Abdominal Radiology (2019) 44:1850–1857
https://doi.org/10.1007/s00261-019-01908-5

KIDNEYS, URETERS, BLADDER, RETROPERITONEUM

# Quantitative contrast-enhanced ultrasound of renal perfusion: a technology for the assessment of early diabetic nephropathy in cynomolgus macaques with type 2 diabetes mellitus

Jieli Luo[1] · Jianshe Chen[1] · Yang Sun[1] · Hang Zhou[1] · Kailun Xu[2] · Fengbo Huang[2] · Pintong Huang[1]

Published online: 29 January 2019


## Abstract

**Purpose** The aim of this study was to investigate the effectiveness of contrast-enhanced ultrasound (CEUS) in predicting early nephropathy in cynomolgus macaques with spontaneous type 2 diabetes mellitus (T2DM).

**Methods** Six cynomolgus macaques with spontaneous T2DM and six normal cynomolgus macaques (Group 1) were included in this study. The time–intensity curve was used to obtain parameters such as peak values, red blood volume (RBV), red blood flow (RBF), time to peak (TTP), and mean transit time (MTT). Biopsy renal tissue samples were assessed histopathologically. Six cynomolgus macaques with spontaneous T2DM were subgrouped into T2DM without nephropathy group (Group 2) and T2DM with nephropathy group (Group 3) based on histopathological findings.

**Results** Peak value had the largest area under the curve comparing with RBF, RBV, TTP, MTT. The sensitivity and specificity of peak value with cut-off value of 38.65 dB for the diagnosis of DN were 98.3% and 83%, respectively. Peak value, RBV, and RBF in Group 3 was significantly decreased compared with Group 1 and Group 2 ($P=0.000$, $x^2=23.99$; $P=0.003$, $x^2=9.14$; $P=0.02$, $x^2=5.14$).

**Conclusions** The perfusion parameter of peak value in CEUS might be useful in predicting early diabetic nephropathy in spontaneous T2DM cynomolgus macaques.

**Keywords** Diabetes · Nephropathy · Cut-off value · Histopathology · Glomerular filtration rate

## Introduction

With the rapid economic development and lifestyle intervention, more and more people lay an emphasis on public health crisis—diabetes, particularly in type 2 diabetes mellitus (T2DM) [1, 2]. One of the most important diabetic microvascular complications is diabetic nephropathy (DN) [3]. The process of renal changes in diabetes mellitus is ranging from early hyperfiltration with an increased glomerular filtration rate (GFR) to late nephrosclerosis and fibrosis with azotemia. An estimated 30–40% of T2DM patients are afflicted with nephropathy [4]. DN is considered one of the risk factors for the happening of cardiovascular events and may cause high mortality and morbidity [5]. Early identification of renal changes increases the chance of preventing overt nephropathy from incipient nephropathy which has important practical implications for improving the outcome [6]. Heart Outcomes Prevention Evaluation (HOPE) study also showed that treatment for incipient or nephropathy was aimed at reducing the risk of disease progression and preventing cardiovascular complications [7].

The definition of early DN is that preclinical is associated with few clinical or laboratory findings. Renal pathology change is the golden standard of DN. Clinical practice guidelines for treatment type 2 diabetes published by the American Diabetes Association and the Canadian Diabetes Association recommend screening for microalbuminuria when diagnosing type 2 diabetes [8]. However, some patients with T2DM had been estimated GFR declined

✉ Pintong Huang
huangpintong@zju.edu.cn

Jieli Luo
2517118@zju.edu.cn

[1] Department of Ultrasound, The Second Affiliated Hospital of Zhejiang University School of Medicine, No. 88 Jiefang Road, Hangzhou 310009, Zhejiang, China

[2] Department of Pathology, The Second Affiliated Hospital of Zhejiang University School of Medicine, Zhejiang 310009, China

to < 60 ml/min/1.73 $m^2$ without going through the stage of microalbuminuria [9]. Two-dimensional ultrasound is not sensitive enough in detecting early renal changes like the renal size and parenchyma flow. Cynomolgus macaque is the best candidate as animal models of human disease and behavior have a close evolutionary relationship.

However, contrast-enhanced ultrasound (CEUS) has significant advantages over CT, ultrasound, and blood test due to the absence of ionizing radiations, lack of the risk of nephrotoxicity, and cost savings [10]. CEUS is established as a significant advancement in imaging and an effective, repeatable, noninvasive, economic imaging technique [11]. Contrast agents are excreted into the respiratory system rather than the collecting system.

Some studies had shown that CEUS had been widely employed in diagnosis and evaluation of renal diseases. But it is uncertain whether CEUS could be sensitive enough to distinguish early DN from T2DM. The goal of our study was to determine the renal CEUS findings in DN to define a possible role for CEUS in the early identification with suspected DN.

## Materials and methods

All applicable national guidelines for the care and use of animals were followed. Six cynomolgus macaques with spontaneous T2DM (males, Guangdong Landao Biological Technology Company, China) and six normal cynomolgus macaques (males, Suzhou Xishan Zhongke Experimental Animal Co., Ltd., China) were selected for the present study. All cynomolgus macaques were fed standard feed pellets and fruit. Six cynomolgus macaques with spontaneous T2DM underwent hyperinsulinemic-euglycemic clamp test prior to the CEUS examination. The body weights of cynomolgus macaques were measured in kilograms by intramuscular injection of 10 mg/kg ketamine for anesthesia.

Traditional B-mode ultrasound was performed successfully on bilateral kidneys in each cynomolgus macaque using a MyLab Twice (Esaote, Genova, Italy) machine equipped with LA332 (3–11 MHz) transducer. The maximum diameters of left and right kidney including length, width, and height were measured in longitudinal and transverse view. The same frequency pulsed Doppler ultrasound was used to examine intrarenal arteries with a standard 90 degree skin-probe angle. The resistive index (RI) = (peak systolic frequency shift − minimum diastolic frequency shift)/peak systolic frequency shift. The RI value of each kidney was calculated as the average value. Three to five waveforms were recorded in three different areas of the kidneys. An average renal RI value for each animal was obtained by averaging the RI of the left and right kidneys. The person who performed ultrasound was blinded to the laboratory findings.

CEUS procedure was performed using MyLab Twice (Esaote, Genova, Italy) machine equipped with Contrast Tuned Imaging technology (CnTI) after Doppler ultrasound. LA332 (3–11 MHz) linear array transducer was used with the acoustic pressure of 50 kPa in each cynomolgus macaque. The contrast agent SonoVue® (Bracco SpA, Milan, Italy) in this study was a lyophilized powder, which was reconstituted by adding 5 ml of 0.9% saline and gently shaking the vial by hand to form a homogeneous microbubble suspension. A 20-gauge needle cannula was inserted into femoral vein and 0.03 ml/kg of SonoVue was injected as a bolus followed by 3 ml of saline flush. All CEUS were performed in the longitudinal view of each kidney and the transducer was manually positioned during each imaging procedure at the same position; the entire movie sequence (at least 3 min) was stored on magnetic optical disks for analysis. After 30 min later, CEUS on another kidney was performed.

The cine loop of each kidney was analyzed three times using Qontrast 4.0 software. The region of interest (ROI) was drawn on the superficial peripheral renal cortex at the same depth and in similar size without including medulla performed by two radiologists. Once the perfusion processing was performed, the arrival of contrast within the analysis ROI was automatically detected. An echo-power signal is a time–intensity curve derived from either a pixel or a region of interest. The quantitative parameters of each kidney were automatically obtained including peak value, time to peak (TTP), red blood volume (RBV) red blood flow (RBF), and mean transit time (MTT) by the system from the time–intensity curve (TIC), and the average value of parameters was calculated. The person who performed CEUS was blinded to the laboratory findings.

Blood samples were collected following an overnight 12-h fast at 8 am, and the animal keeper collected 24-h urine samples. Fast plasma glucose (FPG), blood urea nitrogen (BUN), serum creatinine (s-Cr), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and glycohemoglobin $A_{1c}$ ($HbA_{1c}$) were measured. Urinary albumin excretion (UAE) was measured on at least two out of a total of three occasions (every other day during a week). Animals were stratified as having either nonmicroalbuminuria (UAE < 30 mg/24 h) or microalbuminuria (UAE < 300 mg/24 h and UAE ≥ 30 mg/24 h) according to UAE level.

Ultrasound-guided core needle biopsy was performed in both kidneys of each cynomolgus macaque at the end of examination. Partial renal biopsy samples were prepared for hematoxylin–eosin staining (HE). Other partial renal samples were fixed with 10% neutral formalin for periodic acid Schiff staining (PAS). The slides were observed under light microscopy. The remaining renal biopsy samples were fixed with 2.5% glutaraldehyde in phosphate buffer for over 4 h

which were prepared to transmission electron microscope (TEM) to assess ultrastructural alternations in the glomerulus. The early DN was defined as thickened glomerular basement membranes, the presence of diffuse mesangial expansion, or Kimmelstiel–Wilson nodules histologically. Six cynomolgus macaques with T2DM were subgrouped into two groups according to renal histopathological result, T2DM without nephropathy group (Group 2), and T2DM with nephropathy group (Group 3).

Statistical analysis was performed with SPSS 22.0 software (IBM, USA) and GraphPad Prism 5.0; $P<0.05$ was considered statistically significant. The measurement data were tested for normality using the Kolmogorov–Smirnov test. The Kruskal–Wallis $H$ test was used to compare the different parameters among three groups when the data had a nonnormal distribution. The Mann–Whitney $U$ test was used to compare the different parameters with two groups when the data had a nonnormal distribution. The receiver operating characteristic (ROC) curve was conducted to get cut-off point of different parameters.

## Results

There were no significant differences of renal length, width, height between left and right kidney ($P>0.05$, for all). Also there were no significant differences of renal length, width, height among three groups ($P>0.05$, for all). There was no association of variables with RI at radiologist 1 or radiologist 2. The RI of Group 1, Group 2, and Group 3 were $0.68\pm0.09$, $0.65\pm0.06$, and $0.67\pm0.04$, respectively. There were no significant differences of renal RI between left and right kidney ($P>0.05$, for all). Also there were no significant differences of RI among three groups ($P>0.05$, for all). Renal diameter and RI are shown in Fig. 1a, b.

Representative CEUS perfusion curves in Group 1, Group 2, and Group 3 are shown in Fig. 2. The ROC curves for accuracy of peak value, TTP, RBV, MTT, RBF in diagnosing DN are plotted in Fig. 3. The sensitivity and specificity of peak value with cut-off value of 38.65 dB for the diagnosis of DN were 98.3% and 83%, respectively. While TTP and MTT in Group 3 showed no significant differences compared to Group 1 and Group 2 in CEUS ($P>0.05$, for both), peak value, RBV, and RBF in Group 3 was significantly decreased compared with Group 1 and Group 2 ($P=0.000$, $x^2=23.99$; $P=0.003$, $x^2=9.14$; $P=0.02$, $x^2=5.14$).

There was no significant difference between the BW of each group (Fig. 1c). The general characteristics of cynomolgus macaques in Group 1, Group 2, and Group 3 are summarized in Table 1. One cynomolgus macaque was non-microalbuminuria and another cynomolgus macaque was microalbuminuria in Group 3. There was no microalbuminuria in Group 1 and Group 2. All the cynomolgus macaques in Group 3 were DN.

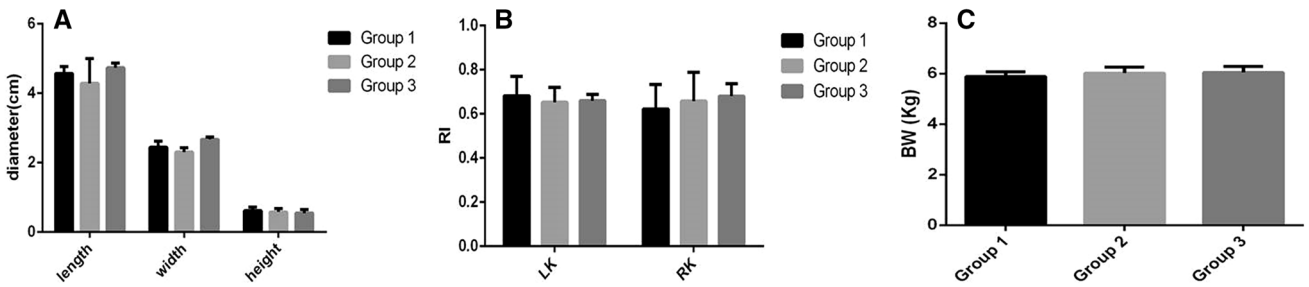


**Fig. 1** The schematic diagram shows the length, width, height of kidney (**a**), bilateral RI (**b**), and weight (**c**) in Group 1, Group 2, and Group 3

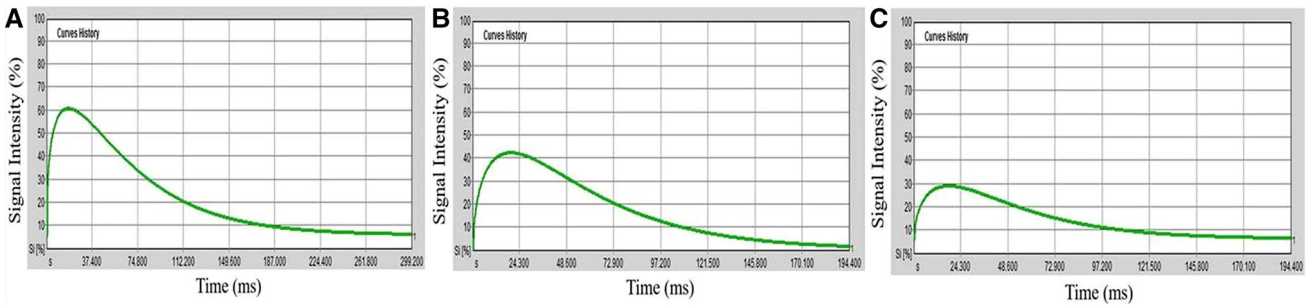


**Fig. 2** Representative CEUS perfusion curves according to intensity signal at different times of perfusion. **a** Group 1, **b** Group 2, **c** Group 3. Each curve contains ascending slope, peak, and descending slope

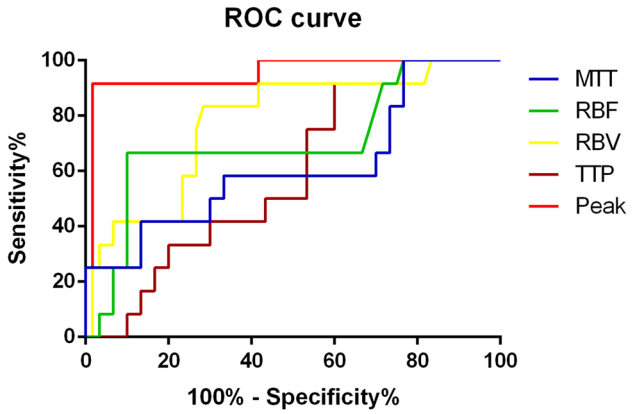


**Fig. 3** ROC of CEUS quantitative index including peak value, TTP, RBV, MTT, RBF (area under curve)

Two cynomolgus macaques in Group 3 (4 kidneys) had diabetic nephropathy. Six cynomolgus macaques in Group 1 (12 kidneys) and four cynomolgus macaques in Group 2 (8 kidneys) had not nephropathy. HE and PAS stain showed normal histological structure of the glomerulus in Group 1 and Group 2. HE and PAS stain showed extracellular matrix expansion, glomerular basement membrane thickening, and Kimmelstiel–Wilson lesion in Group 3. Transmission electron micrographs of glomerulus in monkeys showed podocyte foot process effacement, glomerular basement membrane thickening, and increased lysosomal accumulation in Group 3. Renal histopathological results are illustrated in Figs. 4 and 5.

## Discussion and conclusions

The leading cause of end-stage renal disease throughout the world is DN [12–14]. This life-threatening condition often requires renal dialysis or renal transplantation [15–23]. Early detection of nephropathy and early intervention are the key to improving prognosis for patients with diabetic nephropathy. Many articles had reported that end-stage kidneys were usually small and irregular. However, morphological change was not absolute and specific in the development of DN, and the measurement of renal morphology just played an accessory diagnostic value for DN [24]. Our results also supported the conclusion that the evaluation of renal morphology was not accurate in predicting development and progression of DN.

Microalbuminuria had been considered to be an easy, convenient, noninvasive, and major index to judge the progression of DN; the price of microalbuminuria detection was one-fifth of the CEUS test, but the specificity, accuracy, and sensitivity still remain questionable for early identification of DN [25, 26]. In our study, one cynomolgus macaque in Group 3 was nonmicroalbuminuria. But the pathology showed DN. Sometimes, the progression of DN is not necessarily paralleled with the progression of UAE [27]. DN is theoretically characterized by renal perfusion. RI of renal vessel is an invasive and traditional method to measure renal perfusion which is an indication to renal biopsy. However, distal perfusion index could not be retrieved properly because of reduced peripheral perfusion which was not detected by the ultrasound machine accurately. Therefore, further dynamic renal tissue perfusion measurement analyses were performed solely by proximal perfusion index according to Stoperka et al. study [28]. The vessel-transducer angle impacts on perfusion quantification.

**Table 1** Levels of UAE, FPG, BUN, Cr, TC, LDL, HDL, HbA1c in Group 1, Group 2, and Group 3

| | Group 1 (12 kidneys) | Group 2 (8 kidneys) | Group 3 (4 kidneys) |
|---|---|---|---|
| UAE (mg/24 h) | 16.83 ± 3.68 | 16.58 ± 2.54 | 105.25 ± 96.26[ab] |
| FPG (mmol/l) | 5.33 ± 0.27 | 16.40 ± 3.97[a] | 20.25 ± 1.48[a] |
| BUN (mmol/l) | 5.42 ± 0.35 | 7.70 ± 1.57[a] | 8.15 ± 0.07[a] |
| s-Cr (μmol/l) | 45.33 ± 9.99 | 83.25 ± 4.79[a] | 89.50 ± 2.12[a] |
| TC (mmol/l) | 4.03 ± 0.50 | 6.60 ± 0.51[a] | 7.05 ± 0.49[a] |
| LDL-C (mmol/l) | 2.92 ± 0.08 | 3.60 ± 0.36[a] | 3.65 ± 0.07[a] |
| HDL-C (mmol/l) | 1.33 ± 0.42 | 1.48 ± 0.26 | 1.70 ± 0.14 |
| $HbA_{1c}$(%) | 5.27 ± 0.69 | 5.15 ± 1.16 | 5.75 ± 0.49 |

Values are presented as mean ± standard error

*UAE* urinary albumin excretion, *FPG* fasting plasma glucose, *BUN* blood urea nitrogen, *s-Cr* serum creatinine, *TC* total cholesterol, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *HbA1c* glycohemoglobin A1c

[a]Significant change at $P < 0.05$ in comparison with Group 1

[b]Significant change at $P < 0.05$ in comparison with Group 2

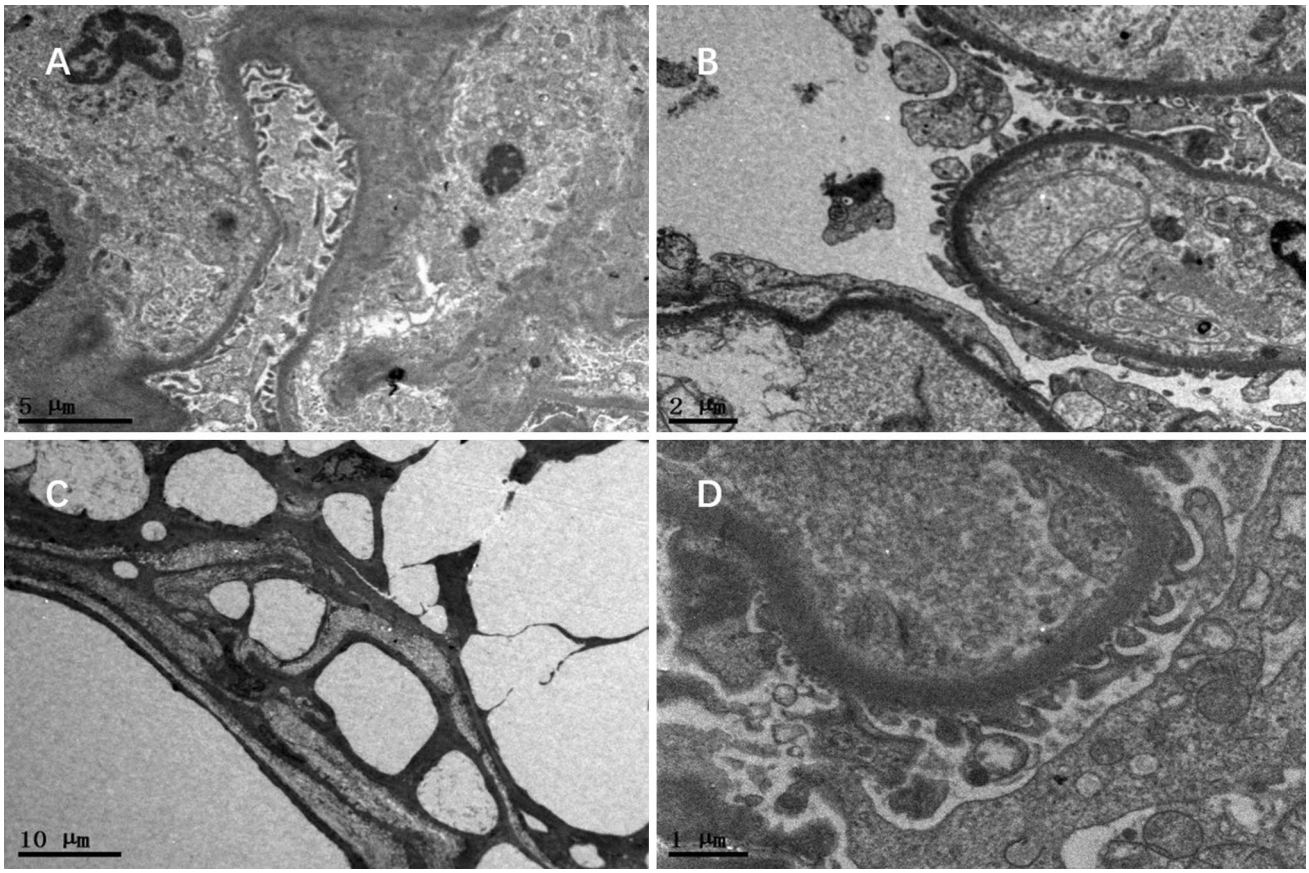


**Fig. 4** **a** Photomicrograph of renal tissue section in Group 1 and Group 2 showing no histopathological alteration and the normal histological structure of the glomerulus (HE × 200). **b** Photomicrograph of renal tissue section in Group 1 and Group 2 showing no histopathological alteration and the morphology of the kidney is normal (PAS × 200). **c** Photomicrograph of renal tissue section in Group 3 showing extracellular matrix expansion, accumulation, and glomerular basement membrane thickening (HE × 200). **d** Photomicrograph of renal tissue section in Group 3 showing glomerular basement membrane and mesangial expansion to the so-called Kimmelstiel–Wilson lesions (arrowhead) (PAS × 200)

Angle differences might impact on the dynamic renal tissue perfusion repeatability because it influenced velocity measurement. We tried to employ a standard 90° skin-probe angle. There was no association of variables with RI in different groups. It is well known that the gold standard for the diagnosis of diabetic nephropathy is pathology, but renal puncture is an invasive method which sometime caused the complications. Moreover, a large amount of patients with renal involvement are not biopsied in clinical [29]. All in all, renal biopsy is not suitable as a screening tool.

The vital importance in the progression of DN was microvascular function and structural damage [30]. CEUS has shown a promising diagnostic imaging technique to noninvasively and repetitively quantify tissue perfusion on a capillary level [31, 32]. Early identification of DN will prompt early referral and initiation of renal protective therapy [33, 34]. Although CEUS is not widely used in nephritic research, CEUS is economical, convenient, and time saving compared to CT and MRI. CEUS opened up a new sight in the characterization of microvasculature [35, 36]. Qontrast 4.0 analysis software allowed precise quantification of the parameters in a certain region of interests accurately and conveniently. The contrast agent used in CEUS consist of tiny gas-filled microbubbles of size similar to red blood cell tracers, which is exhaled through the lung. According to different blood volumes of each kidney, the intensity of the CEUS signal depends on the concentrations of contrast agents that permeate into the kidney tissue. CEUS has been used excellent technique for the assessment for renal microvascular perfusion in patients with chronic kidney injury and guidance for renal biopsy. In this study, we investigated the effectiveness of CEUS in detecting early DN. Our data showed that by comparison with Group 1 and Group 2, quantitative indexes of DN were characterized by reduced peak, indicating that less contrast microbubbles entered the renal cortex microvascular bed. Further, ROC curve indicated that the cut-off value of peak set as 38.65 dB, the diagnostic accuracy, and specificity were satisfactory. Our

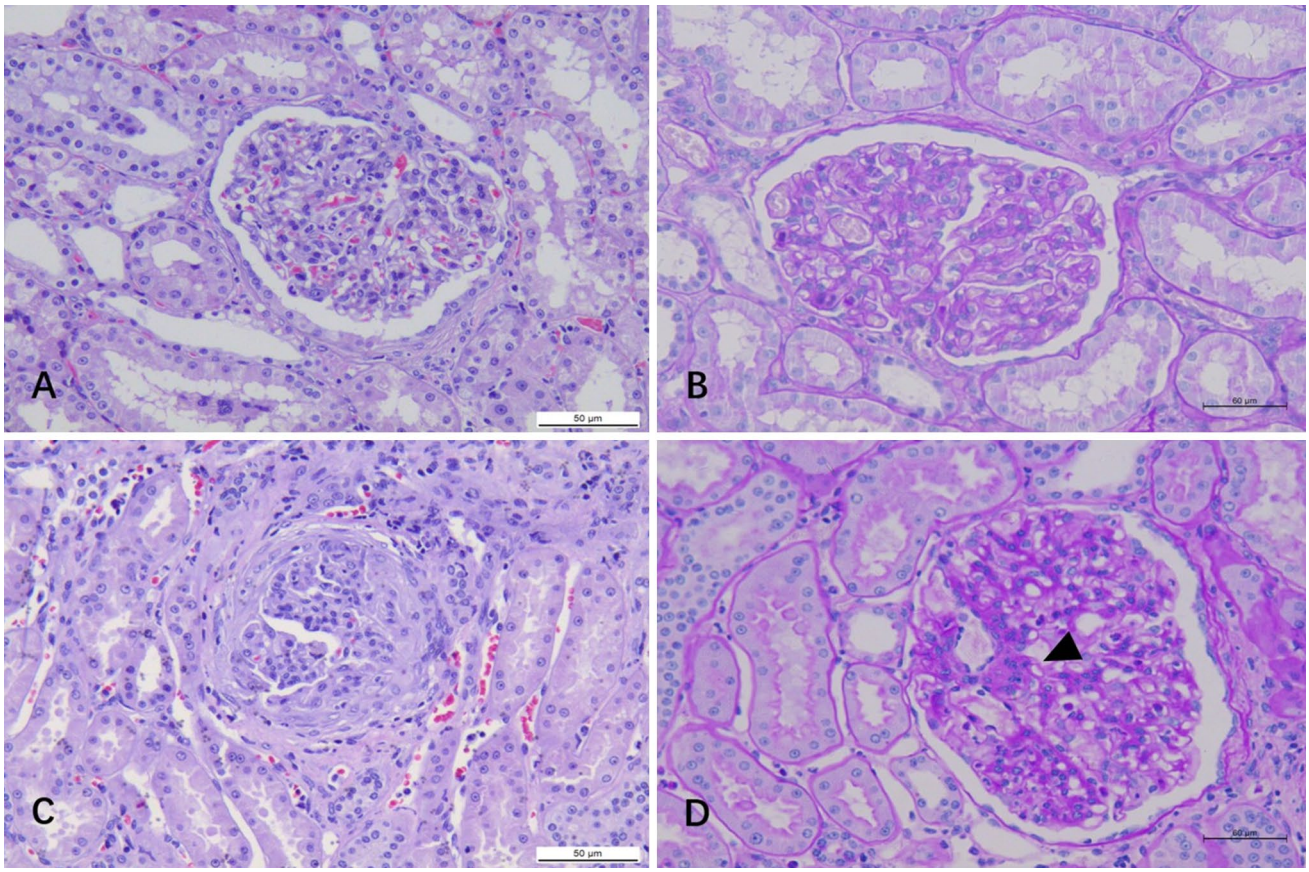


**Fig. 5** Transmission electron micrographs of glomerulus in monkeys. **a** Normal podocyte in Group 1 and Group 2 (×1750). **b** Podocyte foot process effacement in Group 3 (×2500). **c** Glomerular basement membrane thickening in Group 3 (×790). **d** Increased lysosomal accumulation in Group 3 (×5900)

results suggested that quantitative index of peak provided new insight to quantify the impaired microcirculation perfusion in the early stage of DN.

As it has been well known, intra- and inter-species differences make it difficult to extrapolate low ranking animal data to humans. However, the close evolutionary relationship between humans and nonhuman primates suggest that they share many of the specific genetic mechanisms involved in determining differential susceptibility to disease. Cynomolgus macaques are physiologically and genetically very similar to humans [37]. So cynomolgus macaque is the best candidate as animal models of human disease and behavior. There are some limitations of using CEUS to assess renal microvascular perfusion in this study. The glomerular filtration rate of each monkey was not performed in this study and is the subject in our ongoing research. Secondly, though the bilateral kidneys of each monkey were performed using US-guided core needle biopsy for pathogical examination, the sample volume is still small in this study. Thirdly, 0.03 ml/kg of SonoVue was injected in this experiment. TIC and quantitative analysis of CEUS will be affected by the dose of ultrasound contrast agents. The last but not the least, CEUS was performed on both sides in each monkey, but US-guided core needle biopsy for pathological examination was only performed on one side in each monkey. Pathological result could not totally represent CEUS result.

Peak value might be a valuable quantitative index for evaluating early perfusion changes in T2DM with DN. CEUS quantitative analysis will be accepted as the test of choice for detection of T2 with DN.

**Acknowledgements** Dr. Pintong Huang is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Author contributions** PH designed this study. JL, JC, YS, KX, and FH acquired the data. JL, JC, and YS interpreted the data. JL wrote the main manuscript text. HZ edited the manuscript. All authors reviewed the manuscript. All authors have approved of the final version of the manuscript.

**Funding** This study was funded by the National Natural Science Foundation of China (Grant Nos. 81420108018, 81527803) of China.

**Data availability** The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Compliance with ethical standards

**Conflict of interest** The authors declare that there is no duality of interest associated with this manuscript.

## References


1. Rutten G, Alzaid A (2018) Person-centred type 2 diabetes care: time for a paradigm shift. Lancet Diabetes Endocrinol 6 (4):264-266. https://doi.org/10.1016/S2213-8587(17)30193-6
2. Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N, Shaw W, Law G, Desai M, Matthews DR, Group CPC (2017) Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. N Engl J Med 377 (7):644-657. https://doi.org/10.1056/NEJMoa1611925
3. Hanssen NMJ, Scheijen J, Jorsal A, Parving HH, Tarnow L, Rossing P, Stehouwer CDA, Schalkwijk CG (2017) Higher Plasma Methylglyoxal Levels Are Associated With Incident Cardiovascular Disease in Individuals With Type 1 Diabetes: A 12-Year Follow-up Study. Diabetes 66 (8):2278-2283. https://doi.org/10.2337/db16-1578
4. Lipska KJ, Hirsch IB, Riddle MC (2017) Human Insulin for Type 2 Diabetes: An Effective, Less-Expensive Option. JAMA 318 (1):23-24. https://doi.org/10.1001/jama.2017.6939
5. Tanada Y, Okuda J, Kato T, Minamino-Muta E, Murata I, Soga T, Shioi T, Kimura T (2017) The metabolic profile of a rat model of chronic kidney disease. PeerJ 5:e3352. https://doi.org/10.7717/peerj.3352
6. Zhong C, Chen Z, Luo X, Wang C, Jiang H, Shao J, Guan M, Huang L, Huang X, Wang J (2018) Corrigendum to "Barhl1 is required for the differentiation of inner ear hair cell-like cells from mouse embryonic stem cells" [Int. J. Biochem. Cell Biol. 96 (2018) 79-89]. Int J Biochem Cell Biol 97:128-129. https://doi.org/10.1016/j.biocel.2018.02.009
7. Effects of ramipril on cardiovascular and microvascular outcomes in people with diabetes mellitus: results of the HOPE study and MICRO-HOPE substudy. Heart Outcomes Prevention Evaluation Study Investigators (2000). Lancet 355 (9200):253-259
8. Bodzek P, Olejek A, Adamusiak-Kutrowska I, Zamlynski J, Wolnicka B (2002) [Evaluation of the health care of diabetic pregnant women and their newborns in 1992-2001 in the Obstetrics and Gynaecology Department in Bytom]. Wiad Lek 55 Suppl 1:43-49
9. Moriya T, Tanaka S, Sone H, Ishibashi S, Matsunaga S, Ohashi Y, Akanuma Y, Haneda M, Katayama S (2017) Patients with type 2 diabetes having higher glomerular filtration rate showed rapid renal function decline followed by impaired glomerular filtration rate: Japan Diabetes Complications Study. J Diabetes Complications 31 (2):473-478. https://doi.org/10.1016/j.jdiacomp.2016.06.020
10. Derlin T, Gueler F, Brasen JH, Schmitz J, Hartung D, Herrmann TR, Ross TL, Wacker F, Wester HJ, Hiss M, Haller H, Bengel FM, Hueper K (2017) Integrating MRI and Chemokine Receptor CXCR4-Targeted PET for Detection of Leukocyte Infiltration in Complicated Urinary Tract Infections After Kidney Transplantation. J Nucl Med 58 (11):1831-1837. https://doi.org/10.2967/jnumed.117.193037
11. Berzigotti A, Nicolau C, Bellot P, Abraldes JG, Gilabert R, Garcia-Pagan JC, Bosch J (2011) Evaluation of regional hepatic perfusion (RHP) by contrast-enhanced ultrasound in patients with cirrhosis. J Hepatol 55 (2):307-314. https://doi.org/10.1016/j.jhep.2010.10.038
12. Panduru NM, Forsblom C, Saraheimo M, Thorn LM, Gordin D, Elonen N, Harjusalo V, Bierhaus A, Humpert PM, Groop PH, FinnDiane Study G (2017) Urinary liver-type fatty acid binding protein is an independent predictor of stroke and mortality in individuals with type 1 diabetes. Diabetologia 60 (9):1782-1790. https://doi.org/10.1007/s00125-017-4328-x
13. Marso SP, McGuire DK, Zinman B, Poulter NR, Emerson SS, Pieber TR, Pratley RE, Haahr PM, Lange M, Brown-Frandsen K, Moses A, Skibsted S, Kvist K, Buse JB, Group DS (2017) Efficacy and Safety of Degludec versus Glargine in Type 2 Diabetes. N Engl J Med 377 (8):723-732. https://doi.org/10.1056/NEJMoa1615692
14. Malik S, Suchal K, Khan SI, Bhatia J, Kishore K, Dinda AK, Arya DS (2017) Apigenin ameliorates streptozotocin-induced diabetic nephropathy in rats via MAPK-NF-kappaB-TNF-alpha and TGF-beta1-MAPK-fibronectin pathways. Am J Physiol Renal Physiol 313 (2):F414-F422. https://doi.org/10.1152/ajprenal.00393.2016
15. Wen CP, Chang CH, Tsai MK, Lee JH, Lu PJ, Tsai SP, Wen C, Chen CH, Kao CW, Tsao CK, Wu X (2017) Diabetes with early kidney involvement may shorten life expectancy by 16 years. Kidney Int 92 (2):388-396. https://doi.org/10.1016/j.kint.2017.01.030
16. Wekerle T, Segev D, Lechler R, Oberbauer R (2017) Strategies for long-term preservation of kidney graft function. Lancet 389 (10084):2152-2162. https://doi.org/10.1016/S0140-6736(17)31283-7
17. Thrasher J (2017) Pharmacologic Management of Type 2 Diabetes Mellitus: Available Therapies. Am J Cardiol 120 (1S):S4-S16. https://doi.org/10.1016/j.amjcard.2017.05.009
18. Vanhove T, Hasan M, Annaert P, Oswald S, Kuypers DRJ (2017) Pretransplant 4beta-hydroxycholesterol does not predict tacrolimus exposure or dose requirements during the first days after kidney transplantation. Br J Clin Pharmacol 83 (11):2406-2415. https://doi.org/10.1111/bcp.13343
19. den Braver NR, de Vet E, Duijzer G, Ter Beek J, Jansen SC, Hiddink GJ, Feskens EJM, Haveman-Nies A (2017) Determinants of lifestyle behavior change to prevent type 2 diabetes in high-risk individuals. Int J Behav Nutr Phys Act 14 (1):78. https://doi.org/10.1186/s12966-017-0532-9
20. Chen CB, Zhao L, Han M, Wang XP, Zhou J, Yuan XP, Wang CX (2017) Renal Transplantation Using Stone-bearing Deceased Donor Kidneys-Experience of a Transplant Center in China. Urology 107:251-256. https://doi.org/10.1016/j.urology.2017.05.040
21. Carney EF (2017) Transplantation: Survival benefit of accepting a diabetic deceased donor kidney. Nat Rev Nephrol 13 (8):444. https://doi.org/10.1038/nrneph.2017.88
22. Budde K, Zeier M, Witzke O, Arns W, Lehner F, Guba M, Jacobi J, Kliem V, Reinke P, Hauser IA, Vogt B, Stahl R, Rath T, Duerr M, Paulus EM, May C, Porstner M, Sommerer C, Group HS (2017) Everolimus with cyclosporine withdrawal or low-exposure cyclosporine in kidney transplantation from Month 3: a multicentre, randomized trial. Nephrol Dial Transplant 32 (6):1060-1070. https://doi.org/10.1093/ndt/gfx075
23. Allen PJ, Chadban SJ, Craig JC, Lim WH, Allen RDM, Clayton PA, Teixeira-Pinto A, Wong G (2017) Recurrent glomerulonephritis after kidney transplantation: risk factors and allograft outcomes. Kidney Int 92 (2):461-469. https://doi.org/10.1016/j.kint.2017.03.015
24. Sabatino A, Regolisti G, Bozzoli L, Fani F, Antoniotti R, Maggiore U, Fiaccadori E (2017) Reliability of bedside ultrasound for measurement of quadriceps muscle thickness in critically ill patients with acute kidney injury. Clin Nutr 36 (6):1710-1715. https://doi.org/10.1016/j.clnu.2016.09.029

25. Nishimura M, Kato Y, Tanaka T, Taki H, Tone A, Yamada K, Suzuki S, Saito M, Ando Y, Hoshiyama Y (2017) Effect of Home Blood Pressure on Inducing Remission/Regression of Microalbuminuria in Patients With Type 2 Diabetes Mellitus. Am J Hypertens 30 (8):830-839. https://doi.org/10.1093/ajh/hpx050
26. Katahira M, Hanakita M, Ito T, Suzuki M (2013) The ratio of glycosylated albumin to glycosylated hemoglobin differs between type 2 diabetic patients with low normoalbuminuria and those with high normoalbuminuria or microalbuminuria. Diabetes Care 36 (12):e207-208. https://doi.org/10.2337/dc13-1243
27. Lindhardt M, Persson F, Zurbig P, Stalmach A, Mischak H, de Zeeuw D, Lambers Heerspink H, Klein R, Orchard T, Porta M, Fuller J, Bilous R, Chaturvedi N, Parving HH, Rossing P (2017) Urinary proteomics predict onset of microalbuminuria in normoalbuminuric type 2 diabetic patients, a sub-study of the DIRECT-Protect 2 study. Nephrol Dial Transplant 32 (11):1866-1873. https://doi.org/10.1093/ndt/gfw292
28. Stoperka F, Karger C, Beige J (2016) Limited Accuracy of Colour Doppler Ultrasound Dynamic Tissue Perfusion Measurement in Diabetic Adults. PLoS One 11 (12):e0168905. https://doi.org/10.1371/journal.pone.0168905
29. Al-Rubeaan K, Siddiqui K, Al-Ghonaim MA, Youssef AM, Al-Sharqawi AH, AlNaqeb D (2017) Assessment of the diagnostic value of different biomarkers in relation to various stages of diabetic nephropathy in type 2 diabetic patients. Sci Rep 7 (1):2684. https://doi.org/10.1038/s41598-017-02421-9
30. Avgustin N, Rotar Z, Pajek J, Kovac D, Osredkar J, Lindic J (2017) The predictive value of urinary vascular endothelial growth factor (VEGF) on worsening kidney function in proteinuric chronic kidney disease. Clin Nephrol 88 (13):10-13. https://doi.org/10.5414/CNP88FX03
31. Siracusano S, Bertolotto M, Ciciliato S, Valentino M, Liguori G, Visalli F (2011) The current role of contrast-enhanced ultrasound (CEUS) imaging in the evaluation of renal pathology. World J Urol 29 (5):633-638. https://doi.org/10.1007/s00345-011-0699-7
32. Lee G, Jeon S, Lee SK, Cheon B, Moon S, Park JG, Cho KO, Choi J (2017) Quantitative evaluation of renal parenchymal perfusion using contrast-enhanced ultrasonography in renal ischemia-reperfusion injury in dogs. J Vet Sci 18 (4):507-514. https://doi.org/10.4142/jvs.2017.18.4.507
33. Dumont V, Tolvanen TA, Kuusela S, Wang H, Nyman TA, Lindfors S, Tienari J, Nisen H, Suetsugu S, Plomann M, Kawachi H, Lehtonen S (2017) PACSIN2 accelerates nephrin trafficking and is up-regulated in diabetic kidney disease. FASEB J 31 (9):3978-3990. https://doi.org/10.1096/fj.201601265R
34. Dey A, Hao S, Wosiski-Kuhn M, Stranahan AM (2017) Glucocorticoid-mediated activation of GSK3beta promotes tau phosphorylation and impairs memory in type 2 diabetes. Neurobiol Aging 57:75-83. https://doi.org/10.1016/j.neurobiolaging.2017.05.010
35. Sidhu PS, Cantisani V, Deganello A, Dietrich CF, Duran C, Franke D, Harkanyi Z, Kosiak W, Miele V, Ntoulia A, Piskunowicz M, Sellars M, Gilja OH (2017) Authors' Reply to Letter: Role of Contrast-Enhanced Ultrasound (CEUS) in Paediatric Practice: An EFSUMB Position Statement. Ultraschall Med 38 (4):447-448. https://doi.org/10.1055/s-0043-106461
36. Huang L, Chen K, Chen FC, Shen HY, Ye JC, Cai ZP, Lin X (2017) Intraoperative contrast-enhanced ultrasonography for microcirculatory evaluation in rhesus monkey with spinal cord injury. Oncotarget 8 (25):40756-40764. https://doi.org/10.18632/oncotarget.17252
37. Zhang H, Nair V, Saha J, Atkins KB, Hodgin JB, Saunders TL, Myers MG, Jr., Werner T, Kretzler M, Brosius FC (2017) Podocyte-specific JAK2 overexpression worsens diabetic kidney disease in mice. Kidney Int 92 (4):909-921. https://doi.org/10.1016/j.kint.2017.03.027