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Leucosceptroid B from glandular trichomes of *Leucosceptrum canum* reduces fat accumulation in *Caenorhabditis elegans* through suppressing unsaturated fatty acid biosynthesis

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[ABSTRACT] Obesity that is highly associated with numerous metabolic diseases has become a global health issue nowdays. Plant sesterterpenoids are an important group of natural products with great potential; thus, their bioactivities deserve extensive exploration. RNA-seq analysis indicated that leucosceptroid B, a sesterterpenoid previously discovered from the glandular trichomes of *Leucosceptrum canum*, significantly regulated the expression of 10 genes involved in lipid metabolism in *Caenorhabditis elegans*. Furthermore, leucosceptroid B was found to reduce fat storage, and downregulate the expression of two stearoyl-CoA desaturase (SCD) genes f*at-6* and *fat-7*, and a fatty acid elongase gene *elo-2* in wild-type *C. elegans*. In addition, leucosceptroid B significantly decreased fat accumulation in both *fat-6* and *fat-7* mutant worms but did not affect the fat storage of *fat-6*; *fat-7* double mutant. These findings indicated that leucosceptroid B reduced fat storage depending on the downregulated expression of *fat-6*, *fat-7* and *elo-2* and thereby inhibiting the biosynthesis of the corresponding unsaturated fatty acid. These findings provide new insights into the development and utilization of plant sesterterpenoids as potential antilipemic agents.

[KEY WORDS] Sesterterpenoid; Leucosceptroid B; *Leucosceptrum canum*; *Caenorhabditis elegans*; Fat accumulation; Fatty acid biosynthesis; Antilipemic agent

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Introduction

With the improvement of living quality, the prevalence of overweight and obese individuals is increasing at an amazing speed around the world, with approximately 107.7 million children and 603.7 million adults suffering from obesity worldwide in 2015 [1]. Obesity and obesity-related diseases, such as type 2 diabetes (T2D), fatty liver and hyperlipidemia, have gradually become the most common diseases, threatening human health seriously [1-2]. In addition to advocating a balanced diet and physical exercise, a range of anti-obesity drugs and functional foods have been proposed [3]. In particular, natural products have been considered potentially ideal candidates for the development of safe and effective anti-obesity therapies [4].

Sesterterpenoids are an important class of terpenoids possessing complex chemical structures and extensive biological activities [5]. Our previous studies on the chemodiversity and biosynthesis of plant sesterterpenoids led to the discovery of two unique families of defensive sesterterpenoids named leucosceptroids and colquhounoids from two Himalayan Lamiaceae plants *Leucosceptrum canum* and *Colquhounia coccinea* var. *mollis*, respectively, as well as a GFDP synthase catalyzing the formation of the C_{25} prenyl diphosphate

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Dedicated to Professor SUN Han-Dong on the Occasion of His 80th Birthday

precursor from *L. canum* [6-13]. The complex structures and significant defensive functions of these compounds have attracted great attention for chemical synthesis [14-20], but their biological activities have not been well investigated.

The nematode *Caenorhabditis elegans* is a multicell model organism with many advantages, such as a small size, inexpensive/easy culture, short generation cycle, high number of progeny and easy genetic manipulation [21]. The complete sequence of *C. elegans* genome is available, and approximately 60%−80% of the genes have a homologous relationship with human genes [21]. Many aspects, including cellular processes, metabolic pathways and signaling pathways involved in diseases, are well conserved between humans and *C. elegans*, which have made *C. elegans* an ideal tool for human disease investigation and drug discovery [22]. Like in humans, lipids, including fatty acids and triacylglycerides, are the energy reservoirs of *C. elegans*, and lipid metabolism and regulation in worms are similar to that in human, but much simpler and clearer [21, 23]. In order to explore the biological activities of plant sesterterpenoids, their influence on the gene expression of *C. elegans* was analyzed using RNA-seq, an efficient technology that can provide high-throughput and sensitive data at the gene expression level that may eventually reflect an organism's physiology and biochemistry [24]. We observed that the expression of genes involved in lipid metabolism in *C. elegans* was significantly regulated by leucosceptroid B, a major compound in the glandular trichomes of *L. canum*. Through phenotypic observation in combination with qRT-PCR, biochemical and mutant analyses, we found that leucosceptroid B could reduce fat accumulation in wild-type *C. elegans*, and the mechanism was associated with its impact on unsaturated fatty acid biosynthesis.

Materials and Methods

C. elegans strains and culture conditions

The *C. elegans* strains used in this study were wild-type Bristol N2, BX106 *fat-6*(*tm331*), BX153 *fat-7*(*wa36*) and BX156 *fat-6*(*tm331*); *fat-7*(*wa36*). The N2 strain was obtained from the Caenorhabditis Genetics Center (CGC), while the mutant strains were kindly provided by Prof. LIANG Bin at Kunming Institute of Zoology, Chinese Academy of Science. *C. elegans* strains were raised on nematode growth media (NGM) with *Escherichia coli* strain OP50 as a food source at 20 °C in a constant temperature and humidity incubator.

Pharyngeal pumping assay

L1 larval of wild-type worms were hatched from eggs that were prepared by sodium hypochlorite and NaOH treatment of gravid adults and were cultured for approximately 42 h to obtain L4 worms. Synchronized L4 worms were transferred to NGM plates seeded with *E. coli* OP50 containing 6.25, 12.5, 25 or 50 μmol·L^{-1} leucosceptroid B. Meanwhile, DMSO was added as a negative control. After culture for 24 h, the worm pharyngeal pumping frequency was observed under a Lecia DMil microscope according to the description in the literature [25]. At least 15 worms were selected randomly in each group. The experiment was repeated at least twice.

RNA extraction and RNA-seq analysis

Synchronized L1 wild-type worms were grown in NGM plates seeded with *E. coli* OP50 containing 125 μmol·L^{-1} leucosceptroid B or DMSO. Worms were collected at the L4 stage (approximately 42 h) and total RNA was then isolated using TRIzol (Invitrogen, USA) according to the manufacturer's instructions. RNA-seq was performed on a BGISEQ-500 platform at the Beijing Genomics Institute in Shenzhen, China. RNA-seq of each group was repeated with three independent biological replicates.

Oil Red O Staining

Oil Red O staining was performed as described in the literature [26]. Synchronized young adult (YA) nematodes were collected and washed with phosphate buffered saline (PBS). Worms were fixed in 1% paraformaldehyde solution at 4 °C for 1 h. After two freeze-thaw cycles in a liquid nitrogen/ice water bath, the worms were collected by centrifugation and washed three times with PBS. The worms were dehydrated in 60% isopropanol for 15 min at room temperature, and then the supernatant was removed. The nematodes were stained with Oil Red O dye solution (Solarbio, China) for 4 h at room temperature away from light. After that, the nematodes were washed with PBS and observed under a Lecia DMil microscope with differential interference contrast (DIC) optics. Photographs were taken, and the average pixel intensity for a 50 × 50 μm^2 area immediately behind the pharynx was measured using ImageJ software (developed by the National Institute of Health). At least 10 worms were selected randomly in each group, and the experiment was repeated at least three times.

Lipid extraction and analysis

Synchronized YA nematodes with 1−3 eggs were collected and washed with distilled water. Chloroform−methanol (1 : 1, *V/V*) was added to extract lipids. After being frozen overnight at –20 °C, a solution containing 1 mol·L^{-1} KCl and 0.2 mol·L^{-1} H_3PO_4 was added, and the solution was then separated into organic and aqueous phases. The organic layer was isolated and dried with nitrogen gas, and chloroform was added to redissolve the total lipids. We performed thin-layer chromatography (TLC) to separate triacylglycerols (TAGs). Subsequently, the TAG fraction was methylated in methanol solution with 2.5% sulfuric acid at 80 °C for 1 h. Fatty acid methyl ester derivatives were then extracted by *n*-hexane, and analyzed using gas chromatography/mass spectrometry (GC-MS) (Agilent 7890A-5975C, USA). The analysis conditions were as follows: a HP-5 MS quartz capillary column (30 m × 250 μm × 0.25 μm) was used; the injection volume was 5 μL; high purity helium was the carrier gas; the initial temperature was 80 °C which was maintained for 2 min; the temperature was programmed to increase to 190 °C at 10 °C·min^{-1}, then programmed to increase to 200 °C at 2 °C·min^{-1} which was maintained for 5 min; finally the temperature was raised to 250 °C at 10 °C·min^{-1} and maintained for 4 min. The experiment was repeated at least three times.

qRT-PCR analysis

Total RNA was extracted from synchronized L4 stage

worms that were grown on 50 μmol·L^{-1} leucosceptroid B or DMSO as described above. cDNAs were synthesized using a HiFiScript gDNA Removal cDNA Synthesis Kit (CoWin Biosciences, China). UltraSYBR Mixture with Low ROX (CoWin Biosciences, China) was used for the qRT-PCR reaction on an Applied Biosystems 7500 instrument (Life Technologies, USA). Relative expression of genes was calculated according to the $^{\Delta\Delta}$Ct method, and *act-1* was used as the reference gene. Oligonucleotide primers 5′-GAAGACAAACT TGGAATCCGAGC-3′ and 5′-GTAGCCTTTTCCATACTCT CCCAG-3′ for *acdh-1*, 5′-ACAAACAAGTCCAGCAGCCA C-3′ and 5′- CCGAAGTTCCAGAGAGCAAGTG-3′ for *elo-2*, 5′-ACACCATCGTCGGTCTTCCAG-3′ and 5′-GAGGACAA AGGCAATGTAAGCAC-3′ for *fat-2*, 5′-GCCTACAAAGCC ACCCTCTCA-3′ and 5′-TCTTCACCAACAACCATCCCA-3′ for *fat- 5*, 5′-ACTCGTGGATTCTTCTTCGCTCA-3′ and 5′-GCTTGGCTCCTTGTTCCTTAACTT-3′ for *fat-6*, 5′-CAA CAGCGCTGCTCACTATT-3 and 5′-CACCAACGGCTACA ACTG TG-3′ for *fat-7*, as well as 5′-GCTGGACGTGATCTT ACTGATTACC-3′ and 5′-GTAGCAGAGCTTCTCCTTGAT GTC-3′ for *act-1* were used. Each experiment was repeated with three biological replicates, each of which was conducted with three technical duplicates.

Statistical analysis

Statistical analysis of data was performed using *t*-tests or analysis of variance (ANOVA) followed by least significant difference (LSD) multiple comparison tests with Prism-Graph5 software (GraphPad Software Inc. CA, USA).

Results

Leucosceptroid B had no effect on food intake and viability of nematodes

Because leucosceptroid B (Fig. 1A) showed potent antifeedant activity against two generalist insects, beet armyworm (*Spodoptera exigua*) and cotton bollworm (*Helicoverpa armigera*) [6], it was interesting to investigate the impact of this compound on the food intake and viability of nematodes. Synchronized L4 WT nematodes were transferred to NGM medium with *E. coli* OP50 containing leucosceptroid B or DMSO, and the frequency of pharyngeal pumping was observed after 24 hours. The pharyngeal pumping frequency of the control group was 203.2 ± 2.9 times per minute. Meanwhile, the pharyngeal pumping frequency of the treatment groups supplied with 6.25, 12.5, 25, and 50 μmol·L^{-1} leucosceptroid B was 201.6 ± 2.3, 197.1 ± 1.5, 200 ± 2.5 and 205.7 ± 3.1 per minute, respectively (Fig. 1B). There was no significant difference in pharyngeal pumping frequency between the control and treatment groups, indicating that leucosceptroid B had no significant effect on food intake and viability of nematodes.

RNA-seq analysis of C. elegans indicated that leucosceptroid B regulated the expression of genes involved in lipid metabolism

To obtain a comprehensive view of the gene expression affected by leucosceptroid B, RNA-seq was performed using the RNA extracted from L4 nematodes treated with DMSO or 125 μmol·L^{-1} leucosceptroid B. Differentially expressed genes (DEGs) of the two groups were analyzed by the Noiseq method. We obtained 230 DEGs, of which 183 genes were upregulated and 47 genes were downregulated in the treatment group. Subsequently, DEGs were subjected to Gene Ontology (GO) analysis and assigned into 31 categories of GO classification (Fig. 2A). A total of 75 DEGs were involved in binding, 68 in the single-organism process, and 65 in the metabolic process. To better understand the functions of DEGs, the involved pathways were analyzed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. The results showed that the DEGs are mainly involved in metabolism (85 DGEs) and human diseases (70 DGEs) (Fig. 2B).

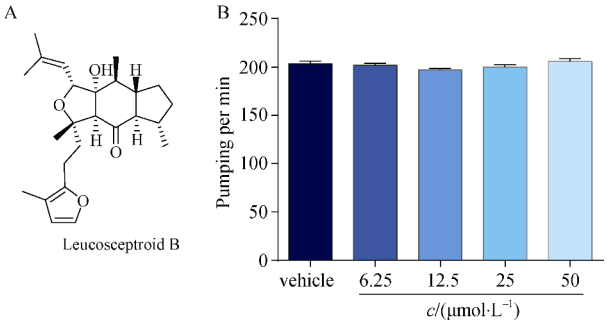

Fig. 1 Leucosceptroid B and its effect on the pharyngeal pumping frequency of worms. (A) Chemical structure of leucosceptroid B. (B) Statistical analysis of pharyngeal pumping rates of wild-type *C. elegans* treated with different concentrations of leucosceptroid B

Significantly, 10 DGEs are involved in the lipid metabolism of nematodes, including fatty acid metabolism, sphingolipid metabolism, and glycerophospholipid metabolism (Table 1). Among them, the expression of *fat-7* encoding a Δ^9 desaturase belonging to a stearoyl-CoA desaturase (SCD) family that catalyzes the formation of unsaturated fatty acids, was downregulated by leucosceptroid B. Moreover, leucosceptroid B also suppressed the transcripts of *elo-2* and *acdh-1*. *elo-2* encodes a fatty acid elongase that is responsible for the biosynthesis of straight long-chain saturated fatty acids and polyunsaturated fatty acids (PUFA) [27-28], while *acdh-1* is involved in the breakdown of fatty acid chains in the *β*-oxidation-like pathway [29]. In contrast, the expression of *fat-2*, which encodes a Δ^{12} desaturase involved in PUFA biosynthesis [30], was upregulated by leucosceptroid B. Therefore, leucosceptroid B affected the expression of *fat-7*, *acdh-1*, *elo-2* and *fat-2*, which play important roles in the biosynthesis and metabolism of fatty acids.

Leucosceptroid B reduced fat accumulation of wild-type nematodes

Since the genes involved in fatty acid biosynthesis and metabolism were regulated by leucosceptroid B, we explored whether leucosceptroid B also affected fat accumulation. In the worm, fat is stored in the form of lipid droplets [23], and the Oil Red O staining is considered a validated method to clearly show the amount of fat in nematodes [31]. We found that the fat content decreased significantly in wild-type nematodes treated with different concentrations of leucoscep-

troid B (Fig. 3A). Fat content in worms treated with 6.25 μmol·L^{-1} leucosceptroid B was 83.7% ± 4.1% of that in the control group, whereas it was reduced to 72.5% ± 2.9%, 70.4% ± 6.5% and 63.3% ± 2.9% after treatment with 12.5, 25 and 50 μmol·L^{-1} leucosceptroid B, respectively (Fig. 3B). These results demonstrated that leucosceptroid B could reduce the fat storage of wild-type nematodes in a dose-dependent manner.

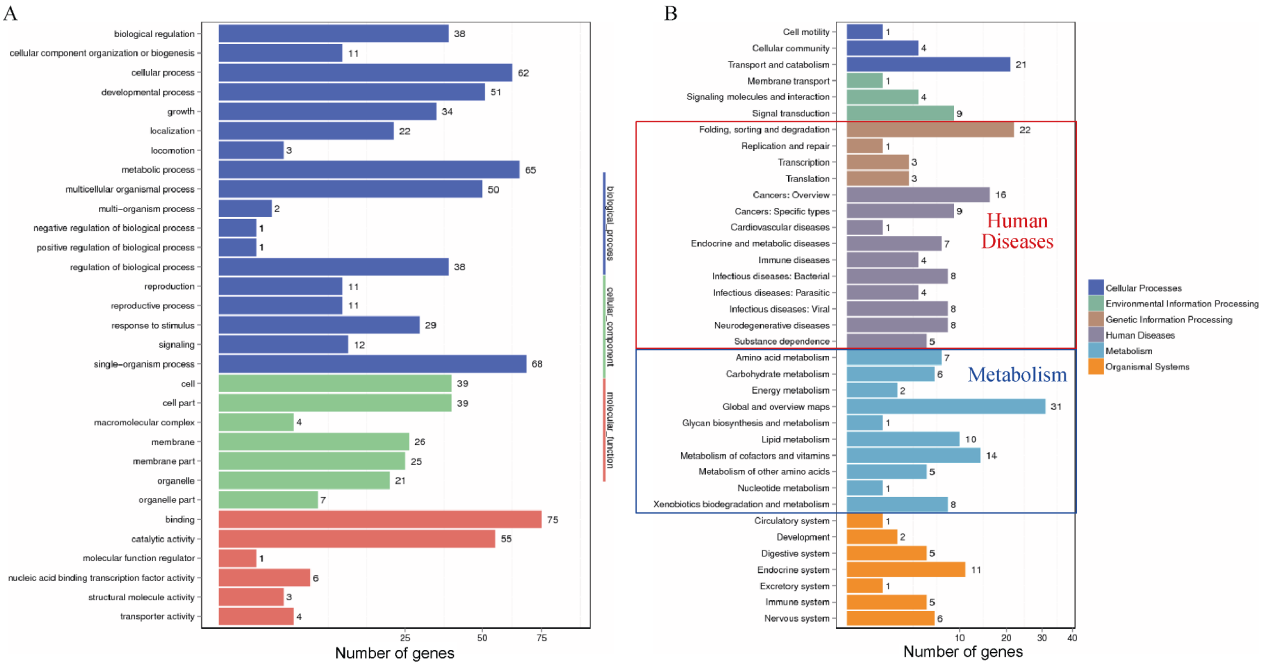

Fig. 2 GO classification and KEGG pathway analyses of differentially expressed genes in *Caenorhabditis elegans* treated with 125 μmol·L^{-1} leucosceptroid B and control. The abscissa is the number of differentially expressed genes (DEGs), and the ordinate represents the secondary GO classification (A) and KEGG pathway (B). Secondary GO classification and KEGG pathways belong to different primary pathways that are represented by different colors

Table 1 Significant difference genes associated with lipid metabolism in leucosceptroid B treated *C. elegans* by RNA-seq analysis

Symbol	Means-vehicle	Means-treatment	log2 Ratio (treatment/vehicle)	Probability	Pathway
gba-2	0.80	39.81	5.64	0.93	Sphingolipid metabolism
F20G2.1	4.56	43.54	3.25	0.88	Synthesis and degradation of ketone bodies
ckb-2	22.15	84.55	1.93	0.85	Glycerophospholipid metabolism
Y65B4BR.1	34.20	98.53	1.53	0.83	Glycerophospholipid metabolism
ZK1290.5	20.56	55.91	1.44	0.81	Glycerolipid metabolism
fat-2	461.42	973.39	1.08	0.82	Fatty acid metabolism
elo-2	176.59	82.75	–1.09	0.80	Fatty acid metabolism
acdh-1	515.35	236.63	–1.12	0.82	Fatty acid degradation
F10D2.8	211.98	77.08	–1.45	0.84	Steroid hormone biosynthesis
fat-7	22.77	3.96	–2.52	0.83	Fatty acid metabolism

Leucosceptroid B affected the fatty acid composition of wild-type nematodes

Considering that FAT-7 mainly acts on C18 : 0 to produce C18 : 1n9, and ELO-2 could elongate C16 : 1n7 to form C18 : 1n7, the fatty acid composition, especially the ratio of C18 : 1n9/C18 : 0 and C18 : 1n7/C16 : 1n7 in nematodes treated with leucosceptroid B, was assessed. Synchronized L1 wild-type nematodes were cultured to the YA stage in NGM medium with *E. coli* OP50 containing 50 μmol·L^{-1} leucosceptroid B or DMSO, and the total lipids of the nematodes were extracted. The triglyceride fraction was separated by TLC, and after methyl esterification treatment, the fatty acid composition was analyzed using GC-MS. As a result, the ratios of C18 : 1n9/C18 : 0 and C18 : 1n7/C16 : 1n7 were significantly decreased in the compound-treated nematodes, while the ratio of C16 : 1n7/C16 : 0 was not changed (Fig. 3C). These results indicated that leucosceptroid B inhibited the conversion of C18 : 0 to C18 : 1n9 and C16 : 1n7 to C18 :

1n7, which were catalyzed by two SCDs, FAT-6 and FAT-7, as well as ELO-2, respectively. However, the conversion of C16 : 0 to C16 : 1n7 catalyzed by another SCD FAT-5 was not affected by leucocepteroid B.

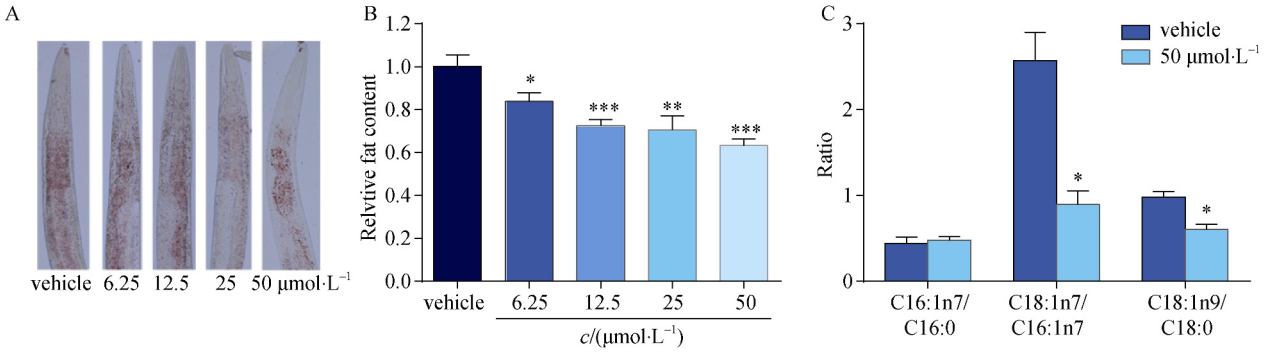

Fig. 3 Effect of leucocepteroid B on fat accumulation and fatty acid composition of wild-type *C. elegans*. (A) Images of lipid droplets stained with Oil Red O in wild-type *C. elegans* treated with different concentrations of leucocepteroid B. (B) Relative quantification of lipid content in wild-type *C. elegans* treated with different concentrations of leucocepteroid B. (C) Ratio of fatty acid in wild-type *C. elegans* treated with 50 μmol·L^{-1} leucocepteroid B and DMSO. Data are presented as the mean ± SEM. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ *vs* the control group

qRT-PCR validated the expression of genes responsible for unsaturated fatty acid biosynthesis

Subsequently, qRT-PCR was carried out to validate the relative expression of four DEGs obtained by RNA-seq analysis, *fat-7*, *elo-2*, *acdh-1* and *fat-2*, along with *fat-5* and *fat-6* which are the other two SCDs involved in unsaturated fatty acid biosynthesis [32]. The results showed that the expression levels of *fat-6*, *fat-7* and *acdh-1* were significantly decreased in worms treated with 50 μmol·L^{-1} leucocepteroid B, but no obvious changes in the expression of *fat-5* and *fat-2* were observed (Fig. 4). Therefore, the results of RNA-seq and qRT-PCR consistently indicated that the transcripts of *fat-6*, *fat-7*, *elo-2* and ac*dh-1* involved in fatty acid biosynthesis and metabolism were downregulated by leucocepteroid B. It was suggested that leucocepteroid B might reduce the synthesis of the unsaturated fatty acids C18 : 1n9 and C18 : 1n7 in nematodes by inhibiting the expression of *fat-6*, *fat-7* and *elo-2*.

Fat-6; fat-7 double mutant nematodes abolished the reduce of fat content by leucocepteroid B

To verify that leucocepteroid B actually acts on *fat-6* and *fat-7*, the fat storage in the *fat-6* mutant, *fat-7* mutant and *fat-6; fat-7* double mutant was further investigated using Oil Red O staining. Leucocepteroid B reduced fat accumulation in both the *fat-6* mutant and *fat-7* mutant, and the decreased fat content corresponded with the increase of compound concentration (Figs. 5A and 5B). In contrast, leucocepteroid B did not affect fat storage in *fat-6; fat-7* double mutant (Fig. 5C) that was unable to biosynthesize normal PUFAs [32]. These findings indicated that the impact of leucocepteroid B on fat storage depended on *fat-6* and *fat-7*. Collectively, leucocepteroid B inhibited the expression of the fatty acid biosynthesis genes *fat-6*, *fat-7* and *elo-2*, resulting in the decrease in the synthesis of unsaturated fatty acid including C18 : 1n7 and 18 : 1n9 and probably that of PUFAs, which ultimately led to reduced fat storage in nematodes.

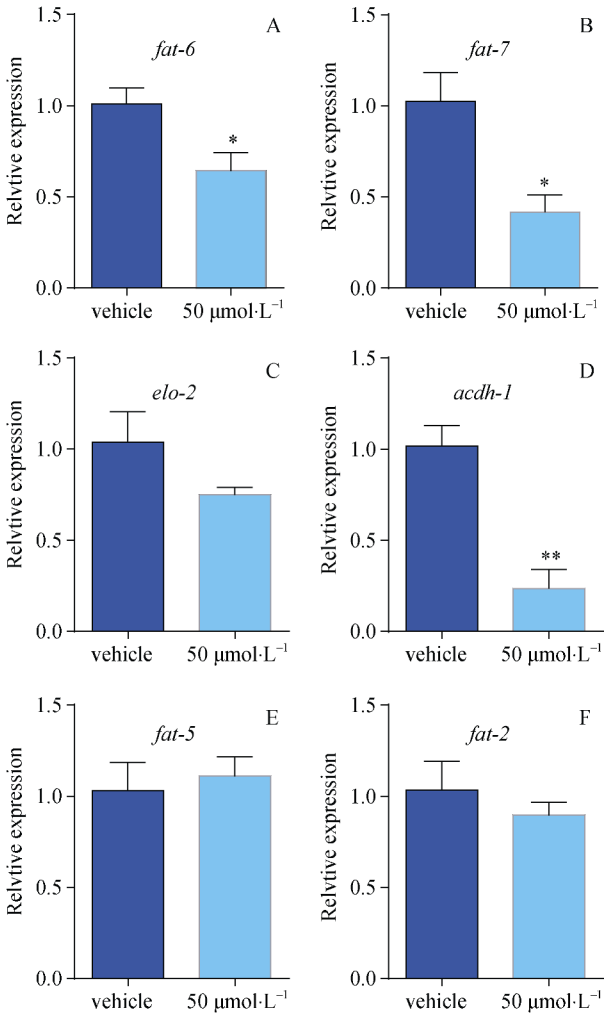

Fig. 4 qRT-PCR analysis of the expression levels of genes involved in fatty acid metabolism in response to leucocepteroid B. Data are presented as the mean ± SEM. $^{*}P < 0.05$ *vs* the control group

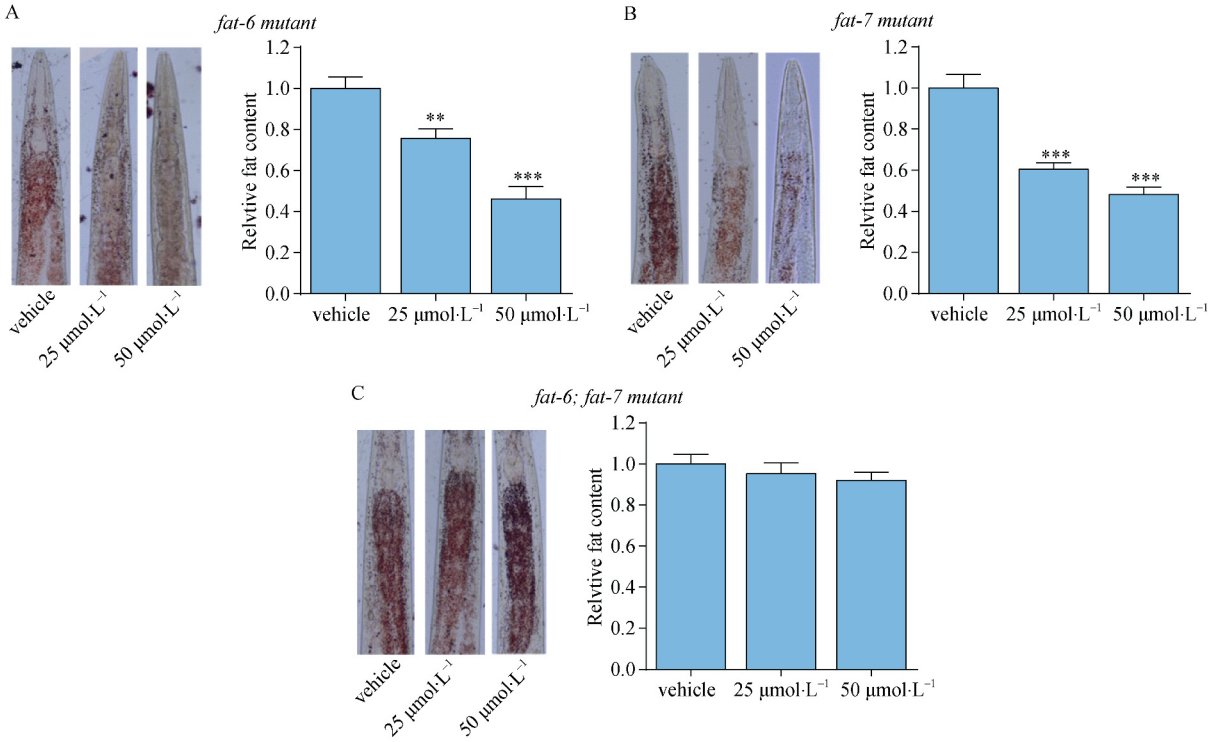

Fig. 5 Effect of leucosceptroid B on fat accumulation of mutant *C. elegans*. (A) Image (left) and relative quantification (right) of lipid droplets stained with Oil Red O in *fat-6* mutant nematodes treated with control and 25 and 50 μmol·L^{-1} leucosceptroid B. (B) Image (left) and relative quantification (right) of lipid droplets in *fat-7* mutant nematodes. (C) Image (left) and relative quantification (right) of lipid droplets in fat-6; *fat-7* double mutant nematodes. Data are presented as the mean ± SEM. $^{***}P < 0.001$, $^{**}P < 0.01$ *vs* the control group

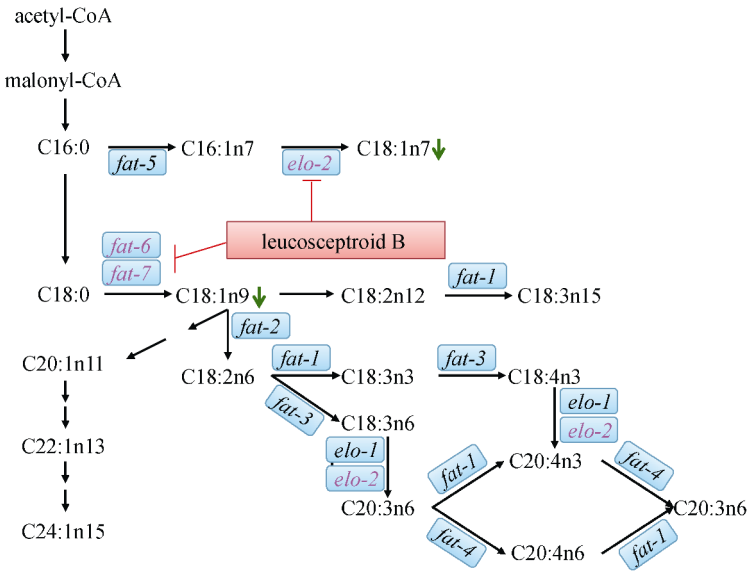

Fig. 6 A model of leucosceptroid B reducing fat storage in *C. elegans*. Leucosceptroid B downregulated the expression of *fat-5*, *fat-6* and *elo-2*, as well as the ratio of oleic acid/stearic acid (C18 : 1n9/C18 : 0) and vaccenic acid/palmitoleic acid (C18 : 1n7/C16 : 1n7) in wild-type *C. elegans*, which ultimately led to reduced fat storage. The green arrow indicates the reduced unsaturated fatty acids. Purple font represents the downregulated gene transcript

Discussion

SCDs are key enzymes catalyzing the biosynthesis of monounsaturated fatty acids from saturated fatty acids. Inhibiting the activity of SCD in mammals (SCD-1) and *C. elegans* (FAT-5, FAT-6 and FAT-7) was reported to be associated with

increased saturated fatty acid accumulation as well as decreased fat storage, and thus SCD has been considered to be one of the targets for the treatment of obesity and metabolic disorders [33-34]. Likewise, ELO-2 is another key enzyme for PUFA biosynthesis, and suppression of *elo-2* causes imbalances in fatty acid composition, thereby resulting in alteration of fat storage [28]. In this study, we found that leucosceptroid B decreased the fat content of wild-type *C. elegans* but did not affect the pharyngeal pumping rate, suggesting that the reduced fat storage did not depend on inhibiting food intake. In addition, leucosceptroid B reduced the transcripts of *fat-6*, *fat-7*, *elo-2* and *acdh-1*, as well as the ratio of C18 : 1n9/C18 : 0 and C18 : 1n7/C16 : 1n7. Coincidentally, both FAT-6 and FAT-7 mainly act on C18 : 0 to yield C18 : 1n9, while ELO-2 is involved in the process of catalyzing C16 : 1n7 to form C18 : 1n7 [27]. Therefore, the activity of FAT-6, FAT-7 and ELO-2 should be suppressed by leucosceptroid B through downregulating the transcription of their encoding genes in wild type worms (Fig. 6). In *fat-6* and *fat-7* single mutants, *fat-7* and *fat-6* can mutually compensate for the loss of one isoform, while *fat-6*; *fat-7* double mutant loses two isoforms and therefore is unable to synthesize C18 : 1n9 precursor for PUFA. Since we have proved that leucosceptroid B inhibited *fat-6* and *fat-7*, it is not surprising that leucosceptroid B significantly reduced the fat content of *fat-6* and *fat-7* single mutants but did not affect fat storage in *fat-6*; *fat-7* double mutant. The mutant analysis results further confirmed that leucosceptroid B decreased fat storage *via* inhibiting *fat-6* and *fat-7*. In addition, due to the decrease in fat accumulation, the expression of *acdh-1* involved in the regulation of fatty acid degradation was also significantly downregulated.

In *C. elegans*, at least five transcription regulators, including NHR-49, NHR-80, SBP-1/SREBP and DAF-16/FOXO, are involved in regulating the transcripts of *fat-5* and *fat-6* [32], but leucosceptroid B did not affect the expression of these regulators by RNA-seq analysis. Intriguingly, the expression of four genes in the MAPK signaling pathway, three genes in the PI3K-AKT signaling pathway, and two genes in the AMPK signaling pathway were significantly regulated by leucosceptroid B. Therefore, further investigation is warranted to determine whether leucosceptroid B downregulated the expression of *fat-6*, *fat-7* and *elo-2* depending on the MAPK, PI3K-AKT and AMPK signaling pathways.

Leucosceptroid B is the major component of the glandular trichomes of *L. canum*, whose leaves are used to extract natural food pigments in China. This work presented new information on the role of leucosceptroid B in the decrease of fat storage, which provides a scientific basis for the development and utilization of leucosceptroids as potential antilipemic agents.

Acknowledgments

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