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Response to letter Re: Dynamic pituitary-adrenal interactions in the critically ill after cardiac surgery

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There are no competing interests.

Dorin *et al* [Ref] ask several questions concerning the modeling of cortisol dynamics in our manuscript(1) and specifically, the potential consequences that Cortisol Binding Globulin (CBG) could have had on the estimates of the cortisol secretion rate and its modulation via ACTH. The responses to these questions require their formulation in the following broader context.

One can formulate models of the cortisol dynamics and the travel of bound / unbound molecules in the blood circulation, at three levels. At the finest level, cortisol molecules rapidly bind and unbind to CBG and albumin. The next level involves the rapid mixing, advection (movement with the inherent blood flow), and diffusion of the molecules within the blood. Blood cycles through the circulation at, roughly, 5L/min, and hence for a 70kg individual, once per minute. After cortisol release, and 1-2 cycles through the heart, the molecules are reasonably well mixed. The other rapid effects, advection and diffusion, generally act on a similar time scale, with fast half-lives for most molecules being in the 2-4 min range. The third level, the coarser scale of irrecoverable elimination, at which molecules exit the blood, the half-lives are generally longer. For cortisol, general population estimates are in the 45-70 min range.

In our manuscript(1), the model of cortisol dynamics in the blood was of two components:

1. A fast rate of elimination that represented the entirety of mixing, advection and diffusion.
2. A slow rate of (irrecoverable) elimination.

The finest level of dynamics, the on- and off-binding to carrier proteins, was not included. The basic model for (1)-(2) involves two coupled differential equations, in which a population-based

fraction of allocation between the two was applied. Under an assumption that the starting concentration is allocated by the same fraction, the evolving concentration can be written as a convolution with a biexponential decay rate. In reply to the first question, the estimation of the kinetics and secretion was, as we would describe it, via a two-component model, which can be represented as bi-exponential convolution.

As mentioned by the authors of the Letter, one of the paper's co-authors (DMK) initiated models and estimation procedures for a three-component model which includes the on- and off-binding to CBG and albumin, and hence estimation of free cortisol concentrations (2). The original formulation of the approach occurred with the modeling of testosterone (T) and its binding to sex-hormone binding globulin (SHBG) and albumin (2). One can imagine a hierarchy of potential data, accompanying LH and T, in this context. This could consist of (a) simultaneous SHBG and albumin sampling at the same rate as LH and T (e.g., every 10 min for 24 hrs); (b) SHBG and albumin sampled less frequently, possibly only once (at the extreme); or, (c) SHBG and albumin are not observed, but rather populational assumptions are made so to achieve estimated fractions of free T, T-SHBG and T-albumin. The original modeling was concerned with the data settings of (a)and (b), and less so that of (c) (3-5). The modeling approach was then applied to the HPA axis (2, 6, 7). The approach involved the construction of values for the off- and on-rate constants for CBG and albumin binding, calculated from literature-based values for the population mean equilibrium association (M^{-1}) and dissociation (M) constants for each of cortisol-CBG and cortisol-albumin, as well as mean binding capacities for CBG and albumin, and (in some cases) the half-lives for the off-rates for each of CBG and albumin.

The above modeling and resulting estimation methodology utilizing all three levels of cortisol dynamics in the blood, is certainly valid, especially in the data setting (a). The issue though is the potential effect of the imposed assumptions on the estimation, as one proceeds from the context of (a), where the carrier proteins are sufficiently sampled, to that of (c) where they are not sampled, but are replaced by populational assumptions. This is the setting of the present manuscript (1). In the Critically Ill (CI) group, CBG and albumin were measured for each subject at the beginning and end of the 24-hr period. The CI group's CBG and albumin mean and SD values are given in Table 1 within the manuscript; the values are in the normal range. For the control group, neither CBG nor albumin were measured.

To have estimated free cortisol in the manuscript would have necessitated different estimation methods for the two groups. One potentially could have first estimated free cortisol in both groups, not utilizing the CI group's CBG and albumin values. Then, one could estimate free cortisol for the CI group, utilizing the carrier protein data. If there was no difference between the two methods for the CI group, then free cortisol could have been estimated for both groups by the same method. This was not done, mainly because of the following reasoning. The perspective of the co-author (DMK) was that the imposition of assumptions on the unobserved on- and off-binding, with no carrier protein data, may well result in estimation bias. It is not unlike an attempt to find a path of least action, where one may have wrongly imposed restrictions on the path passing through specified multiple points at certain times.

The alternative is (and was) a "lumping" of all the dynamical components into just two terms: fast- and slow-rates of elimination; one might further assume a fixed half-life for the fast rate. That is, our conclusion in this context was that we would consider the carrier protein

binding effect as being manifested in the other two rates, and to do the estimation as a two-component model. We fixed the fast rate half-life for cortisol at 2.41min and for ACTH at 3.5min, which are published, population-based estimates. Hence, it was then the slow rates which were estimated. The fast rate dynamics should be reasonably similar for the two groups (this is mainly as a result of re-distribution of the molecules), and that physiological effects. Under these assumptions, with any carrier protein effects being subsumed within the estimates of the slow rate of elimination, one would expect accurate cortisol secretion rate estimates. We observed estimates for the slow rate half-life 53 vs 56 min for the CI and control groups respectively and statistically concluded that there was no difference. In (6-7), CBG and albumin were not observed, and the approach taken was that of the present manuscript.

We did analyze the ACTH data, which is presented in Table 3 in the manuscript. Because of the restriction as to the volume of blood withdrawal in the CI group, ACTH was assayed every hour, not every 10 min. The control group ACTH was assayed every 10 min, and comparisons were done on the control group to see the effects of subsampling ACTH every hour vs every 10 min, for both the estimation of ACTH secretion as well as the estimation of the ACTH-cortisol dose response parameters. Those results are included in the supplementary data. The estimation of the efficacy (parameter) of the ACTH drive on cortisol secretion rate, only involves interpolation in the sense that the ACTH feedforward signal had to be interpolated at the 10 min level, in the case of the CI group. The logistic dose-response function that was utilized was the symmetric logistic and we did not extend the ACTH-cortisol curve in any manner.

As to what effect the potential measurement of CBG and albumin, and the inclusion of a third-component for cortisol could have had on our estimates of cortisol secretion and its

modulation by ACTH, it is difficult to infer. For example, in this context, with the limitations on the amounts of blood that can be withdrawn, one can only assume that any changes in CBG amounts in the critically ill, would be manifested in the slow rate estimates, i.e., the composition. Further, the specific critical illness being studied, could or could not respond differently to variations in CBG and albumin. Hence, in response to the primary question in the Letter to the Editor, it is difficult to infer the potential consequences that CBG and albumin could have had on the estimates of the cortisol secretion rate and its modulation via ACTH, and the comparison of the present manuscript to that of other critical illnesses.

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