Ramnath, R. D., Butler, M. N., Foster, R. R., & Satchell, S. C. (2020). Response to There is little evidence that the endothelial glycocalyx has a specific role in glomerular permeability of albumin. *Kidney International*, *97*(5), 1057–1058. https://doi.org/10.1016/j.kint.2020.02.006

There is little evidence that the endothelial glycocalyx has a specific role in glomerular permeability of albumin

To the editor: It is not commonly appreciated that the most accurate quantitative measure of the interaction of glycosaminoglycan chains (such as in syndecans) with each other or with albumin is through the analysis of their equilibrium thermodynamic interactions.[1,2] There are many ways to do this, say through osmotic pressure analysis. These studies have, for over 70 years, shown that the interaction is not charged-based but one of steric exclusion under physiological conditions. This immediately tells you that charge selectivity, which has been proposed as a major force governing transglomerular transport of albumin, is a flawed concept. This has been shown now in many studies, particularly in the reanalysis of the work of Haraldsson *et al.* and Deen.[2] I would encourage any reader interested in this area to read these critiques and the reexamination of the data. It is also salutary to note that if such an extraordinary force existed it would have been recognized in the general scientific literature; absolutely no recognition, outside nephrology, has ever been made.

There are still deniers of the lack of charge selectivity, although where this may be operating has been a moving target. In the study by Ramnath *et al.*,[3] the mantle has been assigned to the endothelial glycocalyx. With all the controversy of the overturning of the charge selectivity concept (once a basic tenet in nephrology), anyone would have to be concerned that there still remain research groups purely devoted to it and the fact that the glomerular filter offers an extremely restrictive filter to albumin. Ramnath *et al.*[3] feel justified that an increase of 4.9-fold in albumin urinary excretion in 8-week post-streptozotocin diabetic mice can be explained by a 2-fold increase in glomerular permeability for albumin as measured in isolated glomeruli whose syndecan-4 has been shed by a matrix metalloproteinase; these changes can be reversed by treatment with inhibitors to the matrix metalloproteinase. On reading this work, serious ambiguities in interpretation arise from the following:

(i) There is no accounting for tubular uptake of the filtered albumin. The influence of a 2-fold increase in albumin permeability will be reduced and therefore become less of a quantitative explanation for the 4.9-fold increase in albumin excretion. This clearly diminishes the putative role of the glycocalyx and raises doubt as to the site of action of the matrix metalloproteinase inhibitor.

(ii) It is universally accepted that increments in albumin excretion in diabetes occur independently of nonspecific changes in the glomerular filter as measured by glomerular permeability of dextrans and ficolls. In the current study, Ramanth *et al.*[3] do not distinguish whether changes in glomerular permeability are the result of nonspecific or specific alterations to albumin permeability.

(iii) As explained in a previous letter,[2] this group does not measure albumin permeability. They measure a permeability function as well as an albumin-albumin diffusional exchange function, which is not measured in any other studies on glomerular permeability or clearance and has no physiological relevance. Therefore, the changes they measure may not have anything to do with glomerular permeability.

(iv) This group is simply dismissive[4] of important experimental 2-photon data that show that the glomerular sieving coefficient of albumin is governed by size alone; they have not recognized that the 2-photon system can accurately provide time-independent glomerular-sieving coefficient of dextrans of known glomerular-sieving coefficients.[2]

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4. Desideri S, Onions KL, Butler MJ, et al. The authors reply. *Kidney Int*. 2018;94:220.

Wayne D. Comper[1,2]

[1]Salaqua Diagnostic Inc., New York, New York and Los Angeles, California, USA; and [2]Salaqua Australia Pty. Ltd., Melbourne, Australia

Correspondence: *Wayne D. Comper, Salaqua Inc., 888c 8th Avenue, Suite 258, New York, New York 10019, USA. E-mail: wcomper@hotmail.com*

Kidney International (2020) **97,** 1057; https://doi.org/10.1016/j.kint.2020.01.041

The authors reply: We write in response to the letter entitled "There is Little Evidence That the Endothelial Glycocalyx Has a Specific Role in Glomerular Permeability of Albumin,"[1] in reference to our original publication.[2] The title of the letter is very misleading. It represents only the opinion of this author and is diametrically opposed to the data presented in our paper and an accumulating body of evidence including *in vivo* multiphoton measurements.[3] This author's claims are not supported by experimental observations, and we and others have addressed them extensively including in a response[4] to a previous similar letter. It is curious that the major focus of the letter seems to be on charge selectivity when charge is not mentioned in our article.

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Raina D. Ramnath[1], Matthew J. Butler[1], Rebecca R. Foster[1] and Simon C. Satchell[1]

[1]*Bristol Renal, Translational Health Sciences, Bristol Medical School, University of Bristol, Bristol, UK*

Correspondence: *Raina D. Ramnath, Bristol Renal, Translational Health Sciences, Bristol Medical School, University of Bristol, Dorothy Hodgkin Building, Whitson Street, Bristol BS1 3NY, UK. E-mail: mdrdr@bristol.ac.uk*

Kidney International (2020) **97,** 1057–1058; https://doi.org/10.1016/j.kint.2020.02.006

Renal tubular lysosomal vacuoles are a generic toxic manifestation and not particularly associated with agrochemicals and heavy metal toxicity or specific to a disease

To the editor: I congratulate Vervaet *et al.* for their article containing excellent pictures of tubular cells.[1] However, the acronym used, *CINAC* (chronic interstitial nephritis in agricultural communities), is misleading; persons not living in agricultural regions and not exposed to agriculture or agrochemicals can also develop the same chronic kidney disease of unknown etiology, which stigmatizes them.[2] Lysosomal organelles facilitate cellular metabolism, degradation of macromolecules, recycling, and redox regulation, which maintain cell survival.[3] Lysosome tubulopathy includes hereditary and acquired forms. Genetic abnormalities of lysosomal organelles cause life-threatening storage disorders,[3] whereas acquired lysosomopathies manifest as generic tubulointerstitial nephropathies.

Once lysosome membranes are damaged, they release chemical irritants, enzymes, and proteins into cytoplasm, causing cellular disruptions and even apoptosis. In acquired lysosomal diseases, toxic tubular pathologies are the final common pathway but are not specific to particular toxins, agrochemicals, or diseases.

Using ultrastructural studies, lysosomal tubulopathies have been reported in many common and rare diseases.[4] Such ultrastructural studies report similar manifestation of lysosomal vacuoles. Metabolically, highly active tubular cells react to neutralize toxins so, unsurprisingly, manifesting abnormalities are similar, representing lysosomopathies of toxic tubular cell damage. However, these do not point to any particular causative factor, including agrochemicals, and are not novel findings.

As noted in the article,[1] such vacuoles have been reported after exposure to many nephrotoxins, including antibiotics and antirejection agents. Reported lysosomal manifestations, including vacuolation,[1] are nonspecific and the findings are too preliminary and overly theoretical to tie to agrochemicals, as suggested. I suggest that the reported findings are speculative and not novel or confirmatory of any particular cause.

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Sunil J. Wimalawansa[1]

[1]*Department of Medicine and Endocrinology, Cardio Metabolic & Endocrine Institute, North Brunswick, New Jersey, USA*

Correspondence: *Sunil J. Wimalawansa, Department of Medicine and Endocrinology, Cardio Metabolic & Endocrine Institute, North Brunswick 08902, New Jersey, USA. E-mail: suniljw@hotmail.com*

Kidney International (2020) **97,** 1058; https://doi.org/10.1016/j.kint.2020.01.021

The authors reply: We thank Dr. Wimalawansa[1] for the interest in our work[2] and appreciate the critical remarks.

With respect to our proximal tubular histopathological observations, in particular the presence of enlarged dysmorphic lysosomes containing dispersed dark aggregates, we emphasize that they are novel in chronic interstitial nephritis in agricultural communities (CINAC)/chronic kidney disease of unknown etiology (CKDu) and several toxic nephropathies, including transplant patients on calcineurin inhibitor therapy.[2] To the best of our knowledge, electron microscopic (EM) images of similar aberrant lysosomes have only been reported for light chain disease by Herrera *et al.*, which we independently confirmed.[3] The EM lysosomal phenotype we report is unquestionably distinct from those that have been reported for lysosomal storage disorders (both genetic and acquired), in as far as EM images thereof are available in the literature. Lysosomal storage disorders generally present intra-lysosomal multilayered depositions (e.g., Fabry) or alternative phenotypes, but do not resemble the dispersed, dark, fairly uniform aggregates observed in CINAC/CKDu and several toxic nephropathy