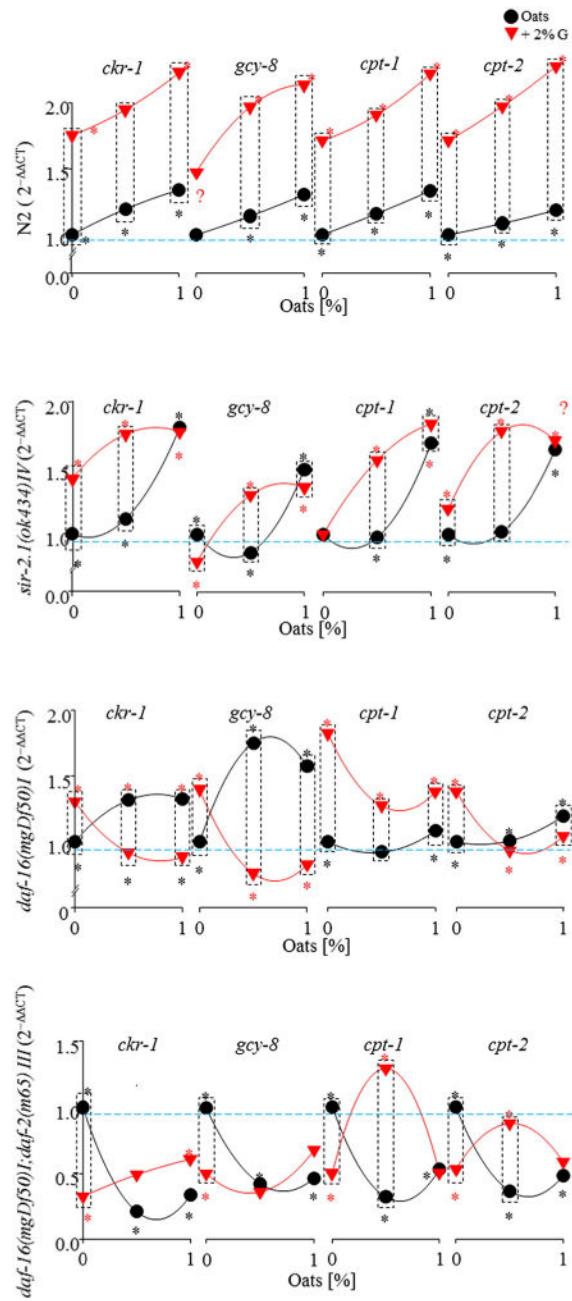


Fig. 3. Oat feeding altered gene expression of *ckr-1*, *gcy-8*, *cpt-1*, and *cpt-2* by Q-RT-PCR in *C. elegans*.

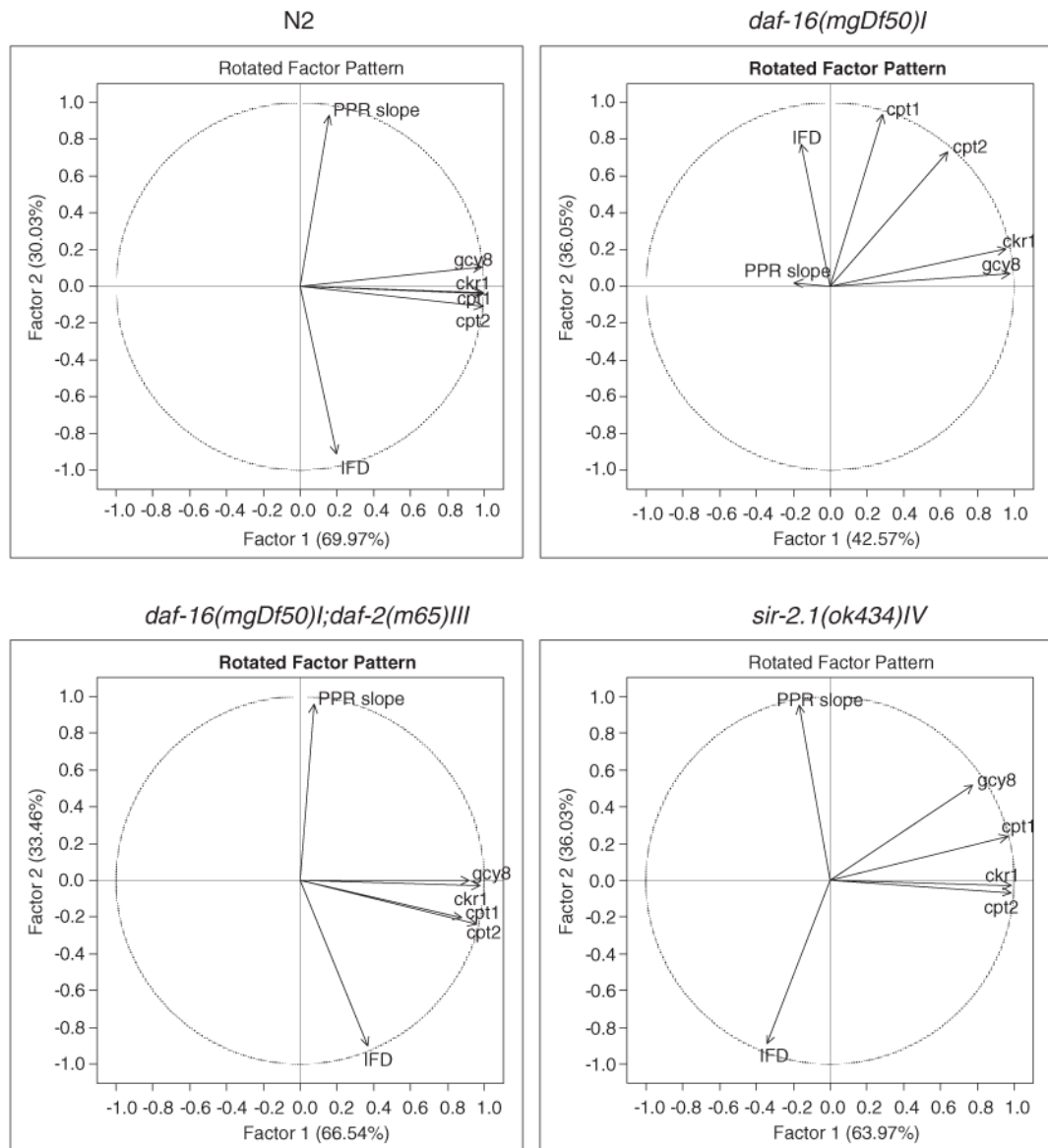


Fig. 4. Scatter plot of PCA showing the 2 strong factors in relationship with *ckr-1*, *cpt-1*, *cpt-2*, *gcy-8*, PPR slope, and IFD.

Table 1Information on *C elegans* strains

Strains	Gene deficient	Human homologs	Functions	References
Wild type (N2)	–	–	–	[7,59]
<i>daf-16(mgDf50)I</i>	<i>daf-16</i>	Forkhead box protein O (FOXO)	<i>Caenorhabditis elegans daf-16</i> has a central role in mediating the downstream insulin signaling pathways and is the major target of the <i>daf-2</i> pathway.	[8]
<i>daf-16(mgDf50)I; daf-2(m65)III</i>	<i>daf-16</i> and <i>daf-2</i>	FOXO and insulin/IGF-1 receptor	The <i>C elegans daf-2</i> gene encodes the homolog of the mammalian insulin receptor, which has preserved ligand-binding and tyrosine kinase domains	[8]
<i>sir-2.1(ok434)IV</i>	<i>sir-2.1</i>	NAD-dependent protein deacetylase sirtuin-1	The <i>sir-2.1</i> transgene functions upstream of <i>daf-16</i> in the insulin-like signaling pathway; regulates lifespan independently.	[42]

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Table 2

Composition of oats (Quaker® Oats)

Content	Quantity (%)	Content	Quantity (ppm)
β -Glucan	4.6	Ave 2c	5.4
Insoluble dietary fiber	7.3	Ave 2f	8.8
Starch	60.9	Ave 2p	5.3
Protein	13	Ave 5p	1.2
Lipids	6.6	Ave total	20.8
Moisture	10.3		

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Table 3

Protocol of experiment

	Dosage (%)									
	Without glucose					With glucose				
Treatments (50 μ L)	0	0.5	1	3	0	0.5	1	3	0	10
OP50 (2×10^9 cfu/mL, μ L)	10	10	10	10	10	10	10	10	10	10
Oats (5%, μ L)	0	5	10	30	0	5	10	30	0	5
Glucose (50%, μ L)	0	0	0	0	0	2	2	2	2	2

Table 4

Diet ingredient composition

Nutrients	Oat diet (mg/mL)	OP50 (mg/mL)	Oat treatment (mg/plate)			
			0	0.5%	1%	3%
β -Glucan	0.046	–	0.000	0.012	0.023	0.069
Insoluble dietary fiber	0.073	–	0.000	0.018	0.037	0.110
carbohydrate	0.609	7.5	0.375	0.527	0.680	1.289
Protein	0.130	37.5	1.875	1.908	1.940	2.070
Lipids	0.066	5.0	0.250	0.267	0.283	0.349

Table 5

PCA description: factor 1 and factor 2

Relation to factors	Factor 1					Factor 2						
	PPR	IFD	<i>ckr-1</i>	<i>gcy-8</i>	<i>cpt-1</i>	<i>cpt-2</i>	PPR	IFD	<i>ckr-1</i>	<i>gcy-8</i>	<i>cpt-1</i>	<i>cpt-2</i>
N2	11	24	99	98	100	100	94	-90	1	15	2	-6
<i>daf-16(mgDf50I)</i>	-20	-15	95	98	29	64	2	77	20	7	93	73
<i>daf-16(mgDf50I);daf-2(m65)III</i>	-24	64	93	87	90	99	93	-73	29	30	9	8
<i>sir-2.1(ok434)IV</i>	-17	-34	98	78	96	98	95	-89	-3	52	24	-7