

## Piceatannol Reduces Fat Accumulation in *Caenorhabditis elegans*

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**ABSTRACT** Excess fat accumulation and abnormal metabolism are involved in numerous diseases and thus the research on identification of compounds that can regulate energy homeostasis could significantly facilitate the current effort to prevent and/or treat metabolic disorders. Piceatannol, one of the natural stilbenes, was previously found to decrease lipid accumulation of 3T3-L1 adipocytes. However, its role in fat metabolism *in vivo* is not known. Thus, *Caenorhabditis elegans* as an animal model was used in the current study to determine the effect of piceatannol on fat accumulation and its underlying mechanisms. The results showed that 50 and 100  $\mu$ M piceatannol significantly reduced fat accumulation of wild-type worms grown in normal and high-glucose conditions without altering the growth rate, worm length, pumping rate, or moving speed. The current study further indicated that piceatannol decreased the expression of *sbp-1* (encodes an ortholog of mammalian sterol regulatory element-binding protein) and its target gene *fasn-1* (encodes an ortholog of fatty acid synthase) as well as increased the expression of *hosl-1* (encodes an ortholog of hormone-sensitive lipase) in glucose-treated worms. These data suggested that piceatannol reduced fat accumulation in *C. elegans* by suppression of genes involved in lipid synthesis and possibly through stimulation of lipolysis. Given that piceatannol exerts similar effects in both *C. elegans* and 3T3-L1 cells, our finding could provide a mechanistic insight into the role of piceatannol in lipid metabolism in mammals.

**KEYWORDS:** *C. elegans* • fat accumulation • lipid metabolism • piceatannol

### INTRODUCTION

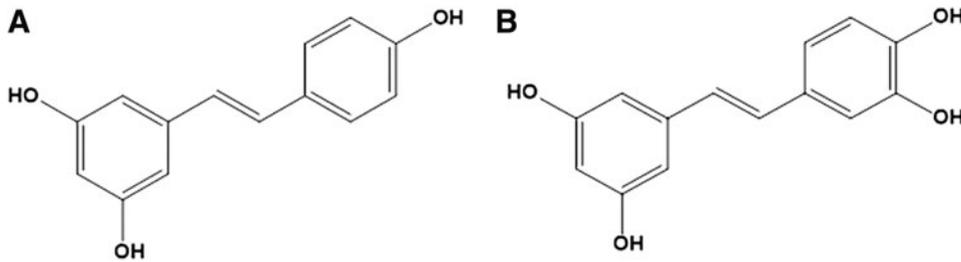
CURRENTLY, MORE THAN TWO-THIRDS of U.S. adults are overweight or obese.<sup>1</sup> As it is known that excess fat accumulation and abnormal metabolism are involved in numerous diseases such as type 2 diabetes,<sup>2</sup> the research on identification of compounds that can regulate energy homeostasis could significantly facilitate prevention and/or treatment of metabolic disorders. However, many compounds that are effective in *in vitro* assays fail to exhibit the desired response *in vivo* because of the sophistication of energy regulatory networks in organisms.<sup>3</sup> One of the approaches to resolve this problem is to establish whole organism models to identify new fat regulatory compounds. *Caenorhabditis elegans* (*C. elegans*), a free-living nematode, has been extensively utilized as an animal model for the research involving aging, obesity, and other physiological processes because of its relatively short life span, quick turnover, easy maintenance, well-known genetic pathways, and large brood size, etc.<sup>4</sup> Studies showed that the core mechanisms of regulatory pathways involving energy ho-

meostasis are largely conserved from mammals to nematodes.<sup>5</sup> *C. elegans* therefore offers great potential as an *in vivo* model to identify compounds that modulate fat storage.

As the best known member of the stilbene family, resveratrol has gained widespread attention because of its diverse bioactivity, including antiobesity.<sup>6</sup> Piceatannol is an analog and a metabolite of resveratrol, which possesses an additional hydroxyl group in position 3 of resveratrol (Fig. 1).<sup>7</sup> Piceatannol can be found in many plant sources, including grapes, white tea, and passion fruits,<sup>8,9</sup> and similar to resveratrol, many studies have reported the beneficial effects of piceatannol, such as estrogenic, anti-inflammatory, antioxidant, and anticancer activities.<sup>7</sup> Moreover, it has been reported that the catechol group present in ring B of piceatannol formed by phenolic groups in positions 3' and 4' significantly increases its antioxidant activity,<sup>10</sup> suggesting a possibility that piceatannol may have a greater beneficial effect than resveratrol. We have previously reported the life span-extending effect of piceatannol on *C. elegans*<sup>11</sup> and piceatannol was previously found to decrease lipid accumulation of 3T3-L1 adipocytes<sup>12</sup> and mice.<sup>13</sup> This study on mice reported that the effects of piceatannol were slightly, although not significantly, more effective than that of resveratrol in mice.<sup>13</sup> However, its role in fat metabolism of whole organisms has not yet been studied. Thus, the purpose of the current study was to determine the effects of piceatannol on fat accumulation of *C. elegans* in

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**FIG. 1.** Structures of (A) resveratrol and (B) piceatannol.

comparison with resveratrol as well as to determine the underlying mechanisms.

## MATERIALS AND METHODS

### Materials

All the strains of *C. elegans* were obtained from the Caenorhabditis Genetics Center, University of Minnesota, USA. Piceatannol (purity >98%) was from TCI America (Portland, OR) and resveratrol (purity ≥98%) was from MP Biomedicals (Santa Ana, CA). D-(+)-glucose (purity 99.5%) was from Sigma-Aldrich Co. (St. Louis, MO). Other chemicals were from Thermo Fisher Scientific, Inc., (Pittsburgh, PA) unless stated otherwise. Household bleach (The Clorox Company, Oakland, CA, USA) was used for bleaching the worms.

### *C. elegans* culture

M9 buffer, S-complete, and nematode growth medium (NGM) used for *C. elegans* culture were prepared as previously described.<sup>14–16</sup> The *C. elegans* strain used in this study was N2, Bristol (wild type). Synchronized populations were obtained according to standard protocols.<sup>17</sup> Treatments were started with synchronized L1 worms in 12-well plates at 20°C. Treatments were piceatannol (50 and 100 μM), resveratrol (50 and 100 μM), or 0.1% dimethyl sulfoxide (DMSO as a vehicle). For inclusion of glucose, additional glucose (0.5%, 0.75%, or 1%) was added during the treatment period. After 48 h of treatment, worms were collected for triglyceride and protein quantification. For Nile red staining, L1 worms were grown in liquid medium with 100 ng/mL Nile red (added as a 0.5 mg/mL stock solution in acetone at final concentration of acetone was 0.02%) and incubated for 48 h at 20°C.<sup>5,18</sup>

### Growth rate, pumping rate, worm size, and locomotive activity

Growth rate was determined by counting the number of worms at each developmental stage after 48 h as previously described.<sup>5</sup> For pumping rate, the number of pharyngeal contractions of 12 randomly selected nematodes was counted under the microscope for 1 min.<sup>19</sup>

Worm size and locomotion behavior were analyzed by WormLab tracking system (MBF Bio-science, Williston, VT), as described previously.<sup>11</sup> Low-peptone NGM plates were covered with a thin layer of *Escherichia coli* OP50 spread at 5 min before tracking. Worms were transferred to

the plates and allowed to acclimate for 30 min before the tracking started. A 2-min recording (7.99 frames/sec) was captured. WormLab software was then used to track and analyze the worm size (length and width) and moving behavior of 40–50 animals per treatment.

### Triglycerides and protein assay

Worms were collected and washed three times with M9 buffer before sonication (Sonicator 505, Fisher Scientific) for 3 min. The homogenized samples were then used for triglyceride and protein determination. The samples were analyzed with commercial kits: Infinity™ Triglyceride Reagent from Thermo Fisher Scientific, Inc., (Middletown, VA) for triglyceride measurement and Bio-Rad DC protein assay kit from Bio-Rad Co., (Hercules, CA) for protein measurement, respectively, according to manufacturers' instructions. Absorbance was measured using the Spectra-Max i3 Platform (Molecular Devices LLC, Sunnyvale, CA) at 510 and 630 nm, respectively, for triglyceride and protein determination. Triglyceride content was normalized to protein concentration.<sup>16,20</sup>

### Nile red staining of *C. elegans*

Worms were collected and washed three times with M9 buffer before being anesthetized in 10 mM sodium azide and then placed on an agarose pad covered with a coverslip; 12–15 randomly selected worms were photographed for each treatment group under a confocal microscope (Eclipse 80i SOP; Nikon, Tokyo, Japan).<sup>5</sup> The dye intensity was measured and quantified by ImageJ (Dr. Wayne Rasband, NIH, USA).

### Real-time polymerase chain reaction

Total RNA was isolated with TRIzol. Reverse transcription to generate cDNA was performed using high-capacity cDNA reverse transcription kit (Thermo Fisher Scientific, Inc., Middletown, VA). Quantitative RT-PCR was carried out using the StepOnePlus™ Real-Time PCR system (Applied Biosystems, Foster City, CA) and results were analyzed using comparative Ct method. TaqMan gene expression assays used for *sbp-1* (Ce02453000-m1), *nhr-49* (Ce02412667-m1), *hosl-1* (Ce02494529-m1), *atgl-1* (Ce02406730-g1), *pod-2* (Ce02427721-g1), *fasn-1* (Ce02411648-g1), *acs-2* (Ce02486193-g1), and *ama-1* (Ce02462726-m1) (internal control) were purchased from Life Technologies Co. (Carlsbad, CA).

*Statistical analysis*

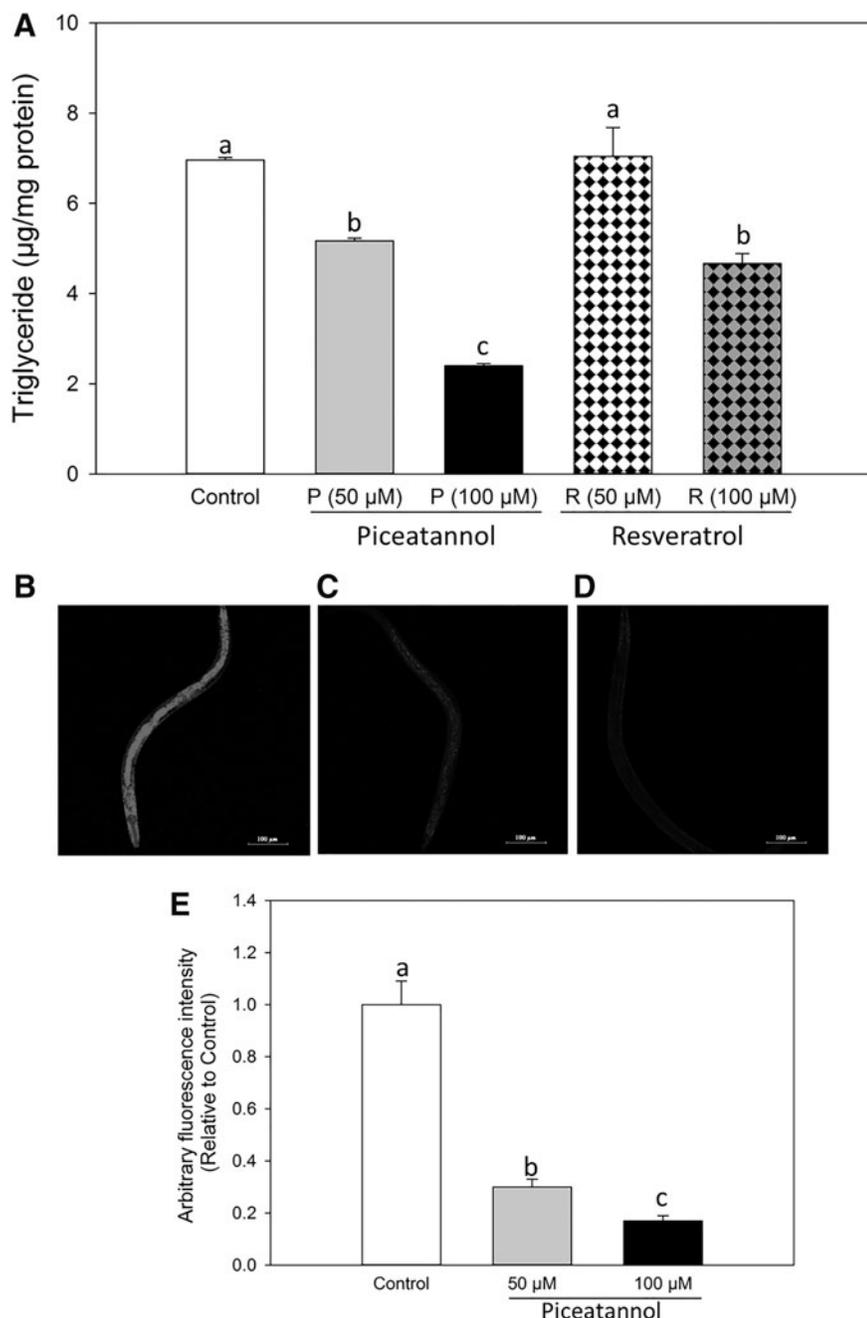
Data are presented as mean ± S.E. and analyzed with the Statistical Analysis System (SAS Institute, Cary, NC). Differences between groups were assessed with one-way analysis of variance, followed by Tukey's multiple range test. Significance of differences was defined at the  $P < .05$  level.

**RESULTS**

*Piceatannol decreased fat accumulation of C. elegans*

Since piceatannol is an analog and a metabolite of resveratrol, we measured the fat-reducing effect of both compounds

at 50 and 100  $\mu\text{M}$ . Figure 2A indicates that 100  $\mu\text{M}$  resveratrol significantly decreased fat accumulation of *C. elegans*, 31% reduction compared with control, while 50  $\mu\text{M}$  did not show any significant effect. In contrast, both 50 and 100  $\mu\text{M}$  piceatannol treatments significantly reduced fat accumulation compared with the control group (25% and 63% reduction over control, respectively), indicating that piceatannol treatments were more effective than the same concentrations of resveratrol. The effect of piceatannol on fat accumulation was also confirmed by Nile red staining. As shown in Figure 2B–E, 50 and 100  $\mu\text{M}$  piceatannol significantly decreased the fluorescence intensity of Nile red-stained intracellular fat by 70% and 83%, respectively, compared with control.



**FIG. 2.** Piceatannol decreased fat accumulation of *C. elegans*. (A) Synchronized L1 worms were treated with 0.1% DMSO as control, piceatannol (50 or 100  $\mu\text{M}$ ), or resveratrol (50 or 100  $\mu\text{M}$ ) for 48 h. Triglyceride content was normalized by protein concentration. (B–E) Synchronized L1 worms were treated with control (B) or piceatannol at 50 (C) or 100  $\mu\text{M}$  (D) and 100 ng/mL Nile Red for 48 h; 12–15 randomly selected worms were photographed for each treatment group under a confocal microscope. (E) The dye intensity was measured and quantified by ImageJ. Values represent different means ± S.Es. ( $n = 3–15$ ). <sup>a–c</sup>Means with different letters are significantly different at  $P < 0.05$ . DMSO, dimethyl sulfoxide.

### Piceatannol decreased fat accumulation of glucose-treated worms

Glucose has been used to increase fat accumulation as an established obesity model in *C. elegans*.<sup>21</sup> We next investigated the effect of piceatannol on glucose-stimulated fat accumulation in *C. elegans*. Our results showed that glucose increased fat accumulation of *C. elegans* (by 1.4 and 2.4-fold at 0.5% and 0.75%, respectively) and reached saturation at levels above 0.75% (Fig. 3A). Thus, we next tested the effect of piceatannol on worms cultured with 0.75% glucose. Piceatannol at both 50 and 100  $\mu\text{M}$  reduced the fat accumulation of glucose-treated worms by 22% and 44% compared with control, respectively, but not to the level in control (Fig. 3B).

### Effect of piceatannol on growth rate, worm size, pumping rate, and locomotive activity of glucose-treated worms

Our previous study showed that piceatannol did not alter any of the physiological states of wild-type nematodes.<sup>11</sup> In the current study, we also determined whether it had any effects on glucose-treated worms, including worm size, growth rate, pumping rate, and locomotive activity. As shown in Figure 4A, piceatannol did not influence the worm length after 48 h of incubation compared with both non-treated worms and glucose-treated worms. Glucose (0.75%) slightly but significantly increased the width of *C. elegans* (Fig. 4B). After coincubation with piceatannol (100  $\mu\text{M}$ ), the worms showed reduced width compared with glucose-treated worms, although no effect was observed upon 50  $\mu\text{M}$  treatment (Fig. 4B). Piceatannol did not influence the growth rate of glucose-treated worms as shown in Figure 4C. After 48 h of incubation, 93–95% of the worms reached late the L4 stage for all the four groups.

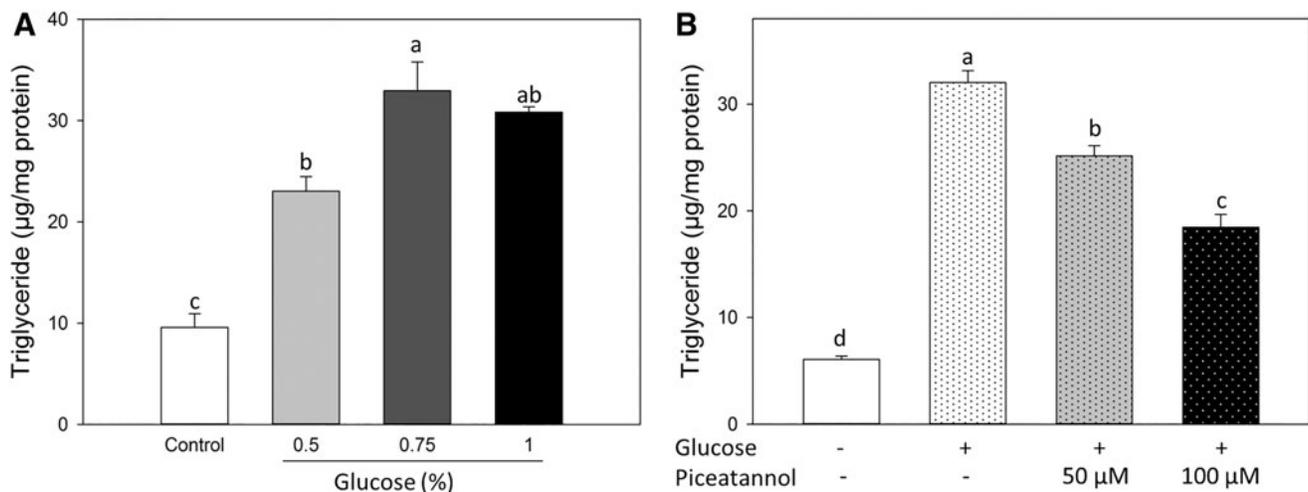
Pharyngeal pumping is used as an indicator of food intake for *C. elegans*.<sup>22</sup> In addition, the automated worm tracking

system was employed to determine moving behavior of worms as an indicator of energy expenditure in *C. elegans*.<sup>20</sup> Our previous report demonstrated that piceatannol did not influence the pumping rate or moving behavior of *C. elegans* after short-term treatment (48 h).<sup>11</sup> Thus, we determined the effects of piceatannol on pumping rate and movement in glucose-treated worms (Fig. 4D, E). Piceatannol, at both 50 and 100  $\mu\text{M}$ , did not affect the pumping rate compared with glucose-treated worms (Fig. 4D), suggesting that there were no changes in food intake due to the treatments. As shown in Figure 4E, both 50 and 100  $\mu\text{M}$  piceatannol did not show any effect on the moving speed of glucose-treated worms, suggesting no effects of piceatannol on energy expenditure.

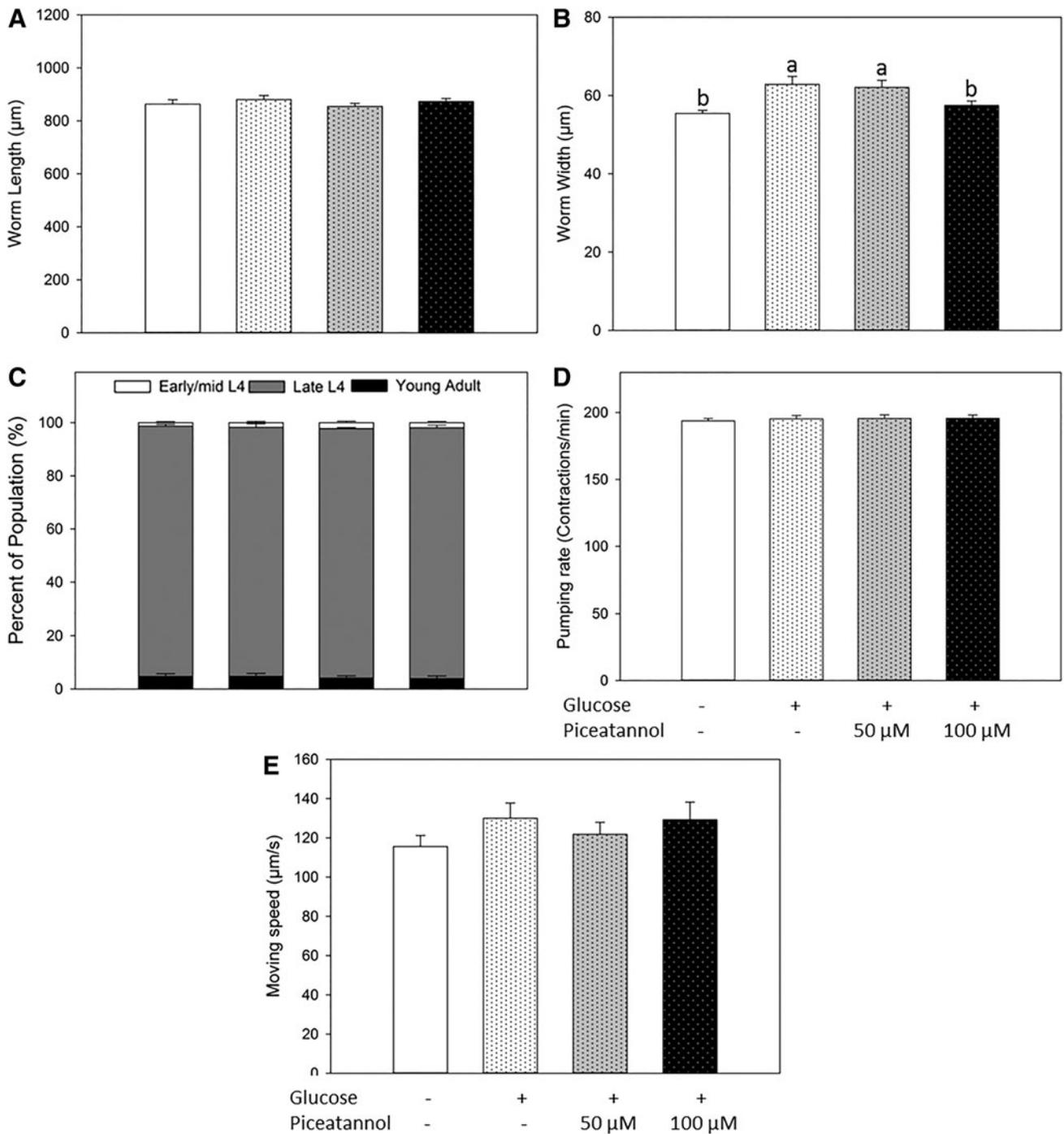
### Effect of piceatannol on the expression of genes involved in lipid metabolism

Real-time PCR was used to investigate the effect of piceatannol on the expression of genes involved in lipid metabolism of *C. elegans*. *sbp-1* encodes a *C. elegans* ortholog of mammalian sterol regulatory element-binding protein (SREBP), which is a crucial regulator of fatty acid synthesis and lipid homeostasis of this organism.<sup>23–25</sup> In *C. elegans*, the function of NHR-49 is close to mammalian peroxisome proliferator-activated receptors, which regulates  $\beta$ -oxidation and the expression of other genes that respond to dietary input.<sup>26</sup> Our results showed that piceatannol did not affect the expression of these two genes (Fig. 5A) in wild-type worms. We also measured the mRNA levels of two lipases in *C. elegans*, adipose triacylglyceride lipase 1 (ATGL-1) and hormone-sensitive lipase (HOSL-1), which are responsible for more than 90% of triacylglyceride hydrolysis.<sup>27</sup> Our results indicated that piceatannol did not affect *atgl-1* expression, but significantly increased the expression of *hosl-1* (Fig. 5A).

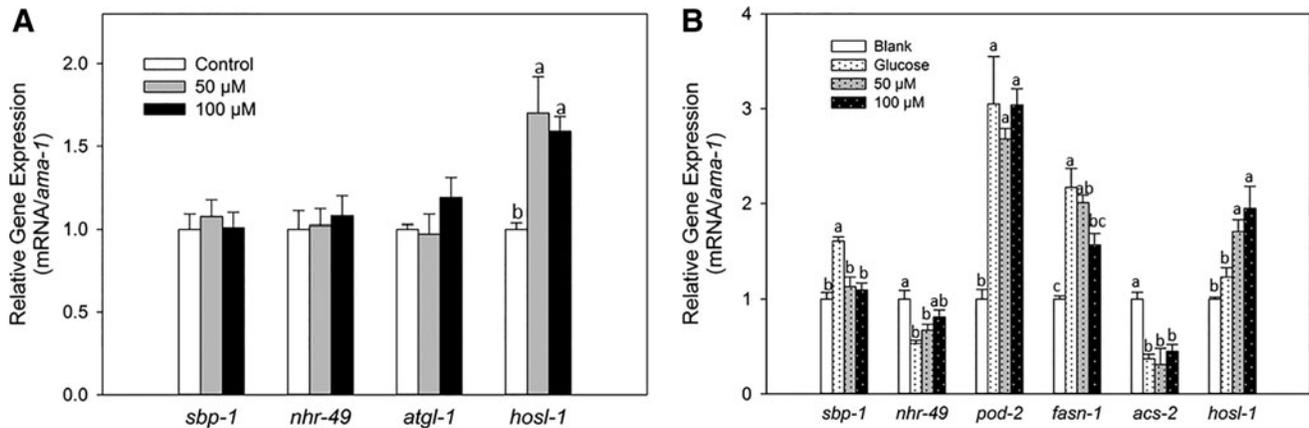
On the other hand, glucose (0.75%) significantly increased the expression of *sbp-1* (by 61%) and its targets, the



**FIG. 3.** Piceatannol reduced fat accumulation of glucose-treated worms. **(A)** Synchronized L1 worms were treated with D-glucose at (0%, 0.5%, 0.75%, or 1%) for 48 h. **(B)** Synchronized L1 worms were treated with 0.1% DMSO as blank, 0.75% D-glucose as positive control, or 0.75% D-glucose with piceatannol (50 or 100  $\mu\text{M}$ ) for 48 h. Triglyceride content was normalized with protein concentration. Values represent different means  $\pm$  S.Es. ( $n=3$ ). <sup>a-c</sup>Means with different letters are significantly different at  $P < .05$ .



**FIG. 4.** Effect of piceatannol on worm size, growth rate, pumping rate, and locomotive activity of glucose-treated worms. Synchronized L1 worms were treated with 0.1% DMSO as blank, 0.75% D-glucose as positive control, or 0.75% D-glucose with piceatannol (50 or 100  $\mu\text{M}$ ) for 48 h. **(A)** Worm length and **(B)** worm width were analyzed by WormLab software. **(C)** The number of worms at each developmental stage was counted. **(D)** The number of pharyngeal contractions of 12 randomly selected nematodes was counted under a microscope for 1 min. **(E)** WormLab software was used to track the average moving speed of each worm and 40–50 animals of each treatment were tracked and values calculated. Values represent different means  $\pm$  S.Es. ( $n=40\text{--}50$  for worm size and moving speed;  $n=3$  plates and  $\approx 100$  worms/plate for growth rate;  $n=12$  for pumping rate). <sup>a-b</sup>Means with different letters are significantly different at  $P < .05$ .



**FIG. 5.** Effect of piceatannol on the expression of fat metabolism-related genes in *C. elegans*. (A) Synchronized L1 worms were treated with 0.1% DMSO as control or piceatannol (50 or 100  $\mu$ M) for 48 h; (B) Synchronized L1 worms were treated with 0.1% DMSO as blank, 0.75% D-glucose as positive control, or 0.75% D-glucose with piceatannol (50 or 100  $\mu$ M) for 48 h. Values represent different means  $\pm$  S.E.s. ( $n=3$ ). <sup>a-c</sup>Means with different letters are significantly different at  $P < .05$ .

lipid synthesis-related genes, *pod-2* and *fasn-1*, by 3- and 2-fold over the control, respectively (Fig. 5B). *C. elegans pod-2* and *fasn-1* encode acetyl-CoA carboxylase and fatty acid synthase that are predicted to catalyze the first and second steps in *de novo* fatty acid biosynthesis, respectively.<sup>28</sup> Glucose (0.75%) also repressed the expression of *nhr-49* (by 47%) and its target gene *acs-2* (by 63%) (Fig. 5B). *acs-2* is predicted to encode a mitochondrial acyl-CoA synthetase, which activates fatty acids for transport into the mitochondrial matrix, where the fatty acids are then subject to  $\beta$ -oxidation.<sup>29</sup> Moreover, an increased mRNA level of *hosl-1* (by 23%) was observed upon glucose incubation (Fig. 5B).

Although piceatannol did not show any effect on *sbp-1* mRNA level of wild-type worms (Fig. 5A), it significantly reduced the expression of *sbp-1* by 30% and 32% at 50 and 100  $\mu$ M compared with control, respectively, in glucose-treated worms (Fig. 5B). Piceatannol did not affect the mRNA level of *pod-2* compared with glucose-treated control, but 100  $\mu$ M treatment reduced the expression of *fasn-1* by 28% (Fig. 5B). Piceatannol at 50 and 100  $\mu$ M slightly increased the expression of *nhr-49*, but did not show any effect on its target gene *acs-2* (Fig. 5B). As observed in nonglucose-treated worms, piceatannol at both 50 and 100  $\mu$ M significantly increased the expression of *hosl-1* (by 39% and 59%, respectively) over glucose-treated control (Fig. 5B).

## DISCUSSION

In the present study, piceatannol (50 and 100  $\mu$ M) significantly reduced fat accumulation in both nonglucose-treated worms and glucose-treated worms. Piceatannol at 100  $\mu$ M reduced the width of glucose-treated nematodes, likely due to reduced fat accumulation, without altering other physiological properties (worm length, growth rate, pumping rate, and locomotive activity). Real-time PCR assay revealed that piceatannol reduced the expression of fatty acid synthesis-related genes, *sbp-1* and *fasn-1*, as

well as increased the expression of *hosl-1* in glucose-treated worms.

Clinical studies on the bioavailability of resveratrol in humans reported that oral administration of resveratrol can achieve 0.1–6  $\mu$ M blood concentrations for several hours.<sup>30</sup> However, little information is known on the physiologically relevant concentrations of piceatannol in humans. In the current study, 50 and 100  $\mu$ M concentrations of piceatannol were used based on a previous study, where 25 and 50  $\mu$ M piceatannol decreased the lipid accumulation of 3T3-L1 adipose without any cytotoxicity.<sup>12</sup> We found that only 50 and 100  $\mu$ M, but not 25  $\mu$ M (data not shown), significantly decreased the fat accumulation in *C. elegans*. This inconsistency may be attributed to the difference of study models and method to quantify fat content that we used and/or chemical stability of piceatannol in treatment conditions.

Glucose has been used to establish the *C. elegans* obesity model in many studies with the concentration ranging from 0.18% to 2%.<sup>21,31–33</sup> In the current study, glucose dose-dependently elevated the fat accumulation of *C. elegans* and reached its maximum effect at 0.75%. Thus, we used this concentration for further research to investigate the anti-obesity property of piceatannol. In mammals, triglyceride levels increase through upregulation of SREBP under the influence of nutritional status, such as glucose treatment.<sup>34,35</sup> The same effect of glucose on the *sbp-1* expression in *C. elegans* was also observed in our study and previous report.<sup>31,32</sup> Together with the elevated expression of *pod-2* and *fasn-1*, these data demonstrated increased fat synthesis in glucose-treated worms. In contrast, as the same effect on PPAR $\alpha$  in mammals,<sup>36,37</sup> glucose also reduced the mRNA levels of *nhr-49* and its target *acs-2* in *C. elegans*, suggesting lower fatty acid  $\beta$ -oxidation in these nematodes. Collectively, increased fat synthesis and reduced  $\beta$ -oxidation upon glucose treatment eventually lead to the nematodes with high fat accumulation.

Piceatannol significantly reduced the mRNA level of *sbp-1* in glucose-treated worms. SBP-1 positively regulates fatty

acid synthesis and lipid homeostasis of this organism.<sup>31</sup> In addition, the expression of its target gene *fasn-1* was also decreased upon 100  $\mu$ M treatment. POD-2 and FASN-1 are predicted to catalyze the first and second step in *de novo* fatty acid biosynthesis, respectively.<sup>28</sup> Since piceatannol only affected the expression of *fasn-1*, but not *pod-2*, it may act mainly on the second step in *de novo* fatty acid biosynthesis, namely the process from malonyl-CoA to saturated fatty acyl-CoAs. However, even though piceatannol had a slight effect on *nhr-49*, piceatannol did not influence its target *acs-2*, indicating that this may not be a significant contribution to overall fat content in this model.

HOSL is a multifunctional enzyme capable of hydrolyzing a variety of acylesters, including triacylglycerol, diacylglycerol, and monoacylglycerol.<sup>38</sup> HOSL-1 is predicted to function in the regulation of energy homeostasis and fat metabolism by catalyzing the first rate-limiting step of triacylglyceride hydrolysis.<sup>39</sup> The activity of HOSL-1 is regulated both post-translationally by phosphorylation and by pretranslational mechanisms.<sup>40</sup> A study found that the amount of HOSL protein and its mRNA levels in subcutaneous adipocytes show a strong correlation with its lipolytic activity.<sup>41</sup> Based on our results, piceatannol may increase the activity of HOSL-1 at the transcriptional level; however, its influence on the post-translational level of HOSL-1 needs to be further investigated.

In summary, the current study shows that piceatannol (50 and 100  $\mu$ M) significantly reduced fat accumulation in *C. elegans* through the reduction of fat synthesis and potential elevation of lipolysis. Given that piceatannol exerts similar effects in both *C. elegans* and 3T3-L1 adipocytes, *C. elegans* can be a useful *in vivo* model to investigate any bioactives that may target lipid metabolism.

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## AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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