The buffer power of blood: a reappraisal of its mathematical expressions with implications on the role of albumin as a buffer. Authors' last word.

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TO THE EDITOR:

We would like to thank the colleagues for their interest in our Viewpoint (1).

The role of albumin in acid-base balance is determined by its structure: ~21 aminoacidic groups with a fixed negative charge behave like strong acids, while ~16 imidazoles with a pH-dependent positive charge behave like buffers (2). When adding albumin to a solution (Viewpoint **Panel A**), its fixed negative charges decrease pH and the buffer power calculated according to Stewart (β_{PS} = $A/d[H^+]$) is reduced (3, 4). Similar acid-base effects can be obtained reducing the SID of a solution by adding strong anions (Viewpoint **Panel B**) (1). Dr. Morgan is therefore right that, in order to study the isolated effects of albumin *using* β_{PS}, SID should be kept constant. *In-vivo*, however, changes in albumin and SID often happen in parallel, the latter likely as a compensation of the former (5). For instance, in septic patients (Viewpoint **Panel C**), the lower concentration of albumin's fixed negative charges with respect to healthy subjects (-9.5 [-10.0;-8.8] *vs.* -14.8 [-15.4;-14.5] mEq/L, calculated as -21·[Alb] in mMol/L (2)) is paralleled by an almost identically lower SID (36.0 [32.1;40.0] *vs.* 41.5 [40.1;43.1] mEq/L) (1). The sum of total strong charges in solution (SID + Albumin's fixed charges) is indeed only slightly lower in septic patients (24.0 [21.8;28.3] *vs.* 25.0 [24.0;26.7] mEq/L), explaining their only minimally lower pH at similar PCO_2 (1), and resulting in similar total CO_2 content. In our opinion, this rules out the difference in SID as the major cause of the observed lower buffer power. On the contrary, the latter is likely explained by the reduced concentration in albumin's imidazole groups (7.2 [6.7;7.6] *vs.* 11.3 [11.1;11.7] mMol/L, calculated as 16·[Alb] in mMol/L (2)). Of note, the positive charges on the imidazoles can be calculated as $(16\cdot[Alb] \cdot [H^+]) /(Ka+ [H^+])$, where Ka is their average dissociation constant (~ $1.78\cdot10^{-7}$) (2). Interestingly, the calculated change in imidazoles' charges (per pH unit) during plasma CO_2 titration in our septic patients and healthy subjects was 2.8 [2.4;3.4] *vs.* 4.1 [3.8;4.2] mEq/L, resembling their buffer power obtained experimentally according to Van Slyke: β_{VS} = 2.4

[1.5;2.7] *vs.* 4.0 [3.0;4.4] mEq/L (1). In our opinion, this strongly suggests that the buffer power of albumin is secondary to its imidazole groups, and that it can be correctly quantified by β_{VS}. Moreover, it confirms what stated by Krbec and colleagues: despite a similar PCO_2 variations, due to the different albumin concentration, the variation in total CO_2 differed markedly. Therefore, when calculating buffer power, it seems reasonable to use total CO_2 variations, *i.e.* the added acid, instead of ΔPCO_2.

Finally, we would like to underline that in our Viewpoint (and previous experiments) β_{VS} *was not* calculated based on albumin concentration, its pK and the prevailing pH. As stated in the manuscript, Equation 1 was only described as an alternative to experimental titration (1), while all reported values of β_{VS} were calculated as $d[HCO_3^-]/dpH$ using values of $[HCO_3^-]$ and pH obtained experimentally through CO_2 tonometry.

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