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Research Article

Hypoglycemic Effects of 17,18-Didehydroloesenerine in High-Fat Diet Induced Diabetic Mice via AMP-Activated Protein Kinase

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Abstract

Background: Skeletal muscle insulin resistance is a major pathological feature of type 2 diabetes mellitus. Although the alkaloid loesenerine has been reported to enhance glucose utilization in muscle cells *in vitro*, the associated *in vivo* efficacy and the dependence of this effect on the AMP-activated protein kinase (AMPK) pathway remain unclear. Therefore, this study aimed to investigate the hypoglycemic activity and underlying mechanism of the associated analog, 17,18-didehydroloesenerine (DHL). **Methods:** Differentiated L6 myotubes were treated with DHL to evaluate glucose utilization and lactate production. The *in vivo* hypoglycemic effect of DHL was assessed in high-fat diet (HFD)-induced diabetic mice using an oral glucose tolerance test. The underlying mechanism was examined by Western blot analysis and AMPK inhibition using dominant-negative adenoviruses. **Results:** DHL dose-dependently increased glucose utilization and lactate production in L6 myotubes and significantly improved glucose tolerance in diabetic mice. DHL activated the AMPK signaling pathway, as indicated by increased phosphorylation of AMPK and the associated downstream target acetyl-CoA carboxylase (ACC). Genetic inhibition of AMPK abolished the stimulatory effect of DHL on glucose utilization. **Conclusions:** DHL exerts potent hypoglycemic effects both *in vitro* and *in vivo*. Under these *in vitro* conditions, DHL treatment activated the AMPK pathway, as evidenced by increased AMPK phosphorylation in L6 myotubes. Furthermore, infection with Ad-AMPK-DN attenuated DHL's effects compared with the Ad-GFP control in L6 myotubes. These findings suggest that AMPK activation contributes to DHL's actions under the present cell-based conditions. It should be noted that AMPK phosphorylation in skeletal muscle and *in vivo* AMPK inhibition were not assessed in the current study, and therefore direct extrapolation to an *in vivo* AMPK-dependent mechanism requires further investigation. These findings identify DHL as a novel AMPK-activating alkaloid and provide a basis for further development of DHL as a potential anti-diabetic lead compound.

Keywords: type 2 diabetes; AMP-activated protein kinase; alkaloids; hypoglycemic agents

1. Introduction

Type 2 diabetes mellitus (T2DM) poses a significant and growing global health challenge, contributing substantially to worldwide morbidity and mortality [1]. The development of T2DM is closely associated with insulin resistance, one of the earliest detectable abnormalities characterized by diminished glucose uptake in peripheral insulin-sensitive tissues [1,2]. Among these tissues, skeletal muscle plays a predominant role in postprandial glucose homeostasis, accounting for up to 80% of insulin-stimulated glucose disposal [2,3,4]. Consequently, the marked reduction in skeletal muscle glucose utilization observed in diabetes represents a key therapeutic target [5,6].

AMP-activated protein kinase (AMPK), a central regulator of cellular and organismal energy metabolism, is activated in response to a decrease in intracellular ATP levels [7]. Crucially, AMPK activation in skeletal muscle stimulates glucose uptake and metabolism through mechanisms that are largely independent of insulin signaling, thereby offering a promising strategy to bypass insulin resistance and lower blood glucose [8,9,10]. Therefore, pharmacological activators of AMPK are considered attractive candidates for the treatment of T2DM.

Natural products have long served as a major source of new drug leads and therapeutic inspiration. Alkaloids, in particular, represent a prominent class of bioactive compounds with diverse pharmacological activities [11]. Notably, several alkaloids have been identified as effective AMPK modulators. A well-established example is berberine (BBR). Extensive research has shown that BBR inhibits mitochondrial respiratory chain complex I, leading to an increased cellular adenosine diphosphate (ADP)/adenosine triphosphate (ATP) ratio and subsequent allosteric activation of AMPK. Because of this robust and well-characterized AMPK-activating effect, BBR is frequently used as a positive control in studies investigating AMPK-related metabolic pathways, providing a reli-

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able benchmark for evaluating novel candidate compounds [8]. Macrocyclic spermidine alkaloids are polyamine heterocycles biosynthetically derived from L-Orn or L-Arg via putrescine, and they mainly occur in a limited number of plant species, such as those in the family Celastraceae [12]. Recently, loesenerine was reported to activate the AMPK pathway by increasing the ADP/ATP ratio through inhibition of mitochondrial respiration, thereby enhancing glucose utilization in C2C12 cells [13].

Given the therapeutic potential of AMPK activation and the promising but incomplete data on loesenerine, we turned our attention to its analog, 17,18-didehydroloesenerine (DHL, Fig. 1). The present study was designed to systematically evaluate the hypoglycemic effects of DHL both *in vitro* and *in vivo* and to explicitly test whether its action depends on AMPK activation. Our findings aim to validate DHL as a novel AMPK-activating alkaloid and provide a foundation for its further investigation as a potential anti-diabetic lead.

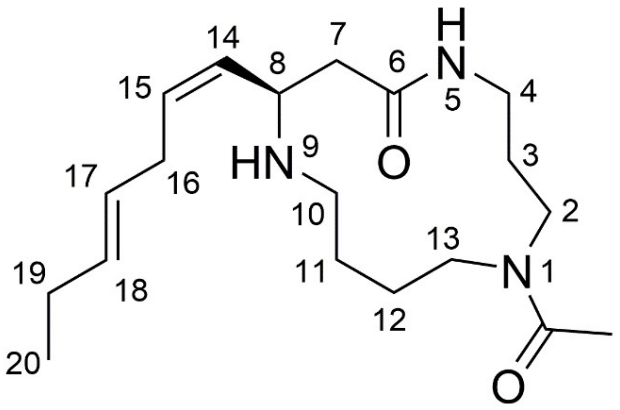

Fig. 1. Chemical structure of 17,18-didehydroloesenerine (DHL).

2. Materials and Methods

2.1 Cell Culture and Differentiation

L6 myoblasts (American Type Culture Collection (ATCC), catalog No. CRL-1458) were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen) supplemented with 10% fetal bovine serum (FBS; Invitrogen) at 37 °C under 5% CO_2. To induce differentiation into myotubes, confluent myoblasts were switched to DMEM containing 2% FBS for 6 days, with the medium changed every 48 h. L6 cells were authenticated by STR profiling (rat STR profile) and confirmed mycoplasma-negative; identity was consistent with the reference profile.

2.2 Measurement of Glucose Consumption and Lactate Production

Differentiated L6 myotubes in 96-well plates were treated with DHL (at the concentrations of 20 and 40 μM; the compound was isolated from Euonymus fortunei in our laboratory, as detailed in the **Supplementary Material**) or 5 μM berberine (BBR; positive control; CAS: 2086-83-1, Lot# B832574, Macklin, Shanghai, China) in serum-free DMEM containing 0.5% BSA and 5.5 mM glucose for 12 h. Glucose and lactate concentrations in the culture medium were determined using commercial kits (Glucose Assay Kit, G016-0600, Shanghai, China; Lactate Assay Kit, 2016032401, Ningbo, China) according to the manufacturers' instructions. Glucose consumption was calculated as the difference between the initial and final glucose concentrations in the medium.

2.3 Measurement of Glucose Uptake

Glucose uptake was assessed using 2-[1,2-^{3}H]-deoxy-D-glucose. After pretreatment with DHL (20 and 40 μM) or BBR (5 μM) in serum-free DMEM for 2.5 h, myotubes were incubated for 15 min at 37 °C in Hanks' Balanced Salt Solution (HBSS) containing 100 μM 2-deoxyglucose and 0.5 μCi/well 2-[1,2-^{3}H]-deoxy-D-glucose (PerkinElmer, NET549A001MC). The reaction was terminated by washing the cells three times with ice-cold PBS. Cells were then lysed with 0.1% Triton X-100, and radioactivity was measured using a scintillation counter (MicroBeta Plate Counter, PerkinElmer).

2.4 Oxygen Consumption Rate (OCR) Assay

OCR was measured in differentiated L6 myotubes seeded in an XF96 microplate (Seahorse Biosciences) using a Seahorse XF96 Analyzer (Agilent). Cells were equilibrated for 1 h at 37 °C in assay medium (DMEM with 25 mM glucose and 2 mM sodium pyruvate, pH 7.4) in a non-CO_2 incubator. After baseline recording, OCR was monitored following the sequential injection of oligomycin (2 μM), carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone (FCCP, CAS No. 370-86-5, 0.5 μM), and DHL (40 μM).

2.5 Measurement of Cellular ADP/ATP Ratio

The intracellular ADP/ATP ratio was quantified using a luminescence-based ADP/ATP Ratio Assay Kit (BioVision, #K255-200) according to the manufacturer's instructions. Differentiated L6 myotubes in 96-well plates were treated with DHL (20 and 40 μM) for 3 h before measurement.

2.6 Immunoblotting Assay

L6 myotubes were incubated with DHL (20 and 40 μM) for 3 h in serum-free DMEM and then lysed in RIPA buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% NP-40, 1 mM Na_3VO_4, 1 mM DTT, 1 mM EGTA) contain-

ing protease inhibitors (Roche, Basel, Switzerland). Total cellular protein was separated by SDS-PAGE after boiling for 10 min in SDS loading buffer. Before incubation with the primary antibodies, membranes were blocked with 5% milk in TBS containing 0.1% (v/v) Tween-20 for 1 h at room temperature. Chemiluminescent detection was performed using enhanced chemiluminescence (ECL) western blotting reagents (GE Healthcare, RPN2236). The primary antibodies used were as follows: AMPKα antibody (#2532, CST, USA), phospho-AMPKα (Thr172) antibody (#5256, CST, USA), ACC antibody (#3662, CST, USA), phospho-ACC (Ser79) antibody (#3661, CST, USA), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (#2118, CST, USA). All primary antibodies were diluted 1:1000 for immunoblotting analysis.

2.7 Adenovirus Infection

Recombinant adenoviruses expressing dominant-negative forms of AMPK, AMPKα1 (D159A) and AMPKα2 (K45R) (α1/α2-dominant negative (DN)), were constructed using the pAdEasy system (Agilent Technologies, New York, NY, USA). L6 myoblasts were infected with adenoviruses expressing either the control green fluorescent protein (GFP) reporter protein or α1/α2-DN before differentiation.

2.8 Animals

All animal experiments were approved by the Animal Ethics Committee of the Shanghai Institute of Materia Medica (2025-04-lj-202). Six-week-old male C57BL/6J mice (Shanghai Shengchang Biotechnology Co., Ltd.) were housed under standard laboratory conditions: a 12 h light/-dark cycle, with room temperature maintained at 22 ± 2 °C. Mice were fed either a normal chow diet (n = 5) or a high-fat diet (HFD; 60% calories from fat; Research Diets, USA; n = 12) for 6 weeks.

2.9 Glucose Tolerance Assay

After the dietary intervention, high-fat diet (HFD)-fed mice were randomly assigned to two groups: HFD-Vehicle (n = 6) and HFD-DHL (n = 6). Following a 6 h fast, mice received a single oral gavage of DHL (100 mg/kg) or vehicle. One hour later, glucose (2 g/kg) was administered intraperitoneally. Blood glucose was measured at 0, 15, 30, 60, 90, and 120 min after injection. At the end of the experiment, mice were euthanized by cervical dislocation.

2.10 Statistical Analysis

All data are expressed as the mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by Dunnett's post hoc test was performed, and $p < 0.05$ was considered statistically significant.

3. Results

3.1 Effects of DHL on Glucose Utilization in L6 Myotubes

Skeletal muscle is one of the primary tissues responsible for glucose disposal; therefore, we first examined the effects of DHL in L6 myotubes. As shown in Fig. 2A, incubation of L6 myotubes with DHL at concentrations of 20 and 40 μM for 12 h increased glucose consumption in a dose-dependent manner. We also evaluated glucose uptake using radiolabeled 2-[1,2-^{3}H]-deoxy-D-glucose in L6 cells after 2.5 h pretreatment with DHL (20 and 40 μM), followed by a 15 min glucose uptake assay. The results showed that DHL increased glucose uptake by 1.11- and 1.26-fold at concentrations of 20 and 40 μM, respectively (Fig. 2B).

3.2 Effects of DHL on Lactate Production in L6 Myotubes

Glucose, the major cellular energy source, is taken up into cells, converted to acetyl-CoA, and enters the tricarboxylic acid (TCA) cycle to generate electron donors that are ultimately delivered to the mitochondrial electron transport chain (ETC), where ATP is produced [14]. When mitochondrial oxidative phosphorylation is inhibited, anaerobic glycolysis is stimulated as a compensatory mechanism for ATP generation, accompanied by increased lactate production [14]. We therefore measured lactate production in the culture medium. The results revealed that DHL markedly promoted lactate production in myotubes, suggesting that this alkaloid increases glucose utilization by enhancing anaerobic glycolysis in L6 cells (Fig. 3).

3.3 Effects of DHL in High-Fat Diet (HFD)-Induced Diabetic Mice

After demonstrating that DHL promoted glucose utilization *in vitro*, we further evaluated its hypoglycemic activity *in vivo*. Mice were fed a high-fat diet (HFD) for six weeks to induce insulin resistance and glucose intolerance, as evidenced by impaired glucose tolerance compared with normal-chow-fed mice following a glucose challenge (Fig. 4A) [15]. Subsequently, to assess its potential therapeutic effect, a single acute dose of DHL (100 mg/kg) was administered orally to the HFD-fed mice 1 h before intraperitoneal glucose injection. Compared with the HFD-vehicle group, the DHL-treated group showed significantly lower blood glucose levels at 90 and 120 min after glucose administration. Consistently, area under the curve (AUC) analysis demonstrated that this single acute treatment improved glucose clearance in HFD-induced diabetic mice (Fig. 4B).

3.4 DHL Activates AMPK Pathway

Once the inhibition of mitochondrial respiration lead to the insufficient of ATP production, cells initiate anaerobic glycolysis as a compensation mechanism for ATP generation [15]. The above results suggest that DHL may increase glucose utilization by promoting anaerobic glycolysis of myotube cells. So we investigated the effects of DHL

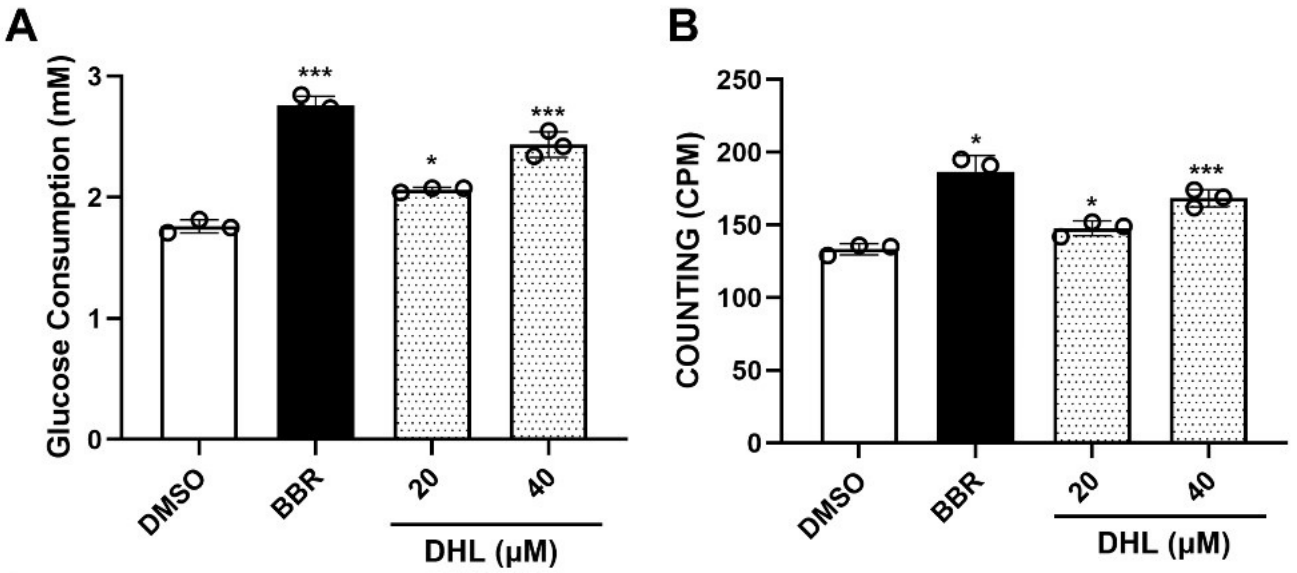

Fig. 2. Effects of DHL on glucose utilization in L6 myotubes. (A) Glucose consumption in L6 myotubes following 12 h of treatment with DHL. (B) Glucose uptake assessed using 2-[1,2-^{3}H]-deoxy-D-glucose after 2.5 h pretreatment with DHL followed by a 15 min uptake assay. Berberine (BBR) (5 μM) was used as a positive control. Data are presented as the mean ± SEM from 3 independent experiments. *$p < 0.05$, ***$p < 0.001$ vs. DMSO group. DMSO, dimethyl sulfoxide; SEM, standard error of the mean.

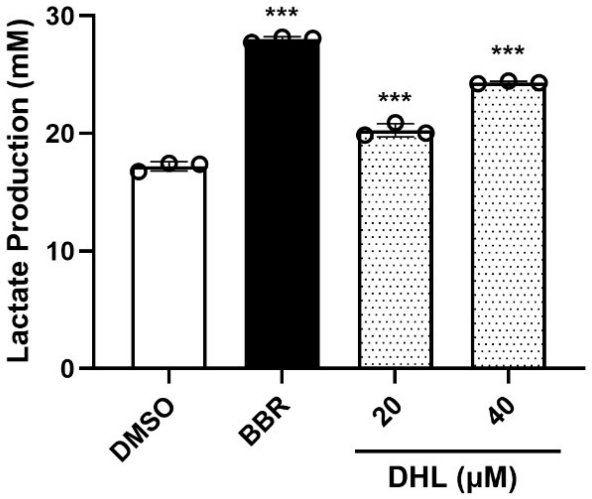

Fig. 3. DHL increases lactate production in L6 myotubes. Lactate levels in the medium were measured after L6 myotubes were treated with DHL for 12 h. BBR (5 μM) was used as a positive control. Data are presented as the mean ± SEM from 3 independent experiments. ***$p < 0.001$ vs. DMSO group.

on mitochondrial respiration in L6 myotubes. As shown in Fig. 5A, cellular oxygen consumption (OCR) rate (primary in mitochondria) was significantly suppressed by DHL in the presence of oligomycin and FCCP. These results indicated that the alkaloid promoted glucose utilization in myotubes via anaerobic glycolysis.

ATP is primarily generated in mitochondria from ADP through TCA cycle and mitochondrial electron transport chain. The inhibition of mitochondrial respiration would lead to the reduction of ATP production, increasing of ADP/ATP ratio, and thus activation of AMPK [16]. Since the mitochondrial respiration was inhibited by DHL, so we examined whether cellular ATP production was affected by this compound. As shown in Fig. 5B, a significant increase of ADP/ATP ratio was found in L6 myotubes incubated with the alkaloid at the concentrations of 20 and 40 μM.

Correspondingly, treatment with DHL increased phosphorylation of both Thr172 on the α-catalytic subunit of AMPK and Ser79 on its canonical downstream target acetyl-CoA carboxylase (ACC) in L6 myotubes (Fig. 5C). This finding suggests that DHL activates AMPK by inhibiting mitochondrial respiration and reducing ATP production efficiency [17].

3.5 Effects of DHL on Glucose Uptake in AMPK α1/α2-DN Adenovirus–Infected L6 Myotubes

To further investigate the role of AMPK in DHL-induced glucose utilization, AMPK α1/α2-DN adenovirus was used to inhibit the expression and activation of AMPKα in L6 myotubes. As shown in Fig. 6A, the DHL-induced increase in phosphorylation of ACC, a downstream target of AMPK, at 40 μM was blocked in myotubes pretreated with AMPK α1/α2-DN adenovirus. Furthermore, DHL failed to stimulate glucose uptake when AMPK activation was blocked by AMPK α1/α2-DN adenovirus (Fig. 6B). These data indicated that DHL-induced glucose uptake in L6 myotubes depends on AMPK activation.

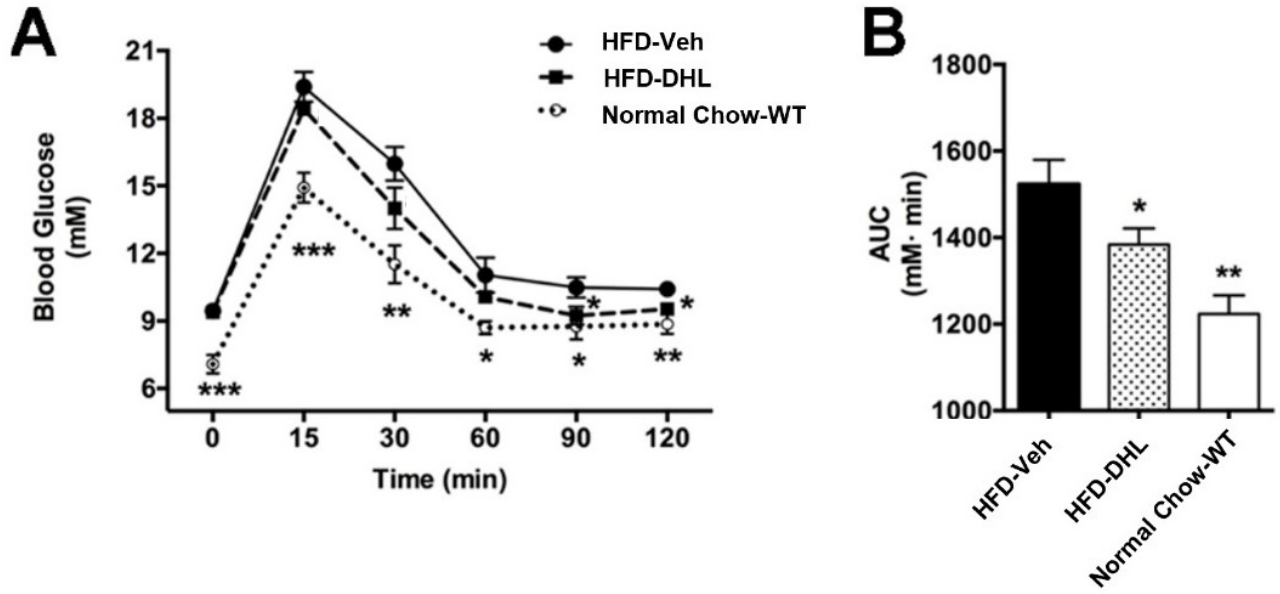

Fig. 4. DHL improves glucose tolerance in high-fat diet (HFD)-induced diabetic mice. (A) Blood glucose levels during an intraperitoneal glucose tolerance test (IPGTT) before (0 min) and at indicated time points after glucose injection. (B) Corresponding area under the curve (AUC) for blood glucose. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ vs. HFD-vehicle group, n = 6 in HFD groups and n = 5 for normal chow group. WT, wild-type.

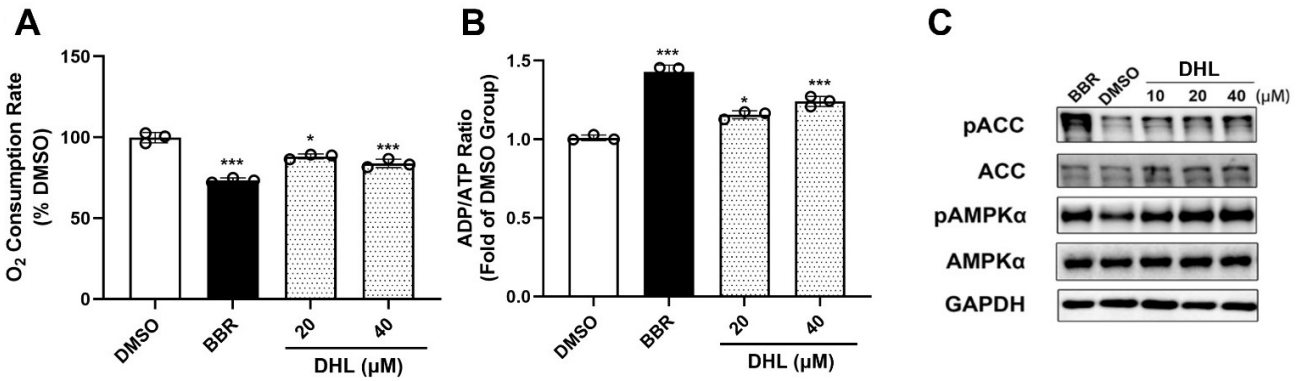

Fig. 5. DHL activates the AMP-activated protein kinase (AMPK) signaling pathway in L6 myotubes. (A) Oxygen consumption rate (OCR) measured in myotubes following sequential treatment with oligomycin, FCCP, and DHL. (B) Cellular ADP/ATP ratio after 3 h of treatment with DHL. (C) Immunoblot analysis of phosphorylated AMPK and acetyl-CoA carboxylase (ACC) (downstream target) following 3 h of treatment with DHL. BBR (5 μM) was used as the positive control. The data are presented as fold of DMSO group and as the means ± SEM. *$p < 0.05$ and ***$p < 0.001$ vs. DMSO group, n = 3 for all groups. pACC, phosphorylated acetyl-CoA carboxylase; pAMPKα, phosphorylated AMP-activated protein kinase α-subunit; ADP, adenosine diphosphate; ATP, adenosine triphosphate; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; FCCP, Carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone.

4. Discussion

We report that DHL, a macrocyclic spermidine alkaloid, enhances glucose utilization in L6 myotubes and improves glucose tolerance in HFD-induced diabetic mice. Crucially, our mechanistic investigations provide compelling evidence that these effects are mediated through activation of the AMPK pathway. Our *in vitro* data establish a clear sequence of events linking DHL treatment to AMPK activation. DHL dose-dependently increased glucose consumption and uptake in differentiated L6 myotubes, a well-established model for studying skeletal muscle metabolism. Concurrently, DHL significantly increased lactate production, suggesting a shift toward anaerobic glycolysis. This metabolic shift was further supported by the observed inhibition of mitochondrial oxygen consumption rate, indicating impaired oxidative phosphorylation. The consequent

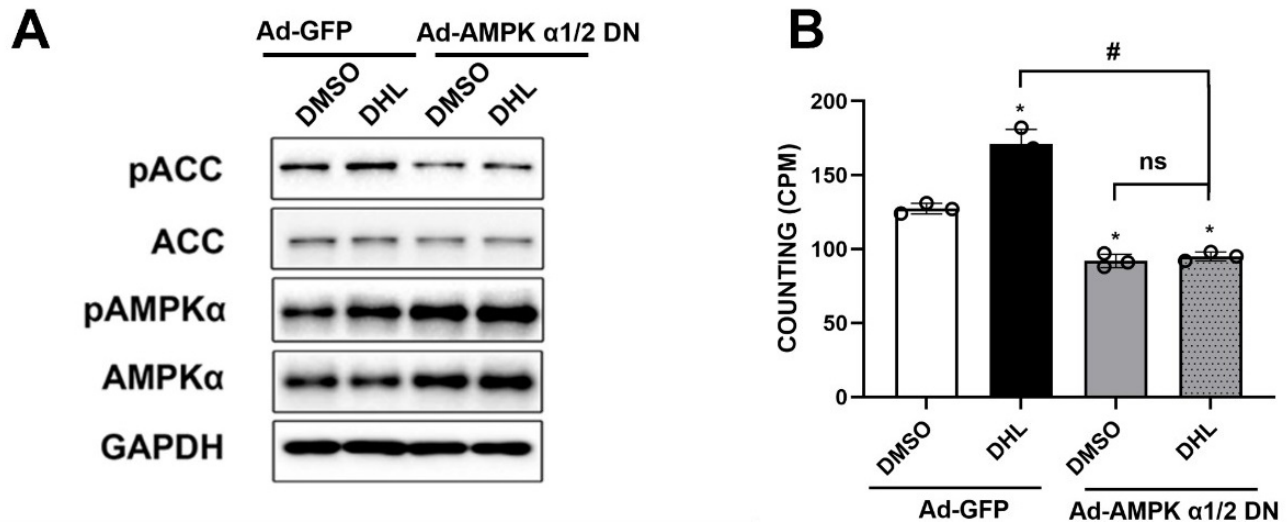

Fig. 6. Genetic inhibition of AMPK abolishes the effect of DHL on glucose uptake. L6 myoblasts were infected with adenoviruses expressing either a control GFP or dominant-negative AMPK α1/α2 (AMPK-DN) for 3 h, followed by treatment with DMSO or 40 μM DHL for 3 h. (A) Immunoblot showing that AMPK-DN infection blocked DHL-induced phosphorylation of ACC. (B) Glucose uptake assessed using 2-[1,2-^{3}H]-deoxy-D-glucose. Data are presented as percentage of the respective DMSO control group (mean ± SEM; n = 3). *$p < 0.05$ vs. Ad-GFP DMSO group; #$p < 0.05$ vs. Ad-GFP DHL group; ns = not significant (Ad-AMPK-DN DHL vs. Ad-AMPK-DN DMSO). Ad-GFP, adenovirus expressing green fluorescent protein; DN, dominant negative.

disruption of cellular energy homeostasis was confirmed by a significant increase in the ADP/ATP ratio, a classic trigger for AMPK activation [18,19]. Consistently, DHL treatment increased phosphorylation of AMPK at Thr172 and of its downstream target ACC at Ser79. The strongest evidence for the requirement of AMPK came from the genetic inhibition experiments, in which dominant-negative AMPK constructs completely abolished the effect of DHL on glucose utilization. This mechanistic cascade—inhibition of mitochondrial respiration, elevation of the ADP/ATP ratio, activation of AMPK, and subsequent enhancement of glucose uptake—is consistent with the established mode of action of several known AMPK activators, such as berberine, which was used as a positive control in this study [8].

The therapeutic relevance of targeting AMPK in skeletal muscle for T2DM management is well established. Skeletal muscle is responsible for the majority of insulin-stimulated glucose disposal, and insulin resistance in this tissue is a primary defect in T2DM [1,2]. AMPK acts as a central cellular energy sensor and, when activated, stimulates glucose uptake through mechanisms that are largely independent of insulin signaling. This offers a promising strategy to bypass insulin resistance. Our *in vivo* experiment provides preliminary proof-of-concept for the efficacy of DHL. A single acute oral administration of DHL significantly improved glucose tolerance in HFD-fed mice, as evidenced by lower blood glucose levels at later time points and a reduced area under the curve (AUC). This suggests that the AMPK-activating property observed *in vitro* translates into a measurable hypoglycemic effect *in vivo*. The delayed onset of action *in vivo* may reflect pharmacokinetic factors such as absorption and distribution, which warrant further investigation.

The discovery of DHL adds to the growing repertoire of natural product-derived AMPK activators being explored for metabolic diseases. Like other bioactive alkaloids and flavonoids, DHL appears to act as an indirect activator, likely by perturbing mitochondrial function and altering cellular adenine nucleotide levels. Although this mechanism is shared with some reference compounds, DHL remains novel because it possesses a distinct macrocyclic spermidine scaffold. This structural uniqueness may offer advantages in terms of specificity, potency, or pharmacokinetic properties and therefore merits further exploration.

5. Limitations

This study was designed as a preliminary investigation to establish proof of concept for the *in vivo* glucose-lowering efficacy of DHL and to explore its AMPK-associated mechanism in L6 myotubes. Although the findings are promising, several important aspects remain to be characterized in future work, which naturally follows from the focused scope of this initial study.

First, the pharmacokinetic (PK) and toxicological profiles of DHL require systematic investigation. The delayed pharmacological effect observed in the intraperitoneal glucose tolerance test (IPGTT) suggests that its *in vivo* PK properties, such as absorption and distribution, significantly influence its temporal profile. Furthermore, comprehensive safety assessments, including evaluation in normoglycemic models to exclude the risk of hypoglycemia and

detailed toxicological studies, were beyond the scope of this efficacy-focused report. Defining the PK profile, therapeutic window, and safety of DHL will be essential steps in its further development.

Second, the precise contribution of systemic insulin sensitivity to the observed *in vivo* effect warrants further study. As the primary aim was to validate efficacy and probe the AMPK pathway, direct measures of insulin sensitivity, such as the insulin tolerance test (ITT), were not included. Therefore, although our *in vitro* data strongly support an AMPK-dependent mechanism in L6 myotubes, the relative contribution of improved peripheral insulin sensitivity versus other potential mechanisms *in vivo* remains to be elucidated. Future studies should directly dissect its effects on whole-body insulin sensitivity.

Third, the lack of a parallel positive control drug, such as metformin, in the animal model limits direct comparison of potency within this study. Our design prioritized establishing the intrinsic activity of DHL in the disease model. A head-to-head comparison with first-line therapies will be a critical component of subsequent efficacy benchmarking.

These limitations highlight clear and logical directions for continued research and do not diminish the central finding of this study: DHL is a novel macrocyclic spermidine alkaloid that exhibits *in vivo* antihyperglycemic activity, mechanistically linked to AMPK activation in skeletal muscle cells.

6. Conclusion

Our *in vitro* mechanistic studies demonstrated that DHL promotes glucose utilization in L6 myotubes in an AMPK-dependent manner. In an HFD-induced diabetic mouse model, DHL also improved glucose tolerance. Taken together, these findings suggest that the hypoglycemic effect of DHL may be associated with activation of the skeletal muscle AMPK pathway. This study provides valuable insight into hypoglycemic alkaloids and may contribute to the development of a new class of anti-diabetic agents.

Abbreviations

AMPK, AMP-activated protein kinase; ACC, acetyl-CoA carboxylase; HFD, high-fat diet; BBR, Berberine; OCR, oxygen consumption rate; DMEM, Dulbecco's modified Eagle's medium.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding authors on reasonable request.

Author Contributions

LZ and DX performed the research. JL and YY analyzed the data. YL and JC designed the research study and wrote the manuscript. All authors read and approved the final manuscript. All authors contributed to editorial changes in the manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All animal experiments were approved by the Animal Ethics Committee of the Shanghai Institute of Materia Medica (2025-04-lj-202). All animal experiments were conducted in accordance with the ARRIVE guidelines and the relevant national or international standards.

Acknowledgment

Not applicable.

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Conflicts of Interest

The authors declare no conflicts of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/IJP48283.

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