which glycolysis suppresses oxidative phosphorylation and mitochondrial respiration, may occur, particularly when higher concentrations of glucose are used.\textsuperscript{13} Thus, our view of one metabolic pathway taking over from another is largely a reflection of the experimental systems that we use to study them, and in reality, these metabolic pathways are concurrently used. Subtle skewing of the balance between them may have significant consequences for differentiation, but understanding this nuanced systems biology of metabolism may require a new generation of technologies, perhaps featuring \textit{in vivo} reporting.

The paper by Liu \textit{et al.}\textsuperscript{4} breaks new ground and forces us to consider metabolic programming and its temporal nature in the process of nephrogenesis. This work is certain to spawn new and exciting studies to help understand not only the physiology and biochemistry of nephron differentiation but also, the potential aberrations that initiate and drive renal neoplasia.

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\section*{DISCLOSURES}
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See related article, “Regulation of Nephron Progenitor Cell Self-Renewal by Intermediary Metabolism,” on pages 3323–3335.

\section*{Extracellular Vesicles in Preeclampsia: Evolving Contributors to Proteinuria}

Elizabeth A. Phipps*†‡ and Eliyahu V. Khankin*†§

*Renal Division, Brigham and Women’s Hospital and †Division of Nephrology, Massachusetts General Hospital, Boston, Massachusetts; ‡Harvard Medical School, Boston, Massachusetts; and §Division of Nephrology, Department of Medicine, Beth Israel Deaconess Medical Center, Boston, Massachusetts


Preeclampsia remains a condition with high morbidity and mortality, with inherent challenges in diagnosis and treatment. Patients are typically referred to nephrologists to rule out preeclampsia in the setting of nephrotic syndrome or new-onset acute kidney failure during pregnancy. Preeclampsia is thought to be a vascular disease with maternal endothelial dysfunction due to circulating placental antiangiogenic factors.\textsuperscript{1} It is currently believed that glomerular capillary damage is the primary factor that leads to proteinuria; however, the precise mechanisms are still being debated. Garovic \textit{et al.} first proposed that loss of glomerular podocytes in the urine that has been observed in many nephrotic syndromes including preeclampsia may be a key factor that contributes to the pathogenesis of proteinuria.\textsuperscript{2}

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Correspondence: Dr. Eliyahu V. Khankin, Beth Israel Deaconess Medical Center, 99 Brookline Avenue, RN-370A, Boston, MA 02215. Email: ekhankin@bidmc.harvard.edu

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The effects of podocyte injury and the characterization of diseased podocytes have therefore emerged as important areas of study. It is known that the expression of podocyte-specific proteins is altered in patients with preeclampsia, with preserved expression of podocin and reduced expression of nephrin in tissue. This difference is maintained in the podocytes isolated from the urine of women with preeclampsia. To date, several research groups have attempted to study podocyte-specific protein shedding in the urine as a potential biomarker for the condition. Interestingly, although the expression of nephrin in podocytes is downregulated in patients with preeclampsia, urinary levels are increased, suggesting that nephrin shedding in the urine (nephrinuria) has mechanistic and diagnostic significance in the disease. Po
docyturia has also been studied as a potential biomarker for preeclampsia, but the identification of urinary extracellular vesicles (EVs) of podocyte origin may hold greater diagnostic significance.

EVs are semispherical structures (nano- to micron-sized) composed of a fluid core encapsulated by a lipid bilayer released by cell membrane surfaces in both physiologic and pathologic conditions. Pisitkun et al. first described EVs in the urine in 2004. Since then, several other studies have demonstrated various urinary EVs to have potential as biomarkers for kidney injury as well as other maladies. The outer shell of the EV is rich in disaturated lipids, such as sphingomyelin and gangliosides, and contains a cytosol-derived fluid core; thus it can deliver proteins, lipids, and nucleic acids specific to the cell type from which they originate in a form protected from surrounding enzymatic degradation. Although their use has emerged as an exciting new investigative tool, laboratory techniques for isolating and processing EVs remain a challenge.

In this issue of the Journal of the American Society of Nephrology, Gilani et al. expand on the above findings with exciting new evidence. They hypothesize that the altered expression of podocyte-specific proteins in patients with preeclampsia would be reflected in urinary EVs and urinary nephrin levels, whereas the formation of EVs is linked to podocyte injury induced by circulating cellfree fetal hemoglobin (HbF) in maternal plasma. The authors studied 91 pregnant women at delivery: 49 with preeclampsia and 42 normoten
tive controls (patients with gestational hypertension, essential hypertension, and gestational diabetes were excluded). They first conducted a validation study to assess various urine-processing techniques and their effects on EV counts (centrifugation of urine samples resulted in significantly decreased numbers of EVs, whereas freezing and thawing the samples did not alter these). Using flow cytometry and utilizing antibodies to annexin-V (a marker for EVs), nephrin, and podocin, the authors demonstrate elevation of annexin-V–positive EV counts in the urine of patients with preeclampsia compared with controls. The ratio of podocin-positive EVs to nephrin-positive EVs was increased in the samples of patients with preeclampsia, whereas urinary nephrin-specific ELISA demonstrated significant nephrinuria in preeclampsia versus normal urine samples. Importantly, the podocin-positive/nephrin-positive EV ratio and nephrinuria correlated positively with proteinuria, cystatin C levels, BP, and uric acid levels, and negatively with plasma hemopexin levels. The mechanistic experiment aimed to elucidate the effects of cell-free HbF in a rabbit preeclampsia model, and demonstrated significantly increased albuminuria and annexin-V– and podocin-positive EV counts in HbF-injected animals compared with controls.

The work of Gilani et al. presents numerous important advances. As EVs emerge as valuable clinical markers of disease, standardized methods for their detection and quantification are gradually becoming more and more essential. The group identifies and thoroughly evaluates the technique for the processing of urine samples that reliably preserves EVs and allows for further quantification and characterization. The study builds on a prior understanding of preeclampsia-specific podocyte protein expression and EV profiling to show that the ratio of podocin-positive/nephrin-positive urinary EVs is increased in preeclampsia, and correlates positively with nephrinuria, proteinuria, and other hallmarks of the condition, including impaired renal function, elevated BP, and elevated serum uric acid. Given the need for robust, reliable biomarkers for preeclampsia, the podocin-positive/nephrin-positive urinary EV ratio may provide clinicians with a more sensitive diagnostic tool allowing for more prompt identification of affected pregnancies, compared with currently used diagnostic tests. The relative simplicity of urine sample collection and recent developments and advances in the field of microfluidics and on-chip system-based microvesicle isolation makes the EV-based method of preeclampsia diagnosis an attractive target for the development of bedside and rapid testing applications.

This study also builds on previous evidence in an attempt to further elucidate a mechanistic link between increased maternal plasma cellfree HbF levels and urine albumin and EV levels. Prior work in this field has suggested hemolysis with subsequent free hemoglobin and heme-related injury to the podocytes and endothelial cells as the underlying mechanism of renal histopathological findings associated with preeclampsia. Naëv et al. demonstrated podocyte mitochondrial and endoplasmic reticulum swelling and apoptosis, along with a prominent degree of obliterated endothelial cell fenestrations and areas of increased but structurally aberrant fenestration in rabbits infused with HbF. This study provides additional compelling evidence for this mechanism via measuring the downstream effects of podocyte injury after HbF infusion (namely albuminuria and annexin-V– and podocin-positive EV formation).

Moving forward, it will be important to replicate and expand on the current processing techniques for EVs as they develop an increasing role as markers of disease. It will also be important to explore the expression of podocin-positive and nephrin-positive EVs at different time points throughout gestation. Does the increased ratio hold true early
in gestation? Also, methods developed here for urinary EV isolation and characterization may be useful for other kidney disorders.

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DISCLOSURES

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REFERENCES


Why Diuretics Fail Failing Hearts

Evan C. Ray,* Cary R. Boyd-Shiwarski,* and Thomas R. Kleyman†‡

*Renal-Electrolyte Division, Department of Medicine and †Departments of Pharmacology and Chemical Biology and ‡Cell Biology, University of Pittsburgh, Pittsburgh, Pennsylvania


Loop diuretics represent a key component of the therapeutic armamentarium used to reduce extracellular fluid volume in heart failure. Unfortunately, resistance to loop diuretics commonly occurs, complicating clinical management. In general, diuretic resistance refers to the inability to reduce extracellular fluid volume, despite maximal therapeutic doses of diuretics. There are several proposed mechanisms through which diuretic resistance in heart failure occurs.1 First, activation of the sympathetic nervous system and release of volume regulatory hormones increase the reabsorption of filtered Na⁺ in the proximal tubule, resulting in reduced Na⁺ delivery to more distal segments.2,3 Second, enhanced Na⁺ reabsorption may occur at sites distal to the thick ascending limb of the loop of Henle, damping potential urinary Na⁺ losses that occur with administration of a loop diuretic. This may reflect activation of distal convoluted tubule (DCT) Na⁺/Cl⁻ cotransporters by diuretic-induced hypokalemia as well as hypertrophy of cells in the DCT.4,5 Recent studies suggest that low serum K⁺ levels, which can occur with administration of a loop diuretic, lead to activation of kinases that eventually result in phosphorylation and activation of the Na⁺/Cl⁻ cotransporter in the DCT.6,7 Furthermore, distal nephron Na⁺ transporters, including the Na⁺/Cl⁻ cotransporter, the epithelial Na⁺ channel, the Na⁺–dependent Cl⁻/HCO₃⁻ exchanger, and pendrin, a Na⁺–independent Cl⁻/HCO₃⁻ exchanger, are activated by volume regulatory hormones (angiotensin II and/or aldosterone) in the context of reduced effective arterial volume.7–11 Third, reduced glomerular filtration diminishes the filtered load of Na⁺.12 Fourth, impaired gastrointestinal absorption or reduced proximal tubular secretion of a loop diuretic can result in reduced delivery of the diuretic to the tubular lumen.12,13 Understanding key mechanisms of diuretic resistance in the human heart failure population is important in determining approaches to overcome resistance.

The work published in this issue of the Journal of the American Society of Nephrology by Rao et al.14 addresses mechanisms of diuretic resistance in an outpatient heart failure population managed at the Yale Transitional Care Center. In


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Correspondence: Dr. Thomas R. Kleyman, Renal-Electrolyte Division, University of Pittsburgh, 3550 Terrace Street, Pittsburgh, PA 15261. Email: kleyman@pitt.edu

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