Hemodialysis Patients Have Impaired Cerebrovascular Reactivity to CO2 Compared to Chronic Kidney Disease Patients and Healthy Controls: A Pilot Study

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Introduction: Recurrent hemodialysis (HD)–induced ischemia has emerged as a mechanism responsible for cognitive impairment in HD patients. Impairment of cerebrovascular function in HD patients may render the brain vulnerable to HD-induced ischemic injury. Cerebrovascular reactivity to CO2 (CVR) is a noninvasive marker of cerebrovascular function. Whether CVR is impaired in HD patients is unknown. In this study, we compared CVR between healthy participants, HD patients, and chronic kidney disease (CKD) patients not yet requiring dialysis.

Methods: This was a single-center prospective observational study carried out at Kidney Clinical Research Unit in London, Canada. We used carefully controlled hypercapnia to interrogate brain vasomotor control. Transcranial Doppler was combined with 10–mm Hg step changes in CO2 from baseline to hypercapnia (intervention) and back to baseline (recovery) to assess CVR in 8 HD, 10 CKD, and 17 healthy participants.

Results: HD patients had lower CVR than CKD or healthy participants during both intervention and recovery (P < 0.0001). There were no differences in CVR between healthy and CKD participants during either intervention (P = 0.88) or recovery (P = 0.99). The impaired CVR in HD patients was independent of CO2-induced changes in blood pressure, heart rate, cardiac output, or dialysis vintage. In the CKD group, CVR was not associated with the estimated glomerular filtration rate.

Conclusions: Our study shows that HD patients have impaired CVR relative to CKD and healthy participants. This renders HD patients vulnerable to ischemic injury during circulatory stress of dialysis and may contribute to the pathogenesis of cognitive impairment.

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vasoactive stimuli, such as changes in CO₂ levels. Normal CVR would oppose HD-induced reductions in CBF and attenuate associated ischemic brain injury. Clinical assessment of CVR requires a method to measure CBF (e.g., using transcranial doppler or magnetic resonance imaging) and a method to induce changes in CO₂. CVR is calculated as a ratio between change in CBF and change in CO₂, with abnormal values associated with cerebrovascular pathology. Impairment in CVR has been associated with increased risk of stroke, cognitive impairment, and cortical thinning. In concussion, CVR can differentiate between concussed and normal patients despite similar anatomic and global resting CBF measurements in both groups. In leukoaraiosis, CVR has emerged as an early functional marker of brain tissue at risk for subsequent injury, with impaired CVR accurately predicting areas of nonaffected white matter that progress to white matter hyperintensities in 1 year.

Whether CVR is impaired in HD patients is not well established. In this prospective observational study, we assessed CVR in a cohort of HD patients and compared it to CVR in CKD patients and healthy controls with no documented history of CKD. We hypothesized that HD patients would have impaired CVR, which would be more severely impaired than in CKD patients owing to additional circulatory injury occurring as a result of recurrent endothelial injury resulting from exposure to intermittent HD. Given that CVR has become an early marker of cerebrovascular dysfunction, establishing whether it is impaired in HD and CKD patients may provide additional insights into the pathophysiology of cerebrovascular disease and cognitive impairment in patients with renal disease.

**MATERIALS AND METHODS**

**Study Design and Participants**

This was a single-center prospective observational study that was approved by Western University Health Sciences Research Ethics Board (protocol number 109548) and carried out at the Kidney Clinical Research Unit in London, Canada. HD and CKD patients were recruited from the prevalent patient population of the HD and CKD programs. A convenience sample of consecutive healthy non-CKD participants were recruited from the general population using poster advertisements. Inclusion criteria varied by participant group. For healthy participants, we included adults (age ≥18 years) with no history of cardiovascular disease, cerebrovascular disease, diabetes or CKD. For CKD patients, we included adults (age ≥18 years) with a diagnosis of stage 4 or 5 CKD. For HD patients, we included adults (age ≥18 years) who were receiving hemodialysis treatment at least 3 times per week at a London Health Sciences Centre facility. Dialysis patients were tested on the day between their dialysis sessions. Outside the current study, all dialysis patients received conventional hemodialysis without dialysate cooling. Exclusion criteria in all participants were as follows: severe chronic obstructive lung disease or asthma, past history of cerebrovascular accident (stroke or transient ischemic attack), carotid stenosis or carotid surgery, prior neurosurgery, history of vasculitis, pregnancy, and mental incapacity for consent. All participants signed an informed consent form prior to participation in the study.

**CBF Monitoring**

We used transcranial Doppler to measures middle cerebral artery blood flow velocity (CBFv) as an indicator of global CBF. We insonated middle cerebral arteries through the transtemporal window with standard technique using 2-MHz transcranial Doppler probes that were fixed in places with a provided head frame (ST3, Spencer Technologies, USA). CBFv was sampled and recorded continuously at 125 Hz on an ST3 device.

**CO₂ Changes**

We used an automated gas blender and gas delivery breathing circuit (RA-MR, RespirAct; Thornhill Medical, Toronto, Canada) to prospectively target and control end-tidal gases. The RespirAct is based on the theory of sequential gas delivery to provide a quantitative and reproducible CO₂ stimuli. In contrast to other vasodilatory stimuli such as transient or pharmacologically induced changes in MAP, administration of acetazolamide, breath holding, or addition of CO₂ to inspired gas, prospective targeting with RespirAct facilitates more accurate measurement of CVR by enabling noninvasive, rapidly reversible and reproducible CO₂ stimuli that reduce between-subject variability.

**Hemodynamic Monitoring**

We used Finapres NOVA (Finapres, Finapres Medical Systems, Netherlands) to record heart rate (HR), systolic and diastolic blood pressure, and mean arterial pressure (MAP), as well as cardiac output (CO) non-invasively during the protocol. The Finapres finger-cuff was placed on the middle finger of the nonfistula arm. Noninvasive methods such as Finapres are validated in hemodialysis settings and have been used in prior CVR studies. Although its accuracy and precision are not interchangeable with direct intra-arterial measurements, it has become an accepted...
noninvasive alternative in physiological and clinical studies.\textsuperscript{23,24}

Study Protocol and Data Collection
Participants were seated in a comfortable recliner chair for the duration of the study. After application of the transcranial Doppler probes, the Finapres probe and a RespirAct facemask, baseline respiratory and hemodynamic data were acquired after 5 minutes of acclimatization to the experimental apparatus. The CO\textsubscript{2} sequence consisting of three 5-minute stages was then applied: (i) patient’s own resting end-tidal partial pressure of CO\textsubscript{2} (PetCO\textsubscript{2}) (baseline); (ii) increase in PetCO\textsubscript{2} by 10 mm Hg above baseline (intervention); and (iii) return to resting PetCO\textsubscript{2} (recovery) (Figure 2). End-tidal partial pressure of O\textsubscript{2} (PetO\textsubscript{2}) was kept constant at normoxia. CBFv, hemodynamics, and changes in end-tidal gases were recorded continuously. Data were exported from monitors into custom Python analysis package as comma-separated values and aligned used time stamps. To allow comparison between participants, CBFv was expressed as percentage change from participant’s baseline. The average values for CBFv, MAP, HR, CO, PetCO\textsubscript{2}, and PetO\textsubscript{2} for each experimental stage were calculated using data from the last minute of each 5-minute stage. To assess for hysteresis, CVR was calculated as the percentage change in CBFv divided by the change in PetCO\textsubscript{2} for both increase in PetCO\textsubscript{2} from baseline to hypercapnia (CVR intervention) and decrease in PetCO\textsubscript{2} from hypercapnia back to baseline (CVR recovery).

Statistical Analysis
Statistical analysis was performed using the GraphPad Prism software version 8.3 for macOS (GraphPad Software, San Diego, CA, USA). Continuous variables were reported using medians and interquartile ranges (IQRs), and categorical variables were reported as frequency (%). Variables were analyzed for normal distribution using the Shapiro-Wilk normality test. To compare differences between participant groups (healthy vs. CKD vs. HD patients), we used 1-way analysis of variance with Tukey multiple comparisons test for normally distributed variables, and Kruskal-Wallis test for non-normally distributed variables. We used Pearson correlation or nonparametric (Spearman rank) correlation analysis to assess the strength of relationship between CVR and change in MAP, HR, CO, estimated glomerular filtration rate (eGFR, for CKD patients only), and dialysis vintage (length of time on dialysis, for HD patients only) within participant groups. We reported correlation coefficients ($r$ values) and the significance level of the $P$ value. A $P$ value of $<0.05$ was considered significant.

RESULTS
A total of 17 healthy non-CKD participants, 10 CKD, and 8 HD patients completed full protocol and were included in the final analysis (Figure 1). Participant baseline characteristics, resting CBFv, end-tidal gases and hemodynamic variables are summarized in Table 1. Healthy participants were younger (median 31.0 years,
than CKD patients (median 71 years, IQR 61, 71, \( P = 0.003 \)). There was no difference in age between CKD and HD patients (\( P > 0.99 \)). Healthy participants also had a lower weight (median 68 kg, IQR 64, 85) than CKD (median 94 kg, IQR 84, 102, \( P = 0.01 \)) and HD (median 91 kg, IQR 85, 105, \( P = 0.04 \)) patients, with no difference noted between CKD and HD patients (\( P = 0.7227 \)). Healthy participants had lower BMI (median 25.0, IQR 22.5, 27.9) than CKD (median 30.8, IQR 27.9, 35.3, \( P = 0.0258 \)) and HD (median 31.4, IQR 28.7, 35.3, \( P = 0.0424 \)) patients, with no difference noted between CKD and HD patients (\( P > 0.9999 \)). There was statistically significant difference in the prevalence of hypertension, hyperlipidemia, diabetes and heart failure between participant groups. CKD patients had higher prevalence of hypertension, hyperlipidemia, diabetes and coronary artery disease compared to healthy participants (Supplementary Table S1). HD patients had a slighter higher baseline end-tidal partial pressure of CO\(_2\) (PetCO\(_2\), median 42 mm Hg, IQR 37, 46) than CKD patients (median 36 mm Hg, IQR 34, 38, \( P = 0.03 \)), but not healthy participants (median 38 mm Hg, IQR 35, 42, \( P = 0.25 \)). There were no differences between participant groups in terms of sex, height, and baseline CBFv, PetO\(_2\), systolic and diastolic blood pressure, MAP, HR, or CO.

Figure 2 shows experimental data from representative participants from each group. Within group change in end-tidal gases, CBFv and hemodynamic variables across experimental stages are summarized in Table 2. Across all participant groups, PetCO\(_2\) and CBFv increased from baseline during intervention and returned back to baseline during recovery, with no corresponding changes in PetO\(_2\). Hemodynamic responses to changes in PetCO\(_2\) varied across participant groups. In all participants, MAP increased from baseline during intervention and did not return back to baseline during recovery, although the former was not statistically significant in CKD group and the latter in the healthy group. HR remained unchanged across experimental stages in healthy participants and HD patients, but increased slightly with intervention in CKD patients. CO remained unchanged across experimental stages in CKD and HD patients but increased during intervention and returned back to baseline in healthy participants.

Table 3 summarizes the magnitude of change in end-tidal gases, hemodynamic parameters, CBFv and CVR across participant groups during CO\(_2\) intervention and recovery. The magnitude of change in end-tidal gases

![Figure 2](image-url)
and hemodynamic parameters were similar across all participant groups during both intervention and recovery. HD patients had a smaller increase in CBFv during intervention compared to healthy participants and CKD patients. The same pattern was observed during recovery, but it only reached statistical significance for healthy versus HD patients.

Figure 3 shows individual participant changes in CBFv, MAP, and PetCO2 during intervention and recovery across participant groups. Compared with healthy and CKD participants, HD patients had a lower CBFv response to a change in PetCO2 during intervention ($P = 0.026$ vs. healthy, $P = 0.047$ vs. CKD) and recovery ($P = 0.035$ vs. healthy, $P = 0.12$ vs. CKD). This translated into significantly lower CVR in HD patients compared with healthy and CKD participants during both intervention ($P = 0.008$ vs. healthy, $P = 0.004$ vs. CKD) and recovery ($P = 0.01$ vs. healthy, $P = 0.024$ vs. CKD) (Figure 4). There were no differences in CVR between healthy and CKD participants during either intervention ($P = 0.78$) or recovery ($P = 0.99$).

Correlation analysis showed that there was no relationship between CVR and change in MAP, HR, CO, and PetO2 during both intervention and recovery within each participant group (Table 4). In the CKD group, there were no correlations between CVR and eGFR. In the HD group, there were no correlations between CVR and dialysis vintage.

**DISCUSSION**

**Main Findings**

Our study shows that HD patients have impaired CVR compared to CKD patients and healthy participants. This impairment was not associated with dialysis vintage, suggesting that CVR impairment may occur early following initiation of HD. In contrast to our second hypothesis, we showed that CVR was not impaired in CKD patients and was not associated with the degree of renal impairment as measured by eGFR. Taken together, these findings suggest that CVR impairment in renal disease is not simply a result of worsening renal function or uremia but potentially also the result of insults specific to HD treatment. Given that HD treatment is associated with intradialytic reduction in CBF, impairment of CVR in HD patients may facilitate ischemic white matter injury and contribute to...

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**Table 1. Participant baseline characteristics**

|                          | Healthy (n = 17) | CKD (n = 10) | HD (n = 8) | P value |
|--------------------------|------------------|--------------|------------|---------|
| **Females, n (%)**       | 8 (47)           | 3 (30)       | 3 (38)     | 0.6785  |
| **Age (yr)**             | 31 (26, 41)      | 71 (56, 73)  | 69 (61, 71)| 0.0002  |
| **Weight (kg)**          | 68 (64, 85)      | 94 (84, 102) | 91 (85, 105)| 0.0077  |
| **Height (cm)**          | 168 (165, 174)   | 175 (163, 182)| 172 (163, 175)| 0.5267  |
| **BMI**                  | 25.0 (22.5, 27.9) | 30.8 (27.9, 35.3)| 31.4 (28.7, 35.3)| 0.0081  |
| **Comorbidities, n (%)** |                  |              |            |         |
| Hypertension             | 1 (6)            | 9 (90)       | 7 (88)     | <0.0001 |
| Hyperlipidemia           | 1 (6)            | 7 (70)       | 8 (100)    | <0.0001 |
| Diabetes                 | 0 (0)            | 5 (50)       | 2 (25)     | 0.0067  |
| CAD                      | 0 (0)            | 3 (30)       | 2 (25)     | 0.0086  |
| Heart Failure            | 0 (0)            | 0 (0)        | 2 (25)     | 0.0279  |
| Asthma                   | 0 (0)            | 1 (10)       | 2 (25)     | 0.1122  |
| COPD                     | 0 (0)            | 1 (10)       | 2 (25)     | 0.1122  |
| Stroke or TIA            | 0 (0)            | 1 (10)       | 1 (13)     | 0.3579  |
| PVD                      | 0 (0)            | 1 (10)       | 0 (0)      | 0.1122  |
| **CBFv (cm/s)**          | 52 (43, 60)      | 48 (46, 56)  | 45 (21, 58)| 0.5956  |
| **PetCO2 (mm Hg)**       | 38 (35, 42)      | 36 (34, 38)  | 42 (37, 46)| 0.0400  |
| **PetO2 (mm Hg)**        | 103 (102, 112)   | 111 (107, 114)| 108 (92, 112)| 0.1739  |
| **SBP (mm Hg)**          | 129 (119, 137)   | 136 (116, 145)| 127 (107, 178)| 0.7120  |
| **MAP (mm Hg)**          | 96 (90, 103)     | 90 (82, 100) | 87 (71, 103)| 0.4861  |
| **DIA (mm Hg)**          | 76 (70, 82)      | 68 (60, 80)  | 63 (56, 70)| 0.0718  |
| **HR (bpm)**             | 76 (67, 83)      | 73 (56, 85)  | 68 (58, 79)| 0.6330  |
| **CO (l/min)**           | 5.7 (5.1, 6.4)   | 5.7 (5.6, 7.4)| 7.8 (6.7, 9.8)| 0.0625  |
| **Hb (g/dL)**            | n/a              | 124 (110, 143)| 114 (103, 123)| 0.1948  |
| **eGFR (ml/min per 1.73 m²)** | 22 (17, 27)     | —            | —           |         |
| **Dialysis vintage (mo)**| 27 (14, 62)      | —            | —           |         |

BMI, body mass index; CAD, coronary artery disease; CBFv, cerebral blood flow velocity; CKD, chronic kidney disease patients; CO, cardiac output; COPD, chronic obstructive pulmonary disease; DIA, diastolic blood pressure; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; HD, hemodialysis patients; HR, heart rate; MAP, mean arterial blood pressure; PetCO2, end-tidal partial pressure of CO2; PetO2, end-tidal partial pressure of O2; PVD, peripheral vascular disease; SBP, systolic blood pressure; TIA, transient ischemic attack.

*P < 0.05.

Values are expressed as counts (percentage) or medians (interquartile range).
acceleration of cerebrovascular disease and cognitive impairment following initiation of HD.

CVR in Patients With Renal Disease
Only a few studies have examined CVR in patients with renal disease with conflicting findings. Skinner et al. used transcranial Doppler in 12 HD patients to show that their CVRs were similar to those from historic healthy controls and were similar before and after dialysis sessions. The difference between these findings and our results is likely due to difference in the CO2 stimulus. Skinner et al. used rebreathing to increase CO2, which is in contrast to our square-wave stimulus results in gradual ramp increase in CO2 that can produce higher CVR values. Given that tolerance of such gradual hypercapnia varies between patients, the

Table 2. Within-group changes in variables between the baseline, intervention, and recovery stages

| Variable | BL | INT | REC | P value |
|----------|----|-----|-----|---------|
| Healthy  |    |     |     |         |
| PetCO2 (mm Hg) | 38 (35, 42) | 47 (44, 51) | 38 (35, 42) | <0.0001 |
| PetO2 (mm Hg)  | 103 (102, 112) | 108 (103, 112) | 106 (102, 112) | 0.4525 |
| CBFv (%) | 0 (0, 0) | 38 (27, 47) | -2 (-7, 4) | <0.0001 |
| MAP (mm Hg) | 96 (90, 103) | 110 (92, 115) | 99 (89, 108) | <0.0001 |
| HR (b/min) | 76 (67, 83) | 78 (67, 88) | 79 (67, 88) | 0.1966 |
| CO (l/min) | 5.7 (5.1, 6.4) | 6.6 (5.3, 7.4) | 5.8 (5.6, 6.8) | 0.0003 |
| CKD patients |    |     |     |         |
| PetCO2 (mm Hg) | 38 (34, 39) | 48 (44, 48) | 38 (34, 39) | <0.0001 |
| PetO2 (mm Hg)  | 108 (105, 114) | 108 (105, 114) | 110 (105, 114) | 0.4040 |
| CBFv (%) | 0 (0, 0) | 40 (34, 51) | -1 (-3, 2) | <0.0001 |
| MAP (mm Hg) | 94 (87, 103) | 104 (93, 114) | 103 (91, 108) | 0.0367 |
| HR (b/min) | 69 (56, 81) | 73 (59, 89) | 69 (59, 84) | 0.0038 |
| CO (l/min) | 5.6 (5.5, 6.4) | 6.6 (6.7, 7.3) | 5.9 (5.2, 6.9) | 0.5933 |
| HD patients |    |     |     |         |
| PetCO2 (mm Hg) | 42 (37, 46) | 51 (47, 55) | 42 (39, 44) | <0.0001 |
| PetO2 (mm Hg)  | 108 (92, 112) | 108 (97, 112) | 108 (98, 112) | 0.3623 |
| CBFv (%) | 0 (0, 0) | 20 (9.37) | -2 (-5, 0) | 0.0039 |
| MAP (mm Hg) | 87 (71, 103) | 102 (82, 111) | 92 (74, 109) | 0.0004 |
| HR (b/min) | 68 (58, 79) | 74 (61, 86) | 70 (59, 78) | 0.2139 |
| CO (l/min) | 7.8 (6.7, 9.8) | 8.4 (7.4, 10.7) | 8.2 (7.1, 10.6) | 0.0901 |

BL, baseline; CBFv, cerebral blood flow velocity expressed as percentage change from participant baseline; CKD, chronic kidney disease patients; CO, cardiac output; HR, heart rate; INT, intervention; MAP, mean arterial pressure; PetCO2, end-tidal CO2; PetO2, end-tidal PO2; REC, recovery.
*P < 0.05 versus BL.
**P < 0.05 versus REC.
Values are expressed as median (interquartile range).

Table 3. Changes in physiologic parameters during intervention and recovery

| Variables | Healthy | CKD | HD | P Value |
|-----------|---------|-----|----|---------|
| Intervention |        |     |    |         |
| ΔPetCO2 (mm Hg) | 9.6 (8.9, 10.1) | 9.6 (9.6, 10) | 9.8 (9.5, 10) | 0.7914 |
| ΔPetO2 (mm Hg)  | 0 (-0.2, 1) | 0.1 (-0.4, 0.5) | 0 (-0.5, 0.3) | 0.4997 |
| ΔMAP (mm Hg) | 8.3 (5, 15.5) | 9.7 (-0.6, 12.7) | 12.6 (7.5, 16.2) | 0.3247 |
| ΔHR (bpm) | 4 (1.6, 6.2) | 4.4 (-0.6, 5.9) | 4.5 (0.7, 7.4) | 0.9026 |
| ΔCO (l/min) | 0.6 (0.1, 1) | 0.7 (-0.2, 0.9) | 0.8 (-0.1, 1.5) | 0.4148 |
| ΔCBFv (%) | 38.5 (27, 46.6) | 36.8 (29.1, 48.7) | 19.7 (8.9, 36.6) | 0.0228 |
| CVR (%/mm Hg) | 4 (2.9, 4.8) | 4 (3.4, 5.1) | 2.1 (0.9, 3.7) | 0.0031 |
| Recovery |        |     |    |         |
| ΔPetCO2 (mm Hg) | -9.6 (-9.9, -8.8) | -9.7 (-9.8, -9.5) | -9.6 (-10, -9.3) | 0.8562 |
| ΔPetO2 (mm Hg)  | 0 (-0.4, 0.7) | 0.1 (-0.2, 0.3) | 0.2 (0.1, 0.4) | 0.6092 |
| ΔMAP (mm Hg) | -7.2 (-11, -2.6) | -3.4 (-6.9, 1.4) | -6.7 (-11.1, -1.3) | 0.1373 |
| ΔHR (bpm) | -0.3 (-4.5, 4.4) | -0.5 (-4.3, 0.9) | -2.3 (-5.5, 2.1) | 0.5942 |
| ΔCO (l/min) | -0.5 (-1, -0.1) | -0.4 (-0.9, 0.1) | -0.3 (-0.7, 0.3) | 0.4857 |
| ΔCBFv (%) | -37 (-49.5, -30.5) | -40 (-44.8, -28.8) | -20 (-39.8, -12.3) | 0.0397 |
| CVR (%/mm Hg) | 4.1 (3.1, 5.2) | 4.3 (3.6, 5) | 2 (1.4, 4.1) | 0.0120 |

ΔCBFv, change in cerebral blood flow velocity expressed as percentage change from participant baseline; CKD, chronic kidney disease patients; ΔCO, change in cardiac output; CVR, cerebrovascular reactivity calculated as ΔCBFv/ΔPetCO2; HD, hemodialysis patients; ΔHR, change in heart rate; ΔMAP, change in mean arterial blood pressure; ΔPetCO2, change in end-tidal CO2, ΔPetO2, change in end-tidal PO2.
Values are expressed as median (interquartile range).
*P < 0.05 versus H.
**P < 0.05 versus CKD.
actual delivered CO₂ stimulus will vary between patients affecting CVR measurements. In another study, Ishida et al. found no difference in CVR during isoflurane-induced anesthesia between nondiabetic patients with chronic renal failure and controls, although there was inverse correlation between CVR and blood urea nitrogen in patients with chronic renal failure. The finding of normal CVR in this study can be explained by the concurrent administration of isoflurane anesthesia, a potent cerebral vasodilator known to increase CBF and affect CVR assessment. Furthermore, only half of patients in this study were receiving HD, with remaining patients receiving other form of dialysis or were predialysis. Similar to our study, there was no difference in CVR between healthy controls and CKD patients. Given older age and higher burden of comorbidities in our CKD group compared with healthy controls, we expected worse CVR in the CKD group. However, this was not the case. Although the relative contribution of age and comorbidities to CVR impairment in patients with renal disease warrants further investigation, it appears that hemodialysis is associated with impairment in CVR despite similar age and comorbidity profile between CKD and HD patients.

Two studies showed impairment of CVR in patients with renal disease. In keeping with our findings, Kuwabara et al. showed impaired CVR in HD patients compared with healthy controls. However, in contrast to our findings, they also showed impaired CVR in CKD patients. Although this discrepancy may be due to differences in CKD severity, we cannot infer this because the Kuwabara et al. study did not report eGFR values. Another explanation is that their study used positron emission tomography instead of transcranial Doppler to assess CBF. Finally, Kuwabara et al. used 5% CO₂ inhalation technique, which resulted in more modest (5–7 mm Hg) increase in CO₂ with large variability between patients compared to precise ~10 mm Hg CO₂ stimulus used in our study. Given that magnitude and reproducibility of CO₂ stimuli can affect CVR measurements, the observed variability of CO₂ stimuli in Kuwabara et al. study may explain the discrepancy from our results. Kuwabara et al. also showed that anemia can attenuate CVR in patients with renal disease a dose-dependent manner. However, given similar hemoglobin values between the CKD and HD patients in our study, anemia does not explain impaired CVR in HD group compared with the CKD group. In another study, Szpryger et al. showed that CVR was impaired in children receiving HD, but not in children receiving peritoneal dialysis or children with CKD treated conservatively. These findings are in keeping with our results, although Szpryger et al. used hyperventilation-induced hypocapnia rather than hypercapnia to assess CVR.

The Implication of Impaired CVR in HD Patients

CVR has emerged as a noninvasive marker of cerebrovascular function that predicts development of subcortical white matter injury in patients with leukoaraiosis. Impaired CVR is associated with increased risk of poor clinical outcomes including concussion, stroke, and cognitive impairment. Our finding of impaired CVR in HD, but not CKD, patients may explain why initiation of HD is associated with accelerated progression of cerebrovascular disease and cognitive decline. Impaired CVR may render the brain vulnerable to repeated ischemic insults associated with intradialytic reduction in CBF. In patients with preserved CVR, these ischemic episodes would be counteracted by vascular dilation in
response to local increase in CO₂ in ischemic regions in order to preserve tissue oxygen delivery. In patients with impaired CVR, this vasodilatory response is attenuated. This may facilitate ischemic injury via 2 mechanisms.

First, failure to vasodilate may prevent local compensatory increase in CBF, further worsening ischemia. Second, vasodilation in brain regions with preserved CVR may divert the blood flow from the regions with impaired CVR that are unable to dilate via vascular steal.33 Interestingly, regional assessment of CVR in healthy participants revealed that such “steal” phenomenon is predominately localized to white matter where elderly patients develop leukoaraiosis (i.e., white matter injury).34 This may explain why HD patients develop new diffuse subcortical white matter injury that is independent of the traditional cardiovascular risk factors (smoking, diabetes, and ischemic heart disease) shortly after initiation of dialysis.3

The mechanism of CVR impairment in HD patients is unclear. Preserved CVR in CKD patients that is independent of eGFR suggests that CVR impairment in HD patients is not simply due to reduction in renal function but is in some way related to the hemodialysis treatment. Impaired CVR is associated with endothelial dysfunction,35 which is common in patient with renal disease and can be directly induced in HD patients by osmotic injury to endothelial glycocalyx. Experimental data on healthy subjects has shown that acute intravenous sodium loading increased microvascular permeability and damaged the endothelial glycocalyx.36 In chronic HD patients, repeated, acute intradialytic sodium loading due to high dialysate sodium concentrations may increase microvascular permeability over time. This is suggested by evidence of impaired microvascular barrier in chronic HD patients.37 Other potential mechanisms for impaired CVR in HD patients include chronic inflammatory state,38 increased vascular stiffness,39 decreased baroreflex sensitivity,40 chronic endotoxemia,41 or chronic uremic stress.

Table 4. Correlation analysis between CVR, CBF, end-tidal gases, hemodynamic variables, and dialysis vintage (for HD patients only) across participant groups

| Variable 1 | Variable 2 | Intervention r | P value | Recovery r | P value |
|------------|------------|----------------|---------|------------|---------|
| CVR        | ΔMAP       | 0.313          | 0.221   | -0.291     | 0.257   |
| CVR        | ΔHR        | -0.256         | 0.322   | -0.270     | 0.296   |
| CVR        | ΔCO        | 0.276          | 0.284   | -0.241     | 0.351   |
| CVR        | ΔPetO₂     | -0.237         | 0.36    | 0.239      | 0.356   |
| CKD        | CVR        | 0.402          | 0.249   | -0.069     | 0.849   |
| CVR        | ΔHR        | 0.207          | 0.565   | -0.352     | 0.318   |
| CVR        | ΔCO        | -0.351         | 0.320   | 0.316      | 0.374   |
| CVR        | ΔPetO₂     | 0.16           | 0.659   | -0.143     | 0.694   |
| CVR        | eGFR       | -0.175         | 0.629   | -0.626     | 0.063   |
| HD         | CVR        | -0.102         | 0.811   | -0.316     | 0.446   |
| CVR        | ΔHR        | 0.275          | 0.509   | -0.143     | 0.736   |
| CVR        | ΔCO        | 0.116          | 0.784   | -0.313     | 0.450   |
| CVR        | ΔPetO₂     | -0.397         | 0.330   | -0.031     | 0.942   |
| CVR        | dialysis vintage | -0.112       | 0.792   | -0.198     | 0.638   |

CKD, chronic kidney disease patients; ΔCO, change in cardiac output; CVR, cerebrovascular reactivity; eGFR, estimated glomerular filtration rate; HD, hemodialysis patients; ΔHR, change in heart rate; ΔMAP, change in mean arterial blood pressure; ΔPetO₂, change in P₂O₂.

Dialysis vintage: duration of time that the patient has been receiving dialysis.
Our sample size was small yet sufficient to demonstrate significant difference in CVR between HD patients, CKD patients, and healthy non-CKD participants. Absence of CKD in the healthy group was determined based on history and chart review only. Given that we did not collect bloodwork in our healthy participants, we did not estimate their eGFR and cannot fully exclude the possibility of CKD in that group. Our healthy participant group was much younger than CKD or HD groups. This age bias was due to a convenience sample recruitment strategy using poster advertisements that we used at our hospital. The effect of age on CVR has been debated in the literature, with some studies reporting no difference in CVR between young and older adults and some reporting reduction in CVR with age. The different results between these prior studies are likely due to differences in the methods used to measure CBF (e.g., transcranial Doppler vs. 4-D MRI) or in the difference between methods used to induce changes in CO2. Despite age difference between the healthy and CKD participants in our study, they had similar CVRs, suggesting that older age in our CKD and HD groups was unlikely to be a significant determinant of impaired CVR in the HD cohort. Future studies should explore the relationship between age and CVR in CKD and HD patient populations.

Although the portable nature of transcranial Doppler makes it useful for assessment of CVR in the clinic, it has several limitations. First, CBV can underestimate changes in CBF by about 8% because of hypercapnia-induced dilation of insonated arteries, which would underestimate CVR. However, this does not preclude comparison of relative differences in CVR between our participant groups given the same method used in all 3 groups. Second, CBV estimates global CBF, and future studies using tomographic modalities will be required to assess regional differences in CVR. Our study provides much needed pilot data to support detailed CVR assessment in renal disease patients with comprehensive neuroimaging modalities. We used PetCO2 as an estimate of partial pressure of CO2 (PaCO2), which is the actual stimulus controlling CBF. However, in contrast to inhaled CO2 methods that result in variable differences between PetCO2 and PaCO2 across participants, the RespirAct method effectively eliminates alveolar dead space such that PetCO2 closely approximates PaCO2.

CONCLUSION

In conclusion, we showed that HD patients have impaired CVR compared to CKD patients and healthy participants. Given that CVR has emerged as a noninvasive marker of cerebrovascular function, its impairment may predispose HD patients to ischemic brain injury and cognitive decline. Future studies should explore whether impaired CVR predicts development of ischemic brain injury and cognitive decline in HD patients and whether it can be used to identify patients who should be considered for alternative modalities of renal replacement therapy.

DISCLOSURE

MS reports a patent US8459258B2 licensed to Thornhill Scientific Inc. The RespirAct is currently a nonclinical research tool approved by Health Canada, assembled and made available by Thornhill Scientific Inc., a spin-off company from the University Health Network, to research institutions to enable CVR studies.

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SUPPLEMENTARY MATERIAL

Supplementary Material (PDF)

Table S1. Healthy versus CKD group—comorbidities.
Table S2. Causes of CKD.

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