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Article

Nuciferine's dual pathway regulation of lipid metabolism: A biomechanical perspective based on HepG2 cells and *Caenorhabditis elegans*

Supplementary file

I. HepG2 cells' ROS content detection

HepG2 cells were cultured in DMEM medium supplemented with 10% FBS. The cell suspension was adjusted to a density of 1.5×10^5 cells/mL and seeded into a six-well plate, with 1 mL of the suspension added to each well. The cells were then cultured under conditions of 37 ℃, 5% CO_2, and saturated humidity. Following cell adherence, treatments were administered to HepG2 cells using OA (soluble in methanol) and nuciferine (soluble in DMSO), either concurrently or sequentially. A preliminary incubation with 0.5 mM OA for 24 h at 37 ℃ was performed, succeeded by exposure to 10, 20, 30, or 40 μM of nuciferine for an additional 24 h at the same temperature. For the control group, cells were initially subjected to treatment with 0.3% methanol for 24 h at 37 ℃, followed by treatment with 0.1% DMSO for another 24 h at 37 ℃. In the OA control group, cells underwent a 24-hour preincubation with 0.5 mM OA at 37 ℃, succeeded by a 24-hour preincubation with 0.1% DMSO at 37 ℃. The positive control group received a 24-hour preincubation with 0.5 mM OA at 37 ℃, then each group of cells was treated with Sim (10 μM) for 24 h at 37 ℃ for replicate analysis, with the entire procedure being conducted in triplicate. After removing the culture medium, 500 μL of 10 μM DCFH-DA solution was added to each well and incubated in the dark at 37 ℃ for 1 h. The excitation and emission wavelengths were set to 485 nm and 525 nm, respectively, and the fluorescence intensity of each well was measured under dark conditions. Finally, fluorescence images of intracellular ROS were captured using an inverted fluorescence microscope in the dark.

II. Molecular docking procedure

The PubChem (https://pubchem.ncbi.nlm.nih.gov) database was queried to retrieve the three-dimensional structural data of SREBP-1c and PPARα. Corresponding PDB format files for these targets were also obtained. The core targets underwent preprocessing using Pymol software, which involved dehydration and ligand removal. Subsequently, molecular docking experiments between nuciferine and the core targets were performed using Autodock tools. In these experiments, a binding energy of −7 kJ/mol or lower between nuciferine and the core targets was deemed indicative of a stable interaction. Finally, the molecular docking results were imported into Pymol software for visualization, providing an intuitive representation of the molecular interactions.

III. Determination of movement and life expectancy in *C. elegans* assays

Based on modifications from the study by Bai et al., an assessment was conducted on the head thrashing and body bending of nematodes [1]. Under the culture conditions described in 2.6, after N2 nematodes were treated with 2% glucose and different concentrations of nucifine (20, 30, 40μM), L4-stage larvae were positioned in a droplet of M9 buffer atop NGM plates without OP50 and allowed to acclimate for 30 s. Subsequently, the frequencies of head thrashing and body bending were separately counted over a 10-second period. The experiment was conducted in triplicate, with at least 15 nematodes per group serving as representative samples.

Regarding lifespan, nematodes were cultured under the conditions described in Section 2.6, using 2% glucose and various concentrations of nuciferine. Starting from day 0 (embryonic stage), approximately 100 nematodes from each group were transferred daily to respective fresh NGM plates until all had died. The count of surviving nematodes was recorded. Slight discrepancies in the actual counts in the statistical results were due to losses from mortality or censored

animals. The criteria for death included the absence of movement, stress-induced movement by parasites, and lack of pharyngeal contraction after one or two gentle touches [2].

IV. Measurement of length and width

Under the culture conditions described in 2.6, after N2 nematodes were treated with 2% glucose and different concentrations of nuciferine (20, 30, 40 μM), the actual somatic dimensions of *C. elegans* L4 larvae were carefully measured by Image J software, thereby assessing the developmental effects of high glucose and nuciferine. This quantitative analysis enabled an objective assessment of growth alterations in the organisms under the experimental conditions.

V. Oil red O staining and triglyceride determination of *C. elegans*

C. elegans lipid content was assessed using the Oil Red O which staining protocol as previously described by Xiao et al., for comparative measurement [3] under the culture conditions described in Section 2.6 for a duration of 3 days, 20 to 40 N2 Bristol *C. elegans* were selected. Initially, age-synchronized nematode population from specimens from each group were gathered and washed three times using M9 buffer, followed by fixation in a 4% paraformaldehyde solution for 20 minutes. Subsequently, the mixture underwent centrifugation, and the supernatant was removed. After incubation in Oil Red O for 2 h, the nematodes were washed three times with M9 buffer to eliminate excess dye. Finally, the stained nematodes were examined using an inverted microscope fitted with a digital camera.

Under the above cultivation conditions, the N2 Bristol *C. elegans* and Mutant *C. elegans* sbp-1 (ep79) III, fat-6 (tm331) IV; fat-5 (tm420), fat-6 (tm331) IV; fat-7 (wa36) V, nhr-49 (ok2165) were rinsed three times with M9 buffer to eliminate any external substances. With the assistance of 0.05% Tween 20, *C. elegans* were subjected to ultrasonic treatment for 2 min to obtain uniform tissue fragments [4].TG contents and protein concentrations were quantified using kits. A microplate reader was employed to accurately measure the absorbance of each sample, with data processing conducted using SoftMax Pro 6.5 software by Molecular Devices, Inc., ensuring precise and consistent outcomes.

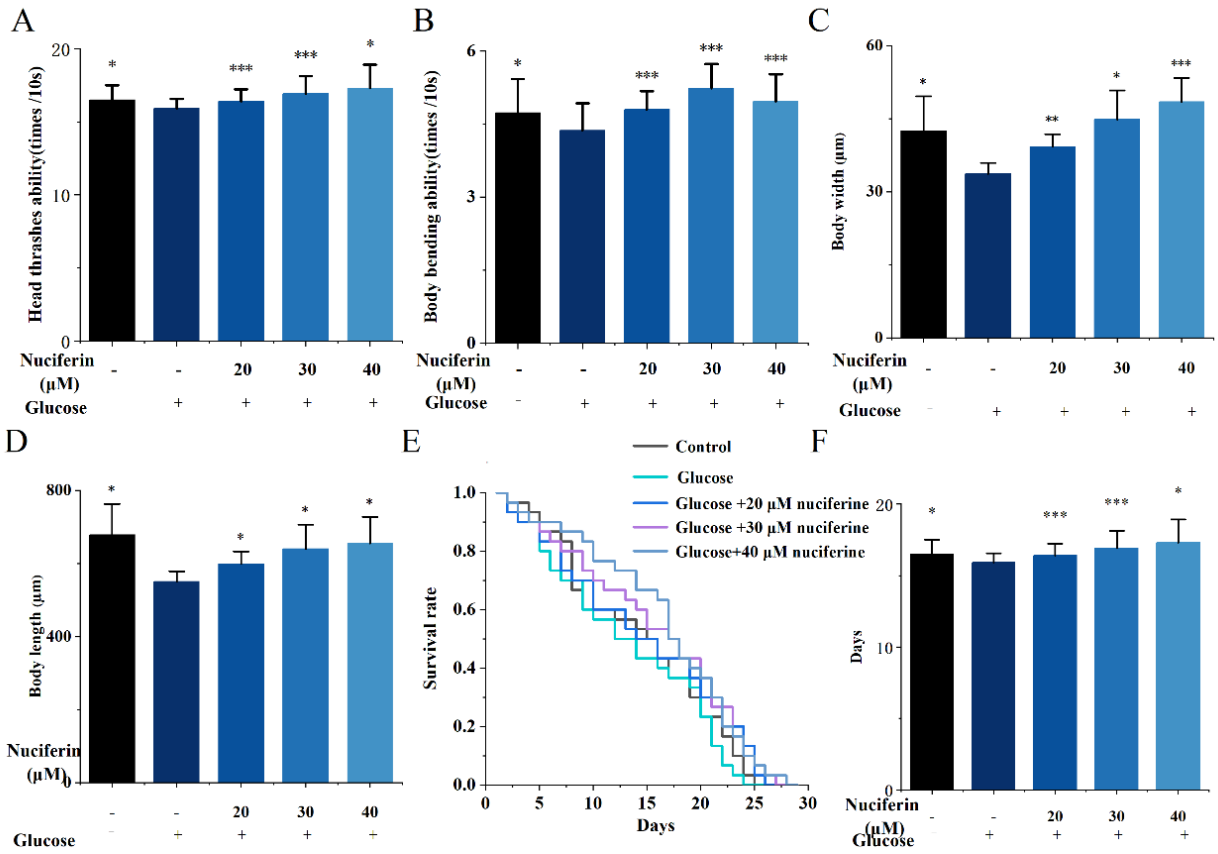

Figure S1. Effects of nuciferine on locomotion ability, growth ability, longevity and other effects of *C. elegans*. **(A)** Head thrashes; **(B)** body movement; **(C)** body width; **(D)** body length; **(E)** median lifespan; **(F)** life expectancy.
$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared between the nuciferine group and the glucose group.

Table S1. Primer sequences used for RT-qPCR amplification.

Gene	Forward (5′-3′)	Reverse (5′-3′)
ACC	ATGTCTGGCTTGCACCTAGTA	CCCCAAAGCGAGTAACAAATTCT
CPT-1	CTGGAGCCAGAAGTGTTCCAC	AGGCACAAAGCGTATGAGTCT
FAS	TGCACAGAATTGAAGGAGTA	ATGGTTTCACGACTGGAGGT
PPAR-α	GGCAATGCACTGAACATCGAG	GCCGAATAGTTCGCCGAAAG
SCD-1	GTGGGTTGGCTGCTTGTGC	TCCCGAGATTGAATGTTCTTGT
SREBP-1c	CAGGACAGGCAGAGGAAGAC	TGAGGACAGCAAGGCAAAG
GADPH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATG
sbp-1	GGCGGCGAAGATTGTGATTC	GGCGGCGAAGATTGTGATTC
mdt-15	CAAAACCAGACAAGCGGTGG	CAAAACCAGACAAGCGGTGG
nhr-49	CTGCAACGGGTGTAAGGGAT	CTGCAACGGGTGTAAGGGAT
nhr-80	ACCATCATGGAGTTGGCTGG	GGCATGATCTCGTCGGTGAT
aak-2	GACACGCGCATCGAAAGTTT	GACACGCGCATCGAAAGTTT
acs-2	GACACGCGCATCGAAAGTTT	GACACGCGCATCGAAAGTTT
fat-5	GGATGGCAGCCATACGATCA	CTCCGACTGCCGCAATAGAT
fat-6	AAGATTGAGAAGGACGGCGG	TTCATTTGGATCCACGGCGA
fat-7	TCGTTGCCATCACAAGTGGA	GAGTTTGCCTCCATGCTCCT
β-actin	ACGAGGTTGCCGCTCTTGTTG	ACGAGTCCTTCTGTCCCATACCG