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Assessing Vascular Markers of Diabetic Disease Progression Through Contrast-Enhanced Ultrasound

Rachel E. White
Joint Department of Biomedical Engineering
University of North Carolina at Chapel Hill
Chapel Hill, NC, USA
rewhite2@ncsu.edu

Kylie Kavanagh, DVM MS MPH
School of Medicine
Wake Forest University
Winston-Salem, NC, USA
kkavanag@wakehealth.edu

Chrissy Sherrill
School of Medicine
Wake Forest University
Winston-Salem, NC, USA
C.Sherrill@wakehealth.edu

Beth Uberseder
School of Medicine
Wake Forest University
Winston-Salem, NC, USA
beuberse@wakehealth.edu

Marwan Bikdash, PhD
Joint Department of Biomedical Engineering
North Carolina Agricultural and Technical State University
Greensboro, NC, USA
bikdash@ncat.edu

James Bain, PhD
Duke Molecular Physiology Institute
Duke University
Durham, NC, USA
james.bain@duke.edu

Emily H. Chang, MD
School of Medicine
University of North Carolina at Chapel Hill
Chapel Hill, NC, USA
emily_chang@med.unc.edu

Kennita A. Johnson, PhD
Joint Department of Biomedical Engineering
University of North Carolina at Chapel Hill
Chapel Hill, NC, USA
kennita@email.unc.edu

***Abstract*—Diabetic disease progression, resulting in diabetic kidney disease (DKD) increases the risk of kidney failure and is a strong predictor for patient mortality. Early detection of DKD could mitigate risks associated with DKD progression, but currently there are no well-established markers that serve this purpose. Healthy, insulin-resistant, and diabetic vervets were imaged using contrast-enhanced ultrasound (CEUS), to quantify kidney perfusion and evaluate CEUS as an early detection method. Time-intensity curve (TIC) data generated by capturing microbubble perfusion in the kidney were collected along with vervet demographic information. The wash-out slope (WOS) for diabetic vervets was significantly steeper than the WOS for healthy vervets ($p < 0.05$), indicating faster microbubble clearance from the kidney in diabetic vervets. Additionally, fasted blood glucose (FBG) levels were significantly different between healthy and diabetic vervets ($p < 0.0001$), which may relate to the differences in WOS. Other TIC metrics, such as area under the curve (AUC) and peak enhancement (PE) did not have significant differences between groups. Overall, CEUS shows potential as a method for detecting changes in blood perfusion that are indicative of disease progression, but further research is necessary to address current limitations with the technique.**

Keywords—diabetic kidney disease, perfusion imaging, contrast enhanced ultrasound

I. Introduction

Diabetes is a prevalent disease, affecting almost 10% of the population in the United States [1]. Diabetes progresses to diabetic kidney disease (DKD) in up to 40% of diabetic patients, increasing their risk for end stage renal failure, dialysis and transplantation [2-4]. Furthermore, DKD has been shown to be a strong predictor of patient mortality [5]. Consequently, early detection and intervention has been recognized as a vital strategy for mitigating diabetic disease progression, however, to date sufficient biomarkers have been lacking [6]. Current markers in blood and urine lag behind diabetic progression, demonstrating a need to identify earlier markers of diabetic disease.

Contrast enhanced ultrasound (CEUS) is an imaging modality that uses signal from microbubbles, small lipid-shelled particles with a gas core, circulating in the blood stream to provide information on blood flow [7]. CEUS has the potential to serve as a method for early detection of diabetic disease by quantifying functional changes in kidney vasculature that arise from the negative influence diabetes exerts on the kidney. The presence of these morphological changes should result in quantifiable differences in perfusion between healthy versus non-healthy populations [7-8].

II. Methods

A. Vervet Colony

Vervets housed at the Wake Forest University Vervet Research Colony were used in this study. Fifteen vervets were divided into cohorts based on health status defined by fasting blood glucose (FBG) and HbA1c levels as healthy, insulin-resistant, or diabetic ($n = 5$ for each cohort). The criteria for FBG and HbA1C levels for each group were as follows: 1) vervets were considered healthy with FBG < 80 mg/dL and HbA1c < 5%, 2) insulin-resistant with FBG between 80-125 mg/dL and HbA1c between 5-6%, and 3) diabetic with FBG > 126 mg/dL and HbA1c > 6%. Each vervet underwent three separate contrast ultrasound imaging sessions (February, May, and July) to quantify kidney perfusion. Vervets were fasted in the morning prior to imaging and blood samples were collected to measure fasted blood glucose levels (mg/dL) and HbA1c levels (%). Catheters were placed in the arms or legs of each vervet to provide access for contrast agent injections. All vervet care was provided by trained veterinarians and technicians in accordance with Wake Forest University Institutional Animal Care and Use

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Committee (IACUC) guidelines for research involving non-human primates.

B. Ultrasound Data Acquisition

Ultrasound imaging was performed using the GE LOGIQ S8 ultrasound scanner (General Electric, Boston, MA, USA) and the C1-5 curvilinear array. Imaging depth, focal depth, transmit frequency, mechanical index (MI), gain, and dynamic range were kept constant across imaging sessions. Table 1 provides an overview of imaging parameters for this study. All vervets received a 0.1 mL bolus dose of Definity (Lantheus Medical Imaging, North Billerica, MA, USA) to observe kidney perfusion, capturing microbubble wash-in and wash-out. Imaging occurred in the transverse plane, with the ultrasound probe positioned over the kidney midpole. Video data of kidney perfusion was captured for up to three minutes at a frame rate of 18 Hz.

C. Image Processing and Statistical Analysis

Data were stored in DICOM format and exported from the scanner for offline processing. Regions of interest (ROIs) were drawn around the kidney using ImageJ (NIH, Bethesda, MD USA). Time-intensity curve (TIC) data were generated by applying the calculation of average pixel intensity in the kidney ROI across time. Specific parameters were extracted from the TICs, including area under the curve (AUC), wash-out slope (WOS), wash-in time (WIT), rise time (RT), peak enhancement (PE), and time to peak (TTP). WOS was calculated by fitting a linear equation (1) to the wash-out data of the following form:

$$y = mx + b, \quad (1)$$

where m represents the slope of the line and b represents the y-axis intercept. The wash-out data was defined as all points past the peak enhancement value. WIT was considered to be the time from the bolus injection to the initial influx of microbubbles in the ROI, RT was defined as the time from wash-in to peak enhancement, PE was the maximum intensity value calculated at the apex of the curve, and TTP was the time from bolus injection to peak enhancement. One-way ANOVA with Tukey's post-hoc multiple comparison analysis and Mann-Whitney t-tests were performed using GraphPad Prism (San Diego, CA, USA) to assess statistical significance of vervet demographics and time intensity curve metrics, respectively.

III. Results

Data on vervet age, body weight, fasted blood glucose (FBG) levels, and HbA1c levels were collected for health classification and to provide demographic information. TIC perfusion metrics were evaluated to differentiate between healthy, pre-diabetic, and diabetic kidney function.

TABLE I. Imaging Parameters

Imaging Parameters					
Imaging depth (cm)	***Focus depth (cm)***	***Frequency (MHz)***	***MI***	***Gain (dB)***	***Dynamic Range (dB)***
7	5.6	Res[a]	0.18	30	57

[a] For the GE LOGIQ S8 Res is one of three frequency settings (Res, Gen, or Pen) that prioritizes resolution (Res) over general imaging (Gen) and depth penetration (Pen).

TABLE II. Vervet Demographics

	Vervet Health Classifications		
	Healthy	***Insulin-Resistant***	***Diabetic***
Age (yrs)	16.04	16.57	20.25
Body Weight (kg)	5.77	6.45	5.83
FBG (mg/dL)[b]	65.40	88.0	355.46
HbA1c (%)[c]	4.22	5.38	8.67

[b] One FBG value was provided as > 600 mg/dL and was set to 600 mg/dL for purposes of calculating the average FBG. [c] Five HbA1c values were provided as < 4 % and were set to 4 % for purposes of calculating the average HbA1c.

A. Vervet Demographics

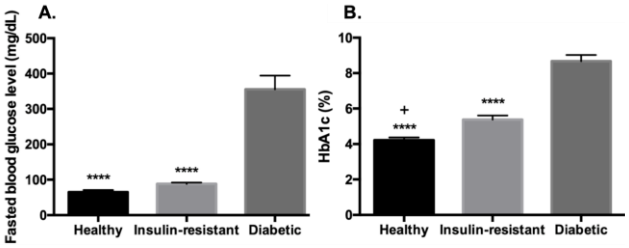

Fig 1. A. Average fasted blood glucose levels for healthy, insulin-resistant, and diabetic vervets. Both healthy and insulin-resistant FBG levels were significantly different from diabetic FBGs, while healthy and insulin-resistant FBG levels were not significantly different from one another. B. Average HbA1c levels for healthy, insulin-resistant, and diabetic vervets. All groups were statistically significant from one another. In both cases, **** represents significance ($p < 0.0001$) compared to diabetic vervets, + represents significance ($p < 0.05$) compared to insulin-resistant vervets, and error bars denote SEM.

Vervet age, body weight, FGB levels, and HbA1c levels were averaged for each group across the three imaging sessions to look at overall demographics for each population. Results are shown in Table 2. One vervet shifted from insulin-resistant to diabetic between the selection of vervet cohorts and the start of imaging. Average vervet FBG levels were 65.4 mg/dL, 88.0 mg/dL, and 355.45 mg/dL with SEMs of 5.48 mg/dL, 4.02 mg/dL, and 38.8 mg/dL for healthy, insulin-resistant, and diabetic vervets, respectively (Fig. 1A). Average HbA1c levels were 4.22%, 5.38%, and 8.67%, with SEMs of 0.15%, 0.24%, and 0.35% for healthy, insulin-resistant, and diabetic vervets, respectively (Fig. 1B).

B. Time-Intensity Curve Parameters

Metrics were calculated individually for each vervet TIC and then averaged across each group. The average and standard error of the mean (± SEM) for each parameter is compiled in Table 3. Pixel intensity for AUC and PE are denoted by arbitrary units [A.U.] and WOS denotes the change in pixel intensity over time [pixels/second].

TABLE III. Time-Intensity Curve Metrics

	Time-Intensity Curve Parameters[d]		
	Healthy	***Insulin-Resistant***	***Diabetic***
AUC	$1.61 \times 10^5 \pm 1.53 \times 10^4$	$1.50 \times 10^5 \pm 1.50 \times 10^4$	$1.45 \times 10^5 \pm 1.32 \times 10^4$
WOS	-0.16 ± 0.044	-0.24 ± 0.065	-0.33 ± 0.053
WIT (s)	9.8 ± 0.961	11.80 ± 1.02	11.88 ± 1.24
RT (s)	3.44 ± 0.333	3.62 ± 0.653	3.60 ± 0.354
PE	89.43 ± 5.22	89.09 ± 7.15	87.36 ± 5.55

	Time-Intensity Curve Parameters[d]		
	Healthy	***Insulin-Resistant***	***Diabetic***
TTP (s)	13.24 ± 0.883	15.43 ± 1.46	15.47 ± 1.38

[d] The first value in the cell is the average and the second value is the standard error of the mean.

C. Statistical Analysis

Both vervet demographics and time intensity curve parameters demonstrated statistical significance between healthy, insulin-resistant, and diabetic populations. Average vervet age was statistically significant for both healthy and insulin-resistant vervets compared to diabetic vervets ($p < 0.01$), but not for healthy compared to insulin-resistant vervets, while average body weight was not stastically signicant between any vervet populations. Average FBG levels were statistically significant for both healthy and insulin-resistant vervets compared to diabetic vervets ($p < 0.0001$), but not between healthy and insulin-resistant vervets, while HbA1c was statistically significant across all populations ($p < 0.0001$ for healthy and insulin-resistant compared to diabetic, $p < 0.05$ for healthy compared to insulin-resistant). Of the TIC parameters, only WOS showed significance between vervet populations. WOS for the diabetic group was significantly different from the healthy group ($p < 0.05$). WIT and TTP showed an increasing trend from healthy to diabetic vervets, but these increases were not statistically significant. AUC, RT, and PE showed no significant differences among vervet populations and the data showed no trends.

IV. Discussion and Conclusions

The significant decrease in WOS between healthy and diabetic vervets was indicative of faster microbubble removal from the kidney for diabetic vervets versus healthy vervets (Fig. 2A). This corresponds to the fact that FBG levels were significantly different between healthy and diabetic vervets, but not between healthy and insulin-resistant vervets. Increased levels of glucose in the blood damage the kidney vasculature, altering the perfusion dynamics. This phenomena could provide an explanation for the faster microbubble wash-out rate in diabetic vervets compared to healthy vervets. Although not significant, there is a trend toward a longer WIT (Fig. 2C) and TTP (Fig. 2D). Because of the direction of renal blood flow entering the cortex before the medulla, the longer WIT and TTP may show changes in the renal cortex while the steeper WOS (faster removal of contrast agent from the kidney) may indicate changes in the renal medulla. In the future, examinations of subsections of the kidney are needed to discern the nature of anatomical and physiological changes caused by diabetes that are reflected in the ultrasound measurements. The lack of significant results for AUC, RT, WIT, and TTP could be a result of the small sample size for each group. With a larger sample size per group, these metrics may have resulted in differences between populations. The relative stability of PE across groups and at different time points indicates consistency in microbubble behavior for a given bolus dose. Information on microbubble behavior, and demonstration of consistent microbubble behavior, is an important consideration when planning longitudinal studies.

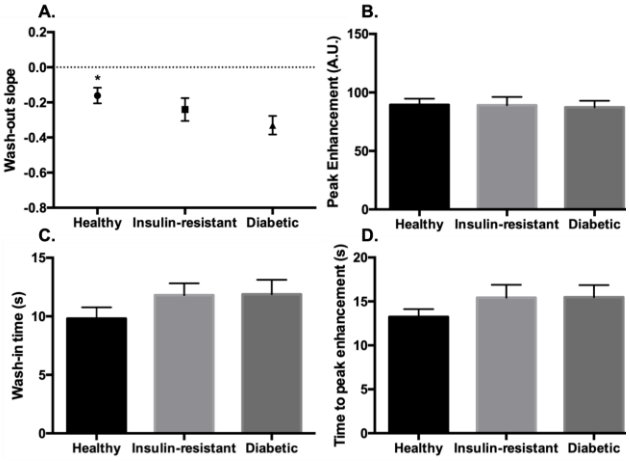

Fig 2. A. Average WOS for healthy, insulin-resistant, and diabetic vervets. Diabetic WOS was significantly different from healthy WOS. B. Average PE; C. Average WIT (s); and D. Average TTP (s) for vervet populations. Where applicable, * represents significance ($p < 0.05$) compared to diabetic vervets and all error bars denote SEM.

An additional study limitation was that stage and rate of insulin-resistance and diabetic progression were unknown for each vervet. Vervets were catergorized in a discrete manner, but it is likely that insulin-resistance and diabetic disease progression were vastly different within these populations. Incorporating metrics characterizing the advancement of diabetic disease would be an important factor to consider for future studies. More TIC metrics may show significant differences when disease advancement is considered as a subgroup for each population. It would be particularly interesting to look at whether or not WIT and TTP became significant with a larger sample size and with further breakdown of disease status, since both showed trends across vervet populations in this study. Vervet body weight was not significantly different among groups, but vervet age was significantly different for diabetic vervets relative to healthy and insulin-resistant vervets. Since diabetes develops naturally over time in these vervets, this is not unexpected, but may be a limitation when comparing vervet diabetic disease progression and human diabetic disease.

This work has shown that CEUS perfusion metrics have the potential to distinguish between healthy, insulin-resistant, and diabetic vervets. However, there are many limitations that need to be addressed with further research. The technique should be explored in greater depth before it could serve as an early detection method for diabetic kidney disease.

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