BMC Veterinary Research

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Camel milk exosomes regulate glucose metabolism by inhibiting mitochondrial complex I in hepatocytes

Bin Yang[1†], Shifeng Du[1†], Ling Liu[1], Jingjing Wang[1] and Demtu Er[1,2*]

Abstract

Background Camel milk is known to have hypoglycemic properties. Previous studies found that camel milk exosomes (CM-exo) may regulate cellular glucose metabolism through the inhibition of mitochondrial complex I, but this hypothesis has not been verified by other experiments. The objective of this study was to verify the hypothesis that CM-exo regulated glucose metabolism in hepatocytes by inhibiting mitochondrial complex I pathway. AML12 cells were treated with extracted exosomes from camel milk and the effect of the CM-exo on cell viability was examined by cell counting kit (CCK)-8 assays. The glucose content of the cell culture medium was measured to determine the glucose consumption of the cells. Lactate release from the cells was determined by measuring the lactate content in the cell culture medium. The glycogen content of AML12 cells was detected. The activity of complex I and the contents of ATP, NAD^+ and NADH were measured. The protein expression levels of adenosine monophosphate-activated protein kinase (AMPK) and phosphorylated AMPK (p-AMPK) were detected by western blotting. The AML12 cells were treated with medium containing CM-exo and gluconeogenic substrates and the glucose content in the cells was determined. The protein expression levels of ten-eleven translocation methylcytosine dioxygenases (TET3), hepatocyte nuclear factor 4α-Promoter 2 (HNF4α-P2), phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G6PC), glycogen synthase kinase 3β (GSK3β) and phosphorylation of GSK3β (p-GSK3β) were detected by western blotting.

Results The results of this study showed that a high dose of CM-exo inhibited the viability of AML12 cells. It promoted glucose consumption, glycogen content and lactate release in AML12 cells, inhibited complex I activity, ATP content, NAD^+ content, and NAD^+/NADH ratio, and increased NADH content. The CM-exo increased the protein levels of p-AMPK, p-GSK3β, the protein expression ratio of p-AMPK/AMPK, p-GSK3β/GSK3β and decreased the glucose content and the protein expression levels of intracellular TET3, HNF4α-P2, PEPCK and G6PC.

Conclusions By inhibiting the activity of mitochondrial complex I in hepatocytes, CM-exo inhibited oxidative phosphorylation, oxidation of NADH to NAD^+ and synthesis of ATP, enhanced glycolysis, activated AMPK and resulted in decreased gluconeogenesis and increased glycogen synthesis.

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Keywords Camel milk exosomes, Complex I, AML12 cells, Gluconeogenesis

Introduction

Camel milk (CM) has been gaining attention due to its ability to treat several diseases, including cancer, autism, hypertension and diabetes [1], where CM has been shown to have hypoglycemic effects in humans and animals [2]. Recent research has shown that CM peptides have hypoglycemic effects [3]. CM lactoferrins exert hypoglycemic effects by activating insulin receptors and their related downstream pathways [4]. Protein hydrolysates from CM also have an anti-hyperglycemic effect and camel milk exosomes (CM-exo) can alleviate diabetic nephropathy in rats [5–6].

Exosomes are abundant in biological fluids, including plasma, urine and milk [7]. Those derived from animal milk have therapeutic effects on some human diseases. Milk exosomes can prevent and treat intestinal diseases [8], bovine milk exosomes (BM-exo) attenuate lung inflammation during necrotizing enterocolitis [9], bovine colostrum exosomes can promote hair regeneration and BM-exo alleviate cardiac fibrosis and enhance cardiac function in cardiac fibrosis rats [10–11]. Studies have shown that CM-exo have anti-tumor effects, but reports on the anti-diabetic effects of camel CM-exo are rare [12].

In previous studies, RNA sequencing was performed on L6 cells after treatment with CM-exo. It was found that the transcription levels of some genes in the treated group were decreased and gene ontogeny (GO) analysis showed that these genes were enriched in mitochondrial complex I. It was hypothesized that CM-exo may regulate glucose metabolism by inhibiting complex I, similar to metformin [13]. In the present study, cellular complex I activity, ATP content, cellular NAD^+ content, nicotinamide adenine dinucleotide (NADH) content and AMPK activity related to complex I activity were detected after treatment of hepatocytes with CM-exo.

Activation of AMPK in the liver leads to inhibition of glucose production and promotion of glycogen synthesis. Activation of AMPK increases the phosphorylation of GSK3β in the liver, which activates glycogen synthase (GS) [14–15]. PEPCK and G6PC are key enzymes that regulate gluconeogenesis [16].

A previous study found that the expression of let-7i in CM-exo ranked second among all microRNAs and it has been reported that let-7 inhibited hepatic glucose production by targeting the TET3/HNF4α-P2 axis and suppressing PEPCK and G6PC gene expression [17–18]. This study speculated that the expression of TET3, HNF4α-P2, PEPCK and G6PC in hepatocytes could be inhibited by CM-exo.

Results

Identification of CM-exo

Transmission electron microscopy showed that CM-exo extracted by Umibio Exosome Extraction Kit ranged in diameter from 30 to 150 nm (Fig. 1A). The expression of extracted CM-exo TSG101 and CD81 was confirmed by WB detection (Fig. 1B).

Effects of different concentrations of CM-exo on cell viability

In this study, a CCK-8 assay was used to detect the effect of 0, 6, 12, 18, 24 and 30 ng/μL CM-exo on the viability of AML12 cells. The results showed that there was no significant change in cell viability after 6, 12 and 18 ng/μL CM-exo treated AML12 cells for 24 h ($P > 0.05$). The viability of cells treated with 24 and 30 ng/μL was significantly decreased for 24 h ($P < 0.01$) (Fig. 2A).

In addition, in the experiment of treating cells with 0, 6, 12 and 18 ng/μL CM-exo for 48 h, 12 and 18 ng/μL CM-exo significantly reduced the cell viability. Therefore, subsequent experiments were performed on cells treated with 0, 6, 12, and 18 ng/μL CM-exo for 24 h (Fig. 2B).

CM-exo promoted hepatocyte glucose consumption and lactate release

In this study, the effects of CM-exo on hepatocyte glucose metabolism were explored using the glucose consumption and lactate release assays. The results of glucose consumption assays showed that 12 and 18 ng/μL CM-exo significantly increased the glucose consumption of AML12 cells for 24 h ($P < 0.01$) (Fig. 3A). The results of lactate release assay showed that 12 and 18 ng/μL CM-exo significantly increased the lactate production of AML12 cells for 24 h ($P < 0.05$) (Fig. 3B).

CM-exo decreased hepatocyte glucose content

The results of cellular glucose content assays showed that 12 and 18 ng/μL CM-exo significantly decreased the glucose production of AML12 cells for 24 h ($P < 0.05$) (Fig. 4).

CM-exo inhibited complex I and ATP synthesis in hepatocytes

To evaluate the effect of CM-exo on the function of complex I, the intracellular complex I activity and ATP content were detected in this study after treatment of AML12 cells with CM-exo. The results showed that 6, 12 and 18 ng/μL of CM-exo significantly reduced complex I activity and 12 and 18 ng/μL of CM-exo significantly reduced ATP content compared to the control group ($P < 0.05$) (Fig. 5A and B).

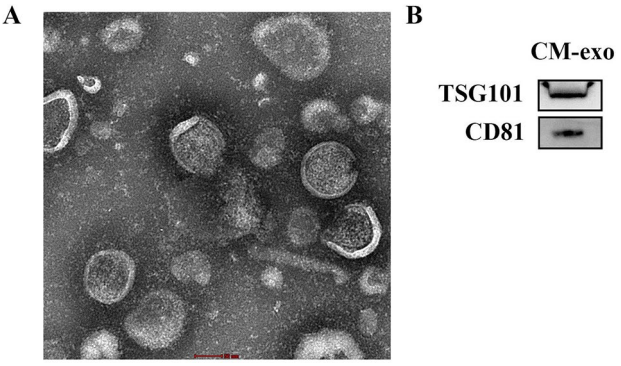

Fig. 1 Identification of CM-exo. The morphology of CM-exo extracted by Umibio Exosome Extraction Kit was observed under transmission electron microscopy (**A**). WB showed that the extracted CM-exo had markers of exosomes TSG101 and CD81 (**B**)

CM-exo increased NADH content and decreased NAD+ content and NAD+/NADH

To explore the effect of CM-exo on NADH and NAD^+ after inhibiting complex I activity, the levels of NADH and NAD^+ in AML12 cells were detected after 24 h treatment with CM-exo. The results showed that 12 and 18 ng/μL CM-exo significantly increased the content of NADH and decreased the content of NAD^+ ($P<0.05$) (Fig. 6A and B). In addition, NAD^+/NADH showed a significant decline after 24 h treatment with CM-exo compared to the control group ($P<0.01$) (Fig. 6C).

AMPK was activated by CM-exo

To explore whether CM-exo can activate AMPK, the expression of AMPK and p-AMPK was detected by western blotting (WB) after AML12 was treated with CM-exo for 24 h. The results showed that p-AMPK/AMPK were significantly increased in CM-exo treated with 6, 12 and 18 ng/μL, compared to the control group ($P<0.01$) (Fig. 7).

CM-exo inhibited the protein expression of TET3, HNF4α-P2, PEPCK and G6PC

The effects of CM-exo on the protein levels of the TET3/HNF4α-P2 axis and rate-limiting enzymes PEPCK and G6PC were examined by WB. The results showed that CM-exo significantly reduced the protein expression of TET3, HNF4α-P2, PEPCK and G6PC ($P<0.05$) (Fig. 8).

CM-exo increased the glycogen content and p-GSK3β protein expression

The glycogen content of AML12 cells was detected after 24 h treatment with CM-exo. The results showed that 6, 12 and 18 ng/μL of CM-exo significantly increased the glycogen content of AML12 cells compared to the control group ($P<0.01$) (Fig. 9A).

To confirm whether CM-exo promoted the expression of p-GSK3β protein, the protein levels of GSK3β and p-GSK3β were detected by WB after CM-exo treated AML12 cells for 24 h. The results showed that 6, 12 and 18 ng/μL of CM-exo significantly increased the ratio of p-GSK3β/GSK3β in AML12 cells compared to the control group ($P<0.01$) (Fig. 9B and C).

Discussion

In this study, CM-exo above 24 ng/μL decreased the viability of AML12 cells and it was hypothesized that CM-exo above this concentration reduced the aerobic metabolism of cells. In contrast to the effect of CM-exo on cell viability, human umbilical cord mesenchymal

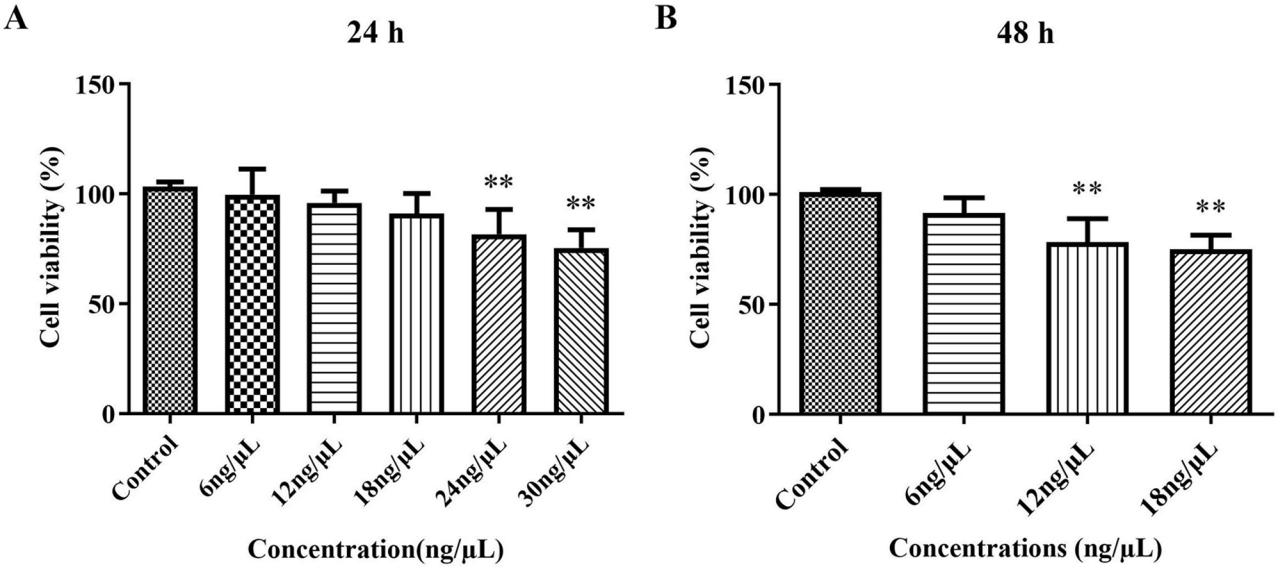

Fig. 2 Effect of CM-exo on viability of AML12 cells. Three independent experiments were performed to obtain similar results. The data are presented as means ± SD ($n=3$). *$p<0.05$ and **$p<0.01$ vs. control

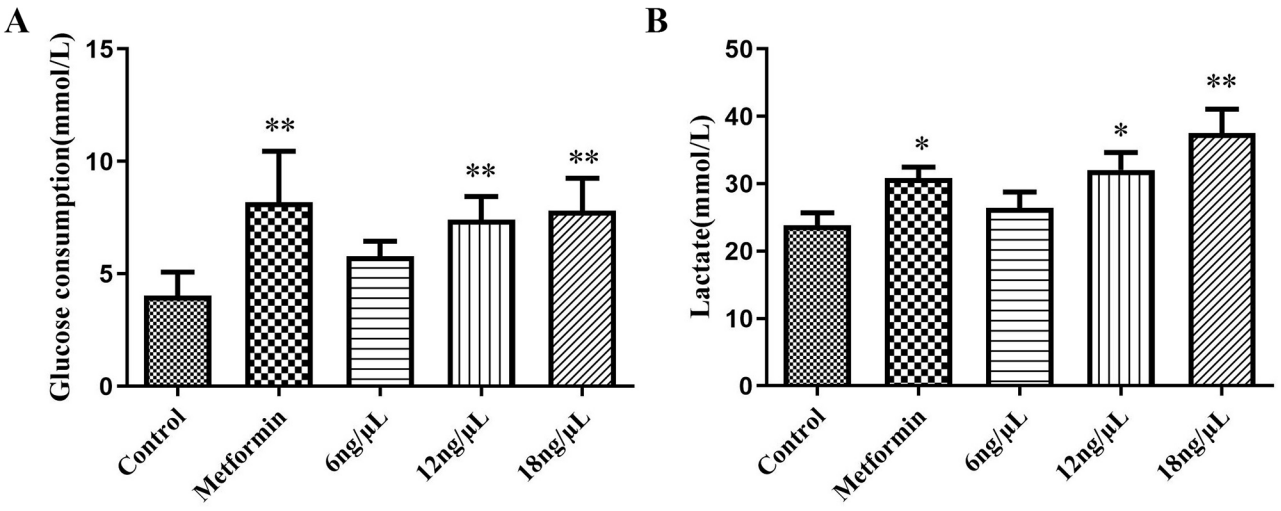

Fig. 3 CM-exo promoted glucose consumption and lactate release in AML12 cells. After treatment of AML12 cells with CM-exo for 24 h, glucose consumption (**A**) and lactate release (**B**) of cell culture medium were measured. Three independent experiments were performed to obtain similar results. The data are presented as means ± SD ($n = 3$). *$p < 0.05$ and **$p < 0.01$ vs. control

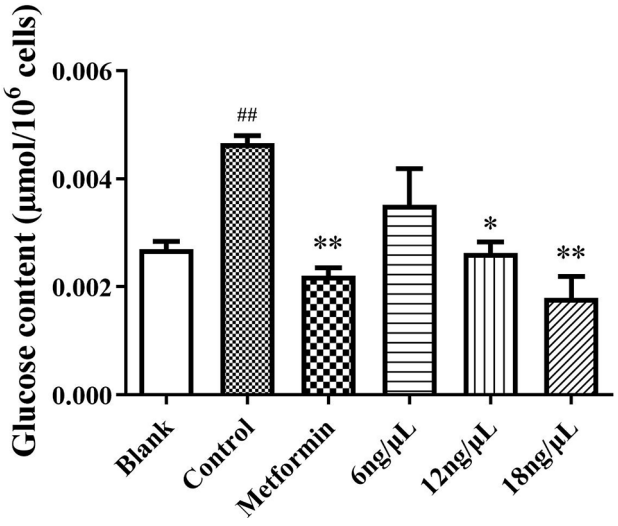

Fig. 4 CM-exo decreased glucose content in AML12 cells. Three independent experiments were performed to obtain similar results. The data are presented as means ± SD ($n = 3$). *$p < 0.05$ and **$p < 0.01$ vs. control

stem cell exosomes can promote the aerobic metabolism of human retinal pigment epithelial cells [19].

Many drugs exert their anti-diabetic effects by regulating glucose consumption in peripheral organs, such as liver and muscle [20]. In vitro cellular glucose consumption is one of the indicators to evaluate glucose metabolism [21]. Lactate is produced by glycolysis and due to the inefficiency of glycolysis in generating ATP, glycolysis requires more glucose consumption than oxidative phosphorylation (OXPHOS) to generate the same amount of ATP [22–23]. The treatment of AML12 cells with CM-exo inhibited OXPHOS and led to the enhancement of cell glycolysis.

Mitochondrial complex I powers ATP synthesis by driving OXPHOS [24], so this study further investigated the activity of complex I and the content of ATP after CM-exo treatment of the cells. The results showed that the inhibitory effect of CM-exo on the activity of complex I was similar to that of the anti-diabetic drug, metformin. Metformin suppresses hepatic gluconeogenesis by inhibiting complex I leading to AMPK activation [25]. Since inhibition of complex I can lead to AMPK activation, this study used WB to examine whether CM-exo can activate cellular AMPK. Activation of AMPK and reduction of ATP production by CM-exo may also be a consequence of complex I inhibition. Complex I is involved in the oxidation of NADH to NAD^+ and it was hypothesized that the inhibition of complex I would lead to an elevation of NADH [26], which was confirmed in this study.

Since both CM-exo and metformin inhibit complex I, the hypothesis that CM-exo also had an inhibitory effect on hepatic gluconeogenesis was investigated by measuring the glucose content in AML12 cells and the protein expression of PEPCK and G6PC, the key rate-limiting enzyme genes of gluconeogenesis and examining the effect of CM-exo on TET3 and HNF4α-P2 protein expression. The reduction of glucose content and protein expression of PEPCK and G6PC in AML12 cells by CM-exo may have been due to the inhibition of complex I by CM-exo. Previous studies found that let-7i could bind to the 3'UTR of TET3 and reduce its protein expression level and the decreased TET3 protein could lead to the decrease of HNF4α-P2, PEPCK and G6PC protein expression levels [18, 27]. The decrease of TET3 and HNF4α-P2 protein expression in this study may be due to the inhibition of TET3 protein expression level by let-7i in CM-exo, so the decrease of PEPCK and G6PC protein

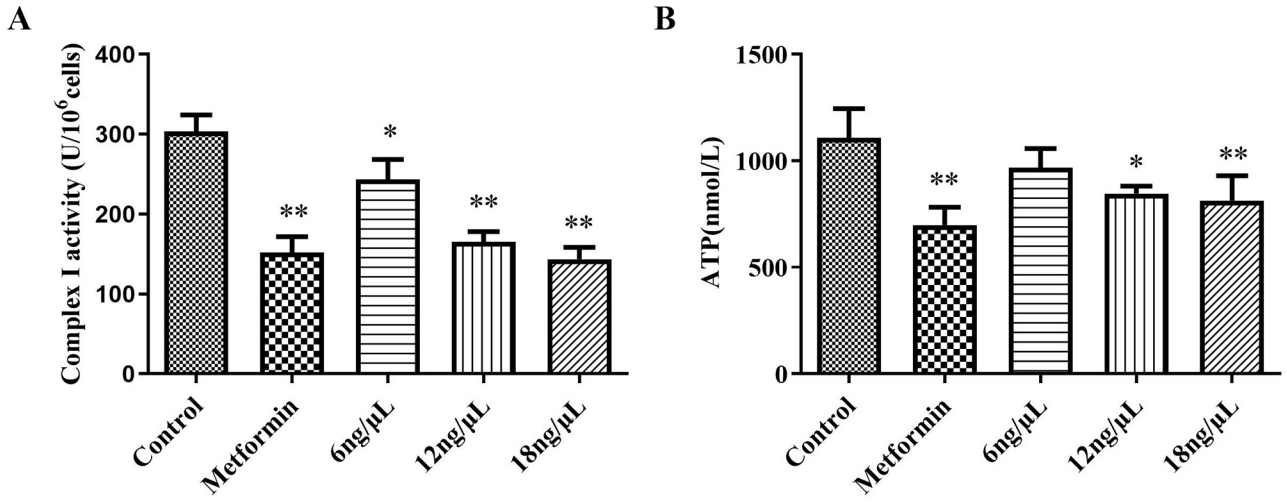

Fig. 5 CM-exo decreased mitochondrial complex I activity and ATP synthesis in AML12 cells. After treatment of AML12 cells with CM-exo for 24 h, the activity of complex I (**A**) and the content of ATP (**B**) in cells were determined. Three independent experiments were performed to obtain similar results. The data are presented as means ± SD ($n = 3$). *$p < 0.05$ and **$p < 0.01$ vs. control

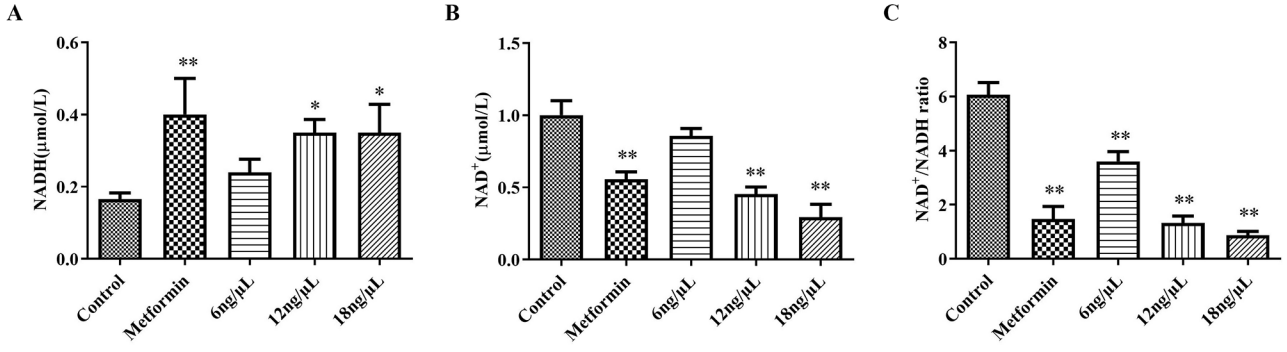

Fig. 6 CM-exo increased NADH content and decreased NAD^+ content and NAD^+/NADH ratio in AML12 cells. After treatment of AML12 cells with CM-exo for 24 h, the NADH (**A**) and NAD^+ content (**B**) in cells were measured and the NAD^+/NADH ratio (**C**) was calculated. Three independent experiments were performed to obtain similar results. The data are presented as means ± SD ($n = 3$). *$p < 0.05$ and **$p < 0.01$ vs. control

expression levels may be partially due to the inhibition of TET3/HNF4α-P2 axis by let-7i contained in CM-exo.

Glucose homeostasis can be maintained by activating AMPK and its downstream substrate GSK3β in the liver [14, 28]. Based on the finding that CM-exo activated AMPK, the effects of CM-exo on glycogen content and p-GSK3β protein expression in hepatocytes were further detected. The results of this study indicated that CM-exo regulate glucose metabolism by increasing glycogen synthesis in hepatocytes in addition to reducing gluconeogenesis.

In addition to metformin as an antidiabetic agent inhibiting complex I, several other drugs or compounds have been found to regulate glucose metabolism or antidiabetic agents by inhibiting complex I. Imeglimin as a novel oral drug for the treatment of type 2 diabetes mellitus (T2DM), rebalances respiratory chain activity by partially inhibiting complex I and restoring complex III activity [29]. The p70 S6 kinase (S6K1) inhibitor, PF-4,708,671, promotes glucose metabolism in muscle and liver cells in part by inhibiting complex I [30]. The protein tyrosine kinase inhibitor, imatinib, promotes the survival of human beta-cells by inhibiting complex I and II [31]. Chickpea flavonoids can alleviate the dysfunction of complex I in pancreas of T2DM rats by inhibiting complex I [32]. It has been found that liver complex I-dependent OXPHOS increased significantly in prediabetes and early stages of type 2 diabetic mice, when rotenone as a specific inhibitor of complex I, significantly inhibited glucose production in primary mouse hepatocytes [33].

Reducing liver glucose output by inhibiting complex I in the liver of diabetic patients is an effective treatment for alleviating hyperglycemia. The present study only investigated the mechanism of CM-exo regulating glucose metabolism at the cellular level. In the future, type 2 diabetic mice should be used to further verify that the mechanism of action of CM-exo against hyperglycemia is

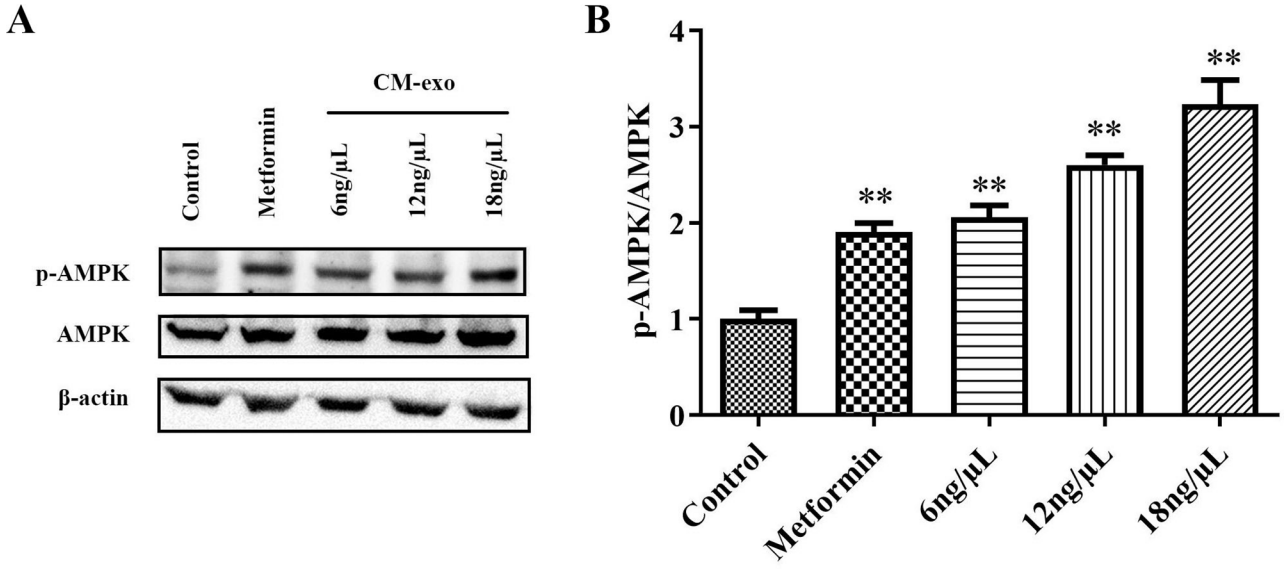

Fig. 7 CM-exo increased the protein expression level of p-AMPK in AML12 cells. After the treatment of AML12 cells with CM-exo for 24 h, the protein expression levels of AMPK and p-AMPK were detected by WB (**A**), and the p-AMPK/AMPK protein expression ratio (**B**) was calculated. Three independent experiments were performed to obtain similar results. The data are presented as means ± SD ($n = 3$). *$p < 0.05$ and **$p < 0.01$ vs. control

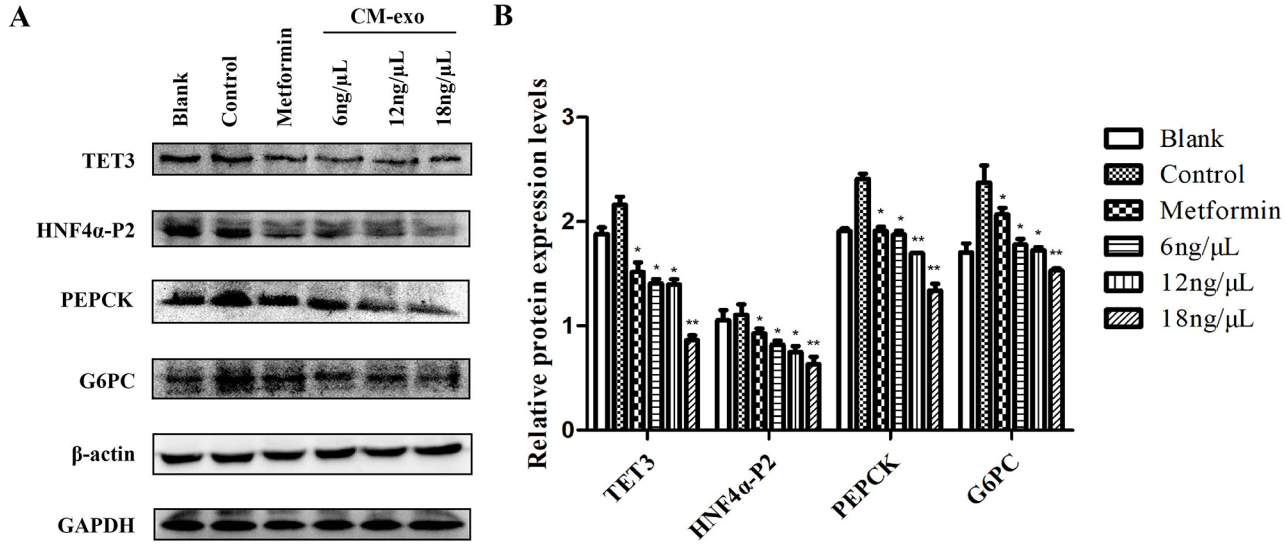

Fig. 8 CM-exo decreased the protein expression levels of TET3, HNF4α-P2, PEPCK and G6PC in AML12 cells. After the treatment of AML12 cells with CM-exo for 12 h, the protein expression levels of TET3, HNF4α-P2, PEPCK and G6PC were detected by WB (**A**) and the relative protein expression levels were calculated (**B**). β-actin and GAPDH were used as a normalization control. Three independent experiments were performed to obtain similar results. The data are presented as means ± SD ($n = 3$). *$p < 0.05$ and **$p < 0.01$ vs. control

through the inhibition of complex I. Further studies are needed to investigate which substances or microRNAs in CM-exo inhibit complex I activity.

Conclusions

Based on the results of previous studies, this study demonstrated for the first time in vitro that CM-exo regulated glucose metabolism, decreased gluconeogenesis and increased glycogen synthesis in hepatocytes by inhibiting complex I activity. This study provides a scientific basis for the use of CM-exo as adjuvant therapy for diabetes.

Methods

Isolation and identification of exosomes

Fresh milk samples were collected from healthy female camels during mid-lactation. Camel milk was centrifuged at 10,000 g for 20 min at 4℃ to remove lipids and some proteins. Exosomes were extracted from skim milk using an Exosome Extraction Kit (Umibio, China).

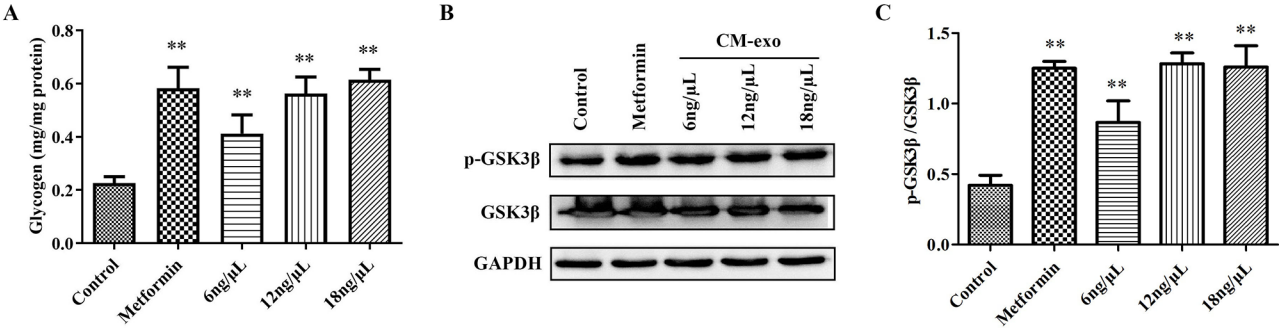

Fig. 9 CM-exo increased glycogen content and the protein expression level of p-GSK3β in AML12 cells. After treatment of AML12 cells with CM-exo for 24 h, glycogen content (**A**), the protein expression levels of GSK3β and p-GSK3β (**B**) in cells were determined. The p-GSK3β/GSK3β protein expression ratio (**C**) was calculated. Three independent experiments were performed to obtain similar results. The data are presented as means ± SD ($n = 3$). *$p < 0.05$ and **$p < 0.01$ vs. control

The exosome particles were suspended in PBS to obtain homogenous suspension. The milk exosomes were filtered by a 0.22 μm filter and then stored in a refrigerator at -80℃. The total protein concentration of milk exosomes was determined by BCA method (Keygen, China). The isolated exosomes were fixed with 2.5% glutaraldehyde for 2 h, stained with phosphotungstic acid, and identified by transmission electron microscopy. The membrane protein markers (CD81 and TSG101) of CM-exo were detected by western blotting. The primary antibodies against TSG101 and CD81 used for WB were purchased from proteintech.

CCK-8 assay

The effect of CM-exo on AML12 cell viability was assessed by CCK-8 assay. The cells were seeded in 96-well plates to a confluence of 70-80%, and then treated with DMEM medium containing different concentrations of CM-exo (0, 6, 12, 18, 24 and 30 ng/μL) for 24 h. In addition, cells were treated with DMEM medium containing 6, 12, and 18 ng/μL CM-exo and cultured for 48 h.10 μL of CCK-8 solution was added to each well, and continue to culture for 1 h. The OD values were read at 450 nm.

Detection of glucose consumption

AML12 cells were seeded in 48-well plates. The cells were treated with different doses of CM-exo in DMEM supplemented with 0.2% BSA for 24 h. The treatment doses were 0, 6, 12 and 18 ng/μL, respectively, metformin (2 mM) was used as the positive control. Glucose concentration of cell culture supernatant was detected by Glucose Oxidase Activity Assay Kit (Applygen, China) according to the manufacturer's instructions, and calculated the glucose consumption.

Detection of lactate release

AML12 cells were seeded in 48-well plates and added with 0, 6, 12 and 18 ng/μL CM-exo or metformin (2 mM) in DMEM supplemented with 0.2% BSA for 24 h. The lactate concentration in the medium was determined with a Lactic Acid Assay Kit (Solarbio, China).

Detection of glucose content in cells

AML12 cells were seeded in 6-well plates and added with 0, 6, 12 and 18 ng/μL CM-exo or metformin (2 mM) in DMEM (glucose free) supplemented with 0.2% BSA, sodium pyruvate (2 mM), sodium lactate (20 mM) and glutamine (10 mM) for 12 h. Glucose content in cells was detected by Glucose Content Detection Kit (Solarbio, China).

Determination of cellular mitochondrial complex I activity

AML12 cells were seeded in 6-well plates and added with 0, 6, 12 and 18 ng/μL CM-exo or metformin (2 mM) in DMEM supplemented with 0.2% BSA for 24 h. The cells were digested with trypsin and collected. The activities of the mitochondrial complex I were determined by the Mitochondrial Respiratory Chain Complex I Activity Assay Kit (Solarbio, China) according to the manufacturer's instructions. Briefly, complex I activity was assayed by monitoring the decrease in NADH at 340 nm for 2 min.

Detection of ATP

AML12 cells were seeded in 12-well plates and added with 0, 6, 12 and 18 ng/μL CM-exo or metformin (2 mM) in DMEM supplemented with 0.2% BSA for 24 h. The cells were digested with trypsin and collected. After adding 50 μl PBS, the cells were lyzed by freeze-thaw method. ATP content was determined by ELISA Kit (Mlbio, China).

Detection of NAD+/NADH

AML12 cells were seeded in 12-well plates and added with 0, 6, 12 and 18 ng/μL CM-exo or metformin (2 mM) in DMEM supplemented with 0.2% BSA for 24 h. The cells were digested with trypsin and collected. Cellular NAD^+ and NADH concentrations were determined

according to the protocol of the NAD^+/NADH Assay Kit (Beyotime, China).

Glycogen content detection

AML12 cells were seeded in 6 cm diameter cell culture plates and added with 0, 6, 12 and 18 ng/μL CM-exo or metformin (2 mM) in DMEM supplemented with 0.2% BSA for 24 h. The cells were digested with trypsin and collected. Glycogen content was detected with the Glycogen Content Detection Kit (Solarbio, China) according to the manufacturer's instructions. Glycogen concentrations were normalized by cellular protein concentration measured with the BCA Protein Assay Kit (Beyotime, China).

Western blotting

In the assay to detect the protein expression of AMPK, p-AMPK, GSK3β and p-GSK3β, AML12 cells were seeded in 6-well plates and added with 0, 6, 12 and 18 ng/μL CM-exo or metformin (2 mM) in DMEM supplemented with 0.2% BSA for 24 h.

In the assay to detect the protein expression of TET3, HNF4α-P2, PEPCK and G6PC, AML12 cells were seeded in 6-well plates and added with 0, 6, 12 and 18 ng/μL CM-exo or metformin (2 mM) in DMEM (glucose free) supplemented with gluconeogenic substrates (2 mM sodium pyruvate, 20 mM sodium lactate and 10 mM glutamine) for 12 h. Except the blank group, the other groups of cells were added with gluconeogenic substrates.

Cells were lysed with RIPA containing PMSF, protease inhibitor and phosphatase inhibitor. The extracted cellular proteins were mixed with loading buffer and heated to 100℃ for denaturation. The protein samples were separated by SDS-PAGE gel electrophoresis, and then the proteins in the gel were transferred onto PVDF membrane. After blocking with TBST containing 5% skim milk powder for 2 h at room temperature, the PVDF membrane was incubated with primary antibodies against AMPK (2532, Cell Signaling Technology), p-AMPK (2535, Cell Signaling Technology), TET3 (ABE290, Millipore), HNF4α-P2 (PP-H6939-00, R&D Systems), PEPCK (ab70358, Abcam), G6PC (PA5-42541,Invitrogen), GSK3β (22104-1-AP, proteintech) p-GSK3β (5558, Cell Signaling Technology), β-actin (GB15003, Servicebio) and GAPDH (GB15004, Servicebio) overnight at 4℃. After rinsing with TBST for 3 times, incubate with HRP-conjugated secondary antibody for 1 h. The membranes were visualized using a Signal Chemiluminescent Detection System (Chem Studio, Jena, Germany). Density of protein bands was quantified by using the Quantity One software.

Statistical analysis

Statistical analysis was performed by ANOVA (GraphPad Prism 5). The data are presented as means ± SD. Significant differences among groups are indicated by $^*p < 0.05$ and $^{**}p < 0.01$.

Abbreviations

CM-exo	Camel milk exosomes
CCK	Cell counting kit
ATP	Adenosine triphosphate
NAD^+	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide
AMPK	Adenosine monophosphate-activated protein kinase
TET3	Ten-eleven translocation methylcytosine dioxygenases
HNF4α-P2	Hepatocyte nuclear factor 4α-Promoter 2
PEPCK	Phosphoenolpyruvate carboxykinase
G6PC	Glucose-6-phosphatase
GSK3β	Glycogen synthase kinase 3β
BM-exo	Bovine milk exosomes
GO	Gene ontogeny
OXPHOS	Oxidative phosphorylation

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-025-04555-9.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Acknowledgements

We thank International Science Editing (http://www.internationalscienceediting.com) for editing this manuscript.

Author contributions

BY designed and performed the experiments, analyzed the data, and drafted the manuscript. SD performed the experiment and analyzed part of the data. LL and JW conducted part of the experiment. DE revised and edited the manuscript.

Funding

This work was supported by Natural Science Foundation Project of Inner Mongolia Autonomous Region (Grant No.2023LHMS03054), Discipline Project of College of Veterinary Medicine, Inner Mongolia Agricultural University (Grant No.SYKJYB202304), Program for improving the Scientific Research Ability of Youth Teachers of Inner Mongolia Agricultural University (BR230157) and Plan (Science and Technology Cooperation) Project of 2023 Autonomous Region Major Research and Development and Achievements Transformation, Science and Technology Agency of Inner Mongolia Autonomous Region (Grant No.2023KJHZ0003).

Data availability

Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participant

The current study was approved by the Experimental Animal Welfare and Ethics Committee of Inner Mongolia Agricultural University (No. NND2021094). Informed consent was obtained from all animal owners for the camel milk samples collected in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Received: 2 August 2024 / Accepted: 31 January 2025
Published online: 22 February 2025

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