Chronic high glucose causes podocyte epithelial-mesenchymal transition through lactate-induced histone lactylation

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Abstract

Background and Purpose: Diabetic nephropathy (DN) closely relates to morphological and functional changes in podocytes, and anaerobic glycolysis represents the predominant energy source of podocytes. However, it is unknown whether lactate accumulation in chronic high glucose caused epithelial-mesenchymal transition (EMT) of podocytes through lactate-derived histone lactylation. Experimental Approach: We examined biomarkers of podocytes and mesenchymal cells as well as lactylation of histone lysine residues (HKla) in mouse MPC cells cultured with high glucose (HG) or lactate (LA). Moreover, these indices were observed in MPCs after HG co-culture with multiple interventions of lactate levels, and differently expressed genes (DEGs) were screened using RNA sequencing. Finally, renal pathological characteristics and histone lactylation were investigated in diabetic mice with lactate-lowering treatments. Key Results: Both HG and LA decreased nephrin levels while increased collagen IV levels in MPCs, and HG and LA stimulation synchronously elevated HKla levels. However, co-treatment with oxamate or dichloroacetate reducing lactate levels alleviated decreases in nephrin and ZO-1 levels and increases in collagen IV and α smooth muscle actin levels as well as HKla levels in HG-cultured MPCs, but co-treatment with rotenone diversely affected these indices. RNA sequencing found eleven DEGs in HG-cultured MPCs after oxamate or dichloroacetate intervention and qPCR experiments validated four of them. Importantly, oxamate or dichloroacetate treatment attenuated renal functions, EMT, and histone lactylation in kidney of diabetic mice. Conclusion and Implications: This study clarified that lactate mediated chronic high glucose-caused podocyte EMT through lactate-induced histone lactylation, and then promoted the pathological process of DN.

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Experimental Approach: We examined biomarkers of podocytes and mesenchymal cells as well as lactylation of histone lysine residues (HKla) in mouse MPC cells cultured with high glucose (HG) or lactate (LA). Moreover, these indices were observed in MPCs after HG co-culture with multiple interventions of lactate levels, and differently expressed genes (DEGs) were screened using RNA sequencing. Finally, renal pathological characteristics and histone lactylation were investigated in diabetic mice with lactate-lowering treatments.

Key Results: Both HG and LA decreased nephrin levels while increased collagen IV levels in MPCs, and HG and LA stimulation synchronously elevated HKla levels. However, co-treatment with oxamate or dichloroacetate reducing lactate levels alleviated decreases in nephrin and ZO-1 levels and increases in collagen IV and α smooth muscle actin levels as well as HKla levels in HG-cultured MPCs, but co-treatment with rotenone diversely affected these indices. RNA sequencing found eleven DEGs in HG-cultured MPCs after oxamate or dichloroacetate intervention and qPCR experiments validated four of them. Importantly, oxamate or dichloroacetate treatment attenuated renal functions, EMT, and histone lactylation in kidney of diabetic mice.

Conclusion and Implications: This study clarified that lactate mediated chronic high glucose-caused podocyte EMT through lactate-induced histone lactylation, and then promoted the pathological process of DN.

Keywords: diabetic nephropathy, epithelial-mesenchymal transition, high glucose, histone lactylation, lactate, monocarboxylate transporter, podocyte

Abbreviations α-SMA α-Smooth muscle actin

Col-IV Collagen IV

DCA Sodium dichloroacetate

DEGs Differentially expressed genes

DM Diabetes mellitus

DN Diabetes nephropathy

EMT Epithelial-mesenchymal transition

FBG Fasting blood glucose

FN Fibronectin

GBM Glomerular basement membrane

GFB Glomerular filtration barrier

HG High glucose

HKla Pan lactylation of histone lysine residues

LA Sodium lactate

LDH Lactate dehydrogenase

MCT Monocarboxylate transporter

MPC Mouse podocyte cell line

Oxa Sodium oxamate

PDH Pyruvate dehydrogenase

PDK Pyruvate dehydrogenase kinase

qPCR Real-time fluorescence quantitative PCR

Rot Rotenone

STZ Streptozotocin

ZO-1 Zonula occludens 1

What is already known

Anaerobic glycolysis represents the predominant energy source of podocytes.

Lactate-derived lactylation modification of histone lysine residues directly stimulates gene transcription from chromatin.

What this study adds

High glucose and exogenous lactate promoted podocyte EMT and increased histone lactylation in podocytes.

Lactate-lowering treatments alleviated podocyte EMT and histone lactylation in *in vitro* and *in vivo* .

Clinical significance

Targeting lactate accumulation provided therapeutic strategy for diabetic microvascular complications, such as DN.

1 Introduction

Breakdown of the glomerular filtration barrier (GFB) is associated with loss of renal functions and is a powerful risk factor for kidney disease progressing to kidney failure (Iseki, Ikemiya, Iseki, & Takishita, 2003). Podocytes situate on the outer surface of the glomerular basement membrane (GBM) and sustain the glomerular structural integrity. Neighbouring foot processes of the podocytes are connected by a continuous adherent junction structure named the slit diaphragm, which forms the huge filtration surface of GFB. Thus, slit diaphragm is critical to the contribution of podocyte functions to the glomerular filtration rate (Daehn & Duffield, 2021).

Diabetic nephropathy (DN) is the major cause of disability and death in patients with diabetes and the leading cause of end-stage renal failure worldwide. DN is characterized clinically by increasing amounts of albuminuria (glomerular origin) and gradually declining renal function. Persistent hyperglycemia has been found to cause podocyte damage, manifested as apoptosis (Z. Chen et al., 2021; Jiang et al., 2022), epithelial-mesenchymal transition (EMT) (Yin et al., 2018; Ying & Wu, 2017), and autophagy (X. Z. Li et al., 2021; X. Q. Liu et al., 2022), consequently resulting in DN (Marshall, 2007; Zhang et al., 2020). For

the EMT of podocytes, the expressions of nephrin, podocin, P-cadherin, and zonula occludens 1 (ZO-1) are downregulated to cause the altered cell-to-cell junctions, and the actin cytoskeleton will be rearranged. Podocyte EMT is still the focus of the pathogenesis of DN.

Lactic acid is an important product of glucose metabolism exerting metabolic and non-metabolic functions. Plasma lactate concentration is highest in obese subjects with type 2 diabetes mellitus (T2DM), which is associated with both fasting plasma glucose and glycated haemoglobin concentrations (Y. D. Chen, Varasteh, & Reaven, 1993), and recent studies confirm the results and such a relationship (Higuchi et al., 2020; Lopez-Cano et al., 2020). Moreover, lactates are increased in kidney and urine of T1DM rats (Zhao et al., 2011). Therefore, lactate increase resulting from prolonged hyperglycemia may be one of the important causes of renal dysfunction. Lactate accumulation can induce EMT of multiple tumor cells and promotes tumor invasion and metastasis (Niu, Luo, Wang, Xia, & Xie, 2021). Importantly, anaerobic glycolysis represents the predominant energy source of podocytes and maintains GFB independent of mitochondrial metabolism and dynamics (Brinkkoetter et al., 2019), offering a strategy to therapeutically interfere with the enhanced podocyte metabolism in various progressive kidney diseases, such as DN. Recently, Zhang et al. establish a new signalling role for lactate, namely, lactate-derived lactylation of histone lysine residues, serving as an epigenetic modification that directly stimulates gene transcription from chromatin (D. Zhang et al., 2019). Furthermore, multiple reports confirm the existence of histone lactylation and its association with transcriptional activation (Cui et al., 2021; Irizarry-Caro et al., 2020). However, whether the excess lactate in podocytes in diabetic conditions produces histone lactylation and contributes to the EMT of podocytes is still unclear.

In this study, we aimed to explore (1) whether exogenetic lactate and lactate resulting from chronic high glucose stimulation caused histone lactylation in podocytes; (2) whether histone lactylation promoted podocyte EMT in high glucose conditions; (3) whether histone lactylation reduction using lactate-lowering interventions ameliorated podocyte EMT and renal functions in diabetic mice.

2 METHODS

2.1 Cell culture and treatments

Mouse podocyte cells (MPCs) were cultured in RPMI-1640 medium containing 10% FBS. After incubation in medium containing 11.1 mM glucose, 10% FBS, penicillin & streptomycin, 5% CO_2 at 37 °C for 24 h, MPCs were grouped: normal glucose (NG, 11.1 mM glucose), high glucose (HG, 30 mM glucose), low, middle, and high concentrations of lactate (LA, NG plus 2.5, 5.0, 10.0 mM sodium lactate, respectively). After 48 h culture, histone lysine lactylation (HKla) levels were determined with extracted histones. Subsequently, NG, HG, and 5 mM sodium lactate (LA-5) groups were adopted to examine protein expressions of a podocyte biomarker nephrin and a mesenchymal cell biomarker collagen IV (Col-IV).

For the mechanism experiments, MPCs were designed as follows: NG, HG, and HG treated with intervening lactate production, including a pyruvate dehydrogenase kinase (PDK) inhibitor sodium dichloroacetate (HG+DCA, 10 mM), a lactate dehydrogenase (LDH) inhibitor sodium oxamate (HG+Oxa, 5mM), and a mitochondrial respiratory chain complex I inhibitor rotenone (HG+Rot, 5 nM) (D. Zhang et al., 2019). After treatments for 48 h, the cells were harvested for index analysis, including lactate and HKla levels as well as protein expressions of biomarkers of podocyte EMT formation, such as nephrin, zonula occludens 1 (ZO-1), Col-IV, and α-smooth muscle actin (α-SMA).

2.2 RNA sequencing

For this experiment, MPCs were designed as NG, LA, HG, HG+Oxa, and HG+DCA groups. Two sets of samples were collected for RNA sequencing (RNA-seq) in GENERGE BIO Company (Shanghai, China). According to purposes of our experiment and grouping, the up-regulated differentially expressed genes (DEGs) were screened between LA and NG as well as between HG and NG, and the down-regulated DEGs between HG+Oxa and HG as well as between HG+DCA and HG. If the p-adjust < 0.05 & $|\log2FC| >= 1$ (up-regulation) or $|\log2FC| <= -1$ (down-regulation), it was accepted to be a significantly different ex-

pression level. Then the intersection genes were found between the up-regulated DEGs in disease and the down-regulated DEGs in drug interventions by using the online platform jvenn.

2.3 Experimental protocol of animals

Male C57BL/6J mice (22-26 g) were bred in the experimental animal room of Jiangsu Key Laboratory of New Drug Research and Clinical Pharmacy, Xuzhou Medical University, China. All mice were housed under controlled humidity (50% ± 10%), temperature (24 ± 1 °C), and light (12 h day/night cycle), freely accessing rodent food and water. The Animal Ethics Committee of Xuzhou Medical University approved mouse experiment. Every effort was made to minimize stress on the mice.

The male mice fasting for more than 12 h were subjected to consecutive five-day intraperitoneal injections of streptozotocin (STZ, 50 mg/kg, Sigma-Aldrich, Shanghai, China), freshly dissolved in 0.1 M sodium citrate at pH 4.5. Age-matched normal mice received sodium citrate buffer alone. Diabetes induction was evaluated using fasting blood glucose (FBG) level with a blood glucose meter. The mice with FBG values more than 11.1 mM were considered diabetic mice on day 7 after the last STZ injection. The diabetic mice were randomly grouped with FBG (10 mice/group) as diabetic mice, diabetic mice treated with DCA (450 mg/kg) or Oxa (125 mg/kg). DCA and Oxa were added into the drinking water with the concentration of 0.562 g/L and 0.104 g/L, respectively.

After treatments for 10 weeks, urine was obtained by squeezing the bladder for albumin assay with a mouse ELISA kit (#EIA06044m, Wuhan Xinqidi Biotech Co. Ltd, Wuhan, China). Blood was collected through the eye vein, and then mice were sacrificed and bilateral kidneys were removed. Half of the left side kidney was placed in 10% formalin for at least 24 h before paraffin embedding, and the cortex of residual kidney was isolated.

2.4 Lactate determination

Lactate levels in blood plasma or renal cortex were determined by using a commercial kit (#A019-2-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, China), and the determination process was performed according to the manufacturer's instructions.

2.5 Histone isolation

Total histone proteins in cells and tissues were extracted by using the acid extraction method as previous report with minor modifications (Baldensperger et al., 2020).

2.6 Protein analysis by immunocytochemistry

Cell immunofluorescence assay was performed according to our previous report (Tang et al., 2020). Briefly, after plated, fixed, permeabilized, and sealing treatment, MPCs were subsequently incubated with anti-nephrin(#38552, Signalway Antibody, USA, 1:250),anti-MCT2 (#46051, Signalway Antibody, USA, 1:250), or anti-Col-IV (#BS1072, Bioworld Technology, USA, 1:250) antibody at 4 degC overnight. Immune-reacted primary antibody was detected after 1 h incubation in a dark place at 37 degC with secondary antibody Dylight 594 Affinipure donkey anti-rabbit IgG (H+L) (#E032421-01, EARTHOX, USA, 1:250). The cells were further stained with DAPI for 2 min in the dark at room temperature and washed, then mounted onto microscope slides in a mounting medium. The cells were viewed with an Olympus BX43F fluorescence microscope.

2.7 Protein analysis by Western blot

Cells were lysed and renal tissue was homogenized in the RIPA buffer with 1 mM phenylmethanesulfonyl fluoride and 1 mM phosphatase inhibitor cocktail at 4 degC for 30 min followed by 12,000 x g centrifugation at 4 degC for 15 min to obtain the supernatant. The BCA protein assay was performed to determine the protein concentration in the homogenate supernatant using a kit (#P0010, Beyotime Institute of Biotechnology, Nantong, China). The protein samples were separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride membrane. The membrane was blocked with

2% milk powder solution for 60 min and incubated overnight at 4 degC with primary antibodies including anti-LDH-A (#21799-1-AP, Proteintech, 1:1000), anti-MCT4 (#22787-1-AP, Proteintech, 1:1000), anti-MCT2 (1:1000), pan anti-L-lactyllysine polyclonal antibody (#PTM-1401, PTM-BIO, Hangzhou, China, 1:1000), anti-nephrin (1:1000), anti-Col-IV (1:1000), anti-ZO-1 (#21773-1-AP, Proteintech, 1:1000), anti-α-SMA (#AF1032, Affinity, 1:1000), and anti-podocin (#BS1072, Bioworld Technology, 1:500). The proteins were detected using goat anti-rabbit IgG (H+L) secondary antibody (#V926-32211, Li-Cor Inc, 1:1000), respectively. An infrared Imaging System was applied to detect immunoreactive blots. Signal densities on the blots were measured with Image J software and normalized using rabbit anti-β-actin antibody (#ET1701-80, HUABIO, Hangzhou, China, 1:1000) or anti-histone H3 antibody (#AF0863, Affinity, 1:1000) as an internal control.

2.8 mRNA assay by real-time qPCR

Changes of some intersection genes from RNA-seq were validated in MPCs with lactate-lowering treatments by qPCR assay. Total RNA was extracted with TRIZOL (#15596-026, Invitrogen, USA). Then mRNA was reversely transcribed by using the ReverTra Ace® kit (#RR037A, TaKaRa, Dalian, China). Real time PCR was performed in a LightCycler 480 system (Roche Applied Science, Mannheim, Germany). The relative mRNA levels were detected and normalized to gene *Actb* as the internal reference through the $2^{-\Delta\Delta^{\hat{}}\tau}$ method. Primers of the associated mouse genes were listed in Table S1.

2.9 Pyruvate dehydrogenase (PDH) activity assay

PDH activity was assessed by using a spectrophotometry assay with a kit (#BC0385, Solarbio Life Science, Beijing, China). Pyruvic acid is decarboxylated by PDH into hydroxyethyl thiamine pyrophosphate, and then dichlorophenolindophenol is reduced leading to a decrease in absorbance at the wavelength of 605 nm. The measurement process was performed according to the manufacturer's instructions.

2.10 Creatinine and blood urea nitrogen (BUN) assay

Besides uric albumin amount and plasma lactate levels, renal functions were also assessed by determining creatinine and BUN levels in plasma using the corresponding commercial assay kits (C011, C013, Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instruction.

2.11 Protein expression in kidney by immunohistochemistry

Protein assays of α-SMA and fibronectin (FN) in glomeruli were performed as previously described (Y. J. Chen et al., 2019). Renal tissue sections were placed in 3% H_2O_2 to eliminate endogenous peroxidase activity, and blocked with 2% bovine serum albumin followed by incubation with the primary antibodies anti-α-SMA (1:200) and anti-FN (#ab45688, Abcam, 1:200) overnight at 4 °C. Then the sections were stained using a polymer HRP detection system (ZSGB-BIO, Beijing, China) and counterstained with hematoxylin. Finally, the sections were examined using an Olympus BX43F microscope, and the stained α-SMA and FN were quantified by Image-Pro Plus 4.0 software.

2.12 Protein expression in glomerulus by immunofluorescence

Protein detection in tissue paraffin section by immunofluorescence assay was conducted as our previous report (Y. W. Liu et al., 2018). Briefly, 4-μm sections were incubated with anti-ZO-1 (1:200), pan anti-L-lactyllysine polyclonal (1:200), anti-MCT2 (1:200) or anti-MCT4 (1:200) antibody overnight at 4 °C followed by the secondary antibody Dylight 594-AffiniPure donkey anti-rabbit IgG(H+L) (1:300). Then, the sections were further stained with DAPI for 3 min in dark place. Finally, the sections were examined using an Olympus BX43F fluorescence microscope.

2.13 Data and Statistical analysis

The data and statistical analysis comply with the recommendations of the British Journal of Pharmacology on experimental design and analysis in pharmacology (Curtis et al., 2018). All statistical analyses were done with GraphPad Prism 6.0 software. Differences between the groups were assessed using a Student's T or

one-way analysis of variance followed by Dunnett's multiple comparisons test. The data in the different experimental groups were expressed as the mean ± SD. $P < 0.05$ was considered statistically significant.

3 Results

3.1 HG Stimulated Lactate Increases in MPCs

Time-dependent changes of lactate were analyzed in HG-cultured MPCs. HG stimulation gradually increased lactate levels after 24, 48, and 72 h culture, with the highest level (4.4 ± 1.14 mM) at 48 h ($p< 0.01$, Figure 1a). Then the potential influencing factors were explored. The protein expressions of LDH-A, a key enzyme of lactate production, and MCT4, an efflux transporter of lactate, were unchanged in the HG-cultured MPCs (Figure 1b, c, d), while the protein expression of MCT2, an uptake transporter of lactate, was significantly increased ($p < 0.01$, Figure 1b, e). Moreover, lack of determination method of LDH-A isoenzyme activity, the enzymatic activity of PDH was measured instead, finding that PDH activity was markedly decreased in the HG-cultured MPCs ($p < 0.01$, Figure 1f). Further study indicated that HG notably increased MCT2 expression in mitochondria by using immunofluorescence co-localization analysis (Figure 1g). Together, HG-stimulated lactate increase in MPCs likely resulted from the upregulated MCT2 in mitochondria and the decreased PDH activity.

3.2 HG and Exogenous Lactate Promoted Histone Lactylation and Induced Podocyte EMT in MPCs

We found that HG and LA at 2.5, 5, and 10 mM all increased HKla levels compared with NG culture for 48 h ($p < 0.05$ or $p< 0.01$, Figure 2a). Combining the lactate levels induced by HG in MPCs, LA at 5 mM was selected to perform the effects on podocyte EMT. HG and LA both notably reduced the protein expression of nephrin while elevated the protein expression of Col-IV compared with NG culture (Figure 2b, c). These data primarily displayed that histone lactylation may cause the EMT of podocytes exposure to chronic high glucose.

3.3 Lactate Decreases Caused the Inhibition of Histone Lactylation in HG-cultured MPCs

Our results showed that co-treatment with DCA or Oxa recovered lactate levels in the HG group into that in the NG group (both $p< 0.01$, Figure 3a), and co-treatment with Rot also significantly decreased lactate levels in the HG group, but still much higher than that in the NG group ($p < 0.01$, Figure 3a). Moreover, co-treatment with Oxa reversed the increased HKla level in the HG group ($p < 0.01$, Figure 3b), while co-treatment with DCA or Rot showed a decreased tendency (Figure 3b). These results demonstrated that lactate mediated HG-induced histone lactylation in podocytes.

3.4 Lactate Decreases Caused the Inhibition of Podocyte EMT in HG-cultured MPCs

Podocyte EMT was examined by using lactate-lowering treatments after HKla levels were decreased. On the one hand, co-treatment of HG and Oxa recovered the reduced nephrin protein in the HG group ($p< 0.01$, Figure 4a, b), co-treatment of HG and DCA did not improve nephrin expression, while co-treatment of HG and Rot further decreased nephrin protein expression ($p < 0.05$, Figure 4a, b). Meanwhile, co-treatment of HG and Oxa or DCA significantly increased ZO-1 expression compared with HG (both $p < 0.05$, Figure 4a, c), and co-treatment with Rot further declined ZO-1 expression in the HG group ($p < 0.01$, Figure 4a, c). On the other hand, co-treatment of HG and Oxa or DCA markedly reduced protein expressions of α-SMA and Col-IV compared with HG ($p< 0.05$ or $p < 0.01$, Figure 4d-f), and co-treatment of HG and Rot also decreased Col-IV expression ($p< 0.05$, Figure 4d, e). Together, the results demonstrated that lactate mediated HG-induced EMT of podocytes.

3.5 Potential Genes Affected by Histone Lactylation on Podocyte EMT in MPCs

RNA-seq technology was used to detect differentially expressed genes after different treatments on podocytes. Compared to NG, LA and HG respectively presented 210 and 594 upregulation genes, obtaining 83 intersection genes (Figure 5a, b), subsequently 9 genes were downregulated in HG+Oxa (Figure 5a), and 11 genes in HG+DCA (Figure 5b). The final overlapped genes were 2900042K21Rik, Gm43597, and Gm12603 (Figure

5c), irrelevant to the concerned phenotype of podocytes (Supplementary Material 2). Disease enrichment in gene percentage and P value barplot showed that DN ranked top 3 (Figure S1). Thus, some intersection genes from Oxa (better effects than DCA) were further to be confirmed by qPCR, indicating that Wnt7b, Jun, and Tbc1d9 were all upregulated in both LA and HG and all downregulated in both HG+Oxa and HG+DCA, while Csn3 was upregulated in only HG and downregulated in both HG+Oxa and HG+DCA. Totally, the results of qPCR were quite consistent with those of RNA-seq.

3.6 Alterations of FBG and Body Weight of Diabetic Mice afterLactate-lowering Treatments

Consecutive five-day STZ stimulation induced a significant ($p< 0.01$) increase in FBG levels in the normal mice, and FBG was still at a higher level after a ten-week duration of diabetes (Table S2). Lactate-lowering treatment with DCA significantly decreased FBG level of diabetic mice, but was still much higher than that of normal mice ($p < 0.01$, Table S2), while Oxa treatment did not improve FBG level of diabetic mice (Table S2). Meanwhile, lactate-lowering treatments with DCA or Oxa did not ameliorate the reduced body weight of diabetic mice (Figure S2). These results indicated that lactate-lowering treatments could not alleviate the basic symptoms of diabetic mice.

3.7 Lactate-lowering Improved the Renal Functions of Diabetic Mice

Plasma lactate level was markedly elevated in diabetic mice compared with that in normal mice ($p < 0.01$, Figure 6a), whereas treatment with DCA or Oxa reversed lactate increase in the plasma of diabetic mice (both $p < 0.01$, Figure 6a). Moreover, plasma Cr and BUN levels as well as urine ALB level were dramatically increased in diabetic mice (all $p < 0.01$, Figure 6b-d), while treatment with DCA or Oxa significantly decreased plasma lactate levels in diabetic mice ($p < 0.05$ or $p< 0.01$, Figure 6b-d). These data indicated that plasma lactate decline improved the renal functions of diabetic mice.

3.8 Lactate-lowering Inhibited Podocyte EMT in Glomeruli of Diabetic Mice

The protein expressions of nephrin and podocin were significantly reduced in the renal cortex of diabetic mice (both $p < 0.01$, Figure 7a, b), while treatment with DCA or Oxa markedly ameliorated the expressions of these two proteins ($p < 0.05$ or $p < 0.01$, Figure 7a, b). Moreover, treatment with DCA or Oxa notably attenuated the decline in ZO-1 expression in the renal glomeruli of diabetic mice (Figure 7c). Meantime, the expressions of FN and α-SMA were remarkably raised in the glomeruli of diabetic mice (both $p < 0.01$, Figure 7d, e), whereas treatment with DCA or Oxa markedly alleviated or even recovered FN and α-SMA expressions (both $p < 0.01$, Figure 7d, e). These results demonstrated that decreasing lactate levels suppressed EMT in renal glomeruli of diabetic mice.

3.9 Lactate-lowering Inhibited HKla Formation in Kidney of Diabetic Mice

Lactate and HKla levels were significantly elevated in the renal cortex of diabetic mice ($p < 0.05$ or $p < 0.01$, Figure 8a, b), while treatment with DCA or Oxa markedly declined lactate and HKla levels in diabetic mice ($p < 0.05$ or$p < 0.01$, Figure 8a, b). Furthermore, HKla level was remarkably increased in the glomeruli of diabetic mice also by using immunofluorescence analysis, while treatment with DCA or Oxa notably attenuated HKla increase (Figure 8c). These results displayed that decreasing lactate levels blocked lactylation of histone lysine residues in glomeruli of diabetic mice.

3.10 Lactate-lowering Differently Affected MCT2 and MCT4 Expressions in Glomeruli of Diabetic Mice

MCT2 expression was significantly increased but MCT4 unchanged in the glomeruli of diabetic mice (Figure 8d, e), whereas treatment with DCA notably decreased not only MCT2 but also MCT4 expression in diabetic mice (Figure 8d, e), and Oxa treatment only decreased MCT2 expression (Figure 8d). These results displayed that renal lactate accumulation resulted from the enhanced MCT2 function in the glomeruli of diabetic mice, and inhibiting PDK attenuated MCT2 and MCT4 expressions in kidney of diabetic mice.

4 Discussion

Podocyte EMT is the main pathological pathway leading to podocyte dysfunction and proteinuria in DN (Y. Li et al., 2008; Yamaguchi et al., 2009). Anaerobic glycolysis and its product lactate are the major energy source of podocytes (Brinkkoetter et al., 2019; Ozawa et al., 2015), and lactate levels are elevated in blood and kidney in diabetic conditions (Y. D. Chen et al., 1993; Higuchi et al., 2020; Lopez-Cano et al., 2020; Zhao et al., 2011). Based on these reports, in the current study, firstly we found that lactate levels were increased in high glucose-stimulated mouse podocytes as well as blood and kidney of diabetic mice, which possibly was due to the upregulated MCT2 in mitochondria and the decreased PDH activity. Secondly, both chronic high glucose and exogenous lactate promoted podocyte EMT and enhanced pan lactylation of histone lysine residues in podocytes. Thirdly, lactate-lowering treatments alleviated podocyte EMT and histone lactylation in not only high glucose-stimulated podocytes but also renal cortex of diabetic mice, and improved renal functions of diabetic mice. Finally, four DGEs were screened and validated in podocytes after oxamate treatment. This study clarified that lactate accumulation mediated the podocyte-undergoing EMT caused by chronic high glucose through lactate-triggered histone lactylation, which contributed to the pathogenesis of DN.

The EMT of podocyte is one of the most phenotypes of podocyte injury caused by high glucose in DN (Ying & Wu, 2017). In our study, chronic high glucose reduced protein expressions of slit diaphragm associated protein ZO-1 and nephrin in MPCs and induced protein expressions of interstitial matrix components Col-IV and α-SMA, leading to the EMT of podocyte. Yamaguchi et al. find that in addition to loss of nephrin and ZO-1, mesenchymal markers such as desmin, fibroblast-specific protein-1, and matrix metalloproteinase-9 are observed in the glomerular podocytes of STZ-induced diabetic mice (Yamaguchi et al., 2009). In our study, podocin as well as nephrin and ZO-1 declined while FN and α-SMA raised in the glomeruli of diabetic mice. Our study confirms that podocytes undergo EMT in prolonged high glucose conditions.

Lactates mediate podocyte EMT caused by prolonged high glucose. Burns et al. find that EMT stimulation correlates with the increased lactate production in bladder cancer cell models (Burns, Hurst, Knowles, Phillips, & Allison, 2021). Miranda-Goncalves et al. report that lactate increases renal cell carcinoma aggressiveness through sirtuin 1-dependent EMT regulation (Miranda-Goncalves et al., 2020). In the current study, extrinsic lactate induced podocyte EMT in MPCs similar to prolonged high glucose elevating lactate levels in MPCs. At the meantime, lactate-lowering treatments inhibited the EMT of podocytes in high glucose-stimulated MPCs, accompanied by decreases in lactate levels. Moreover, lactate-lowering interventions also suppressed podocyte EMT in the glomeruli of diabetic mice with drinking water added oxamate and dichloroacetate. Our findings demonstrate that lactate accumulation contributes to the podocyte EMT caused by prolonged high glucose stimulation.

Lactate-triggered histone lactylation results in podocyte EMT in prolonged high glucose condition. Lactate as a signaling molecule can modify histone by adding a lactyl group onto the lysine residues, called histone lactylation (D. Zhang et al., 2019), a new acylation modification of histone. Moreover, histone lactylation is positively correlated with lactate levels (D. Zhang et al., 2019). In the current study, both exogenous lactate and high glucose increased histone lactylation in MPCs, whereas lactate-lowering treatments with inhibition of lactate dehydrogenase or pyruvate dehydrogenase kinase decreased histone lactylation resulting from high glucose in MPCs. Furthermore, lactate-lowering interventions also attenuated histone lactylation in the renal cortex of diabetic mice. Acylation modification of histone has been reported to participate in the formation of EMT in some diseases (Nam et al., 2020; Peng, Lai, Chang, Hsu, & Wu, 2021). These findings illuminate that prolonged high glucose exposure induces histone lactylation in podocytes due to accumulation of lactates and subsequent podocyte EMT formation.

Studies on biological functions confirm that histone lactylation stimulates gene transcription in disease states, such as histone lactylation induces M2-like genes in M1 macrophages subjected to bacterial infections (D. Zhang et al., 2019), and lactate-induced histone lactylation promotes pro-fibrotic gene expressions of macrophage in lung myofibroblast (Cui et al., 2021). Moreover, deficiency of toll-like receptor signaling adapter B-cell adapter for phosphoinositol-3 kinase decreases the expression of reparative genes in macrophage following microbial stimulation due to the reduced histone lactylation as well as the defective

aerobic glycolysis and reduced lactate production (Irizarry-Caro et al., 2020). Given that lactate increases in plasma and kidney in DN, we thought that this was achieved by lactate-induced histone lactylation for mesenchymal biomarker genes, leading to formation of podocyte EMT. In our study, four candidate genes Wnt7b, Jun, Tbc1d9, and Csn3 were screened and might involve in histone lactylation-induced podocyte EMT in prolonged high glucose conditions. Literatures display that Wnt7b can cause EMT, migration, and invasion of glioma cells (Meng, Tian, Guo, & Wang, 2021), and a conserved mechanism is provided that connects c-Jun amino-terminal kinase and Wnt signaling in regulating EMT and tumor progression in drosophila tumor models (S. Zhang et al., 2019), but TBC1D9 expression is inversely correlated with grade and proliferative index in triple-negative breast cancer (Kothari et al., 2021). However, whether these genes involve in podocyte EMT formation induced by chronic high glucose needs to be further investigated.

Potential influence factors were explored for lactate elevation caused by long-term high glucose in podocytes. Increased intracellular lactate may result from an increase in production or decreases in removal (efflux and conversion), or both. LDH-A is the key enzyme of lactate production and MCT4 is a high-affinity efflux transporter of lactate (Contreras-Baeza et al., 2019; Luo et al., 2017), perplexingly, high glucose unchanged the protein expression of LDH-A and MCT4 in MPCs. We were puzzled. Considering lactate shuttle theory including cell-cell and intracellular shuttle (Brooks, 2009), we examined alterations of lactate influx transporters. MCT1 and MCT2 participate in the uptake of intracellular lactate, but no MCT1 has been found in kidney (Lin, Vera, Chaganti, & Golde, 1998), and MCT2 can be expressed in kidney (Adeva-Andany et al., 2014; Lin et al., 1998). Interestingly, high glucose increased MCT2 expression in MPCs, and further study indicated that MCT2 was expectedly raised in mitochondria. Moreover, we found that PDH activity was reduced in high glucose-stimulated MPCs, decreasing pyruvate transformation and subsequent acetate entry into the tricarboxylic acid cycle. Nevertheless, these require being further clarified and confirmed.

In conclusion, our study reveals that chronic high glucose causes lactate accumulation in podocytes and subsequent epithelial-mesenchymal transition of podocytes through lactate-derived histone lactylation, which then promotes the pathological process of DN. Furthermore, our study can provide a reference for the study of the relationship between the lactate-triggered histone lactylation modification and diabetic chronic complications.

Supplementary information

The manuscript contains supplementary materials.

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CONFLICTS OF INTEREST

None of the authors has any conflict of interests.

Authors Contributions

Yao-Wu Liu: conceptualization, project administration, funding acquisition, and writing-review and editing. Ting Zheng: investigation, methodology, and visualization. Yan-Ping Gu: investigation and writing-original draft preparation. Jiang-Meng Wang: visualization and data curation. Pan-Pan Gu: methodology and data curation. Ting-Ting Huang: supervision and resources. Ling-Shan Gou: funding acquisition and writing-review and editing. All authors read and approved the final manuscript.**Data availability STATEMENT**

All data generated or analysed during this study are included in the published article (and its online supplementary files). The files are available from the corresponding author upon reasonable request.

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References

Adeva-Andany, M., Lopez-Ojen, M., Funcasta-Calderon, R., Ameneiros-Rodriguez, E., Donapetry-Garcia, C., Vila-Altesor, M., & Rodriguez-Seijas, J. (2014). Comprehensive review on lactate metabolism in human health. *Mitochondrion, 17* , 76-100. doi:10.1016/j.mito.2014.05.007

Baldensperger, T., Eggen, M., Kappen, J., Winterhalter, P. R., Pfirrmann, T., & Glomb, M. A. (2020). Comprehensive analysis of posttranslational protein modifications in aging of subcellular compartments. *Sci Rep, 10* (1), 7596. doi:10.1038/s41598-020-64265-0

Brinkkoetter, P. T., Bork, T., Salou, S., Liang, W., Mizi, A., Ozel, C., ... Huber, T. B. (2019). Anaerobic Glycolysis Maintains the Glomerular Filtration Barrier Independent of Mitochondrial Metabolism and Dynamics. *Cell Rep, 27* (5), 1551-1566 e1555. doi:10.1016/j.celrep.2019.04.012

Brooks, G. A. (2009). Cell-cell and intracellular lactate shuttles. *J Physiol, 587* (Pt 23), 5591-5600. doi:10.1113/jphysiol.2009.178350

Burns, J. E., Hurst, C. D., Knowles, M. A., Phillips, R. M., & Allison, S. J. (2021). The Warburg effect as a therapeutic target for bladder cancers and intratumoral heterogeneity in associated molecular targets. *Cancer Sci, 112* (9), 3822-3834. doi:10.1111/cas.15047

Chen, Y. D., Varasteh, B. B., & Reaven, G. M. (1993). Plasma lactate concentration in obesity and type 2 diabetes. *Diabete Metab, 19* (4), 348-354. Retrieved from*http://www.ncbi.nlm.nih.gov/pubmed/8293860*

Chen, Y. J., Kong, L., Tang, Z. Z., Zhang, Y. M., Liu, Y., Wang, T. Y., & Liu, Y. W. (2019). Hesperetin ameliorates diabetic nephropathy in rats by activating Nrf2/ARE/glyoxalase 1 pathway. *Biomed Pharmacother, 111* , 1166-1175. doi:10.1016/j.biopha.2019.01.030

Chen, Z., Tian, L., Wang, L., Ma, X., Lei, F., Chen, X., & Fu, R. (2021). TRIM32 Inhibition Attenuates Apoptosis, Oxidative Stress, and Inflammatory Injury in Podocytes Induced by High Glucose by Modulating the Akt/GSK-3beta/Nrf2 Pathway.*Inflammation* doi:10.1007/s10753-021-01597-7

Contreras-Baeza, Y., Sandoval, P. Y., Alarcon, R., Galaz, A., Cortes-Molina, F., Alegria, K., ... Barros, L. F. (2019). Monocarboxylate transporter 4 (MCT4) is a high affinity transporter capable of exporting lactate in high-lactate microenvironments. *J Biol Chem, 294* (52), 20135-20147. doi:10.1074/jbc.RA119.009093

Cui, H., Xie, N., Banerjee, S., Ge, J., Jiang, D., Dey, T., ... Liu, G. (2021). Lung Myofibroblasts Promote Macrophage Profibrotic Activity through Lactate-induced Histone Lactylation. *Am J Respir Cell Mol Biol, 64* (1), 115-125. doi:10.1165/rcmb.2020-0360OC

Curtis, M. J., Alexander, S., Cirino, G., Docherty, J. R., George, C. H., Giembycz, M. A., ... Ahluwalia, A. (2018). Experimental design and analysis and their reporting II: updated and simplified guidance for authors and peer reviewers. *Br J Pharmacol, 175* (7), 987-993. doi:10.1111/bph.14153

Daehn, I. S., & Duffield, J. S. (2021). The glomerular filtration barrier: a structural target for novel kidney therapies. *Nat Rev Drug Discov, 20* (10), 770-788. doi:10.1038/s41573-021-00242-0

Higuchi, I., Kimura, Y., Kobayashi, M., Narumi, K., Furugen, A., Miyoshi, H., ... Iseki, K. (2020). Relationships between plasma lactate, plasma alanine, genetic variations in lactate transporters and type 2 diabetes in the Japanese population.*Drug Metab Pharmacokinet, 35* (1), 131-138. doi:10.1016/j.dmpk.2019.10.001

Irizarry-Caro, R. A., McDaniel, M. M., Overcast, G. R., Jain, V. G., Troutman, T. D., & Pasare, C. (2020). TLR signaling adapter BCAP regulates inflammatory to reparatory macrophage transition by promoting histone lactylation. *Proc Natl Acad Sci U S A, 117* (48), 30628-30638. doi:10.1073/pnas.2009778117

Iseki, K., Ikemiya, Y., Iseki, C., & Takishita, S. (2003). Proteinuria and the risk of developing end-stage renal disease. *Kidney Int, 63* (4), 1468-1474. doi:10.1046/j.1523-1755.2003.00868.x

Jiang, L., Liu, X., Hu, X., Gao, L., Zeng, H., Wang, X., ... Wu, Y. (2022). METTL3-mediated m(6)A modification of TIMP2 mRNA promotes podocyte injury in diabetic nephropathy. *Mol Ther* doi:10.1016/j.ymthe.2022.01.002

Kothari, C., Clemenceau, A., Ouellette, G., Ennour-Idrissi, K., Michaud, A., R, C. G., ... Durocher, F. (2021). TBC1D9: An Important Modulator of Tumorigenesis in Breast Cancer. *Cancers (Basel), 13* (14)doi:10.3390/cancers13143557

Li, X. Z., Jiang, H., Xu, L., Liu, Y. Q., Tang, J. W., Shi, J. S., ... Yin, X. X. (2021). Sarsasapogenin restores podocyte autophagy in diabetic nephropathy by targeting GSK3beta signaling pathway. *Biochem Pharmacol, 192* , 114675. doi:10.1016/j.bcp.2021.114675

Li, Y., Kang, Y. S., Dai, C., Kiss, L. P., Wen, X., & Liu, Y. (2008). Epithelial-to-mesenchymal transition is a potential pathway leading to podocyte dysfunction and proteinuria.*Am J Pathol, 172* (2), 299-308. doi:10.2353/ajpath.2008.070057

Lin, R. Y., Vera, J. C., Chaganti, R. S., & Golde, D. W. (1998). Human monocarboxylate transporter 2 (MCT2) is a high affinity pyruvate transporter. *J Biol Chem, 273* (44), 28959-28965. doi:10.1074/jbc.273.44.28959

Liu, X. Q., Jiang, L., Li, Y. Y., Huang, Y. B., Hu, X. R., Zhu, W., ... Qi, X. M. (2022). Wogonin protects glomerular podocytes by targeting Bcl-2-mediated autophagy and apoptosis in diabetic kidney disease. *Acta Pharmacol Sin, 43* (1), 96-110. doi:10.1038/s41401-021-00721-5

Liu, Y. W., Hao, Y. C., Chen, Y. J., Yin, S. Y., Zhang, M. Y., Kong, L., & Wang, T. Y. (2018). Protective effects of sarsasapogenin against early stage of diabetic nephropathy in rats. *Phytother Res, 32* (8), 1574-1582. doi:10.1002/ptr.6088

Lopez-Cano, C., Gutierrez-Carrasquilla, L., Barbe, F., Sanchez, E., Hernandez, M., Marti, R., ... Lecube, A. (2020). Effect of Type 2 Diabetes Mellitus on the Hypoxia-Inducible Factor 1-Alpha Expression. Is There a Relationship with the Clock Genes? *J Clin Med, 9* (8)doi:10.3390/jcm9082632

Luo, F., Zou, Z., Liu, X., Ling, M., Wang, Q., Wang, Q., ... Zhang, A. (2017). Enhanced glycolysis, regulated by HIF-1alpha via MCT-4, promotes inflammation in arsenite-induced carcinogenesis. *Carcinogenesis, 38* (6), 615-626. doi:10.1093/carcin/bgx034

Marshall, S. M. (2007). The podocyte: a potential therapeutic target in diabetic nephropathy? *Curr Pharm Des, 13* (26), 2713-2720. doi:10.2174/138161207781662957

Meng, X., Tian, H., Guo, W., & Wang, Z. (2021). circ_0082375 promotes the progression of glioma by regulating Wnt7B. *Transl Neurosci, 12* (1), 456-468. doi:10.1515/tnsci-2020-0181

Miranda-Goncalves, V., Lameirinhas, A., Macedo-Silva, C., Lobo, J., P, C. D., Ferreira, V., ... Jeronimo, C. (2020). Lactate Increases Renal Cell Carcinoma Aggressiveness through Sirtuin 1-Dependent Epithelial Mesenchymal Transition Axis Regulation. *Cells, 9* (4)doi:10.3390/cells9041053

Nam, M. H., Smith, A. J. O., Pantcheva, M. B., Park, K. U., Brzezinski, J. A., Galligan, J. J., ... Nagaraj, R. H. (2020). Aspirin inhibits TGFbeta2-induced epithelial to mesenchymal transition of lens epithelial cells: selective acetylation of K56 and K122 in histone H3. *Biochem J, 477* (1), 75-97. doi:10.1042/BCJ20190540

Niu, D., Luo, T., Wang, H., Xia, Y., & Xie, Z. (2021). Lactic acid in tumor invasion. *Clin Chim Acta, 522* , 61-69. doi:10.1016/j.cca.2021.08.011

Ozawa, S., Ueda, S., Imamura, H., Mori, K., Asanuma, K., Yanagita, M., & Nakagawa, T. (2015). Glycolysis, but not Mitochondria, responsible for intracellular ATP distribution in cortical area of podocytes. *Sci Rep, 5* , 18575. doi:10.1038/srep18575

Peng, P. H., Lai, J. C., Chang, J. S., Hsu, K. W., & Wu, K. J. (2021). Induction of epithelial-mesenchymal transition (EMT) by hypoxia-induced lncRNA RP11-367G18.1 through regulating the histone 4 lysine 16 acetylation (H4K16Ac) mark. *Am J Cancer Res, 11* (6), 2618-2636. Retrieved from*http://www.ncbi.nlm.nih.gov/pubmed/34249418*

Tang, Z. Z., Zhang, Y. M., Zheng, T., Huang, T. T., Ma, T. F., & Liu, Y. W. (2020). Sarsasapogenin alleviates diabetic nephropathy through suppression of chronic inflammation by down-regulating PAR-1: In vivo and in vitro study. *Phytomedicine, 78* , 153314. doi:10.1016/j.phymed.2020.153314

Yamaguchi, Y., Iwano, M., Suzuki, D., Nakatani, K., Kimura, K., Harada, K., ... Saito, Y. (2009). Epithelial-mesenchymal transition as a potential explanation for podocyte depletion in diabetic nephropathy. *Am J Kidney Dis, 54* (4), 653-664. doi:10.1053/j.ajkd.2009.05.009

Yin, J., Wang, Y., Chang, J., Li, B., Zhang, J., Liu, Y., ... Zeng, X. (2018). Apelin inhibited epithelial-mesenchymal transition of podocytes in diabetic mice through downregulating immunoproteasome subunits beta5i. *Cell Death Dis, 9* (10), 1031. doi:10.1038/s41419-018-1098-4

Ying, Q., & Wu, G. (2017). Molecular mechanisms involved in podocyte EMT and concomitant diabetic kidney diseases: an update. *Ren Fail, 39* (1), 474-483. doi:10.1080/0886022X.2017.1313164

Zhang, D., Tang, Z., Huang, H., Zhou, G., Cui, C., Weng, Y., ... Zhao, Y. (2019). Metabolic regulation of gene expression by histone lactylation. *Nature, 574* (7779), 575-580. doi:10.1038/s41586-019-1678-1

Zhang, L., Wen, Z., Han, L., Zheng, Y., Wei, Y., Wang, X., ... Tong, X. (2020). Research Progress on the Pathological Mechanisms of Podocytes in Diabetic Nephropathy.*J Diabetes Res, 2020* , 7504798. doi:10.1155/2020/7504798

Zhang, S., Guo, X., Wu, H., Sun, Y., Ma, X., Li, J., ... Xue, L. (2019). Wingless modulates activator protein-1-mediated tumor invasion. *Oncogene, 38* (20), 3871-3885. doi:10.1038/s41388-018-0629-x

Zhao, L., Gao, H., Lian, F., Liu, X., Zhao, Y., & Lin, D. (2011). (1)H-NMR-based metabonomic analysis of metabolic profiling in diabetic nephropathy rats induced by streptozotocin. *Am J Physiol Renal Physiol, 300* (4), F947-956. doi:10.1152/ajprenal.00551.2010

Figure legends

Figure1 Effects and causes of HG on lactate levels in podocytes. (a) Lactate levels in MPC cells after HG culture at 24, 48, and 72 h, respectively. (b) Representative Western blot bands for LDHA, MCT4, MCT2, and β-actin in MPC cells in NG (11.1 mM) and HG (30 mM) groups. (c) Protein expression of LDHA. (d) Protein expression of MCT4. (e) Protein expression of MCT2. (f) Enzymatic activity of PDH. (g) Protein expression of MCT2 in mitochondria. Mean ± SD, n=3 independent experiments (lactate, LDHA, MCT4, and MCT2), n=5 independent experiments (PDH), $^{**}P < 0.01$, vs. NG group. Scale bar: 20 μm.

Figure 2 Effects of HG and lactate on HKla levels and EMT in podocytes. (a) HKla levels in MPC cells after culture with HG and sodium lactate (LA) at 2.5, 5, 10 mmol/L for 48 h, respectively. (b) Protein expression of Nephrin in MPC cells in HG (30 mM) and LA (5 mM) groups. (c) Protein expression of Col-IV in MPC cells in HG and LA groups. Mean ± SD, n=5 independent experiments, $^{*}P< 0.05$, $^{**}P < 0.01$, vs. NG group. Scale bar: 20 μm.

Figure 3 Effects of HG and lactate production intervention on lactate levels and HKla levels in podocytes. (a) Lactate levels in MPC cells after culture with HG (30 mM) as well as HG plus sodium dichloroacetate (HG+DCA, 10 mM), sodium oxamate (HG+Oxa, 5 mM), or rotenone (HG+Rot, 5 nM) for 48 h, respectively. (b) HKla levels in MPC cells after culture with HG as well as HG plus DCA, Oxa, or Rot for 48 h, respectively. Total histone collected was by using acid extraction, and HKla levels were determined with pan lysine lactylation antibody. Mean ± SD, n=3 independent experiments (lactate), n=4 (HKla) independent experiments, $^{**}P < 0.01$,$^{\#\#}P < 0.01$.

Figure 4 Effects of HG and lactate production intervention on EMT in podocytes. (a) Representative Western blot bands for Nephrin, ZO-1, and β-actin in MPC cells. Protein expression of (b) Nephrin and (c) ZO-1 in MPC cells after culture with HG (30 mM) as well as HG plus sodium dichloroacetate (HG+DCA, 10 mM), sodium oxamate (HG+Oxa, 5 mM), or rotenone (HG+Rot, 5 nM) for 48 h, respectively. (d) Representative Western blot bands for Col-IV, α-SMA, and β-actin in MPC cells. Protein expression of (e)

Col-IV and (f) α-SMA in MPC cells after culture with HG as well as HG plus DCA, Oxa, or Rot for 48 h, respectively. Mean ± SD, n=3 independent experiments, $^{**}P< 0.01$, $^{\#}P < 0.05$,$^{\#\#}P < 0.01$.

Figure 5 Results of RNA sequencing and validation with qPCR in podocytes in conditions of HG and lactate-lowering intervention. Numbers of intersection genes among upregulation in HG & LA and downregulation in HG+Oxa (a), HG+DCA (b), and HG+Oxa & HG+DCA (c). Two different batches of cell samples were used for RNA sequencing. The mRNA levels of Wnt7b (d), Jun (e), Tbc1d9 (f), Csn3 (g) in MPC cells after culture with LA (5 mM), HG (30 mM) as well as HG plus sodium oxamate (HG+Oxa, 5 mM) or sodium dichloroacetate (HG+DCA, 10 mM) for 48 h, respectively. Mean ± SD, n=5 independent experiments, $^{*}P < 0.05$, $^{**}P < 0.01$, vs. NG;$^{\#}P < 0.05$,$^{\#\#}P < 0.01$, vs. HG.

Figure 6 Effects of lactate-lowering on renal functions of diabetic mice. (a) Lactate levels, (b) creatinine levels, (c) blood urea nitrogen (BUN) levels in plasma, and (d) urinary albumin levels in normal mice (N), STZ-induced diabetic mice (DM), diabetic mice treated with sodium dichloroacetate (DM+DCA, 450 mg/kg) or sodium oxamate (DM+Oxa, 125 mg/kg), respectively. Mean ± SD, n = 7 (lactate, creatinine, and BUN), n = 9 (albumin),$^{**}P$ <0.01, vs. N group;$^{\#}P$ <0.05.$^{\#\#}P$ <0.01, vs. DM group.

Figure 7 Effects of lactate-lowering on EMT in the kidney of diabetic mice. Protein expressions of (a) Nephrin, (b) Podocin, (c) ZO-1, (d) FN, and (e) α-SMA in normal mice (N), STZ-induced diabetic mice (DM), diabetic mice treated with sodium dichloroacetate (DM+DCA, 450 mg/kg) or sodium oxamate (DM+Oxa, 125 mg/kg), respectively. Mean ± SD, n=6 (nephrin, podocin, and ZO-1), n = 3 (FN and α-SMA),$^{**}P$ <0.01, vs. N group;$^{\#}P$ <0.05.$^{\#\#}P$ <0.01, vs. DM group. Scale bar: 20 μm.

Figure 8 Effects of lactate-lowering on levels of HKla as well as lactate and its transporters in the kidney of diabetic mice. (a) Lactate levels in the renal cortex, (b) HKla levels in the renal cortex by Western blot, (c) renal glomeruli by immunofluorescence, and the protein expressions of (d) MCT2 and (e) MCT4 in normal mice (N), STZ-induced diabetic mice (DM), diabetic mice treated with sodium dichloroacetate (DM+DCA, 450 mg/kg) or sodium oxamate (DM+Oxa, 125 mg/kg), respectively. Total histone collected was by using acid extraction, and HKla levels were determined with pan anti-lysine lactylation antibody. Mean ± SD, n=6,$^{*}P$ <0.05,$^{**}P$ <0.01, vs. N group;$^{\#}P$ <0.05.$^{\#\#}P$ <0.01, vs. DM group. Scale bar: 20 μm.