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Glomerular and tubular effects of dapagliflozin, eplerenone and their combination in patients with chronic kidney disease: A post-hoc analysis of the ROTATE-3 study

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Abstract

Aim: Sodium-glucose co-transporter 2 inhibitors and mineralocorticoid receptor antagonists reduce albuminuria and the risk of kidney failure. The aim of this study was to investigate the effects of both agents alone and in combination on markers of the glomerular endothelial glycocalyx and tubular function.

Methods: This post-hoc analysis utilized data of the ROTATE-3 study, a randomized cross-over study in 46 adults with chronic kidney disease and urinary albumin excretion ≥100 mg/24 h, who were treated for 4 weeks with dapagliflozin, eplerenone or its combination. The effects of dapagliflozin, eplerenone and the combination on outcome measures such as heparan sulphate, neuro-hormonal markers and tubular sodium handling were assessed with mixed repeated measures models.

Results: The mean percentage change from baseline in heparan sulphate after 4 weeks treatment with dapagliflozin, eplerenone or dapagliflozin-eplerenone was −34.8% (95% CI −52.2, −10.9), −5.9% (95% CI −32.5, 31.3) and −28.1% (95% CI −48.4, 0.1) respectively. The mean percentage change from baseline in plasma aldosterone was larger with eplerenone [38.9% (95% CI 2.8, 87.7)] and dapagliflozin-eplerenone [32.2% (95% CI −1.5, 77.4)], compared with dapagliflozin [−12.5% (95% CI −35.0, 17.8)], respectively. Mean percentage change from baseline in copeptin with dapagliflozin, eplerenone or dapagliflozin-eplerenone was 28.4% (95% CI 10.7, 49.0), 4.2% (95% CI −10.6, 21.4) and 23.8% (95% CI 6.6, 43.9) respectively. Dapagliflozin decreased proximal absolute sodium reabsorption rate by 455.9 mmol/min (95% CI −879.2, −32.6), while eplerenone decreased distal absolute sodium reabsorption rate by 523.1 mmol/min (95% CI −926.1, −120.0). Dapagliflozin-

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eplerenone decreased proximal absolute sodium reabsorption [−971.0 mmol/min (95% CI −1411.0, −531.0)], but did not affect distal absolute sodium reabsorption [−9.2 mmol/min (95% CI −402.0, 383.6)].

Conclusions: Dapagliflozin and eplerenone exert different effects on markers of glomerular and tubular function supporting the hypothesis that different mechanistic pathways may account for their kidney protective effects.

KEYWORDS
albuminuria, chronic kidney disease, dapagliflozin, eplerenone, mineralocorticoid receptor antagonist, sodium-glucose co-transporter 2 inhibitors

1 | INTRODUCTION

Sodium-glucose co-transporter 2 inhibitors (SGLT2i) and mineralocorticoid receptor antagonists (MRAs) slow the progression of kidney function decline and reduce the risk of kidney failure. However, despite their clinical benefits, the risk of kidney failure remains high in a substantial proportion of patients. The high risk of kidney failure is associated with a persistently high albuminuria.[1] The ROTATE-3 study showed that a combination of the SGLT2i dapagliflozin with the MRA eplerenone resulted in a robust additive reduction in albuminuria compared with treatment with either agent alone.[2] These data suggest that the albuminuria and potentially long-term kidney protective effects of both drug classes are independent and complementary.

The albuminuria-lowering effects of SGLT2i and MRAs are incompletely understood and may involve effects on glomerular and tubular function. With respect to glomerular function, the endothelial glycocalyx of glomerular capillaries is the first barrier against urinary albumin leakage throughout the systemic vasculature including the glomerular arterioles.[3] Impairment of the endothelial glycocalyx reduces the charge-selective properties of the glomerular capillaries and has been shown to be associated with increased albuminuria in various pathophysiological conditions, including diabetes and CKD.[4] Experimental studies have suggested that SGLT2i and the MRA spironolactone ameliorate the endothelial glycocalyx, but clinical evidence is scarce.[5–7] Furthermore, the tubular system is essential for sodium handling and regulation of the renin-angiotensin-aldosterone system (RAAS) in renal physiology. SGLT2i primarily act in the proximal tubule while MRAs exert their effects mainly in the distal tubule potentially leading to different effects on tubulo-glomerular feedback and other compensatory effects to prevent dehydration.[8]

Various biomarkers of glomerular and tubular function are presently available. The aim of this study was to characterize the effects of the SGLT2i dapagliflozin and MRA eplerenone on markers of glomerular and tubular function.

2 | METHODS

2.1 | Study design and patients

This was a post-hoc analysis of the ROTATE-3 study, a prospective randomized open-label cross-over clinical trial. The study design, patient population and primary results have been described previously.[2] In short, the study was conducted at three clinical trial sites in Italy (Naples and Catanzaro) and Spain (Valencia). Forty-six patients aged ≥18 years with urinary albumin excretion ≥100 mg/24 h and ≤3500 mg/24 h, estimated glomerular filtration rate (eGFR) >30 ml/min/1.73 m^2 and <90 ml/min/1.73 m^2, serum potassium ≤5.0 mmol/L and who were on stable doses of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers for more than 4 weeks were eligible for participation. The study involved three consecutive open-label cross-over treatment periods of 4 weeks each, in which patients were randomly assigned to a treatment with eplerenone 50 mg once daily, dapagliflozin 10 mg once daily, or the combination of eplerenone 50 mg once daily and dapagliflozin 10 mg once daily in random order, with wash-out periods of 4 weeks following each active treatment period. Plasma and 24-h urine samples were collected at the start and end of each treatment period.

2.2 | Measurements and outcomes

Surrogate measures for the endothelial glycocalyx were heparan sulphate (HS) and the HS-degrading enzyme heparinase. HS and heparinase levels were determined using an enzyme-linked immunosorbent assay kit according to the manufacturer guideline (catalogue number MBS267373 and MBS764335 respectively; MyBioSource.com). Plasma renin and aldosterone were measured to reflect RAAS activity. Monocyte chemoattractant protein-1 (MCP-1) levels were measured as a surrogate measure for inflammation. MCP-1 levels were determined using an enzyme-linked immunosorbent assay kit according to the manufacturer guideline (catalogue number MBS719631; MyBioSource.com). Copeptin was measured as a surrogate marker for antidiuretic hormone release, as antidiuretic hormone is difficult to measure accurately because of its short half-life and rapid degradation.[9] To characterize the effects on proximal and distal tubular sodium handling, various equations were used to calculate proximal and distal sodium delivery and reabsorption. More specifically, these equations were applied using standard measurements of urinary lithium, sodium and chloride clearances, along with the eGFR. From these measurements, fractional excretion rates of lithium, sodium and chloride (FELi = CLi/eGFR; FENa = CNa/eGFR; and FECl = CCl/eGFR) were derived, as well as the estimated 'proximal' [occurring before

the macula densa) fractional sodium reabsorption rate = (eGFR-CLi)/eGFR]. In addition, the estimated 'distal' absolute sodium reabsorption rates were also calculated: (plasma sodium × CLi) – (urine sodium × urine flow rate) (Table S1).

2.3 | Statistical analysis

We used a mixed repeated measures model, with an unstructured covariance matrix, to assess mean percentage changes in HS, heparinase, renin, aldosterone, copeptin, and proximal and distal sodium reabsorption rates during and at the end of each active treatment period. The mixed repeated measures model included both within-subject and between-subject variability. Treatment type was included as a fixed effect, while subject was considered a random effect. The analyses were performed in the overall population as well as in subjects with microalbuminuria as damage to the endothelial glycocalyx and reduced charge selective barrier function is thought to be a determinant of urinary albumin leakage in subjects with microalbuminuria. We also performed Pearson correlations to assess the correlation between changes from baseline in urine albumin-to-creatinine ratio (UACR), eGFR and systolic blood pressure (SBP). Statistical analyses were performed with R version 4.10 (R Statistical Computing).[10]

3 | RESULTS

Between May 2019 and April 2021, 57 patients were assessed for eligibility, of whom 48 were enrolled in the run-in period. Ultimately, 46 patients were randomized into the study. During the follow-up period, two patients died, and two others withdrew consent, leaving a total of 42 patients who completed all treatment periods (Figure S1). Patients with non-missing baseline data and at least one follow-up assessment were eligible for inclusion in the analysis. The baseline characteristics of the 46 patients who were randomized into the study are reported in Table 1. The proportion of patients who adhered to study medication was 86% (SD 11).

3.1 | Effects on markers of glomerular albumin permeability

The mean percentage change from baseline in HS after 4 weeks treatment with dapagliflozin, eplerenone or dapagliflozin-eplerenone was −34.8% (95% CI −52.2, −10.9), −5.9% (95% CI −32.5, 31.3) and −28.1% (95% CI −48.4, 0.1). The mean percentage change from baseline in heparinase with treatment with dapagliflozin, eplerenone or dapagliflozin-eplerenone was −5.9% (95% CI −14.1, 3.1), 4.6% (95% CI −5.3, 15.6) and 4.4% (95% CI −4.7, 14.5) (Table 2 and Figure 1). The effects of the three treatments in reducing heparinase appeared to be more pronounced in subjects with microalbuminuria compared with those with macroalbuminuria (Figure S2) although formal tests for interaction did not reach statistical significance.

TABLE 1 Baseline characteristics of patients.

Age	69.5 (7.6)
Sex, n (%)	
Female	11 (23.9)
Male	35 (76.1)
Type 2 diabetes, n (%)	
No	14 (30.4)
Yes	32 (69.6)
HbA1c, mmol/mol	50.3 (13)
Without type 2 diabetes	39.0 (6)
With type 2 diabetes	55.5 (12)
Blood pressure	
Systolic	136.3 (9.0)
Diastolic	80.0 (7.5)
Estimated GFR, ml/min/1.73 m^2	58.1 (18.6)
UACR[a], mg/g	401 [225, 629]
Medication, n (%)	
Antihypertensive medication use	
ACEi	17 (37.0)
ARB	29 (63.0)
β-blocker	17 (37.0)
Calcium channel blockers	24 (52.2)
Diuretics[b]	22 (47.8)
Thiazide	12 (26.1)
Loop	11 (23.9)
Metformin use	22 (47.8)
Insulin use	11 (23.9)
Statin use	38 (82.6)

Note: Data are given as mean (SD).
Abbreviations: ACEi, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocker; HbA1c, glycated haemoglobin; UACR, urine albumin-to-creatinine ratio.
[a]Geometric mean (25th-75th percentile).
[b]One patient was using both a thiazide and loop diuretic.

3.2 | Effects on tubular neuro-hormonal and inflammation biomarkers

The mean percentage change from baseline in renin with dapagliflozin, eplerenone or dapagliflozin-eplerenone was 4.9% (95% CI −16.7, 32.0), 46.5% (95% CI 15.4, 86.1) and 43.0% (95% CI 13.2, 80.5) respectively. The mean percentage change from baseline in aldosterone was −12.5% (95% CI −35.0, 17.8), 38.9% (95% CI 2.8, 87.7) and 32.2% (95% CI −1.5, 77.4), respectively. Dapagliflozin and dapagliflozin-eplerenone significantly increased copeptin with 28.4% (95% CI 10.7, 49.0) and 23.8% (95% CI 6.6, 43.9) respectively. This effect was not seen with eplerenone [4.2% (95% CI −10.6, 21.4)] (Table S2). The mean percentage change from baseline in MCP-1 with dapagliflozin, eplerenone or dapagliflozin-eplerenone was −7.5% (95% CI −20.9, 8.2), 1.8% (95% CI −13.6, 19.9) and −16.8% (95% CI −29.0, −2.6) respectively (Table S2).

TABLE 2 Changes in biomarkers of glomerular albumin permeability from baseline to week 4 by treatment allocation.

	Dapagliflozin	Eplerenone	Combination
Heparan sulphate, pg/ml			
N	39	36	38
Baseline mean	64.4 (81.0)	55.3 (43.9)	52.9 (40.6)
Week 4 mean	35.0 (26.7)	50.6 (36.8)	43.2 (36.2)
Change from baseline	−34.8 (−52.2, −10.9)	−5.9 (−32.5, 31.3)	−28.1 (−48.4, 0.1)
p-Value	<.01	.72	.051
Heparinase, pg/ml			
N	44	41	43
Baseline mean	2344.2 (983.9)	2241.7 (966.7)	2261.4 (917.8)
Week 4 mean	2253.1 (1174.5)	2361.8 (1084.0)	2345.5 (973.3)
Change from baseline	−5.9 (−14.1, 3.1)	4.6 (−5.3, 15.6)	4.4 (−4.7, 14.5)
p-Value	.19	.36	.35

Note: Shown are mean and standard errors. Change from baseline is given in geometric mean percentage change and 95% CI.

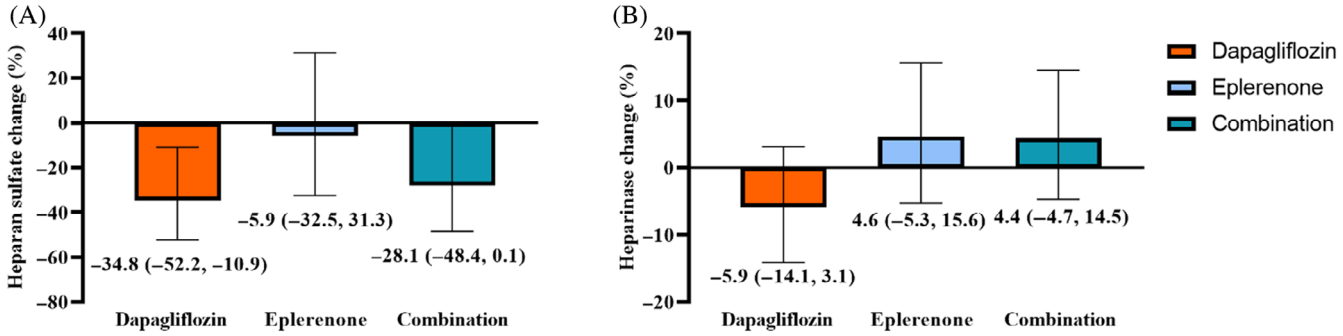

FIGURE 1 Geometric mean percentage change in (A) heparan sulphate and (B) heparinase by treatment allocation. Numbers below each graph indicate the geometric mean percentage change (95% CI) from baseline.

3.3 | Effects on tubular sodium handling

The mean percentage change from baseline in fractional lithium excretion with dapagliflozin, eplerenone or dapagliflozin-eplerenone was 20.5% (95% CI 3.8, 39.8), −1.1% (95% CI −15.7, 16.1) and 3.4% (95% CI −11.2, 20.3). Absolute proximal sodium reabsorption changed with −455.9 mmol/min (95% CI −879.2, −32.6) and −971.0 mmol/min (95% CI −1411.0, −531.0) during dapagliflozin and dapaglifl ozin-eplerenone respectively, but not during eplerenone. Absolute distal sodium reabsorption did not change during dapagliflozin or dapagliflozin-eplerenone treatment but changed with −523.1 mmol/min (95% CI −926.1, −120.0) during eplerenone treatment (Table 3).

3.4 | Correlation between changes in systolic blood pressure, estimated glomerular filtration rate and urine albumin-to-creatinine ratio

As reported previously, all treatments reduced UACR, eGFR and SBP.[2] Changes in UACR correlated with changes in eGFR during dapagliflozin treatment but not during eplerenone or dapagliflozin-eplerenone treatment. Changes in SBP correlated with changes in UACR during eplerenone treatment but not during dapagliflozin or dapagliflozin-eplerenone treatment (Table 4).

4 | DISCUSSION

In this post-hoc analysis of the ROTATE-3 study we showed that dapagliflozin, eplerenone and their combination exert differential effects on glomerular and tubular biomarkers. Moreover, the albuminuria-lowering effects of dapagliflozin correlated with acute reductions in eGFR but not SBP while albuminuria-lowering effects during eplerenone treatment correlated with acute reduction in SBP but not eGFR.[2] These differential effects support the notion that SGLT2i and MRAs exert different mechanistic pathways, which may explain their additive albuminuria-lowering effects.

This study showed that HS levels significantly reduced during treatment with dapagliflozin. This suggests that dapagliflozin positively affects the preservation of the endothelial glycocalyx barrier function. Eplerenone did not reduce HS. Dapagliflozin was associated with a modest reduction in heparinase, but this effect was more

TABLE 3 Changes in biomarkers of tubular sodium handling from baseline to week 4 by treatment allocation.

	Dapagliflozin	Eplerenone	Combination
Fractional lithium excretion, %			
Change from baseline	20.5 (3.8, 39.8)	−1.1 (−15.7, 16.1)	3.4 (−11.2, 20.3)
p-Value	.015	.89	.66
Proximal absolute sodium reabsorption, mmol/min			
Change from baseline	−455.9 (−879.2, −32.6)	−375.2 (−826.1, 75.7)	−971.0 (−1411.0, −531.0)
p-Value	.035	.10	<.01
Distal absolute sodium reabsorption, mmol/min			
Change from baseline	−9.5 (−388.1, 369.0)	−523.1 (−926.1, −120.0)	−9.2 (−402.0, 383.6)
p-Value	.96	.012	.96

Note: Fractional lithium excretion change from baseline is given in geometric mean percentage change and 95% CI. Absolute sodium reabsorption is given in mmol/min and 95% CI.

TABLE 4 Correlations between changes in SBP, eGFR and UACR for different treatments.

Treatment	ΔUACR−ΔeGFR	ΔUACR−ΔSBP	ΔSBP−ΔeGFR
Dapagliflozin	$R = 0.49$, $p < .01$	$R = -0.017$, $p = .92$	$R = 0.15$, $p = .33$
Eplerenone	$R = 0.09$, $p = .56$	$R = 0.40$, $p = .011$	$R = 0.28$, $p = .08$
Combination	$R = 0.12$, $p = .40$	$R = -0.19$, $p = .26$	$R = 0.23$, $p = .13$

Note: Shown are the correlation coefficient (R) and the statistical significance (*p*) of the presented correlations. Δ is the change from baseline until the end of the treatment.
Abbreviations: eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; UACR, urine albumin-to-creatinine ratio.

pronounced in patients with microalbuminuria, consistent with the notion that endothelial glycocalyx damage is primarily linked to microalbuminuria.[11,12] Other studies showed that SGLT2i improved endothelial glycocalyx function.[7] While we did not observe an effect of eplerenone on HS or heparinase, experimental studies reported that spironolactone ameliorated endothelial glycocalyx function.[5] These experimental studies used direct measurements of the endothelial glycocalyx in the glomeruli while our study was limited by the measurement of surrogate markers of the glycocalyx, which may explain these different findings.

Eplerenone showed the greatest effect on the hormones involved in the RAAS, as evidenced by a substantial and significant increase in both renin and aldosterone levels. The increase in renin and aldosterone during eplerenone treatment is probably attributed to feedback loops as a result of the inhibition of the mineralocorticoid receptors. In contrast, dapagliflozin exhibited little effect on the RAAS hormones. Dapagliflozin and dapagliflozin-eplerenone combination therapy showed an increase in copeptin levels. This effect has been observed previously and is probably a secondary effect explained by the relative fluid deficit resulting from SGLT2-induced glycosuria.[13,14]

As anticipated, eplerenone showed a substantial and significant reduction in fluid and sodium reabsorption within the distal tubule. However, dapagliflozin seemed partially to inhibit this effect as the pronounced decline observed during eplerenone monotherapy was substantially diminished during combination therapy. Dapagliflozin and particularly the combination of both dapagliflozin and eplerenone showed a substantial and significant decline in sodium resorption in the proximal tubule, which is anticipated to activate tubulo-glomerular feedback resulting in a decrease in glomerular hyperfiltration and an acute reduction in GFR as reported previously.[2] The correlation between UACR and eGFR during dapagliflozin treatment can be explained (at least partly) through activation of tubulo-glomerular feedback and glomerular haemodynamic effects, which leads to a reduction in intraglomerular pressure and decrease in albuminuria. This has been observed in the DAPA-CKD trial as well.[15] There was no correlation between acute changes in GFR and UACR during eplerenone treatment. However, interestingly, during eplerenone treatment, reductions in SBP correlated with UACR. Eplerenone counteracts the effects of aldosterone, including sodium and water retention and blood vessel constriction leading to a reduction in blood pressure. The observed correlation between SBP and UACR suggests that systemic blood pressure reduction is transmitted into the glomeruli and may alleviate stress on the glomerular filtration system, potentially resulting in decreased UACR. However, further mechanistic studies are warranted to validate these speculative explanations. Further research is also required around the safety of these therapies, in particular potential acute kidney injury, as both dapagliflozin and eplerenone reduce blood pressure and acutely decrease GFR, an effect that is reversible after discontinuation of both agents.

To the best of our knowledge, our study is the first to investigate the independent and complementary nephroprotective effects of both SGLT2i and MRAs. Dapagliflozin and particularly the combination of both medications showed a positive effect on the renal proximal tubule by reduction of the fluid and sodium reabsorption. This

increases the delivery of sodium to the distal tubules and inhibits the release of renin and aldosterone. These changes in tubular function and the RAAS may help to preserve the integrity of the glomerular filtration barrier and reduce albuminuria. Furthermore, during dapagliflozin, a decreased breakdown of the glycocalyx was noted by reducing HS. The greatest effect was most pronounced in patients with microalbuminuria during the combination therapy, which is consistent with the notion that glycocalyx damage is primarily linked to microalbuminuria.[11,12] In addition, the effect of eplerenone on the RAAS and renal distal tubule, by blocking the aldosterone receptors, results in a decrease in both blood pressure and blood volume, consequently leading to a reduction of albuminuria. Although previous studies showed that both eplerenone and dapagliflozin exert anti-inflammatory effects,[16–18] we did not observe a reduction in MCP-1 in the current study. It is possible that the treatment period was too short to detect a reduction in urinary MCP-1. When dapagliflozin and eplerenone were combined we observed a reduction in urinary MCP-1. As increased albuminuria has been associated with inflammation, beneficial glycocalyx effects coupled with downstream anti-inflammatory effects might explain the robust additive UACR-lowering effect when combining dapagliflozin with eplerenone.

This study has limitations. First, it was performed as a post-hoc analysis of a study with a follow-up period of only 4 weeks. It was not possible to investigate if the reductions in albuminuria and changes in the parameters continue over a longer time period. Secondly, the biomarkers HS and heparinase and MCP-1 were used as surrogate outcome measures to examine the effect of the therapies on the endothelial glycocalyx. Further research is needed to determine the impact of the therapies on the glycocalyx by direct measurement of the glycocalyx and more direct measurement of arterial inflammation using imaging techniques such as positron emission tomography imaging.[19] We also recognize that MCP-1 is just one of multiple inflammatory markers. Further research is warranted to elucidate the broader inflammatory pathways affected by these therapies and to assess their clinical significance in a larger patient population. Finally, a placebo treatment period was not included. We were therefore only able to compare parameter responses during active treatment.

In conclusion, this study aimed to explore the mechanisms behind the albuminuria-lowering effects of dapagliflozin, eplerenone and the combination therapy, and by doing so, initiating the development of novel therapeutic strategies to further reduce albuminuria and lower the risk of kidney failure in patients with CKD. This study supports the hypothesis that SGLT2i and MRAs reduce albuminuria through different mechanistic pathways supporting the hypothesis that combined SGLT2i and MRAs may result in additive long-term kidney protective effects.

AUTHOR CONTRIBUTIONS

TTGFL collected the data, performed the analysis and wrote the manuscript. MJP collected the data and wrote the manuscript. UDJM designed the study and wrote the manuscript. MP collected the data and wrote the manuscript. GK designed the study, collected the data and wrote the manuscript. NJ performed the analysis and wrote the manuscript. SEW collected the data and wrote the manuscript. RHJAS designed the study and wrote the manuscript. MA collected the data and wrote the manuscript. LDM collected the data and wrote the manuscript. LDN collected the data and wrote the manuscript. JLG collected the data and wrote the manuscript. HJLH designed the study, collected the data, performed the analysis and wrote the manuscript.

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CONFLICT OF INTEREST STATEMENT

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PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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