Letter to the editor: Angiotensin quantification by mass spectrometry

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TO THE EDITOR: With great interest, we have read Mark Chappell’s (4) comprehensive overview on molecular components of the renin-angiotensin system (RAS). It focused on the analytical methods that can be used to detect the activity or concentration of these molecules, with special emphasis on RIA and ELISA.

We fully agree that the sampling procedure is critical to obtain reliable data on angiotensin levels, independent of the method that is used for detection. Obtaining blood samples for angiotensin quantification requires the immediate addition of an efficient protease inhibitor cocktail ensuring efficient stabilization of angiotensin metabolites by instantly blocking all proteases involved in their metabolism. The sampling time has to be kept as short as possible, and an immediate and efficient mixture of the blood sample and the inhibitor cocktail is essential to avoid artifacts, which is particularly challenging when collecting blood samples from mice. The importance of the sampling time is often underestimated and is likely the cause of unexpected shifts of peptide metabolite patterns despite the use of appropriate protease inhibitor cocktails.

The review also discussed mass spectrometry (MS)-based quantification of angiotensin metabolites. Yet, in our opinion, the technical advantages of this novel technology were incompletely addressed. It is true that MS analysis might result in complex spectra, as shown by Lortie and colleagues (7). Current MS methods for angiotensin quantification therefore exclusively employ liquid chromatography-tandem MS (LC-MS/MS), detecting peptide fragmentation signatures (mass transitions) that are highly specific for individual angiotensin metabolites (8, 10).

To correct for incomplete recovery, RIA/ELISA-based angiotensin quantification methods usually apply labeled angiotensin I or II as internal standards. However, their recovery does not necessarily mimic that of other angiotensin metabolites (3). In contrast, in the LC-MS/MS approach, stable isotope-labeled internal standards of each individual angiotensin metabolite are added to each sample before preparation (1, 5, 6, 9, 11), thus ensuring the highest accuracy. Here it should be noted that the stable isotope-labeled peptides used in LC-MS/MS are chemically identical to the endogenous target peptides and do not interfere with final detection of the corresponding endogenous angiotensin metabolites.

Chappell suggested that the sensitivity of the LC-MS/MS technique currently is such that angiotensin levels can only be detected reliably when increasing their levels by adding exogenous renin. Here we strongly disagree. It should be noted that LC-MS/MS methods are at least as sensitive as the classical RIA/ELISA-based quantification methods, yielding endogenous angiotensin levels in plasma and various tissues that are identical to those observed earlier with the classical methods (2, 3, 12, 13). Current lower limits of quantification for angiotensin metabolites by LC-MS/MS reach 1 fmol/ml in plasma and 5 fmol/g in tissue samples (1, 5–9, 11).

Clearly, the results of LC-MS/MS and classical RIA/ELISA-based quantification methods should now be rigorously compared in order to further establish LC-MS/MS as a valuable alternative. This time-saving and highly sensitive new technology, combined with appropriate sampling conditions, has brought the biochemical evaluation of the RAS to the next level. Rigorous and accurate quantification is even more important now that multiple, novel angiotensin metabolites have been described in recent years, at levels in the femtomolar range. Without reliable quantification, these findings result in confusion rather than a better understanding of the complexity of the RAS.

DISCLOSURES
M. Poglitsch is working for Attoquant Diagnostics, a company developing mass spectrometry-based quantification methods.

AUTHOR CONTRIBUTIONS
M.P., E.S., and A.J.D. drafted manuscript; M.P., E.S., and A.J.D. edited and revised manuscript; M.P., E.S., and A.J.D. approved final version of manuscript.

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