Reply to “Letter to the editor: Does low-frequency power of heart rate variability correlate with cardiac sympathetic tone in normal sheep?”

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REPLY: We thank Pellegrino and Schiller (3) for their detailed critique and their suggestions for further analysis concerning our recent article (2) published in in American Journal of Physiology-Heart and Circulatory Physiology.

These authors raised the general issue that “the idea that no relationship exists simply because a regression model does not yield a statistically significant correlation coefficient is problematic.” Obviously, we did not claim that low-frequency (LF) power of heart rate variability (HRV) bears no relationship with the tone of cardiac sympathetic nerve activity (CSNA). This would have been equivalent to claiming that we had proven our null hypothesis. Rather, we concluded that our results did not support the use of LF HRV as a noninvasive estimate of either CSNA or cardiac baroreflex sensitivity. In contrast, we found that baroreflex sensitivity outperformed LF HRV in tracking CSNA. Pellegrino and Schiller then raised several specific issues concerning our statistics and data handling which, they argue, could have led us to false positive and false negative conclusions.

Possible false positive. They argued that the significant negative correlation that we found between heart period (HP) and CSNA (Fig. 4, A and B) was a false positive result driven by the significant differences between control and heart failure sheep (Table 1). We agree and stated this in the discussion: “the correlation between HP and CSNA tone was relatively weak . . . and mainly reflected differences between groups.”

Possible false negatives. The main specific contention made by Pellegrino and Schiller was that we had failed to detect a real link between LF HRV and CSNA tone (only in control not in heart failure sheep) for three reasons: 1) we did not test the control and heart failure sheep separately, 2) we did not exclude outliers, 3) we normalized LF HRV to the high-frequency (HF) power of HRV rather than to total power minus its very LF component.

In response to point 1, we tested separately in each sheep group whether there was a significant correlation between LF HRV and CSNA tone. With the unaltered data, there was no significant correlation in either group. In response to the request in the letter we provide here $r^2$ and $P$ values for these correlations:

- Log LF HRV vs. Log CSNA spike frequency in control sheep, $r^2 = 0.002, P = 0.894$
- Log LF HRV vs. Log CSNA burst incidence in control sheep, $r^2 = 0.13, P = 0.344$
- Log LF HRV vs. Log CSNA spike frequency in heart failure sheep, $r^2 = 0.02, P = 0.714$

In point 2, they suggested that an outlying data point could have obscured a real correlation between LF HRV and CSNA tone in control sheep. With this point removed, there is indeed a correlation in control sheep between LF HRV vs. CSNA spike frequency ($r^2 = 0.61, P = 0.022$) and vs. CSNA burst incidence ($r^2 = 0.62, P = 0.022$). But is it correct to remove that point? When all groups were considered together, the point in question failed the suggested outlier test (Cook’s distance $>1$, although we appreciate that this threshold choice is arbitrary). When considering control sheep alone, the point also failed the outlier test for the correlation between LF HRV and CSNA burst incidence. The point was an outlier only when considering the correlation between LF HRV and CSNA spike frequency in control sheep alone. Without strong independent justification, our view is that such data selection is arbitrary.

As for point 3, Pellegrino and Schiller stated that our choice of the LF-to-HF index was “a straw man” because they were “not aware of any claims that the LF-to-HF ratio is specific for sympathetic tone.” A statement that the LF-to-HF ratio is considered by some investigators to mirror sympathetic/vagal balance or to reflect sympathetic modulations can be found in the 1996 Task Force report on HRV, probably the most authoritative source on HRV analysis (4). A correspondence between results obtained with LF HRV in normalized units and with LF-to-HF is expected, as these two indexes have been shown to be mathematically equivalent carriers of information (1). In response, however, we have now computed correlations between LF HRV in normalized units (as suggested by Pellegrino and Schiller) and either CSNA spike frequency or CSNA burst incidence. None of these correlations was statistically significant; their coefficient of determination was invariably low ($<0.07$), and no data point had a Cook’s d value $>1$, regardless of whether computations were performed on all sheep together or separately on each sheep group.

We acknowledge that the measures of baroreflex sensitivity between the two groups did not reach statistical significance. However, there were clear significant changes in other variables such as CSNA and HP in the HF group. Type II statistical errors (false negatives) cannot be ruled out in any statistical analysis. However, it should be noted that coefficient of determinations between indexes of LF HRV and CSNA tone were invariably $=0.13$ (when no data were excluded, see above), meaning that at best, LF HRV captured 13% of the variance of CSNA tone. This would seem too low to support the use of LF HRV as a measure of CSNA tone, even if a larger study with greater statistical power were able to uncover a statistically significant relation.
In conclusion, we thank Peter Pellegrino and Alicia Schiller for this discussion. Scientific debate is paramount to progress. Yet, after careful consideration of all of the issues raised by these authors, we remain convinced of the final conclusion of our paper: perhaps it is time to accept that changes in HRV may never be a substitute for direct recordings of CSNA.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

D.M. and A.S. drafted manuscript; D.M., A.S., R.M.M., C.M., and R.R. edited and revised manuscript; D.M., A.S., R.M.M., C.M., and R.R. approved final version of manuscript.

REFERENCES