Original Article

β-Elemene inhibits adipogenesis in 3T3-L1 cells by regulating AMPK pathway

Xiang Deng,[1,*] Zhenmin Liu,[1] and Sen Yang[1,2]

[1]Department of Pediatrics, Chengdu Fifth People's Hospital, No. 33, Mashi street, Wenjiang District, Chengdu, Sichuan 611130, China
[2]Department of Pediatrics, School of Medical and Life Sciences, Chengdu University of Traditional Chinese Medicine, 1166 Liutai Avenue, Wenjiang District, Chengdu, Sichuan, 611137, China

(Received 16 October, 2024; Accepted 16 November, 2024; Released online in J-STAGE as advance publication 24 December, 2024)

The prevalence of childhood obesity in global is quickly augmented, resulting into grievous public health problems and influencing adolescent development. β-Elemene is a sesquiterpene, and can extracted from traditional Chinese medicine-*Curcuma longa* L. β-Elemene has been discovered to display regulatory functions in multiple diseases, but it's roles in obesity need further investigations. The purpose of this work is to investigate the regulatory impacts of β-elemene on obesity progression and associated pathways. In this study, it was revealed that the heightened lipid accumulation in 3T3-L1 cells triggered by 3-isobutyl-1-methylxanthine + dexamethazone + insulin (MDI) can be restrained by β-elemene. Furthermore, β-elemene can modulate lipid metabolism in 3T3-L1 cells mediated by MDI. The glucose consumption was descended after insulin resistance treatment, but this impact was reversed after β-elemene treatment. At last, it was illustrated that the AMPK pathway was retarded after β-elemene induction, but this change was offset after β-elemene treatment. To sum up, our results manifested that β-elemene inhibited adipogenesis in 3T3-L1 cells, and evoked the AMPK pathway. This project may supply serviceable insights of β-elemene in the progression of obesity.

***Key Words*: adipogenesis, AMPK pathway, β-elemene, obesity**

Obesity is occurred due to the imbalance of energy ingestion and utilization in white adipose tissue.(1) The prevalence of childhood obesity has been augmented year by year.(2) The childhood obesity is tanglesome, and environmental and genetic factors both participate into the pathogenesis of this disease.(3) The obesity in children is one primary global health problem, and it can result into the markedly increased risk to happen morbid obesity or diabetes mellitus in later life.(4) Besides, obesity acts as the most familiar cause for insulin resistance (IR) in children, that is also correlative with dyslipidemia and vascular complications.(5,6) Therefore, ameliorating obesity has become the key point, and searching useful drugs for obesity treatment is deemed as the top priority.

Natural products from herbs have become hopeful drugs, and own improvement roles in obesity.(7) β-Elemene is one sesquiterpene, and can extracted from traditional Chinese medicine-*Curcuma longa* L.(8) β-Elemene has been ascertained to display regulatory functions in diversified diseases. For instance, β-Elemene can strengthen lncRNA H19 in non-small cell lung cancer to intensify erlotinib sensitivity.(9) Moreover, β-elemene modulates the JAK/STAT3/NF-κB pathway to improve cardiac inflammation and remodeling evoked by hyperglycemia.(10) β-Elemene restrains HSP70 to accelerated hyperthermia-triggered cell apoptosis in colorectal carcinoma.(11) In addition, β-elemene regulates autophagy to facilitate M2 polarization, thereby ameliorating ischemic stroke.(12) Importantly, it has been testified that β-Elemene can refrain imbalance of microbiota/gut/brain in obesity.(13) Besides, β-elemene can regulate pro-inflammatory factors to modulate M1/M2 macrophage balance in obesity.(14) However, the regulatory impacts and interrelated pathways of β-elemene on lipogenesis and IR in obesity progression remain indistinct.

In this work, it was disclosed that β-elemene inhibited adipogenesis in 3T3-L1 cells, and evoked the adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) pathway. This work suggested that β-elemene may be helpful on improving obesity progression.

Materials and Methods

Cell lines and cell culture. The mouse 3T3-L1 cells bought from iCell Bioscience Inc. (Shanghai, China) were cultivated in Dulbecco's Modified Eagle Medium (DMEM, Gibco; Thermo Fisher Scientific, Waltham, MA) with 10% Newborn Calf Serum (NBS) in a humidified incubator of 5% CO_2 at 37°C. 3T3-L1 cells were treated with 3-isobutyl-1-methylxanthine + dexamethazone + insulin (MDI) (0.5 mM 3-isobutyl-1-methylxanthine, 1 μM dexamethasone, 10 μg/ml insulin). The 3-isobutyl-1-methylxanthine and dexamethasone were withdrawn at day 2, and then insulin was withdrawn at day 4. Next, 3T3-L1 cells were incubated in DMEM (changed every 2 days) until day 8.

β-Elemene (0, 5, 10, 20, 40, and 80 μM; Apexbio, Boston, MA) was employed to treat 3T3-L1 cells.

For IR cell model, on day 8, 3T3-L1 cells were treated with 1 μM dexamethasone for 72 h. The IR-3T3-L1 cells were treated with β-elemene (5, 10, and 20 μM) for 48 h.

CCK-8 assay. 3T3-L1 cells (1,000 cells/well) placed in the 96-well plate were cultivated. CCK-8 solution (10 μl; Dojindo Laboratories, Kumamoto, Japan) was mixed into each well for 2 h. Eventually, cell viability was determined through the spectrophotometer (Thermo Fisher Scientific).

Oil red O staining. After washing, 3T3-L1 cells were fixed by 4% formalin. Next, further washing, 3T3-L1 cells were subjected to air drying. Then, oil red O solution was mixed into each well for 30 min. Post being washed with distilled water, images of stained cells were gained through the inverted fluorescence microscopy (Nikon, Tokyo, Japan).

Detection of TG. 3T3-L1 cells were lysed, next the TG level was measured through the intracellular TG kit (Nanjing Jiancheng, Nanjing, China).

RT-qPCR. The RNAs from 3T3-L1 cells was obtained through using TRIzol reagent (Invitrogen, Carlsbad, CA). The cDNA was generated from RNAs under the SuperScript™ II Reverse

*To whom correspondence should be addressed.
E-mail: dengxiang_666@163.com

doi: 10.3164/jcbn.24-179

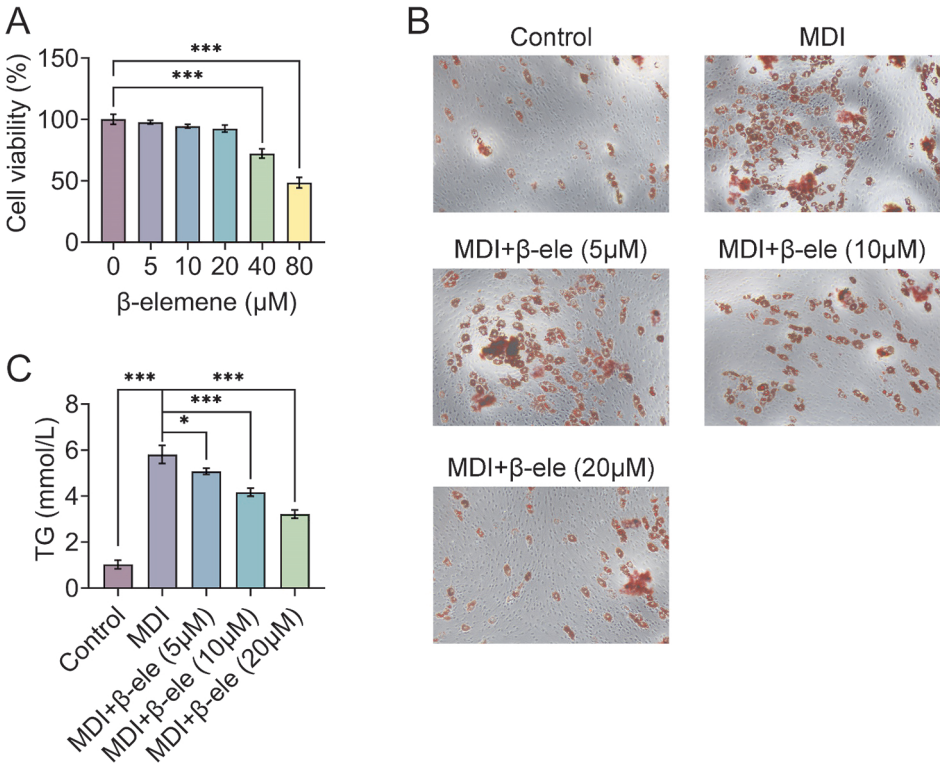

Fig. 1. β-Elemene restrained lipid accumulation in 3T3-L1 cells. (A) The cell viability was confirmed with different concentrations of β-elemene (0, 5, 10, 20, 40, and 80 μM) through CCK-8 assay. (B) The adipogenesis was examined through oil red O staining in the Control, MDI, MDI + β-ele (5 μM), MDI + β-ele (10 μM), and MDI + β-ele (20 μM) groups. (C) The TG level was tested through the TG kit in the Control, MDI, MDI + β-ele (5 μM), MDI + β-ele (10 μM), and MDI + β-ele (20 μM) groups. *p<0.05, ***p<0.001.

Transcriptase Kit (Invitrogen). Then, qRT-PCR was executed through the SYBR Premix Ex Taq™ Kit (Takara, Shanghai, China). At last, the mRNA expressions were determined through the $2^{-\Delta\Delta Ct}$ method.

The primer sequences were as followed:
FABP4: F: 5'-GAGGCGGATGAGAACAAGCA-3', R: 5'-CTCCCAGCAGCTACCATGGA-3';
C/EBPα: F: 5'-CAAGAACAGCAACGAGTACCG-3', R: 5'-GTCACTGGTCAACTCCAGCAC-3';
PPARγ: F: 5'-TCTCCTGTTGACCCAGAGCAT-3', R: 5'-TGGGCCAGAATGGCATCT-3';
ACC: F: 5'-GCCTCAGGAGGATTTGCTGT-3', R: 5'-AGGATCTACCCAGGCCACAT-3';
FAS: F: 5'-GGAAGTTGCCCGAGTCAGAG-3', R: 5'-CTTTCCAGACCGCTTGGGTA-3';
ATGL: F: 5'-ACTCACATCTACGGAGCCTCG-3', R: 5'-TCCTTGGACACCTCAATAATGTTG-3';
HSL: F: 5'-TCAATGGAGACACTTGGCCC-3', R: 5'-TTGGCTTCAGCCTCTTCCTG-3';
GLUT-4: F: 5'-GTTCTTTCATCTTCGCCGCC-3', R: 5'-TTCCCCATCTTCGGAGCCTA-3';
IRS-1: F: 5'-AGAGGACCGTCAGTAGCTCA-3', R: 5'-ACTGAAATGGATGCATCGTACC-3';
Adiponectin: F: 5'-ACTGCAGTCTGTGGTTCTGA-3', R: 5'-CATGACCGGGCAGAGCTAAT-3';
β-actin: forward, 5'-TCTGGCACCACACCTTCTACAA-3', reverse, 5'-TTTTCACGGTTGGCCTTAGG-3'.

Detection of glucose uptake. The glucose consumption was confirmed through the Glucose oxidase-peroxidase kit (Shanghai Rongsheng Biotech, Shanghai, China). The non-esterified fatty acid (NEFA) level was determined through NEFA kit (Nanjing Jiancheng).

Western blot. Proteins from 3T3-L1 cells were gained through the RIPA buffer (Beyotime, Shanghai, China). SDS-PAGE (10%) was adopted for proteins' separation. Then, proteins were put onto PVDF membranes (Beyotime). Post sealing (non-fat milk), primary antibodies were placed into membranes. Post 12 h incubation, the appropriate secondary antibody (1:1,000, ab7090) was also appended. Finally, the chemiluminescence detection kit (Thermo Fisher Scientific) was utilized for measuring the protein bands.

The primary antibodies: GLUT-4 (1:1,000, ab313775; Abcam, Shanghai, China), Adiponectin (2 μg/ml, ab22554), IRS-1 (1:500, ab131487), p-AMPK (1:500, ab131357), AMPK (1:1,000, ab32047) and β-actin (1:1,000, ab8227).

Statistical analysis. Data were expressed as mean ± SD. GraphPad Prism Software 9 (GraphPad Software, San Diego, CA) was utilized for performing statistical analysis. The comparisons were executed through one-way analysis of variance (ANOVA). The p<0.05 was thought as statistically significant.

Results

β-Elemene restrained lipid accumulation in 3T3-L1 cells. The cell viability was receded after β-elemene treatment (40 and 80 μM), but it was not changed after β-elemene treatment (5, 10, and 20 μM) (Fig. 1A). Furthermore, β-elemene treatment (5, 10, and 20 μM) was utilized for next experiments. Through oil red O staining, it was uncovered that adipogenesis was strengthened after MDI treatment, but this phenomenon was offset after β-elemene treatment (5, 10, and 20 μM) (Fig. 1B). In addition, the TG level was risen after MDI induction, but this change was alleviated after β-elemene treatment (Fig. 1C). In short, β-elemene restrained lipid accumulation in 3T3-L1 cells.

doi: 10.3164/jcbn.24-179

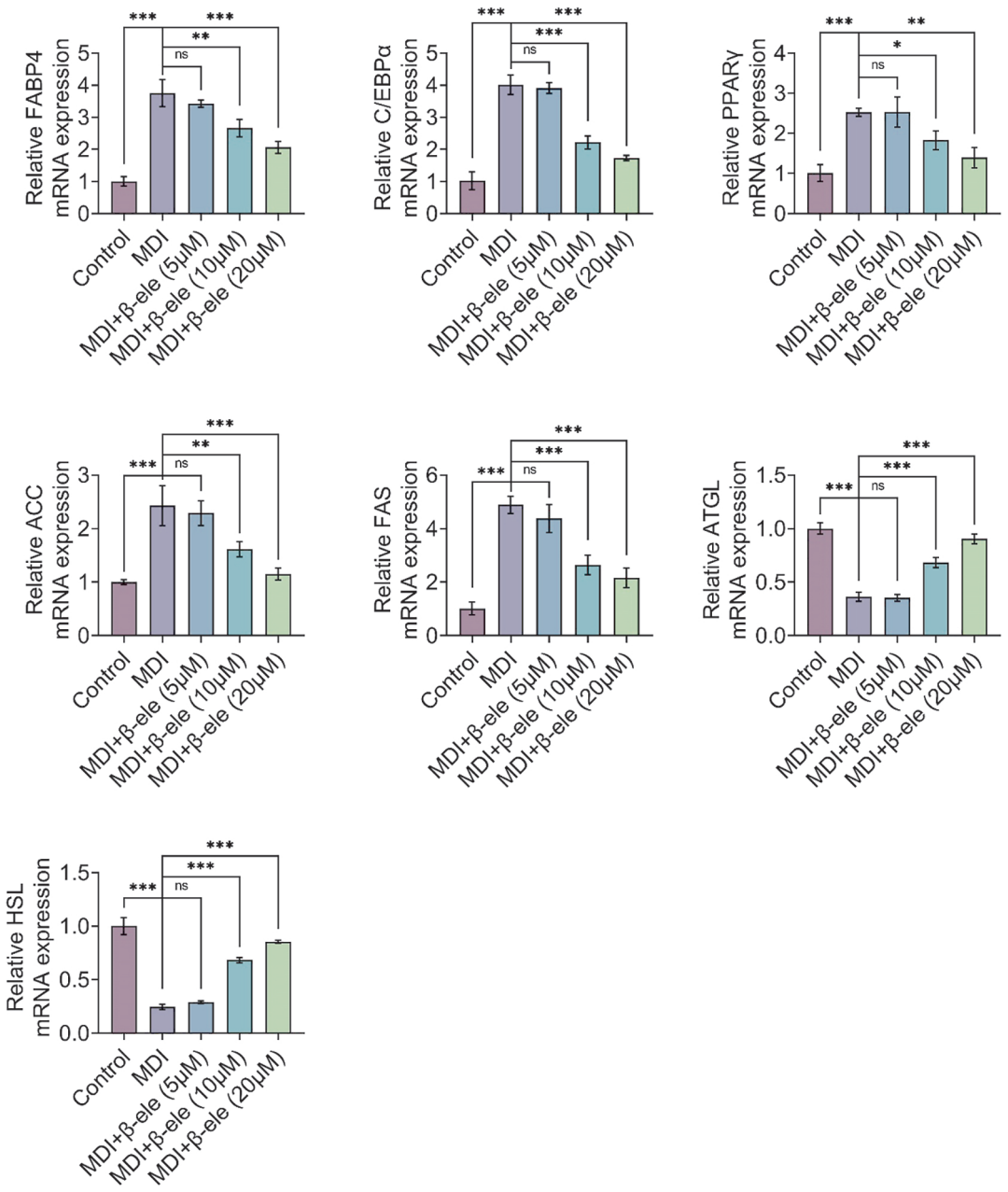

Fig. 2. Effects of β-elemene on lipid metabolism in 3T3-L1 cells. Groups were separated into the Control, MDI, MDI + β-ele (5 μM), MDI + β-ele (10 μM), and MDI + β-ele (20 μM) group. The mRNA expressions of FABP4, C/EBPα, PPARγ, ACC, FAS, ATGL, and HSL were tested through RT-qPCR. *p<0.05, **p<0.01, ***p<0.001.

Effects of β-elemene on lipid metabolism in 3T3-L1 cells. The mRNA expressions of FABP4, C/EBPα, PPARγ, ACC, and FAS were all elevated as well as ATGL and HSL were declined after MDI stimulation, but these impacts were counteracted after β-elemene treatment (10 and 20 μM) (Fig. 2). In general, β-elemene can modulate lipid metabolism in 3T3-L1 cells mediated by MDI.

β-Elemene aggrandized glucose consumption in IR-3T3-L1 cells. The glucose consumption was descended and NEFA was ascended after IR treatment, but these changes were reversed after β-elemene treatment (10 and 20 μM) (Fig. 3A). Besides, the mRNA expressions of GLUT-4, IRS-1, and adiponectin were all decreased after IR induction, but these influences were rescued after β-elemene treatment (Fig. 3B). The same changes of protein expressions for GLUT-4, Adiponectin and IRS-1 were found in Fig. 3C and D. Taken together, β-elemene aggrandized glucose consumption in IR-3T3-L1 cells.

β-Elemene evoked the AMPK pathway. The protein level of p-AMPK/AMPK was lessened after MDI stimulation, but this impact was neutralized after β-elemene treatment (10 and 20 μM) (Fig. 4), hinting that β-elemene can evoke the AMPK pathway.

Discussion

Obesity is generally correlated with lipid accumulation in adipose tissues.[15] Many reports have concentrated on the modulation of lipid accumulation in obesity. For example, in obesity, berberine can positively regulate PPARδ expression to attenuate lipid accumulation.[16] Additionally, liquiritigenin modulates autophagy mechanism in 3T3-L1 cells to against lipid accumula-

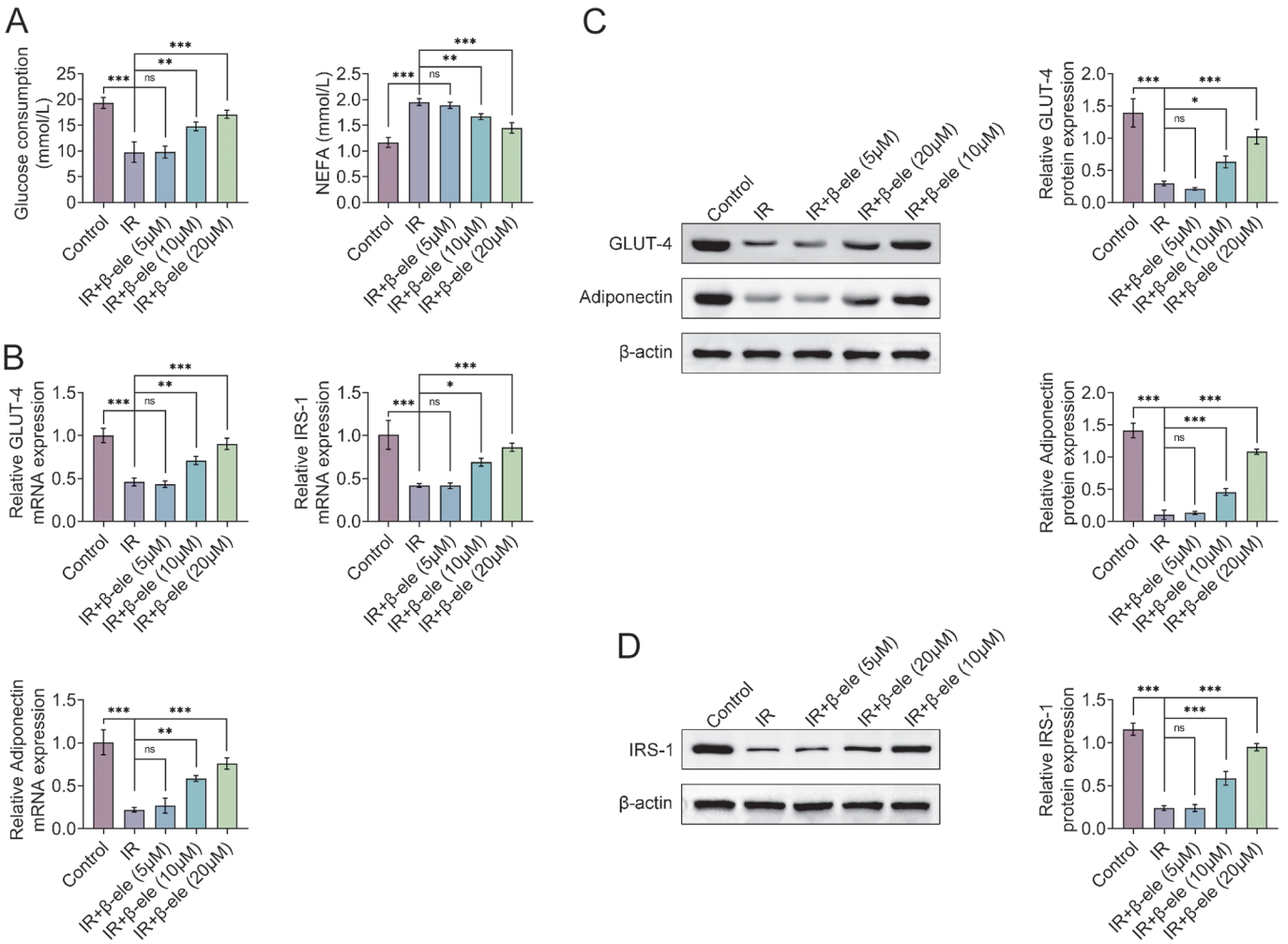

Fig. 3. β-Elemene aggrandized glucose consumption in IR-3T3-L1 cells. Groups were separated into the Control, IR, IR + β-ele (5 μM), IR + β-ele (10 μM), and IR + β-ele (20 μM) group. (A) The glucose consumption and NEFA were measured through the commercial kits. (B) The mRNA expressions of GLUT-4, IRS-1, and adiponectin were assessed through RT-qPCR. (C) The protein expressions of GLUT-4 and Adiponectin were evaluated through Western blot. (D) The protein expression of IRS-1 was verified through Western blot. *p<0.05, **p<0.01, ***p<0.001.

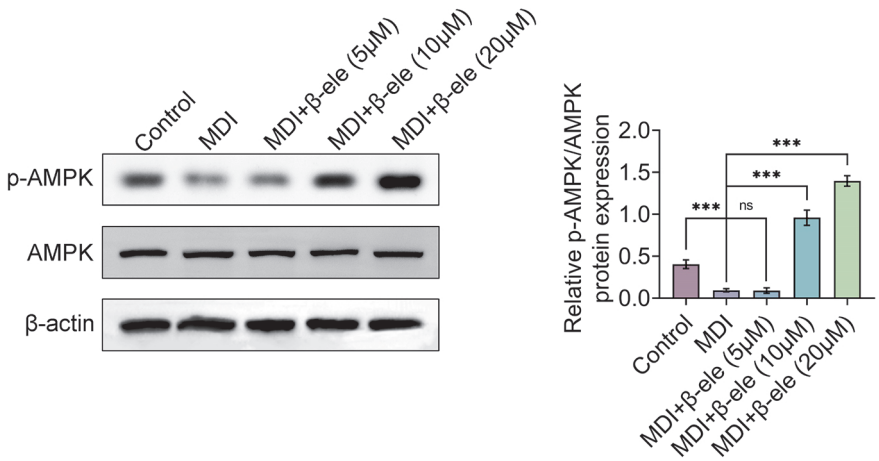

Fig. 4. β-Elemene evoked the AMPK pathway. Groups were separated into the Control, MDI, MDI + β-ele (5 μM), MDI + β-ele (10 μM), and MDI + β-ele (20 μM) group. The protein expressions of p-AMPK and AMPK were determined through Western blot. ***p<0.001.

doi: 10.3164/jcbn.24-179

tion.[17] Geraniin inhibits lipogenesis to cut down lipid accumulation in 3T3-L1 adipocytes through affecting CaMKK2 expression.[18] Furthermore, papain can evoke AMPK to weaken lipid accumulation in obesity.[19] β-Elemene exhibits ameliorative properties in multiple diseases,[9–12] can take part into obesity,[13,14] and its' roles in lipid accumulation and metabolism need further investigations. In this work, it also was revealed that the heightened lipid accumulation in 3T3-L1 cells triggered by MDI can be restrained by β-elemene. Furthermore, β-elemene can modulate lipid metabolism in 3T3-L1 cells mediated by MDI.

IR and glucose consumption are closely involved into obesity, and they have gained more and more attentions. For instance, cistanche tubulosa phenylethanoid glycosides alleviates adipogenesis and improved IR in obesity.[20] Additionally, Bax inhibitor-1 can ameliorate IR and glucose intolerance in obesity.[21] The JNK-1 signaling can influence glucose homeostasis and IR in obesity mice.[22] In addition, miR-592/FOXO1 relieves hyperglycemia and IR in obesity.[23] Similarly, in this work, it was uncovered that the glucose consumption was descended after IR treatment, but this impact was reversed after β-elemene treatment.

AMPK can join into multiple lipid metabolic pathways, making it to be one potential target for intervention in childhood obesity.[24,25] AMPK phosphorylates multiple substrates to intensify fatty acid oxidation, thereby receding lipid storage.[26] AMPK directly phosphorylates and restrains sterol regulatory element binding protein 1C (SREBP1C), and SREBP1C largely contributes to adipogenesis.[27] Therefore, AMPK can serve as one pivotal upstream regulator to suppress lipogenesis and adipogenesis, thereby joining into obesity.[28] Interestingly, it has been proved that β-elemene can stimulate the AMPK pathway.[29,30] However, the regulatory impacts on the AMPK pathway in obesity keep unclear. In this work, it also was illustrated that the AMPK pathway was retarded after β-elemene induction, but this change was offset after β-elemene treatment, manifesting that β-elemene can evoke the AMPK pathway.

In conclusion, it was testified manifested that β-elemene inhibited adipogenesis in 3T3-L1 cells, and evoked the AMPK pathway. Nevertheless, in this project, some limitations are still exhibited. Deeply investigations for β-elemene in obesity progression will be executed in the future.

Author Contributions

All authors contributed to the study conception and design. Material preparation and the experiments were performed by XD. Data collection and analysis were performed by ZL. The first draft of the manuscript was written by SY and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by the Scientific Research Fund of Chengdu Fifth People's Hospital (Grant No. KYJJ2021-036).

Availability of Data and Materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Conflict of Interest

No potential conflicts of interest were disclosed.

References

1 Koenen M, Hill MA, Cohen P, Sowers JR. Obesity, adipose tissue and vascular dysfunction. *Circ Res* 2021; **128**: 951–968.
2 Thomas-Eapen N. Childhood obesity. *Prim Care* 2021; **48**: 505–515.
3 Weihrauch-Blüher S, Wiegand S. Risk factors and implications of childhood obesity. *Curr Obes Rep* 2018; **7**: 254–259.
4 Piché ME, Tchernof A, Després JP. Obesity phenotypes, diabetes, and cardiovascular diseases. *Circ Res* 2020; **126**: 1477–1500.
5 Wu S, Gao H, Ma Y, Fu L, Zhang C, Luo X. Characterisation of betatrophin concentrations in childhood and adolescent obesity and insulin resistance. *Pediatr Diabetes* 2016; **17**: 53–60.
6 Deng L, Zhou R, Jiang W, *et al*. Efficacy and safety of remimazolam besylate in patients with obesity undergoing painless colonoscopy: a prospective, double-blind, randomized controlled trial. *Signa Vitae* 2024; **20**: 76–85.
7 Zhang Q, Bai Y, Wang W, *et al*. Role of herbal medicine and gut microbiota in the prevention and treatment of obesity. *J Ethnopharmacol* 2023; **305**: 116127.
8 Bai Z, Yao C, Zhu J, *et al*. Anti-tumor drug discovery based on natural product β-elemene: anti-tumor mechanisms and structural modification. *Molecules* 2021; **26**: 1499.
9 Xu C, Jiang ZB, Shao L, *et al*. β-Elemene enhances erlotinib sensitivity through induction of ferroptosis by upregulating lncRNA H19 in EGFR-mutant non-small cell lung cancer. *Pharmacol Res* 2023; **191**: 106739.
10 Wang J, Qian C, Chen Y, *et al*. β-Elemene alleviates hyperglycemia-induced cardiac inflammation and remodeling by inhibiting the JAK/STAT3-NF-κB pathway. *Phytomedicine* 2023; **119**: 154987.
11 Zhang C, Li S, Zhao Z. β-Elemene promotes apoptosis induced by hyperthermia via inhibiting HSP70. *Dis Markers* 2022; **2022**: 7313026.
12 Zhao Q, Chen L, Zhang X, Yang H, Li Y, Li P. β-Elemene promotes microglial M2-like polarization against ischemic stroke via AKT/mTOR signaling axis-mediated autophagy. *Chin Med* 2024; **19**: 86.
13 Zhou Y, Qiu W, Wang Y, *et al*. β-Elemene suppresses obesity-induced imbalance in the microbiota-gut-brain axis. *Biomedicines* 2021; **9**: 704.
14 Zhou Y, Takano T, Li X, *et al*. β-Elemene regulates M1-M2 macrophage balance through the ERK/JNK/P38 MAPK signaling pathway. *Commun Biol* 2022; **5**: 519.
15 Nussbaumerova B, Rosolova H. Obesity and dyslipidemia. *Curr Atheroscler Rep* 2023; **25**: 947–955.
16 Shou JW, Shaw PC. Berberine reduces lipid accumulation in obesity via mediating transcriptional function of PPARδ. *Int J Mol Sci* 2023; **24**: 11600.
17 Qin H, Song Z, Zhao C, *et al*. Liquiritigenin inhibits lipid accumulation in 3T3-L1 cells via mTOR-mediated regulation of the autophagy mechanism. *Nutrients* 2022; **14**: 1287.
18 Li J, Wang X, Meng X, *et al*. Geraniin targeting CaMKK2 inhibits lipid accumulation in 3T3-L1 adipocytes by suppressing lipogenesis. *Chem Biol Interact* 2023; **372**: 110364.
19 Kang YM, Kang HA, Cominguez DC, Kim SH, An HJ. Papain ameliorates lipid accumulation and inflammation in high-fat diet-induced obesity mice and 3T3-L1 adipocytes via AMPK activation. *Int J Mol Sci* 2021; **22**: 9885.
20 Abudujilile D, Wang W, Aimaier A, *et al*. Cistanche tubulosa phenylethanoid glycosides suppressed adipogenesis in 3T3-L1 adipocytes and improved obesity and insulin resistance in high-fat diet induced obese mice. *BMC Complement Med Ther* 2022; **22**: 270.
21 Bailly-Maitre B, Belgardt BF, Jordan SD, *et al*. Hepatic Bax inhibitor-1 inhibits IRE1alpha and protects from obesity-associated insulin resistance and glucose intolerance. *J Biol Chem* 2010; **285**: 6198–6207.
22 Pal M, Wunderlich CM, Spohn G, Brönneke HS, Schmidt-Supprian M, Wunderlich FT. Alteration of JNK-1 signaling in skeletal muscle fails to affect glucose homeostasis and obesity-associated insulin resistance in mice. *PLoS One* 2013; **8**: e54247.
23 Song Y, Wu L, Li M, *et al*. Down-regulation of MicroRNA-592 in obesity contributes to hyperglycemia and insulin resistance. *EBioMedicine* 2019; **42**: 494–503.
24 Wang Q, Liu S, Zhai A, Zhang B, Tian G. AMPK-mediated regulation of lipid metabolism by phosphorylation. *Biol Pharm Bull* 2018; **41**: 985–993.

25 Shi L, Jiang Z, Zhang L. Childhood obesity and central precocious puberty. *Front Endocrinol (Lausanne)* 2022; **13**: 1056871.
26 Fang C, Pan J, Qu N, *et al*. The AMPK pathway in fatty liver disease. *Front Physiol* 2022; **13**: 970292.
27 Li Y, Xu S, Mihaylova MM, *et al*. AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice. *Cell Metab* 2011; **13**: 376–388.
28 Canbolat E, Cakıroglu FP. The importance of AMPK in obesity and chronic diseases and the relationship of AMPK with nutrition: a literature review. *Crit Rev Food Sci Nutr* 2023; **63**: 449–456.
29 Wang GY, Zhang L, Geng YD, *et al*. β-Elemene induces apoptosis and autophagy in colorectal cancer cells through regulating the ROS/AMPK/mTOR pathway. *Chin J Nat Med* 2022; **20**: 9–21.
30 Wang J, Xu C, Chen Y, *et al*. β-Elemene enhances the antitumor activity of erlotinib by inducing apoptosis through AMPK and MAPK pathways in TKI-resistant H1975 lung cancer cells. *J Cancer* 2021; **12**: 2285–2294.

doi: 10.3164/jcbn.24-179