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trans-Trismethoxy resveratrol decreased fat accumulation dependent on *fat-6* and *fat-7* in *Caenorhabditis elegans*

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Abstract

trans-Trismethoxy resveratrol (TMR) is a methyl analog of resveratrol. It is found to exhibit enhanced biological effects compared to resveratrol, such as inhibition of cancer cell growth and pro-apoptotic activities. However, the role of TMR in lipid metabolism is not fully understood. In this study, we used *Caenorhabditis elegans*, an *in vivo* nematode model which has been widely applied in disease research, including research on obesity, to investigate the effect of TMR on lipid metabolism. Treatment with TMR (100 and 200 μM) for 4 days significantly reduced triglyceride accumulation (14% and 20% reduction over the control, respectively) of *C. elegans*, without affecting nematode growth, food intake and reproduction. Treatment with TMR significantly downregulated stearoyl-CoA desaturase genes, *fat-6* and *fat-7,* accompanied by a decrease in the desaturation index of fatty acids, the ratio of oleic acid to stearic acid. These results suggest that TMR inhibits fat accumulation by downregulating stearoyl-CoA desaturase in *C. elegans*.

Keywords: *trans*-Trismethoxy resveratrol, *C. elegans*, lipid metabolism, stearoyl-CoA desaturase

Introduction

trans-Trismethoxy resveratrol (TMR, (E)-5-[2-(4-hydroxyphenyl)ethenyl]-1,3-benzene diol) is a naturally occurring organic compound found in plants, including *Pterobolium hexapetallum* and *Virola cuspidate.*[1, 2] TMR is derived from resveratrol by the addition of three methyl esters. Resveratrol is known to be metabolized under a detoxification pathway through sulfation and glucoronidation, which impact its bioavailability.[3] The conversion of three active hydroxy groups into methyl esters in TMR is thought to make it more stable and exhibit higher bioavailability compared to resveratrol.[4-6] TMR has been reported to have many biological properties, such as protection against oxidative stress-induced DNA damage,[7] anti-inflammatory effects,[8] and anti-cancer effects.[4] However, the question of whether this compound also exhibits anti-obesity effects similar to resveratrol remains understudied.

Caenorhabditis elegans (*C. elegans*) is a free-living nematode that has been widely used for many studies, including studies of obesity.[9] It has a compact body size – the adult worm is only 1mm in length. In the laboratory, it can be cultured on either an agar plate or a liquid medium, and non-pathogenic *Escherichia coli* OP50 as the food source. *C. elegans* also has a short lifecycle of approximately 3 days at 25°C, a significant advantage for shortening experiment duration.[10] Moreover, many lipid-metabolism-related signaling pathways are highly conserved from humans to *C. elegans*, which makes *C. elegans* highly suitable as a model for obesity research.[9] Thus, the purpose of the present study was to examine the role of TMR in lipid metabolism using *C. elegans* as an *in vivo* animal model.

Materials and Methods

Materials

trans-Trismethoxy resveratrol (TMR) was purchased from Cayman Chemical (≥98%, Ann Arbor, MI). Chemicals that included biological agar, peptone, LB broth used for *C. elegans* maintenance were purchased from Fisher Scientific Inc. (Pittsburgh, PA). Fluorodeoxyuridine (FUDR) and carbenicillin were purchased from Sigma-Aldrich Co. (St. Louis, MO). Reverse transcription kit, TaqMan gene expression assays and master mix for real time PCR assays were purchased from Applied Biosystems (Carlsbad, CA, USA). Commercial kits used for triglycerides (TG, Infinity™ Triglycerides Reagent) and protein (Bio-Rad DC protein assay kit) quantification were purchased from Thermo Fisher Scientific Inc. (Middletown, VA) and Bio-Rad Co. (Hercules, CA), respectively. *Escherichia coli* OP50 and nematode strains, including N2, Bristol (wild type), *daf-16* (*mgDf50*) *I*, *tub-1* (nr2044) *II*, *nhr-49* (ok2165) *I*, *sbp-1*(ep79) *III*, *fat-5* (tm420) *V*, *fat-6* (tm331) *IV*, *fat-7* (wa36) *V*, *fat-6* (tm331) *IV*; *fat-7* (wa36) *V*, *lin-15B&lin-15A(*n765*) X; waEx15*, *aak-2* (ok524) *X* were obtained from Caenorhabditis Genetics Center (CGC).

Caenorhabditis elegans Maintenance and TG Quantification

Caenorhabditis elegans was maintained as previously described [10] with freshly prepared *Escherichia coli* OP50 as a food source. Unless otherwise noted, synchronized L1 worms [10] were supplemented with 0.1% dimethyl sulfoxide (DMSO) as vehicle or different doses of TMR for 4 days at 20°C in S-complete liquid media before measurements. TG content was determined as previous reported.[9, 11-13] Worms were collected in tubes and washed trice with M9 buffer to eliminate *E. coli* and treatment. After washing, the samples were prepared by sonication in 0.05% Tween 20 and were quantified for TG and protein using commercial kits: Infinity™

Triglycerides Reagent and Bio-Rad DC protein assay kit, respectively. TG content was normalized by protein level.

Growth Rate, Locomotive assay, Progeny and Pumping Rate

Growth rate, body size, and locomotive activity were determined after two days of treatment with TMR as previously described.[14] For growth rate, the number of worms at each developmental stage was recorded. Results were presented as a % of worms at each stage. Body size and the locomotive activity of worms were measured by using a WormLab tracking system (MBF Bioscience, Williston, VT).[11] *C. elegans* were transferred to a fresh *E. coli* OP50-seeded low peptone plate and allowed to acclimate for 10 min before recording. Each video was filmed for 1 min, then analyzed with WormLab software (MBF Bioscience version 3. 1. 0, Williston, VT) for average speed, worm length, and worm width.

Progeny and pumping rate were determined after two days' treatment. Worms were picked onto fresh *Escherichia coli* OP50-seeded nematode growth media (NGM) plates to lay eggs. Parent worms were transferred to new plates every day until the reproduction period was ceased. Daily brood size was recorded. Pumping rate was monitored by counting the pharyngeal contraction of randomly selected worms for 30 sec.[14]

Fatty Acid Composition Analysis

Fatty acid composition was determined as previously described.[12] Pre-treated worms were collected and washed three times to eliminate *E. coli* and treatment. Fatty acid methyl esters were subjected to GC/MS analysis (Shimadzu GC/MS-QP2010 SE, Tokyo, Japan). Both injector and detector temperatures were 250°C, and helium was used as a carrier gas with splitless

injection. Oven conditions were as follows: initial temperature of 50°C rose at a rate of 20°C/min to 190°C, was held for 30 min, then increased at a rate of 2°C /min to 220°C, and then held for 130 min. Identification of fatty acid methyl esters was conducted by comparing with fatty acid standards and/or their mass spectra according to the American Oil Chemists' Society mass spectra data or the NIST Mass Spectral library.

Fluorescence Imaging and Quantification

Fluorescence imaging and quantification were conducted as previously described.[15] After treatment, approximately 20~30 worms were anesthetized with 10 mM NaN_3, then mounted on microscopic slides with a 3% agarose layer and capped with covered slides. Imaging was performed by using confocal microscopy (Nikon Eclipse 80i Microscope SOP, Tokyo, Japan). Pictures of 20-40 worms were taken for each treatment group. The GFP intensity of the first anterior pair of intestinal cells was quantified by using Image J software.[15]

Quantitative Real-Time Reverse Transcription PCR (qRT-PCR)

Real time PCR was performed as previously described.[14] Pre-treated nematodes were collected, and total RNA was extracted by Trizol. A high-capacity cDNA reverse transcription kit (Thermo Fisher Scientific Inc, Middletown, VA) was used for cDNA generation. The StepOnePlus™ Real-Time PCR system (Applied Biosystems, Foster City, CA) was used to perform qRT-PCR. Taqman gene expression assays used in this study were: *daf-16* (Ce02422838_m1), *fat-5* (Ce02488494_m1), *fat-6* (Ce02465318_g1), *fat-7* (Ce02477066_g1), *sbp-1* (Ce02453000_m1), *tub-1* (Ce02435686_m1), *nhr-49* (Ce02412667_m1), *mdt-15* (Ce02406575_g1), *acs-2* (Ce02486193_g1), *kat-1* (Ce02434540_g1), *fasn-1* (Ce02411648_g1), *acs-2* (Ce02486193_g1),

pod-2 (Ce02427721_g1)**,** *nhr-80* (Ce02421189_g1)**,** *atgl-1* (Ce02406733_g1), *hosl-1* (Ce02494529_m1), *aak-1* (Ce02406989_g1), *aak-2* (Ce02406989_g1) and *ama-1* (Ce02462726_m1, an internal control).

Statistical analyses

Statistical analyses were performed by using one-way ANOVA model with homogeneous/heterogeneous variance or generalized one-way ANOVA model with homogeneous/heterogeneous variance, followed by the Tukey's multiple comparison test for the comparisons among groups. For Data in Fig. 2B, 2C, 2F and 3C, the normality assumption did not hold under one-way ANOVA model (with homogeneous/heterogeneous variance), thus, generalized one-way ANOVA model with homogeneous/heterogeneous variance was employed. The statistical analyses were performed using PROC MIXED (for one-way ANOVA) and PROC GLIMMIX (for generalized one-way ANOVA) in SAS statistical software (SAS Institute version 9.4, Cary, NC, USA), and $P < 0.05$ was considered statistically significant.

Results

trans-Trismethoxy resveratrol decreased TG accumulation in C. elegans

As shown in Figure 1, TMR significantly reduced TG accumulation, representative of body fat, in *C. elegans* at both concentrations of 100 and 200 μM, with 14% (P=0.0305) and 20% (P=0.0036) reductions compared to the control, whereas 50 μM TMR did not show any difference from the control. Therefore, a follow-up procedure used 100 and 200 μM of TMR. A similar result was observed for the resveratrol treatment, which exhibited a significant reduction

of TG accumulation in *C. elegans*, approximately 10-20% reductions over control at 100 and 200 μM, which were comparable to those of TMR (Supplementary Figure S1).

Effect of trans-trismethoxy resveratrol on physiological functions

To determine if TMR altered the basic physiological functions of *C. elegans*, several parameters – including growth rate, body size, food intake, reproductive capacity and locomotive activity – were examined. Results in Figure 2A show that TMR at 100 and 200 μM did not influence the growth rate of *C. elegans*. Consistently, no change in body length (Figure 2B) and width (Figure 2C) was observed under TMR treatment. To determine whether the reduced fat accumulation by TMR resulted from the change in food intake, we monitored the pharyngeal pumping rate, a widely-used indicator of food intake in *C. elegans.*[16] TMR did not influence the pumping rate of worms (Figure 2D), suggesting TMR's fat reduction did not result from the change in food intake. Additionally, TMR did not affect the progeny number of worms (Figure 2E), which indicates that TMR did not influence reproduction. Locomotive activity was monitored by measuring the worms' average moving speed, which also serves as an energy expenditure indicator.[17] Our results showed that at 200 μM TMR, moving speed was reduced by 10% compared to the control (Figure 2F), suggesting a reduced energy expenditure. Collectively, these results suggest that TMR, at 100 or 200 μM, did not influence development, food intake and reproduction, but reduced locomotive activity of *C. elegans*.

Effect of trans-trismethoxy resveratrol on lipid metabolism

To explore the underlying mechanisms of TMR's fat reduction effect, we examined the mRNA expression level of lipid metabolism-related genes with real time PCR analysis, as well as the genetic requirements of certain genes with mutant strains.

First, we determined the effect of TMR on lipogenesis. Sterol regulatory element-binding proteins (SREBPs) are the major transcription factors regulating the biosynthesis of cholesterol, fatty acids, and triglycerides, and therefore are considered the therapeutic target for many metabolic diseases, including obesity.[18] In *C. elegans*, SBP-1 is the single homolog of SREBPs, and plays a crucial role in regulating lipogenesis by targeting fatty acid biosynthesis genes: *pod-2*, *fasn-1* and stearoyl-CoA desaturases (SCDs) genes: *fat-5*, *fat-6* and *fat-7*.[19, 20] Loss of function mutation of *sbp-1* will lead to a low fat phenotype in *C. elegans*.[21] However, our results showed that TMR did not influence *sbp-1*, as the mRNA expression of *sbp-1* remained unchanged under TMR treatment (Figure 3A). Moreover, mutation of *sbp-1* failed to abrogate TMR's fat reduction effect as shown in Figure 3B, which suggests that *sbp-1* may not be involved in TMR's fat reduction effect.

Although TMR did not influence *sbp-1*, its known downstream targets, *pod-2*, *fat-6* and *fat-7* were significantly altered by TMR treatment. Indeed, TMR significantly reduced the expression level of *pod-2* by 24% (P=0.0469) at 100 μM, and 26% (P=0.0355) at 200 μM (Figure 3A) compared to the control, indicating a role for *pod-2* in TMR's fat-lowering effect.

SCDs are responsible for catalyzing the rate-limiting step in the formation of monounsaturated fatty acids, which have been suggested as potential drug targets for obesity treatment.[22, 23] Inhibition of SCDs is associated with reduced overall fat accumulation in both mammals[22, 24] and *C. elegans*.[19] Our results showed that SCD genes, *fat-6* and *fat-7,* were significantly downregulated by TMR, in which the *fat-6* mRNA level was reduced by 29% at

100 μM (P=0.0417) compared to the control, and the *fat-7* transcription level was decreased by 89% (P=0.0481) at 100 μM and 81% (P=0.0468) at 200 μM when compared to the control (Figure 3A). This was consistent with the results obtained in *fat-7*::*gfp* transgenic worms, in which there was significant reduction of *fat-7* expression by TMR treatments (P<0.0001 for 100 and 200 μM, respectively, Figure 3C). To further delineate the role of TMR in *fat-6* and/or *fat-7*, we examined the effect of TMR on fat accumulation in *fat-6* and *fat-7* single/double mutants. Results showed that TMR's fat lowering effects were only abolished in the *fat-6*; *fat-7* double mutant, but not the *fat-6* or *fat-7* single mutant (Figure 3B), suggesting the fat reduction effect of TMR was via both *fat-6* and *fat-7*-dependently.

Additionally, DAF-16, the sole *C. elegans* forkhead box O (FOXO) homologue, and NHR-80, the nuclear hormone receptor in *C. elegans,* are known to regulate lipid metabolism by targeting fatty acid desaturation (*fat-5*, *fat-6* and *fat-7*).[25] However, TMR did not affect *daf-16* and *nhr-80*, as TMR did not influence the mRNA expression level of either *daf-16* and *nhr-80* and/or the loss of function mutant of *daf-16* failed to abolish the fat reduction effect of TMR (Figures 3A & 3B). This suggests that TMR's regulation of *fat-6* and *fat-7* may not depend on *daf-16* and *nhr-80*. Taken together, our results suggest that TMR inhibited lipogenesis, possibly through the modulation of *pod-2*, *fat-6* and *fat-7*.

Next, we examined the role of TMR in beta-oxidation. *tub-1* encodes a TUBBY homolog that regulates fat storage across species, including *C. elegans.*[26-28] Mutation of *tub-1* exhibits a high fat phenotype, which appears to be linked with impaired β-oxidation.[19] *kat-1* encodes a β-oxidation enzyme, 3-ketoacyl-CoA thiolase, which acts in a synergistic manner with *tub-1* in regulating β-oxidation.[29] Results (Figure 3A) shows that 100 μM TMR significantly elevated the expression of *tub-1* by 70% (P=0.0005), but not *kat-1* compared to the control. However,

215 treatment of TMR significantly reduced fat reduction in *tub-1* mutant worms (Figure 3B), which
216 suggests that *tub-1* is potentially a contributing factor, but not a requirement, for TMR's fat-
217 reduction effect.

218 *nhr-49* encodes a functional homolog of peroxisome proliferator-activated receptor α.[30] It
219 works with mediator subunit MDT-15 to regulate β-oxidation by targeting genes involved in fat
220 oxidation signaling, such as *acs-2* (encodes an acyl-CoA synthetase). Worms treated with TMR
221 did not show any effects on gene expression of *nhr-49, mdt-15* nor *acs-2* compared to the control
222 (Figure 3A). In addition, TMR significantly reduced fat accumulation in *nhr-49* mutants (Figure
223 3B), which suggests the independence of *nhr-49* in TMR's function. Collectively, these results
224 suggest that TMR may enhance fatty acid beta-oxidation via a *tub-1*-mediated pathway, but not
225 *nhr-49*.

226 Adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) are the major
227 rate-determining enzymes for lipolysis in adipocytes.[31] In *C. elegans*, ATGL and HSL homologs
228 are encoded by *atgl-1* and *hosl-1*, respectively [9]. TMR did not change the expression of *atgl-1*
229 and *hosl-1* (Figure 3A), suggesting that TMR's fat-lowering effect might be independent of its
230 effect on lipolysis.

231 AMP-activated kinase (AMPK) is an energy sensor that modulates metabolic energy
232 balance at the whole-body level.[32] It is known to modulate energy homeostasis by negatively
233 regulating fat synthesis and positively regulating fatty acid β-oxidation and glycolysis.[9, 32] In *C.*
234 *elegans*, there are two genes that encode the homolog of the catalytic alpha subunit of AMPK:
235 *aak-1* and *aak-2*.[19] in which AAK-2 is the subunit thought to be responsible for the kinase
236 activity of AMPK, to regulate lifespan, dauer formation, fat metabolism.[19] TMR did not
237 influence *aak-1* expression, but significantly increased *aak-2* transcription levels (Figure 3A),

238 suggesting the possible involvement of AMPK in TMR's fat reduction effect. We further
239 determined the role of *aak-2* in TMR's function by using the *aak-2* mutant strain. However, the
240 fat reduction effect of TMR was remained in *aak-2* mutants (Figure 3B), suggesting that *aak-2*
241 may not be required for TMR's fat-lowering effect.
242
243 *trans-Trismethoxy resveratrol significantly reduced desaturation index*
244 Based on results that TMR reduces fat accumulation via a *fat-6*- and *fat-7*-dependent manner, we
245 further examined the effect of TMR on the desaturation index of oleic vs. stearic acids, as FAT-6
246 and FAT-7 are responsible for the conversion of stearic acid to oleic acid.[33] Results showed, as
247 expected, that TMR significantly decreased the desaturation index, and lead to 28% and 36%
248 reductions at 100 and 200 μM, respectively, compared to the control (Figure 4).
249
250 **Discussion**
251 TMR, a methyl derivative of resveratrol, significantly decreased the fat accumulation of *C.*
252 *elegans*. Moreover, TMR significantly down-regulated the expression of lipogenesis-related
253 genes *fat-6*, *fat-7*, and *pod-2,* and upregulated the fat oxidation-related gene *tub-1*, which
254 suggests a potential role for TMR in lipogenesis and fat oxidation. It was further determined that
255 fat reduction from TMR was dependent on *fat-6* and *fat-7*, homologs of SCDs. This research is
256 the first to report on the role of TMR in lipid metabolism in *C. elegans*.
257 SCDs have been suggested as a potential drug target for obesity treatment.[22, 24] Mice
258 deficient in SCDs have increased energy expenditure, reduced body adiposity, increased insulin
259 sensitivity, and are resistant to diet-induced obesity and liver steatosis.[24] Our results showed that
260 TMR significantly downregulated SCDs and lead to a reduction in overall fat accumulation in *C.*

elegans. Similar to TMR, other functional food components, including hesperidin,[34] pu-erh tea water extract,[35] and N-γ -(l-glutamyl)-l-selenomethionine,[36] were also found to inhibit fat accumulation in *C. elegans* via a *fat-6*- and *fat-7*-dependent process. The modulation of *fat-6* and *fat-7* was proposed via the regulation of *sbp-1* by hesperidin [34] and *mdt-15* by pu-erh tea extract [35] and N-γ -(l-glutamyl)-l-selenomethionine.[36] However, none of these genes are involved in the effect of TMR investigated in this study. In addition, other SCD regulators NHR-49, NHR-80 and DAF-16 [33] were not influenced by TMR. Therefore, whether TMR's effect was by acting directly on *fat-6* and *fat-7,* or indirectly through the regulation of an alternative upstream modulator, is not clear, and needs to be further elucidated.

pod-2 encodes the acetyl-CoA carboxylase (ACC) homolog responsible for catalyzing the first step of *de novo* fatty acid biosynthesis. In this report TMR significant modulated *pod-2*, but not *sbp-1*, which suggests the modulation of *pod-2* by TMR might be independent of *sbp-1*. In addition to *sbp-1*, POD-2/ACC is known to be regulated by other factors, such as AMPKs.[37] Thus, increased AAK-2/AMPKs might play a role in the regulation of *pod-2* by TMR treatment. Additionally, the reduced *fat-6* and *fat-7* might be contributing to TMR's effect on *pod-2*, as the inhibition of SCDs leads to the accumulation of saturated fatty acyl-CoA, which may result in a feedback inhibition of ACC (*POD-2* in *C. elegans*).[38]

TUB-1, the TUBBY homolog, functions with KAT-1, a 3-ketoacyl-coA thiolase, to modulate fat metabolism, which is known to be linked to fatty acid β-oxidation.[26-28] In addition, RAB-7 (Ras-related protein Rab-7a homolog), a mediated endocytic pathway, might be a target for *tubby* to regulate fat storage.[26-28] In the current study, TMR activated *tub-1*, which suggests a possible role for TMR in fatty acid β-oxidation and the endocytic pathway. Along with TUB-1, NHR-49, a functional homolog of PPARα, is also reported to regulate β-oxidation.[30] However,

284 current results showed that TMR did not influence *nhr-49*, which is consistent with a previous
285 finding that TMR is not acting as a PPARα agonist.[39] Since *nhr-49* and *tub-1* are both able to
286 modulate fatty acid β-oxidation, whereas TMR only modulates *tub-1*, but not *nhr-49*, we
287 speculate that *tub-1* and *nhr-49* may regulate β-oxidation through distinct mechanisms. Further
288 studies will be needed to determine the role of TMR, particularly in *tub-1*-mediated fatty acid β-
289 oxidation.

290 It was previously reported that the fat-lowering effect of resveratrol was via the activation
291 of AMPKs and Sirtuin 1 (SIRT 1) to promote adipocyte browning.[40, 41] Resveratrol derivatives,
292 piceatannol (hydroxylated derivative) and pterostilbene (demethylated derivative), were also
293 reported to activate AMPKs and SIRT 1.[42-45] Our study found that TMR increases expression of
294 *aak-2,* and it was previously reported that TMR also activates SIRT1.[46] In addition, it was
295 reported that resveratrol regulated lipid metabolism by regulating SCDs (*fat-6* and *fat-7*).[47-49]
296 Although this is the first report on the role of SCDs (*fat-6* and *fat-7*) in TMR's impact on fat
297 reduction, results suggest that resveratrol and its derivatives not only share structural homology,
298 they also may share common mechanisms regulating lipid metabolism.

299 Previously TMR was reported to have better bioactivities than resveratrol, especially
300 anticancer properties in numerous cancer cells.[50-55] One study directly compared the same dose
301 of TMR and resveratrol (50 mg/kg dose, orally administered every other day for 52 days) in nude
302 mice, and showed that resveratrol undergoes more extensive metabolic degradation than TMR,
303 which resulted in a higher serum level of TMR (0.94±0.55 μg/mL) compared to that of
304 resveratrol (0.02±0.01 μg/mL).[50, 51] Consistently, Lin et al. reported that the clearance for TMR
305 was found to be 8- to 9-times slower than that of resveratrol in rats.[5] Thus, it was suggested that
306 the improved bioactivities of TMR over resveratrol are due in part to methoxylation in TMR that

may hinder conjugation under detoxification metabolism, and/or alternatively to the greater lipophilicity of TMR, which leads to greater cell membrane permeability than resveratrol. In addition to the TMR studied in this research, which is a *trans*-isomer, TMR also has a *cis*-isomer, which has shown greater potency in inhibiting cancer cell growth compared to *trans*-TMR, although with higher cytotoxicity.[56] However, in the current study, we observed similar effects of TMR and resveratrol on body fat reduction (Supplementary Figure S1). Although *C. elegans* possess the conserved detoxification pathways to the mammals,[57, 58] the metabolic fate of the xenobiotics between *C. elegans* and humans may still be different, including the metabolism of bioactives by gut microbiota in the intestines.[59, 60] Moreover, *C. elegans* can absorb bioactives through cuticles along with ingestion,[19] and these differences may lead to different overall responses. Thus, it would still need to be determined if TMR exhibits enhanced potency over resveratrol and/or other cognates in fat reduction, particularly in humans.

According to our results, TMR at 100 and 200 μM did not significantly impact the normal physiological parameters of growth rate, body size, food intake and reproduction, while the locomotive activity, represented as average moving speed, was reduced by TMR at 200 μM, although with a relatively small reduction of 10% over the control. The reduced locomotive activity by TMR treatment might be an indicator for potential toxicity when the dosage of TMR is greater than 200 μM. Alternatively, as locomotive activity is regulated by neural networks,[61] it may indicate the potential effects of TMR on the nervous system. However, future research would be needed to confirm the significance of reduced locomotive activity by TMR observed in the current study.

To conclude, our data suggests that TMR, a methylated derivative of resveratrol, significantly reduced fat accumulation in *C. elegans*. The fat reduction induced by TMR requires

steroyl-CoA desaturase, *fat-6* and *fat-7,* along with the contributions of *tub-1, pod-2,* as well as *aak-2*. Although it is not currently possible to translate doses used in *C. elegans* directly to animals or humans, the current study may still provide the foundation for future studies with rodents and human clinical studies.

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Reference

1. B. B. Aggarwal, A. Bhardwaj, R. S. Aggarwal, N. P. Seeram, S. Shishodia and Y. Takada, Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies, *Anticancer Res.*, 2004, **24**, 2783-2840.
2. G. Blair, J. M. Cassady, J. Robbers, V. E. Tyler and R. F. Raffauf, Isolation of 3, 4', 5-trimethoxy-trans-stilbene, otobaene and hydroxyotobain from Virola cuspidata, *Phytochemistry*, 1969, **8**, 497-500.
3. T. Walle, F. Hsieh, M. H. DeLegge, J. E. Oatis and U. K. Walle, High absorption but very low bioavailability of oral resveratrol in humans, *Drug Metab. Dispos.*, 2004, **32**, 1377-1382.
4. S. Fulda, Resveratrol and derivatives for the prevention and treatment of cancer, *Drug Discovery Today*, 2010, **15**, 757-765.
5. H.-s. Lin and P. C. Ho, Preclinical pharmacokinetic evaluation of resveratrol trimethyl ether in sprague-dawley rats: the impacts of aqueous solubility, dose escalation, food and repeated dosing on oral bioavailability, *J. Pharm. Sci.*, 2011, **100**, 4491-4500.
6. K. De Vries, M. Strydom and V. Steenkamp, Bioavailability of resveratrol: possibilities for enhancement, *J. Herb. Med.*, 2018, **11**, 71-77.
7. M. Rossi, F. Caruso, R. Antonioletti, A. Viglianti, G. Traversi, S. Leone, E. Basso and R. Cozzi, Scavenging of hydroxyl radical by resveratrol and related natural stilbenes after hydrogen peroxide attack on DNA, *Chem. Biol. Interact.*, 2013, **206**, 175-185.
8. Y. Son, H. T. Chung and H. O. Pae, Differential effects of resveratrol and its natural analogs, piceatannol and 3, 5, 4'-*trans*-trimethoxystilbene, on anti-inflammatory heme oxigenase-1 expression in RAW264. 7 macrophages, *Biofactors*, 2014, **40**, 138-145.
9. P. Shen, Y. Yue and Y. Park, A living model for obesity and aging research: *Caenorhabditis elegans*, *Crit. Rev. Food Sci. Nutr.*, 2018, **58**, 741-754.
10. T. Stiernagle, Maintenance of *C. elegans* (February 11, 2006), *in WormBook, ed. The C. elegans Research Community, WormBook, doi/10.1895/wormbook.1.101.1, http://www.wormbook.org, (accessed April 2019)*
11. Q. Sun, Y. Yue, P. Shen, J. J. Yang and Y. Park, Cranberry product decreases fat accumulation in *Caenorhabditis elegans*, *J. Med. Food*, 2016, **19**, 427-433.
12. D. Colmenares, Q. Sun, P. Shen, Y. Yue, D. J. McClements and Y. Park, Delivery of dietary triglycerides to *Caenorhabditis elegans* using lipid nanoparticles: nanoemulsion-based delivery systems, *Food Chem.*, 2016, **202**, 451-457.
13. P. Shen, Y. Yue, K.-H. Kim and Y. Park, Piceatannol reduces fat accumulation in *Caenorhabditis elegans*, *J. Med. Food.*, 2017, **20**, 887-894.
14. P. Shen, Y. Yue, Q. Sun, N. Kasireddy, K. H. Kim and Y. Park, Piceatannol extends the lifespan of *Caenorhabditis elegans* via DAF-16, *Biofactors*, 2017, **43**, 379-387.
15. G. A. Lemieux, J. Liu, N. Mayer, R. J. Bainton, K. Ashrafi and Z. Werb, A whole-organism screen identifies new regulators of fat storage, *Nat. Chem. Biol.*, 2011, **7**, 206-213.
16. D. Raizen, B.-m. Song, N. Trojanowski and Y.-J. You, Methods for measuring pharyngeal behaviors (December 18, 2012), *in WormBook, ed. The C. elegans Research Community, WormBook, doi/10.1895/wormbook.1.154.1, http://www.wormbook.org, (accessed April 2019)*.
17. P. Shen, J. C. Kershaw, Y. Yue, O. Wang, K.-H. Kim, D. J. McClements and Y. Park, Effects of conjugated linoleic acid (CLA) on fat accumulation, activity, and proteomics analysis in *Caenorhabditis elegans*, *Food Chem.*, 2018, **249**, 193-201.
18. X. Xiao and B.-L. Song, SREBP: a novel therapeutic target, *Acta Biochim. Biophys. Sin.*, 2013, **45**, 2-10.

19. P. Shen, Y. Yue, J. Zheng and Y. Park, *Caenorhabditis elegans*: a convenient *in vivo* model for assessing the impact of food bioactive compounds on obesity, aging, and Alzheimer's disease, *Annu. Rev. Food Sci. Technol.*, 2018, **9**, 1-22.
20. J. L. Watts and M. Ristow, Lipid and carbohydrate metabolism in *Caenorhabditis elegans*, *Genetics*, 2017, **207**, 413-446.
21. T. Nomura, M. Horikawa, S. Shimamura, T. Hashimoto and K. Sakamoto, Fat accumulation in *Caenorhabditis elegans* is mediated by SREBP homolog SBP-1, *Genes Nutr.*, 2010, **5**, 17-27.
22. P. Cohen, J. M. Ntambi and J. M. Friedman, Stearoyl-CoA desaturase-1 and the metabolic syndrome, *Endocr. Metab. Immune Disord. Drug Targets*, 2003, **3**, 271-280.
23. Y. Choi, Y.-C. Kim, Y.-B. Han, Y. Park, M. W. Pariza and J. M. Ntambi, The *trans*-10, *cis*-12 isomer of conjugated linoleic acid downregulates stearoyl-CoA desaturase 1 gene expression in 3T3-L1 adipocytes, *J. Nutr.*, 2000, **130**, 1920-1924.
24. A. Dobrzyn and J. Ntambi, Stearoyl-CoA desaturase as a new drug target for obesity treatment, *Obes. Rev.*, 2005, **6**, 169-174.
25. S. S. Lee, S. Kennedy, A. C. Tolonen and G. Ruvkun, DAF-16 target genes that control *C. elegans* life-span and metabolism, *Science*, 2003, **300**, 644-647.
26. K. Carroll, C. Gomez and L. Shapiro, Tubby proteins: the plot thickens, *Nat. Rev. Mol. Cell Biol.*, 2004, **5**, 55-63.
27. P. W. Kleyn, W. Fan, S. G. Kovats, J. J. Lee, J. C. Pulido, Y. Wu, L. R. Berkemeier, D. J. Misumi, L. Holmgren and O. Charlat, Identification and characterization of the mouse obesity gene tubby: a member of a novel gene family, *Cell*, 1996, **85**, 281-290.
28. A. Mukhopadhyay, B. Deplancke, A. J. Walhout and H. A. Tissenbaum, *C. elegans* tubby regulates life span and fat storage by two independent mechanisms, *Cell Metab.*, 2005, **2**, 35-42.
29. I. C. Elle, L. C. Olsen, M.-B. Mosbech, S. V. Rødkær, D. Pultz, S. G. Boelt, J. Fredens, P. Sørensen and N. J. Færgeman, *C. elegans*: a model for understanding lipid accumulation, *Lipid Insights*, 2008, **1**, LPI. S1057.
30. M. R. Van Gilst, H. Hadjivassiliou, A. Jolly and K. R. Yamamoto, Nuclear hormone receptor NHR-49 controls fat consumption and fatty acid composition in *C. elegans*, *PLoS Biol.*, 2005, **3**, e53.
31. H. Miyoshi, J. W. Perfield, M. S. Obin and A. S. Greenberg, Adipose triglyceride lipase regulates basal lipolysis and lipid droplet size in adipocytes, *J. Cell. Biochem.*, 2008, **105**, 1430-1436.
32. D. G. Hardie, F. A. Ross and S. A. Hawley, AMPK: a nutrient and energy sensor that maintains energy homeostasis, *Nat. Rev. Mol. Cell Biol.*, 2012, **13**, 251-262.
33. J. L. Watts, Fat synthesis and adiposity regulation in *Caenorhabditis elegans*, *Trends Endocrinol. Metab.*, 2009, **20**, 58-65.
34. H. Peng, Z. Wei, H. Luo, Y. Yang, Z. Wu, L. Gan and X. Yang, Inhibition of fat accumulation by hesperidin in *Caenorhabditis elegans*, *J. Agric. Food Chem.*, 2016, **64**, 5207-5214.
35. Y. Ding, X. Zou, X. Jiang, J. Wu, Y. Zhang, D. Chen and B. Liang, Pu-erh tea down-regulates sterol regulatory element-binding protein and stearyol-CoA desaturase to reduce fat storage in *Caenorhaditis elegans*, *PloS ONE*, 2015, **10**, e0113815.
36. C. H. Chang, H. X. Q. Liao, F. L. Hsu, C. T. Ho and V. H. C. Liao, N-Ɣ-(l-glutamyl)-l-selenomethionine inhibits fat storage via the stearoyl-CoA desaturases FAT-6 and FAT-7 and the selenoprotein TRXR-1 in *Caenorhabditis elegans*, *Mol. Nutr. Food Res.*, 2018, **61**, 1800784.
37. C. Cantó and J. Auwerx, AMP-activated protein kinase and its downstream transcriptional pathways, *Cell. Mol. Life Sci.*, 2010, **67**, 3407-3423.
38. K. Ashrafi, Obesity and the regulation of fat metabolism (March 9, 2007), *in WormBook, ed. The C. elegans Research Community, WormBook, doi/10.1895/wormbook.1.130.1, http://www.wormbook.org, (accessed April 2019)*.

39. A. M. Rimando, R. Nagmani, D. R. Feller and W. Yokoyama, Pterostilbene, a new agonist for the peroxisome proliferator-activated receptor α-isoform, lowers plasma lipoproteins and cholesterol in hypercholesterolemic hamsters, *J. Agric. Food Chem.*, 2005, **53**, 3403-3407.
40. S. Wang, X. Liang, Q. Yang, X. Fu, C. J. Rogers, M. Zhu, B. Rodgers, Q. Jiang, M. V. Dodson and M. Du, Resveratrol induces brown-like adipocyte formation in white fat through activation of AMP-activated protein kinase (AMPK) α1, *Int. J. Obes.*, 2015, **39**, 967-976.
41. N. L. Price, A. P. Gomes, A. J. Ling, F. V. Duarte, A. Martin-Montalvo, B. J. North, B. Agarwal, L. Ye, G. Ramadori and J. S. Teodoro, SIRT1 is required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function, *Cell Metab.*, 2012, **15**, 675-690.
42. S. Kawakami, Y. Kinoshita, H. Maruki-Uchida, K. Yanae, M. Sai and T. Ito, Piceatannol and its metabolite, isorhapontigenin, induce SIRT1 expression in THP-1 human monocytic cell line, *Nutrients*, 2014, **6**, 4794-4804.
43. S. Gómez-Zorita, A. Fernández-Quintela, A. Lasa, L. Aguirre, A. M. Rimando and M. P. Portillo, Pterostilbene, a dimethyl ether derivative of resveratrol, reduces fat accumulation in rats fed an obesogenic diet, *J. Agric. Food Chem.*, 2014, **62**, 8371-8378.
44. K. Nagao, T. Jinnouchi, S. Kai and T. Yanagita, Pterostilbene, a dimethylated analog of resveratrol, promotes energy metabolism in obese rats, *J. Nutr. Biochem.*, 2017, **43**, 151-155.
45. Y.-C. Tung, Y.-H. Lin, H.-J. Chen, S.-C. Chou, A.-C. Cheng, N. Kalyanam, C.-T. Ho and M.-H. Pan, Piceatannol exerts anti-obesity effects in C57BL/6 mice through modulating adipogenic proteins and gut microbiota, *Molecules*, 2016, **21**, 1419.
46. Q. Li, K. Wang, Y. Ma, C. Qin, C. Dong, P. Jin, Y. Wu, X. Xiong, N. Li and C. Hu, Resveratrol derivative BTM-0512 mitigates obesity by promoting beige remodeling of subcutaneous preadipocytes, *Acta Biochim. Biophys. Sin.*, 2017, **49**, 318-327.
47. C. Rojas, B. Pan-Castillo, C. Valls, G. Pujadas, S. Garcia-Vallve, L. Arola and M. Mulero, Resveratrol enhances palmitate-induced ER stress and apoptosis in cancer cells, *PLoS ONE*, 2014, **9**, e113929.
48. X.-H. Zhang, B. Huang, S.-K. Choi and J.-S. Seo, Anti-obesity effect of resveratrol-amplified grape skin extracts on 3T3-L1 adipocytes differentiation, *Nutr. Res. Pract.*, 2012, **6**, 286-293.
49. K. Svensson, S. Schnyder, V. Albert, B. Cardel, L. Quagliata, L. M. Terracciano and C. Handschin, Resveratrol and SRT1720 elicit differential effects in metabolic organs and modulate systemic parameters independently of skeletal muscle peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α), *J. Biol. Chem.*, 2015, **290**, 16059-16076.
50. F. S. Aldawsari and C. A. Velázquez-Martínez, 3, 4', 5-*trans*-Trimethoxystilbene; a natural analogue of resveratrol with enhanced anticancer potency, *Invest. New Drugs*, 2015, **33**, 775-786.
51. S. J. Dias, K. Li, A. M. Rimando, S. Dhar, C. S. Mizuno, A. D. Penman and A. S. Levenson, Trimethoxy-resveratrol and piceatannol administered orally suppress and inhibit tumor formation and growth in prostate cancer xenografts, *Prostate*, 2013, **73**, 1135-1146.
52. Y. Bader, S. Madlener, S. Strasser, S. Maier, P. Saiko, N. Stark, R. Popescu, D. Huber, M. Gollinger and T. Erker, Stilbene analogues affect cell cycle progression and apoptosis independently of each other in an MCF-7 array of clones with distinct genetic and chemoresistant backgrounds, *Oncol. Rep.*, 2008, **19**, 801-810.
53. D. Simoni, M. Roberti, F. P. Invidiata, E. Aiello, S. Aiello, P. Marchetti, R. Baruchello, M. Eleopra, A. Di Cristina and S. Grimaudo, Stilbene-based anticancer agents: resveratrol analogues active toward HL60 leukemic cells with a non-specific phase mechanism, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 3245-3248.

54. M. Belleri, D. Ribatti, S. Nicoli, F. Cotelli, L. Forti, V. Vannini, L. A. Stivala and M. Presta, Antiangiogenic and vascular-targeting activity of the microtubule-destabilizing *trans*-resveratrol derivative 3, 5, 4'-trimethoxystilbene, *Mol. Pharmacol.*, 2005, **67**, 1451-1459.
55. L. Kai, J. Wang, M. Ivanovic, Y. T. Chung, W. B. Laskin, F. Schulze-Hoepfner, Y. Mirochnik, R. L. Satcher and A. S. Levenson, Targeting prostate cancer angiogenesis through metastasis-associated protein 1 (MTA1), *Prostate*, 2011, **71**, 268-280.
56. C. S. Mizuno, W. Ampomaah, F. R. Mendonça, G. C. Andrade, A. M. N. d. Silva, M. O. Goulart and R. A. d. Santos, Cytotoxicity and genotoxicity of stilbene derivatives in CHO-K1 and HepG2 cell lines, *Genet. Mol. Biol.*, 2017, **40**, 656-664.
57. T. H. Lindblom and A. K. Dodd, Xenobiotic detoxification in the nematode *Caenorhabditis elegans*, *J. Exp. Zool., Part A*, 2006, **305**, 720-730.
58. T. Fukushige, H. E. Smith, J. Miwa, M. W. Krause and J. A. Hanover, A genetic analysis of the *Caenorhabditis elegans* detoxification response, *Genetics*, 2017, **206**, 939-952.
59. E. M. Wilkinson, Z. E. Ilhan and M. M. Herbst-Kralovetz, Microbiota–drug interactions: impact on metabolism and efficacy of therapeutics, *Maturitas*, 2018, **112**, 53-63.
60. H. Remmer, The role of the liver in drug metabolism, *Am. J. Med.*, 1970, **49**, 617-629.
61. J. Gjorgjieva, D. Biron and G. Haspel, Neurobiology of Caenorhabditis elegans locomotion: where do we stand?, *Bioscience*, 2014, **64**, 476-486.

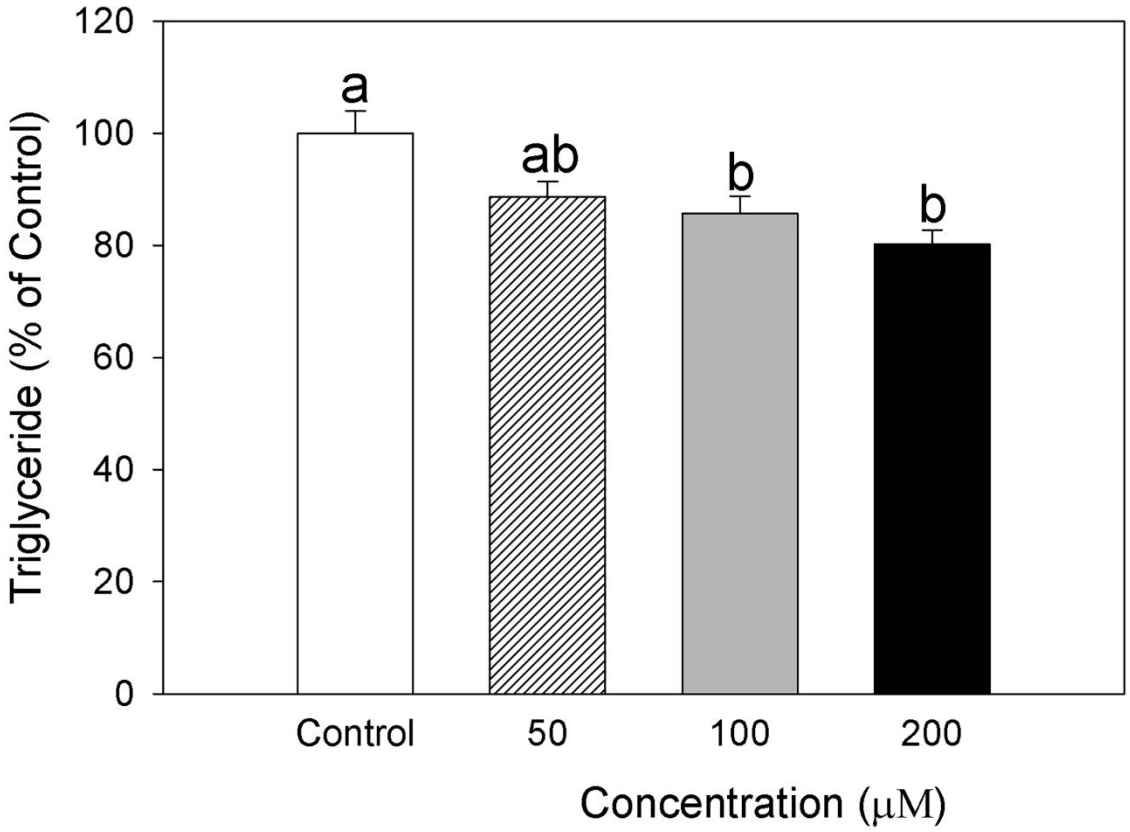
Figure 1. *trans*-Trismethoxy resveratrol significantly decreased fat accumulation in wild type *C. elegans*. Synchronized L1 worms were treated with the control (0.1% DMSO) or *trans*-trismethoxy resveratrol (50, 100 and 200 μM) for 4 days in 12-well plate with liquid medium at 20°C. Triglyceride content was measured and normalized by protein level. Results are expressed as mean±S.E (n=4 wells, each well contained >1000 nematodes). [a,b] Means with different letters are significantly different (P<0.05).

Figure 2. Influence of *trans*-trismethoxy resveratrol on physiological parameters. Synchronized L1 worms were treated with the control (0.1% DMSO) or *trans*-trismethoxy resveratrol (100 and 200 μM) for 2 days in liquid medium at 20°C. Growth rate (A) was scored as the percentage of worms at different developmental stages (n=3 plates, each plate >50 worms). Worm size, including length (B) and width (C), and locomotive activity, as average moving speed (F), were measured and analyzed by using the WormLab Tracking System (n=150-161). Food intake (D) was monitored by counting the pharyngeal pumping rate per 30s (n=12). Reproduction assay (E) was conducted by counting the number of progenies per worm until the reproduction period was ceased (n=5-6). Results are expressed as mean ±S.E. [a,b] Means with different letters are significantly different (P<0.05).

Figure 3. *trans*-Trismethoxy resveratrol regulates lipid metabolism-related genes. (A) Effect of *trans*-trismethoxy resveratrol on the mRNA expressions of lipid metabolism related genes. Synchronized L1 worms were treated with control (0.1% DMSO) or *trans*-trismethoxy resveratrol (100 and 200 μM) for 2 days in 60 mm dish with liquid medium at 20°C (n=3, each plate contained >8000 nematodes). Tested genes are *sbp-1* (sterol regulatory element binding

525 protein-1), *pod-2* (polarity and osmotic sensitivity defect-2), *fasn-1* (fatty acid synthase-1), *fat-5*
526 (fatty acid desaturase-5), *fat-6* (fatty acid desaturase-6), *fat-7* (fatty acid desaturase-7), *daf-16*
527 (abnormal dauer formation-16), *nhr-80* (nuclear hormone receptor-80), *tub-1* (tubby related-1),
528 *kat-1* (3-ketoacyl-coa thiolase), *nhr-49* (nuclear hormone receptor-49), *mdt-15* (mediator-15),
529 *acs-2* (fatty acid CoA synthetase-2), *atgl-1* (adipose triglyceride lipase-1), *hosl-1* (hormone
530 sensitive lipase-1), *aak-1* (AMP-activated kinase-1), and *aak-2* (AMP-activated kinase-2). (B)
531 Effect of *trans*-trismethoxy resveratrol on different mutant worms (n=4 wells, each well
532 contained >1000 nematodes). Synchronized L1 worms were treated with control (0.1% DMSO)
533 or *trans*-trismethoxy resveratrol (100 and 200 μM) for 4 days in 12-well plate with liquid
534 medium at 20°C. Triglyceride content was measured and normalized by protein level. (C)
535 Representative images of FAT-7::GFP expression. FAT-7::GFP expression was analyzed by
536 Image J software by quantifying fluorescence intensity in the first anterior pair of intestinal cells
537 (n=23-42). White arrow points out the first anterior pair of intestinal cells. Results are expressed
538 as mean ±S.E. ^{a,b} Means with different letters are significantly different (P<0.05).
539
540 **Figure 4.** *trans*-Trismethoxy resveratrol significantly decreased the desaturation index.
541 Synchronized L1 worms were administrated with control (0.1% DMSO) or *trans*-trismethoxy
542 resveratrol (100 and 200 μM) for 4 days in 100 mm dish with liquid medium at 20°C. After
543 treatment, total fatty acids were extracted, methylated and then analyzed by GC/MS (n=3 plates,
544 each plate >10000 worms). Results are expressed as mean ±S.E. ^{a,b} Means with different letters
545 are significantly different (P<0.05).

546 Figure 1



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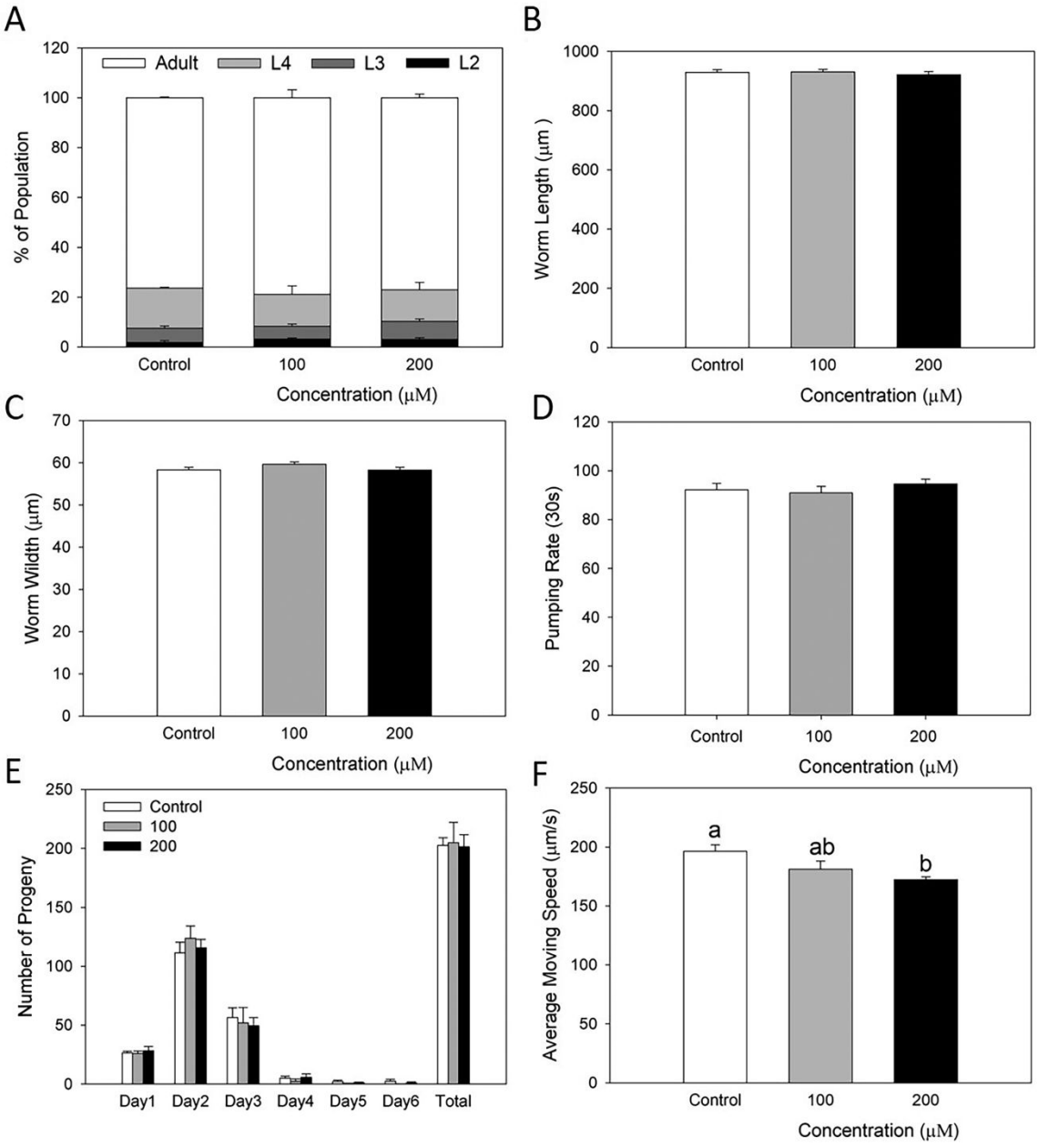
548 Figure 2



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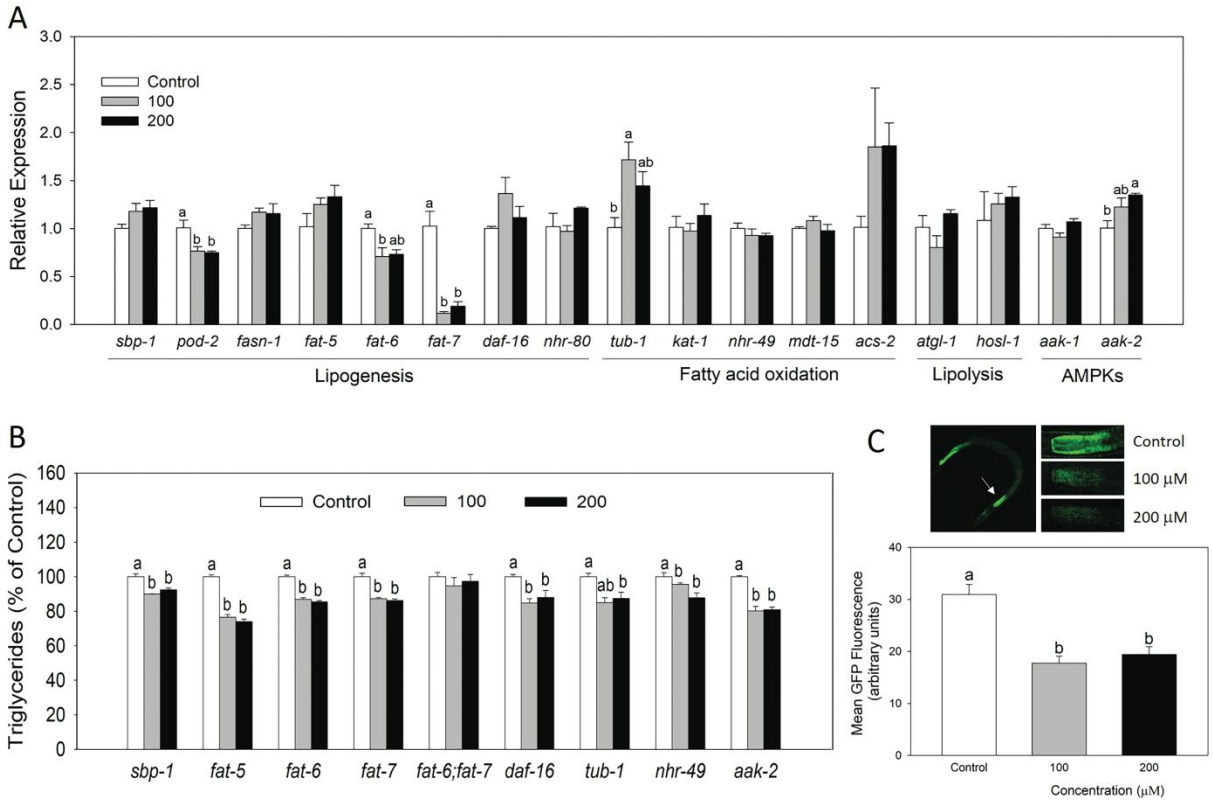

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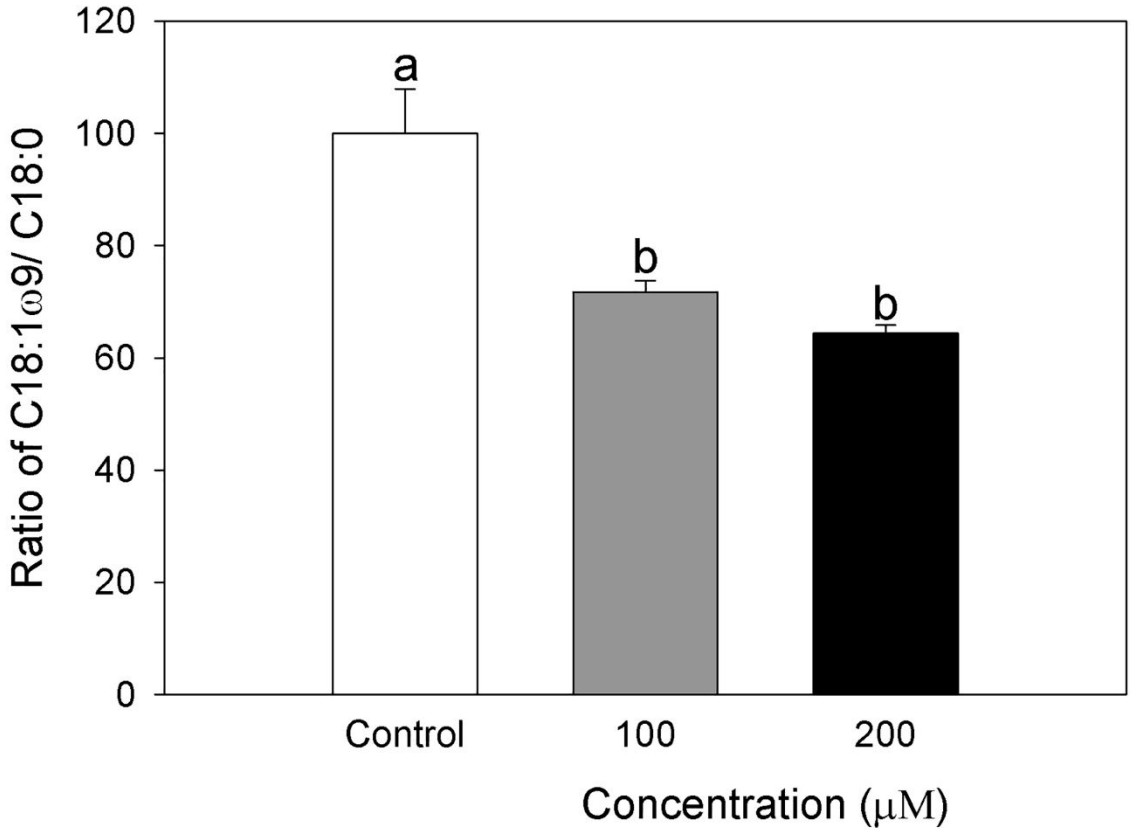
552 Figure 4



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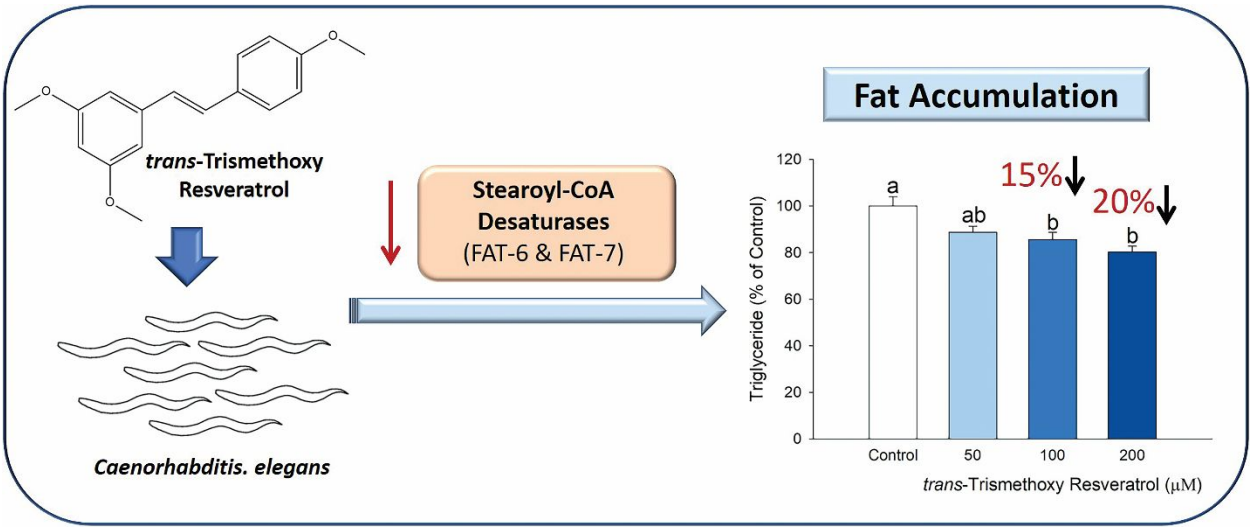

trans-Trismethoxy resveratrol reduced fat accumulation via the regulation of FAT-6 and FAT-7, stearoyl-CoA desaturases homologs, in *Caenorhabditis elegans*.