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Original Article

Effects of different concentrations of berberine on 3T3-L1 adipocyte glycometabolism and inflammatory cytokine expression

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Abstract: Purpose: The goal of this study was to investigate the effects of the concentration of berberine on 3T3-L1 adipocytes glycometabolism and inflammatory cytokines expression. Method: After incubation for 12 days, 3T3-L1 adipocytes were cultured in 0 μmol/L of berberine (vehicle group), 5 μmol/L of berberine (ulatralow dose group), 10 μmol/L of berberine (low dose group), 20 μmol/L of berberine (middle dose group) and 40 μmol/L of berberine (high dose group) for 24 hours. mRNA levels of IL-6, TNF-α, adipocyte factor leptin, visfatin, and adiponectin in each group were detected by PCR method, and glucose consumption and glucose transporting rate were detected by glucose oxidase method and liquid scintillation counter. Result: There was no significant difference in the mRNA level of adiponectin among groups (P>0.050). mRNA levels of IL-6, TNF-α, and leptin in groups intervened with berberine were significantly lower than those in the vehicle group (P<0.001). Among them, the mRNA levels of IL-6, TNF-alpha and leptin of middle dose group were the lowest (P<0.050). The glucose consumption, glucose transporting rate and visfatin of groups treated with berberine were significantly higher than those in the vehicle group (P<0.050), and the glucose consumption, glucose transporting rate and visfatin in the middle dose group were the highest (P<0.050). Conclusion: In summary, berberine can effectively improve the glycometabolism of 3T3-L1 adipocytes and reduce the mRNA expression of inflammatory factors.

Keywords: Berberine, 3T3-L1 adipocyte, glycometabolism, IL-6, TNF-α

Introduction

Type 2 diabetes mellitus is the most common chronic disease among middle-aged and elderly patients [1, 2]. Type 2 diabetes mellitus is closely related to human's insulin resistance [3]. It has been proven that the main promoter of insulin resistance is inflammation. Adipocyte participate in the development of insulin resistance by secreting a large number of inflammatory factors (such as IL-6, TNF-alpha, etc.) [4, 5]. As the most active endocrine organ in human body, adipose tissue interferes with expression of adipokines and inflammatory factors, and it may be of great significance for the future targeted treatment on type 2 diabetes mellitus. Therefore, domestic and overseas researchers have been conducting research in this direction in recent years [6-8]. As the main component of traditional Chinese medicine Coptis chinensis, berberine has excellent blood sugar-lowering effects, lipid-lowering and improving insulin sensitivity. Berberine is often used to treat diabetes, hypertension and other diseases in traditional Chinese medicine [9-12]. It has been reported that berberine can reduce the metabolism of aliphatic acid and accelerate the glucose transport of adipocytes, and it also has certain effects on inflammatory factors [13-15]. This paper analyzed the effects of different concentrations of berberine on glycometabolism function and inflammatory factors release in 3T3-L1 adipocytes.

Materials and methods

Reagent

3T3-L1 cell line was purchased from Shanghai Aolu Biotechnology Co. LTD, and the item number was XB-3248. The high sugar medium (12-741F) was purchased from Beijing Zeping

Table 1. Primer sequences

	R	F
IL-6	5'-GGATGCTACCAAACTGGATA-3'	5'-CTCTGGCTTTGTCTTTCTTG-3'
TNF-α	5'-CGTCGTAGCAAACCACCAAG-3'	5'-GTCCCTTGAAGAGAACCTGG-3'
Linostatin	5'-TCGGTTCTGGTGGCGCTTTGCTAC-3'	5'-AAGTTCCCCGCTGGTGTCCTGTGT-3'
Leptin	5'-AGCCTGCCTTCCCAAAATGT-3'	5'-TGTGGAGTAGAGTGAGGCTT-3'
Adiponectin	5'-ACGACCAGTATCAGGAAAAG-3'	5'-GCCAGTAAATGTAGAGTCGT-3'
β-actin	5'-GGCTGTATTCCCCTCCATCG-3'	5'-CCAGTTGGTAACAATGCCAT-3'

Table 2. mRNA expression levels of IL-6, TNF-α

	IL-6	TNF-α
Microdose group	0.38 ± 0.06	0.57 ± 0.08
Low dose group	0.28 ± 0.07*	0.41 ± 0.07*
Middle dose group	0.22 ± 0.06$^{*,\#}$	0.21 ± 0.06$^{*,\#}$
High dose group	0.26 ± 0.05$^{*,\#,\Delta}$	0.36 ± 0.04$^{*,\#,\Delta}$
Vehicle group	1.02 ± 0.04$^{*,\#,\triangledown}$	1.02 ± 0.09$^{*,\#,\Delta,\triangledown}$
F	54.661	162.854
P	<0.001	<0.001

*means comparison with microdose group, P<0.050; $^{\#}$means comparison with low dose group, P<0.050; $^{\Delta}$means comparison with middle dose group, P<0.050; $^{\triangledown}$means comparison with high dose group, P<0.050.

Technology Co., Ltd. IL-6 PCR kits (K089) were purchased from Beijing Bomaisi Technology Co., Ltd. TNF-α PCR kits (YS04315P) were purchased from Shanghai Caiyou industrial Co., Ltd. Trizol (15596-026) was purchased from Wuhan Kehaojia Biotechnology Co., Ltd. Fetal bovine serum (11011-8611) was purchased from Zhejiang Tianhang Biotechnology Co. LTD.

3T3-L1 cell culture

The 3T3-L1 cell line was cultured in high sugar medium containing 10% of FBS under 37°C and 5% of CO_2. When the cells filled the bottom, cells were inoculated into 6-well culture plate, and standing for 2 hours. The cells were added into the medium containing 10% of FBS, 10 mg/L of insulin, 0.25 μmol/L of dexamethasone and 0.5 mmol/L of Xanthine differentiation solution, and left for 48 hours, and then 10 mg/L of insulin was added to the medium, and left for 48 hours. Thereafter, 10% of FBS DMEN medium was added every 2 days to induce differentiation for totally 12 days. When more than 90% of 3T3-L1 cells showed fat cell phenotype, subsequent treatment was continued. This study has been approved by the Ethics Committee of First Affiliated Hospital, Heilongjiang University of Chinese Medicine.

Berberine treatment

Berberine was dissolved in dimethyl sulfoxide, with a concentration of 0.04 mol/L. 5 μmol/L of berberine (microdose group), 10 μmol/L of berberine (low dose group), 20 μmol/L of berberine (middle dose group) and 40 μmol/L of berberine (high dose group) were respectively added into culture plate. The wells without added berberine were the vehicle group. Three repeated wells for every groups. After 24 hours of culture, qRT-PCR method was used to detect the expression level of related factors in each group.

PCR detection

A TRIzol method was used to extract total RNA of adipocyte, and RNA was transcribed to cDNA according to the instructions of the kit. After the reaction, the cDNA sample was transported into the refrigerator as a template for RT-PCR reaction. The RT-PCR primer sequence was synthesized by China Thermo Fisher Company. See **Table 1** for details. The reaction conditions were as follows: 1 μL of cDNA, 0.4 μL of Forward Primer, 0.4 μL of Universal miRNA qPCR Primer, 10 μL of 2 × TransStart Tip Green qPCR SuperMix, 0.4 μL of Passive Reference Dye (50×) (Optional), and the total volume was brought to 20 μL with RNase-free water. The reaction condition was optimized to as pre-denature at 95°C for 10 minutes, denature at 95°C for 15 seconds, anneal at 60°C for 30 seconds and extension at 72°C for 30 seconds for 45 cycles. A reaction system SYBR Green and fluorescence intensity of double-stranded DNA were used to do quantitative detection of PCR amplification products. All tests were repeated for 3 times, and take the average value as the results. The RNA quantity was normalized against the β-actin content, and gene expression was quantified according to the $2^{-\Delta Ct}$ method. The primers are shown in **Table 1**.

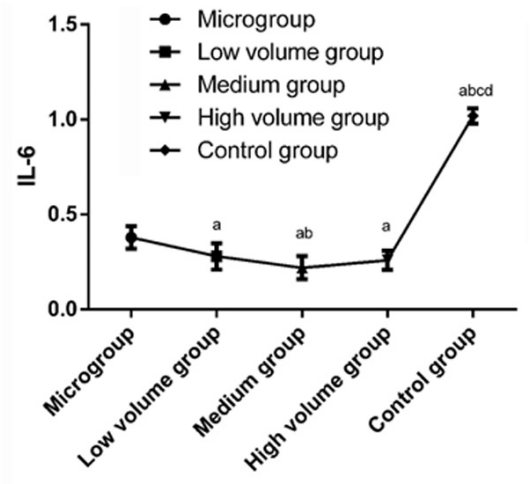

Figure 1. mRNA expression level of IL-6 in each group. *means comparison with microdose group, P<0.050; #means comparison with low dose group, P<0.050; △means comparison with middle dose group, P<0.050; ▽means comparison with high dose group, P<0.050.

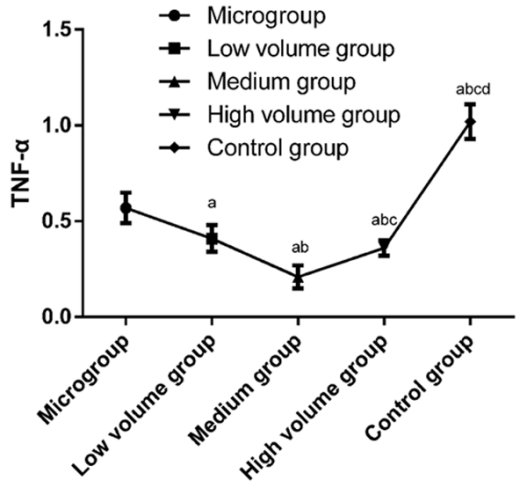

Figure 2. mRNA expression level of TNF-α in each group. *means comparison with microdose group, P<0.050; #means comparison with low dose group, P<0.050; △means comparison with middle dose group, P<0.050; ▽means comparison with high dose group, P<0.050.

Glucose detection and transport rate

The mature 3T3-L1 adipocytes were added into medium containing 1 μmol/L of Dexamethasone, and cultured for 20 hours. After culturing for 12 hours in 0.5% BSA Serum-free DMEM culture medium, 5 μmol/L of berberine (microdose group), 10 μmol/L of berberine (low dose group), 20 μmol/L of berberine (middle dose group) and 40 μmol/L of berberine (high dose group) were respectively added into culture plate. All plates were cultured for 24 hours, and a vehicle group was set up. The glucose oxidase method was used to detect glucose consumption amount, and the vehicle group was taken as base value of glucose. Glucose consumption amount = base value - glucose amount of inspection hole.

After the glucose consumption experiment, 3T3-L1 cells were incubated with 0.5% of BSA buffer (at 37°C) for 15 minutes, then 10 nmol/L of insulin buffer was added, and 3T3-L1 cells were incubated for 20 minutes. At last, 2-deoxy-[3H]-D-glucose was added, and 10 μmol/L of cytochalasin B was added in another hole. The liquid was sucked out after incubation for 10 minutes, and the plates were washed with PBS for three times. Add 1 mL of 0.1 mol/L NaOH and standing for 2 hours. The scintillation solution was added, and the liquid scintillation counter was used to measure the results of each hole. Glucose uptake was calculated by subtracting blank pore (the pore of cytochalasin B) number from each pore number. The glucose uptake of each pore was compared with the value pore without insulin as the basic value, and the ratio of each pore was the glucose transporting rate.

Outcome measurement

Including: mRNA expression levels of IL-6, TNF-α, visfatin, leptin and adiponectin; Glucose consumption; Glucose transport rate.

Statistical methods

SPSS 24 Statistical software (from Beijing Sichuang Weida Information Technology Co., Ltd.) was used to analyze and process the data. All the results were expressed in the form of (Mean ± standard deviation). Comparison among the group were performed using one-way ANOVA and then proofread by post hoc Bonferroni pairwise comparison. If P<0.050, the difference was considered statistically significant.

Results

mRNA expression levels of IL-6 and TNF-α

mRNA expression levels of IL-6 and TNF-alpha were significantly different among groups

Table 3. Comparison of sugar metabolism

	Glucose consumption (mol/L0)	Glucose transport rate (%)
Microdose group	2.47±0.05	46.54±5.07
Low dose group	2.51±0.09	57.08±6.59*
Middle group	3.38±0.12*,#	77.36±10.82*,#
High dose group	2.92±0.07*,#,Δ	70.54±7.15*,#,Δ
Vehicle group	1.52±0.03*,#,Δ,▽	39.82±4.15*,#,Δ,▽
F	469.621	320.650
P	<0.001	<0.001

*means comparison with microdose group, P<0.050; #means comparison with low dose group, P<0.050; Δmeans comparison with middle dose group, P<0.050; ▽means comparison with high dose group, P<0.050.

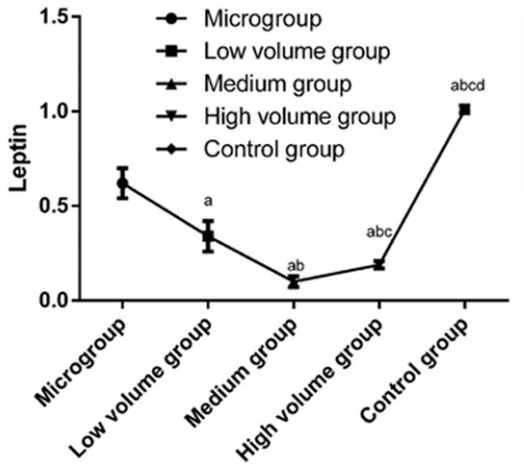

Figure 3. Comparison of mRNA expression of leptin among groups. *means comparison with microdose group, P<0.050; #means comparison with low dose group, P<0.050; Δmeans comparison with middle dose group, P<0.050; ▽means comparison with high dose group, P<0.050.

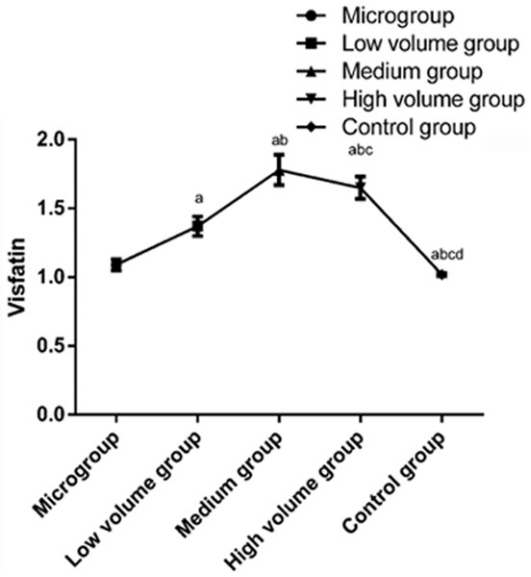

Figure 4. Comparison of mRNA expression of visfatin among groups.*means comparison with microdose group, P<0.050; #means comparison with low dose group, P<0.050; Δmeans comparison with middle dose group, P<0.050; ▽means comparison with high dose group, P<0.050.

(P<0.001). mRNA expression levels of IL-6 and TNF-α in the microdose, low dose, middle dose, and high dose groups were significantly lower than vehicle group (P<0.050). The mRNA expression level of IL-6 in the microdose group was higher than the low dose, middle dose, and high dose groups (P<0.050) and there was no significant difference between the low dose and high dose groups (P>0.050), but mRNA expression levels of both groups were higher than the middle dose group (P<0.050). The mRNA expression level of TNF-α of the low dose group was higher than the high dose group (P<0.050) and the value in the middle dose group was the lowest (P<0.050) (**Table 2, Figures 1**, **2**).

mRNA expression of adipocyte factors

There was no significant difference in adiponectin among groups (P>0.050), but there were significant differences in leptin and visfatin among groups (P<0.001). The leptin in microdose, low dose, middle dose, and high dose groups were significantly lower than vehicle group (P<0.050), but the visfatin were significantly higher than the vehicle group (P<0.050). The level of leptin in the microdose group was significantly higher than low dose, middle dose, and high dose groups (P<0.050). The level of leptin in the low dose group was significantly higher than the middle dose and high dose groups (P<0.050), and the level of leptin in the middle dose group was the lowest (P<0.050). Visfatin in the microdose group was significantly lower than low dose, middle dose, and high dose groups (P<0.050). The visfatin in the low dose group was significantly lower than the middle dose and high dose groups (P<0.050), and visfatin in the middle dose group was the highest (P<0.050) (**Table 3**, **Figures 3**, **4**).

Glycometabolism

There were significant differences in glucose consumption and glucose transport rates among groups (P<0.001). The glucose consump-

Table 4. mRNA expression levels of adipokine mRNAs

	Leptin	Adiponectin	Linostatin
Microgroup	0.62±0.08	0.95±0.14	1.09±0.04
Low dose group	0.34±0.08*	0.88±0.08*	1.37±0.07*
Middle group	0.10±0.03$^{*,\#}$	0.92±0.06$^{*,\#}$	1.78±0.11$^{*,\#}$
High dose group	0.19±0.02$^{*,\#,\Delta}$	0.85±0.05$^{*,\#,\Delta}$	1.65±0.08$^{*,\#,\Delta}$
Vehicle group	1.01±0.02$^{*,\#,\Delta,\triangledown}$	0.86±0.04$^{*,\#,\Delta,\triangledown}$	1.02±0.01$^{*,\#,\Delta,\triangledown}$
F	162.84	1.624	184.762
P	<0.001	0.399	<0.001

*means comparison with microdose group, P<0.050; $^{\#}$means comparison with low dose group, P<0.050; $^{\Delta}$means comparison with middle dose group, P<0.050; $^{\triangledown}$means comparison with high dose group, P<0.050.

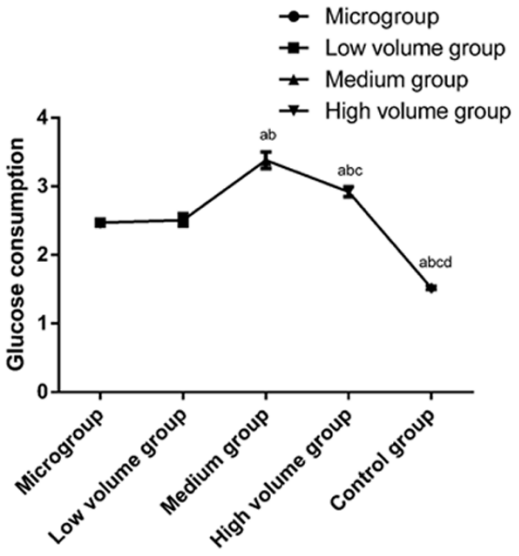

Figure 5. Comparison of glucose consumption among groups.*means comparison with microdose group, P<0.050; $^{\#}$means comparison with low dose group, P<0.050; $^{\Delta}$means comparison with middle dose group, P<0.050; $^{\triangledown}$means comparison with high dose group, P<0.050.

tion and glucose transport rates in the microdose, low dose, middle dose, and high dose groups were significantly higher than the vehicle group (P<0.050). In the berberine intervention groups, there was no significant difference in glucose consumption between the microdose and low dose groups (P>0.050), which were both lower than the middle dose and high dose groups (P<0.050). The glucose consumption in the middle dose group was significantly higher than the high dose group (P<0.050). The glucose transport rate of microdose group was the lowest (P<0.050), followed by the low dose group (P<0.050). The glucose transport rate of the high dose group was the highest (P<0.050) (**Table 4**, **Figures 5**, **6**).

Discussion

Berberine is an isquinoline alkaloid and it is widely found in Berberis [16]. As a traditional Chinese medicine ingredient, berberine has good inhibitory effects on gram-positive and gram-negative bactria [17]. With the development of research, berberine has been found good effects on reducing blood sugar and lipid in recent years [18]. By detecting the pharmacokinetics of berberine, some studies have found that berberine plays a degradation role in adipose tissue, but the underlying mechanism is not clear yet [19]. Currently, there is no effective and permanent cure for type 2 diabetes mellitus [20], so it is particularly important to explore its targeted therapy.

The results show that the relative expression levels of IL-6 mRNA and TNF-α mRNA in berberine groups were lower than those in the vehicle group, while the relative expression levels of IL-6 and TNF-alpha in middle dose group were the lowest, which indicated that berberine could inhibit inflammatory factors in adipocytes. The inhibitory effect of 20 μmol/L of berberine on 3T3-L1 cells was the strongest. At present, the correlation between inflammatory factors and insulin resistance has been recognized clinically. IL-6 is an inflammatory factor secreted by monocyte-macrophage, which has dual functions of causing inflammation and anti-inflammation. Once it exceeds the normal value, it will cause damage to human tissues and cells [20, 21]. By reducing insulin stimulation on receptor substrates, IL-6 weakens PI-3K activity and causes insulin resistance [22]. Therefore, berberine may inhibit differentiation of adipocytes and reduce the expression of IL-6 in adipocytes, thus reducing the production of insulin resistance. TNF-α is a kind of cytokine with many biological activities, which mainly regulates the immune function and inflammatory reaction of the body [23, 24]. In the process of TNF-α pathogenesis, phosphorylation of nuclear factor-KB (NF-kB) promotes the transcription of TNF-α and forms a positive feedback loop of inflammatory signals, and

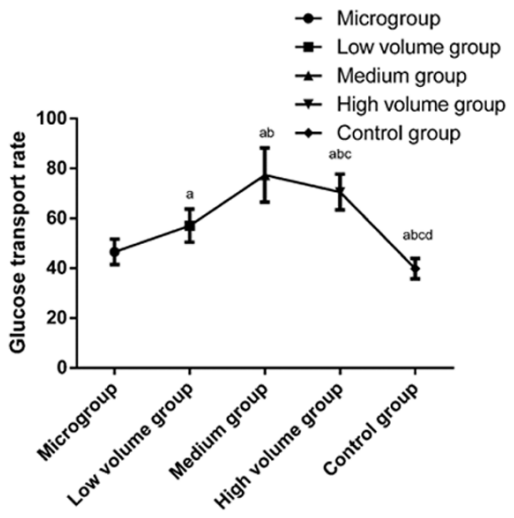

Figure 6. Comparison of glucose uptake rate among groups.* means comparison with microdose group, P<0.050; #means comparison with low dose group, P<0.050; △means comparison with middle dose group, P<0.050; ▽means comparison with high dose group, P<0.050.

causes insulin resistance [25, 26]. It is presumed that berberine changes the inflammatory morphology of adipokines and reduces the expression of TNF-α during berberine application, which reduces the effect of TNF-α on NF-kB, thus maintaining the normal immune function and causing insulin resistance.

Visfatin, leptin and adiponectin are the main factors involved in obesity and insulin resistance [27]. The results of this study showed that there were no significant differences in adiponectin expression among the five groups. But the leptin levels of rats treated with berberine were significantly lower than vehicle group, while the visfatin levels were significantly higher than the vehicle group, indicating that berberine has an intervention effect on leptin and visfatin. As a protein hormone secreted by fat, leptin can inhibit the synthesis of adipocyte [28]. However, the leptin level of experimental rats treated with berberine decreased significantly, which indicated that berberine can inhibit the expression of leptin. It has been reported that the increase of leptin level results in feedback downgrading of leptin receptor level, or signal transmission blocked after the receptor, which is also one of the main causes of obesity [29]. The increase of leptin can promote the production of a large number of oxidative free radicals. It is presumed that berberine intervention can reduce the activation function of endothelin receptor on reductase, and the metabolism of oxidase can be smoother, thus inhibiting the expression of leptin. As a glucose uptake promoting factor, visfatin can be used as simulated insulin to reduce blood sugar [30]. Through the intervention of berberine, the expression of visfatin in experimental rats was significantly increased. Berberine may accelerate the translocation effect of visfatin by activating the P13K-Akt signaling pathway, which increases the visfatin secretion and inhibits the metabolism of blood sugar and reduces the expression of blood sugar. However, due to the limited experimental conditions, the mechanism of berberine on visfatin still needs further in-depth experiments to prove our conjecture.

Comparing the glucose consumption and transporting rate of all groups, glucose consumption and transporting rate of groups added with berberine were significantly higher than vehicle group, and the glucose consumption and transporting rate of middle dose group was the highest. The results indicated that berberine can effectively promote the glucose metabolism function of adipocytes, and the effect will be the best when applying 20 μmol/L of berberine. Some studies have pointed out that Chinese medicine does not rely on the presence of insulin when reducing blood sugar [31]. Therefore, the mechanism of fat and sugar metabolism of berberine may be related to receptors or downstream factors of insulin receptors. Berberine affects the expression and translocation of glucose transporter 4 by acting on receptors, to reduce blood glucose. The results of Guo et al. are consistent with this experiment [32], which can corroborate the results of this experiment.

In this experiment, the effects of berberine on 3T3-L1 cells glucose metabolism and inflammatory factors were studied, and the mechanism of berberine on insulin resistance was preliminarily discussed.

However, due to the limited experimental conditions, the molecular mechanism of berberine has not been clarified, and further experiments are needed for confirmation. In addition, the number of selected cells for the study was limited in this experiment, and it is not excluded differences in other adipocytes. Also, inadequate experimental funds result in the impos-

sibility to detect the protein levels of the various research indicators in this paper.

In conclusion, berberine can effectively improve the glycometabolism of 3T3-L1 adipocytes and reduce the mRNA expression of inflammatory factors, but the exact intervention effect still needs to be confirmed by further study.

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Disclosure of conflict of interest

None.

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