RESEARCH ARTICLE

Urinary exosomes as a novel biomarker for evaluation of α -lipoic acid's protective effect in early diabetic nephropathy

Hanxiao Sun^{1*} | Weifeng Yao^{2*} | Yubin Tang³ | Wenfang Zhuang¹ | Dan Wu¹ | Shan Huang³ | Huiming Sheng¹

¹Department of Laboratory Medicine, Tongren Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

²Department of Endocrine, Wuxi No. 2 Hospital Affiliated to Nanjing Medical University, Wuxi, China

³Department of Endocrine, Tongren Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Correspondence

Huiming Sheng, Department of Laboratory Medicine, Tongren Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China.

Email: poloshm@aliyun.com

Funding information

Shanghai Municipal Health Bureau Project, Grant/Award Number: 15ZR1437900; Natural Science Research Project of Changning District, Grant/Award Number: CNKW2014Z01; Leading Disciplines of Medical Laboratory in Changning District, Grant/Award Number: 2012QZK01; Tongren Hospital grant, Grant/Award Number: TR201407, TRYJ201506

Background: Long-term administration of α -lipoic acid (α -LA) is proved to ameliorate renal impairment. Herein we assessed serum, urinary biomarkers and vascular endothelium function to evaluate its short-period therapeutic effect and identify novel biomarkers for diabetic nephropathy (DN).

Methods: Sixty-two microalbuminuria-stage DN patients were randomly divided into two groups and received the following treatment for 8 weeks: (1) routine treatment (DM group); (2) routine treatment with 600 mg/d α -lipoic acid intravenously (α -LA group). Another total of 21 patients were recruited for the second-stage study and randomly divided into two groups: normoalbuminuria (UAER <30 mg/24 h) and microalbuminuria (UAER from 30–300 mg/24 h).

Results: With α -LA treatment, urinary albumin excretion rates (UAER), serum creatinine (SCr) and malonaldehyde (MDA) declined significantly, whereas plasma superoxide dismutase (SOD) activity increased and endothelium-dependent flow mediated vasodilation (FMD) flexibility improved dramatically. Furthermore, the improvement of FMD showed positive correlation with the variation in MDA and SOD as well (r values are .516 and .435, $P < .01$ and $P < .05$, respectively). In contrast, these markers have no significant difference in the DM group with routine treatment. Notably, the CD63 expressing of exosomes in urine was found higher in the normoalbuminuria patients compared with those in microalbuminuria, parallelly only declined markedly after α -LA administration in normoalbuminuria patients.

Conclusion: In summary, we emphasize short-term α -LA could protect the kidney in the early DN against general oxidative stress, particularly the urinary CD63-positive exosome could be a potential sensitive and therapeutic indicator.

KEYWORDS

α -lipoic acid, diabetic nephropathy, exosomes

1 | INTRODUCTION

Diabetic nephropathy (DN) is one of the most common chronic complications of diabetes and the leading cause of end-stage renal disease associated with considerable mortality and morbidity.¹

The current paradigm suggests oxidative stress and subsequent impairment of vascular endothelial function play important roles in development of diabetic nephropathy.^{2,3} However, the definite mechanism is complex and not yet fully elucidated. Recent research has found that oxidative stress initiates the cascade and contributes to the development of pathogenesis of this debilitating disease, so blockade of certain checkpoint(s) in the process could ameliorate

*These two authors contributed equally to this work.

the intensity of pathophysiology or even restore physiological balance.

It is well-known that α -lipoic acid (α -LA), a powerful anti-oxidant, plays an important role in delaying and protecting against DN as well.^{1,4} The α -LA can quench singlet oxygen, which was applied in clinical treatment of disorders of the peripheral nerves.⁵ Evidence from rodents and human studies indicates that long-term α -LA supplementation effectively attenuates the development and progression of DN through its antioxidant effect.^{6,7} However, the effect of daily administration of α -LA intravenous in a short period in early stage of DN is not clear.

The traditional predictive, diagnostic, and monitoring biomarkers for diabetic nephropathy, such as microalbuminuria, are limited not only in monitoring of stage-dependent pathogenesis but also in predicating late clinical outcomes.⁸ Exosomes are vesicles with a lipid bilayer membrane that are 30–120 nm in diameter and are enriched in endosome-derived components.⁹ Since urine is a noninvasive and readily available body fluid, the discovery of urine exosomes derived from the kidney and urinary tract has become a novel biomarker in DN research.

The aim of this study was to explore the protective effect of α -LA on diabetic nephropathy by monitoring oxidative stress related serum parameters, endothelial function, and evaluate the potential role of urine exosomes as early diagnostic biomarkers.

2 | MATERIALS AND METHODS

2.1 | Subjects and treatments

A total of 62 early diabetic nephropathy subjects were recruited into the first-stage study from the in-patient department of the Department of Endocrinology at the Wuxi No.2 hospital affiliated with Nanjing Medical University. The average age is 56.1 ± 10.4 years old and average disease course is 7.9 ± 4.9 years. The subject inclusion criteria are as follows: (1) The diagnosis of diabetic mellitus was based on the guideline of World Health Organization (WHO). (2) Three consecutive measurements of $30 \text{ mg}/24 \text{ h} < \text{UAER} < 300 \text{ mg}/24 \text{ h}$ during the proximate 1 month with normal renal function (serum $\text{Cr} \leq 133 \mu\text{mol/L}$, $\text{BUN} \leq 7.1 \text{ mmol/L}$). (3) Fasting blood glucose $< 7.0 \text{ mmol/L}$, postprandial blood glucose $< 10 \text{ mmol/L}$, and with stable blood pressure within the range $140 \sim 160 \text{ mm Hg}/85 \sim 100 \text{ mm Hg}$. The exclusion criteria are: (1) No obvious symptoms of cardio-cerebrovascular and liver disease; (2) No hyperthyroidism, concurrent infection, and other renal disorders; (3) No anti-oxidative or nephrotoxic drugs administration in the recent one month [2].

The subjects were randomly divided into either control or therapy group. The control group (DM group) comprised of 29 subjects, 16 male and 13 female; whereas the therapy group (α -LA group) comprised of 33 subjects, 18 male and 15 female. Both control and therapeutic group subjects received regular hypoglycemic therapy and strict diabetes diet, moreover, during the treatment, no Angiotensin-Converting Enzyme Inhibitors (ACEI) drugs were administered. The α -lipoic acid (Alpha-Lipon, Eisai China Inc., Suzhou, Jiangsu Province, China) was given intravenously with 600 mg/d for 2 weeks in the therapeutic group only.

Another total of 21 patients were recruited for the second-stage study from the in-patient department of the Department of Endocrinology at the Shanghai Tongren hospital affiliated to Shanghai Jiaotong University. The criteria were similar as above except that $\text{UAER} < 300 \text{ mg}/24 \text{ h}$. The subjects were divided into two groups: normoalbuminuria ($\text{UAER} < 30 \text{ mg}/24 \text{ h}$) and microalbuminuria (UAER from $30 \sim 300 \text{ mg}/24 \text{ h}$). The normoalbuminuria group comprised of 13 subjects with average age of 65.15 ± 14.58 years old, seven male and six female; whereas the microalbuminuria group comprised of eight subjects with average age of 67.88 ± 13.84 years old, four male and four female.

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethical Committee of the Wuxi No. 2 Hospital and Shanghai Tongren hospital. All participants provided informed consent prior to entering the study.

2.2 | Sample collection and clinical laboratory analysis

Fasting venous blood and total 24-hour urine samples were collected in the first-stage study subjects, and the first morning urine samples in the second-stage study subjects before and after the treatment. The samples were stored at -80°C until analysis. Subsequently glucose, triglyceride (TG), totalcholesterol (TC), low-density lipoprotein (LDL-C), high-density lipoprotein (HDL-C), and serum creatinine (SCr) were detected by the automatic biochemical analyzer (Olympus AU1000, Olympus Medical Systems Co., Tokyo, Japan). Urinary albumin excretion rates (UAER) were evaluated by radioimmunoassay kit (Chinese Nuclear Institute, Beijing, China). The content of malondialdehyde (MDA) was assayed by thiobarbituric acid. The activity of Superoxide Dismutase (SOD) was measured by spectrophotometer assays. All assays were performed according to the manufacturer's instructions (Jiancheng Limited Company, Nanjing, Jiangsu, China). Endothelium-dependent vasodilator function was assessed by brachial arterial flow-mediated dilation (FMD) designed by Corretti.MC. The inner diameter of brachial artery in a resting state (D_0) and after reaction hyperemia (D_1) were measured by the ultrasonic cardiograph (Sequoia 512 model; Siemens, Berlin, German), eventually, FMD was calculated according to the formula, $\text{FMD} = (D_1 - D_0) / D_0 \times 100\%$.¹⁰

2.3 | Exosome isolation

Urine was defrosted at 37°C and vigorously vortexed before used to isolate exosome by Total Exosome Isolation Reagent (from urine; Life Technology, #4484452; Thermo Fisher Scientific, Waltham, MA, USA). Exosome was isolated according to manufacturers' instructions. Isolated exosomes were kept at $2 \sim 8^\circ\text{C}$ for electron microscopy or flow cytometric analysis immediately.

2.4 | Electron microscopy

Exosome pellets were resuspended in $100 \mu\text{L}$ of PBS (pH 7.2). $20 \mu\text{L}$ of sample was spotted on parafilm. A Formvar/carbon coated EM grid (Ted Pella Inc., Redding, CA, USA) was floated on the sample droplet

for 10 minutes. The excess fluid from the grid was eliminated using filter paper. The grid was floated on 15 μ L ddH₂O water for 5 minutes. For the negative stain, the grid was floated on 20 μ L of 2% uranyl acetate (Sigma-Aldrich, Shanghai, China) in ddH₂O for 30 seconds. The excess fluid from the grid was eliminated using filter paper and the grid was then deposited on filter paper (coated side up) for 5 minutes to dry out.¹¹ The results were visualized using a FEI Tecnai G2 Spirit transmission electron microscope (Hillsboro, OR, USA).

2.5 | Flow cytometric analysis

Anti-Human CD63 PE (eBioscience, #12-0639-42, San Diego, CA, USA) was used to stain the exosome pellets for 30 min at 4°C protected from light. The corresponding isotype control for IgG1 (PE, # 12-4714-42) was also from eBioscience. Thereafter, the exosome pellets were resuspended and analyzed with Beckman Coulter Gallios Flow Cytometer (Beckman Coulter, Brea, CA, USA) and Flowjo V10.

2.6 | Statistical analysis

All quantitative data are reported as means $\pm$ standard deviation of the mean (SD) and analyzed using SPSS 18.0 software (SPSS Inc, Chicago, IL, USA). Statistical analyses were performed using the Student *t* test for comparisons of two groups, and *P* values $<.05$ were considered statistically significant. Correlations were performed using Pearson's correlation analysis.

3 | RESULTS

3.1 | Baseline characteristics

During the whole study, side effects were observed in only one patient in α -LA group had mild nausea without vomiting or drug withdrawal and no other apparent.

Clinical data and biomarker concentrations were available for all 62 patients who underwent early stage diabetic nephropathy. No significant differences in clinical parameters were detected between the DM group and the α -LA group. All clinical data are summarized in Table 1.

TABLE 1 Comparison of demographic and clinical characteristics

Parameters	DM group (n=29)	α -LA group (n=33)	Univariate test
Age (y)	55.6 $\pm$ 6.4	57.2 $\pm$ 8.2	NS
Female/male	13/16	15/18	NS
Duration of diabetes (y)	7.7 $\pm$ 4.2	8.0 $\pm$ 4.8	NS
Fasting glucose (mmol/L)	6.34 $\pm$ 0.76	6.38 $\pm$ 0.65	NS
Hemoglobin A1c (%)	7.26 $\pm$ 0.62	7.20 $\pm$ 0.57	NS
Serum creatinine (μ mol/L)	121.4 $\pm$ 10.6	118.6 $\pm$ 10.2	NS
Systolic blood pressure (mm Hg)	145.6 $\pm$ 15.8	148.3 $\pm$ 16.2	NS
Diastolic blood pressure (mm Hg)	91.3 $\pm$ 6.4	90.2 $\pm$ 5.7	NS

NS, not significant; α -LA group, α Lipoic Acid plus therapy; DM group, Diabetic Mellitus routine therapy. Mean $\pm$ standard deviation (SD).

3.2 | α -lipoic acid rescued renal function with reduced oxidative stress and improved endothelium-dependent vasodilator function

As displayed in Table 2, the levels of serum creatinine and UAER in the α -LA group were significantly decreased after treatment ($P<.05$), whereas no significant difference was observed in DM group ($P>.05$). There was no significance difference either intra- or inter-group ($P>.05$) for TG, TC, and LDL-C.

To evaluate the status of oxidative stress and endothelium-dependent vasodilator function, MDA, SOD, and FMD were measured. As shown in Table 3, the level of MDA was significantly decreased in α -LA group ($P<.05$) after one-week treatment, whereas the levels of both SOD and FMD were significantly increased ($P<.05$). Similar results were also observed between the α -LA and DM group ($P<.05$). In contrast, no significant changes were observed in DM group with routine hypoglycemic therapy in any one of MDA, SOD, and FMD ($P>.05$).

3.3 | Correlation between the improvement of FMD and the variation in MDA and SOD

We analyzed the correlation between FMD with age, disease course, systolic blood pressure (SBP), TG, UAER, MDA, and SOD. The positive correlation was observed only in SOD ($r=.578$). While, all other markers showed negatively correlation with FMD ($r=-.286$, $-.417$, $-.325$, $-.521$, $-.392$, $-.489$, respectively. $P<.05$, data not shown). After two-week treatment with α -LA, the improvement of FMD was positively correlated with the variation in MDA ($r=.516$, $P<.01$) and SOD ($r=.435$, $P<.05$; Figure 1).

3.4 | α -lipoic acid affects urinary exosomes

We analyzed isolated exosomes with electron microscopy (EM) to determine exosome quality. As shown in Figure 2A, exosomes with size in the range 50-150 nm were imaged, and exosomes with size among 100-120 nm were mostly captured. To assess the quantity of urinary exosome (uExo), the mean fluorescent intensity of CD63, one of the

Parameters	α -LA group (n=33)		DM group (n=29)	
	Before	After	Before	After
UAER (mg/24h)	147.6 $\pm$ 13.6	106.5 $\pm$ 10.1***	154.1 $\pm$ 17.8	145.3 $\pm$ 13.4
SCr (μ mol/L)	93.7 $\pm$ 13.2	76.6 $\pm$ 11.8***	92.3 $\pm$ 14.5	94.1 $\pm$ 12.9
TG (mmol/L)	1.86 $\pm$ 0.67	1.94 $\pm$ 0.56	1.91 $\pm$ 0.58	1.83 $\pm$ 0.71
TC (mmol/L)	6.63 $\pm$ 1.58	6.24 $\pm$ 1.30	6.52 $\pm$ 1.41	6.87 $\pm$ 1.20
LDL-CH (mmol/L)	4.17 $\pm$ 1.50	4.62 $\pm$ 1.43	4.58 $\pm$ 1.21	4.23 $\pm$ 1.14

UAER, Urinary Albumin Excretion Rates; α -LA group, α lipoic acid therapy; DM group, Diabetic Mellitus routine therapy; SCr, Serum Creatinine; TG, Triglyceride; TC, Total Cholesterol; LDL-CH, Low-Density Lipoprotein.

Mean $\pm$ standard deviation (SD).

* P <.05 vs intra-group corresponding item.

** P <.05 vs inter-group corresponding item.

TABLE 2 Comparison of markers of UAER, renal function and serum lipid

Parameters	α -LA group (n=33)		DM group (n=29)	
	Before	After	Before	After
MDA (μ mol/L)	6.14 $\pm$ 0.58	4.02 $\pm$ 0.52***	5.84 $\pm$ 0.46	5.37 $\pm$ 0.50
SOD (U/mL)	68.78 $\pm$ 8.82	128.81 $\pm$ 4.40***	63.24 $\pm$ 8.26	67.40 $\pm$ 7.71
FMD (%)	9.14 $\pm$ 2.09	14.8 $\pm$ 1.85***	8.22 $\pm$ 2.13	8.73 $\pm$ 1.80

α -LA group, α Lipoic Acid therapy; DM group, Diabetic Mellitus routine therapy; SOD, Superoxide Dismutase; MDA, malondialdehyde; FMD, Flow-Mediated Dilation.

Mean $\pm$ standard deviation (SD).

* P <.05 vs intra-group corresponding item.

** P <.01 vs inter-group corresponding item.

TABLE 3 Comparison of markers of MDA, SOD and FMA

FIGURE 1 Correlation between the improvement of FMD and variation in MDA and SOD. The improvement of FMD was evaluated by FMD after α -LA treatment deducting FMD before treatment, Δ FMD = FMD_{after} - FMD_{before}. The variations in MDA and SOD were calculated in the same way (Δ MDA = MDA_{after} - MDA_{before}, Δ SOD = SOD_{after} - SOD_{before}). The correlation analysis between mentioned Δ FMD vs Δ MDA and Δ SOD were carried out with the SPSS 18.0 software, respectively. (A) Correlation of Δ FMD and Δ MDA with α -LA treatment; (B) Correlation of Δ FMD and Δ SOD with α -LA treatment

"household" uExo markers, was further measured. We found that the expression of CD63 is much higher in normoalbuminuria group compared with microalbuminuria group. Interestingly, we also observed that upon administration of α -lipoic acid, the expression of CD63 in uExos was significantly decreased only in normoalbuminuria group but not in microalbuminuria group (Figure 2B-D).

4 | DISCUSSION

Diabetes mellitus is a multi-faceted metabolic disorder involving an increased oxidative stress which contributes to the pathogenesis of this debilitating disease.⁵ Furthermore, the increase in reactive oxygen species (ROS) will in turn drive vascular complications in DN.³

α -Lipoic acid is a characteristic anti-oxidant with both hydrophilic and hydrophobic properties. Currently, α -LA is widely used in the clinic for diabetic peripheral neuropathy, whose renoprotection has been widely recognized. Based on the strengthening effect in antioxidant ability, oral administration of α -LA can provide some protection

effect against glomerular podocyte injury in type 2 diabetics.⁶ In our study, the therapeutic strategy of intravenous administration of α -LA for 14 days demonstrated similar promising results. The individuals in the α -LA group showed significant clinical improvement, such as a decrease in serum Cr, UAER, and MDA, and conversely, an increase in

SOD. All results mentioned above indicated that α -LA could improve early general oxidative stress level and reduce the urinary microalbumin in a short period.

As a broadly applicable method used for the examination of endothelial function, flow-mediated dilation (FMD) is predominantly dependent on the endothelium-derived NO and related subsequent biochemical events, which are key factors in oxidative stress.¹⁰ In this study, we found that FMD was associated with age, disease course, systolic pressure (SBP), TG, UAER, MDA, and SOD, which was consistent with previous reports.^{12,13} Moreover, FMD recover was observed profoundly in the α -LA group, and the improvement of FMD was positively correlated with MDA and SOD. Dugan and colleagues demonstrated that mitochondrial-derived ROS, which is maintained by a feed-forward AMP kinase activation cascade, is reduced in diabetes but the total urinary ROS was increased.^{14,15} Recently, the role of NADPH oxidase (NOX) isoform NOX4 as a key factor in kidney disease associated with podocyte dysfunction was demonstrated in podocyte-specific inducible NOX4 transgenic mouse.¹⁶ Combined with our results, it is clear that short-term administration of α -LA could protect the kidney in early DN stage, reduce general oxidative stress and inhibit the pathogenesis of DN by attenuating renal NOX4 activity and recovering endothelium-dependent vasodilator function.

Microalbuminuria is widely used as a predictive and prognostic biomarker for DN. However, it is still necessary to identify novel biomarkers which can both monitor the progression in early stage and predicate clinical outcomes.^{17,18} A key finding in the present study is that the level of CD63-positive exosome was significantly increased in urine at early stage of diabetic renal injury. Exosomes are characterized by several molecules as tetraspanins (CD9, CD63), ALIX (PDCD6IP), and ceramides.¹⁹ We observed that the expression of CD63 is significantly increased in normoalbuminuria group than that in microalbuminuria group, which might be a result of slight compensatory increase in GFR in the very beginning of DN. Our result indicates that the decreased urine exosome level might indicate an unfavorable prognosis of DN. After administration of α -lipoic acid, the glomerular function improves to some extent so that the expression of CD63 in uExos plunged in the normoalbuminuria group. However, there is no significant difference in the microalbuminuria group with or without α -lipoic acid treatment, possibly because the limited improvement in GFR was observed.

This study has several limitations. First, proteins and nucleic acids, such as Wilm's tumor (WT)-1, podocalyxin, α 1-antitrypsin, aminopeptidase N, vasorin precursor, ceruloplasmin, and miR-145 were not examined in our study. These can be found in urinary exosomes and could reflect the status of glomerular disease like DN.^{9,20} So, the detailed changes in the microalbuminuria group with or without α -lipoic acid treatment were not observed. Second, sample size should be enlarged before a firm conclusion is reached. Thirdly, the exact mechanism of how the lessening general oxidative stress affecting the CD63-positive exosomes is still unclear. Further studies are necessary to confirm our result.

In summary, our study provides evidence that short-term α -LA could protect the kidney in early DN stage by attenuating general

oxidative stress. Furthermore, the appearance of urine CD63-positive exosomes could be a novel biomarker for both early diagnosis and treatment predication in diabetes. It would be helpful for the prevention, treatment, and earlier diagnosis of diabetic individuals at risk of developing nephropathy.

ACKNOWLEDGMENTS

This work was supported by the Shanghai Municipal Health Bureau Project (15ZR1437900), Natural Science Research Project of Changning District (CNKW2014Z01), Leading Disciplines of Medical Laboratory in Changning District (2012QZK01) and Tongren Hospital grant (grant TR201407, TRYJ201506).

REFERENCES

- Wang L, Wu CG, Fang CQ, et al. The protective effect of alpha-Lipoic acid on mitochondria in the kidney of diabetic rats. *Int J Clin Exp Med*. 2013;6:90–97.
- Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes*. 2005;54:1615–1625.
- Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature*. 2001;414:813–820.
- Shao N, Kuang HY, Wang N, et al. Relationship between oxidant/antioxidant markers and severity of microalbuminuria in the early stage of nephropathy in type 2 diabetic patients. *J Diabetes Res*. 2013;2013:232404.
- Golbidi S, Badran M, Laher I. Diabetes and alpha lipoic Acid. *Front Pharmacol*. 2011;2:69.
- Lin H, Ye S, Xu J, Wang W. The alpha-lipoic acid decreases urinary podocalyxin excretion in type 2 diabetics by inhibiting oxidative stress in vivo. *J Diabetes Complications*. 2015;29:64–67.
- Yi X, Nickleleit V, James LR, Maeda N. alpha-Lipoic acid protects diabetic apolipoprotein E-deficient mice from nephropathy. *J Diabetes Complications*. 2011;25:193–201.
- Glasscock RJ. Debate: CON position. Should microalbuminuria ever be considered as a renal endpoint in any clinical trial? *Am J Nephrol*. 2010;31:462–465; discussion 6–7.
- Salih M, Zietse R, Hoorn EJ. Urinary extracellular vesicles and the kidney: biomarkers and beyond. *Am J Physiol Renal Physiol*. 2014;306:F1251–F1259.
- Corretti MC, Anderson TJ, Benjamin EJ, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol*. 2002;39:257–265.
- Zubiri I, Posada-Ayala M, Sanz-Maroto A, et al. Diabetic nephropathy induces changes in the proteome of human urinary exosomes as revealed by label-free comparative analysis. *J Proteomics*. 2014;96:92–102.
- Knight DR Jr, Smith AH, Schroeder RL, et al. Effects of age on noninvasive assessments of vascular function in nonhuman primates: implications for translational drug discovery. *J Transl Med*. 2013;11:101.
- Moens AL, Goovaerts I, Claeys MJ, Vrints CJ. Flow-mediated vasodilation: a diagnostic instrument, or an experimental tool? *Chest*. 2005;127:2254–2263.
- Dugan LL, You YH, Ali SS, et al. AMPK dysregulation promotes diabetes-related reduction of superoxide and mitochondrial function. *J Clin Invest*. 2013;123:4888–4899.
- Sharma K, Karl B, Mathew AV, et al. Metabolomics reveals signature of mitochondrial dysfunction in diabetic kidney disease. *J Am Soc Nephrol*. 2013;24:1901–1912.

16. You YH, Quach T, Saito R, Pham J, Sharma K. Metabolomics reveals a key role for fumarate in mediating the effects of NADPH oxidase 4 in diabetic kidney disease. *J Am Soc Nephrol*. 2016;27:466–481.
17. Delic D, Eisele C, Schmid R, et al. Urinary exosomal miRNA signature in Type II diabetic nephropathy patients. *PLoS ONE*. 2016;11:e0150154.
18. Lambers Heerspink HJ, de Zeeuw D. Debate: PRO position. Should microalbuminuria ever be considered as a renal endpoint in any clinical trial?. *Am J Nephrol*. 2010;31:458–461; discussion 68.
19. Junker K, Heinzelmann J, Beckham C, Ochiya T, Jenster G. Extracellular vesicles and their role in urologic malignancies. *Eur Urol*. 2016;70:323–331.
20. Erdbrugger U, Le TH. Extracellular vesicles in renal diseases: more than novel biomarkers? *J Am Soc Nephrol*. 2016;27:12–26.

How to cite this article: Sun H, Yao W, Tang Y, et al. Urinary exosomes as a novel biomarker for evaluation of α -lipoic acid's protective effect in early diabetic nephropathy. *J Clin Lab Anal*. 2017;31:e22129. <https://doi.org/10.1002/jcla.22129>