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Reply

Reply to Kovacs *et al.*: Concerning acute inflammatory response following focused ultrasound and microbubbles in the brain

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We thank Kovacs *et al*. for their editorial [15] on our recent publication [1] regarding the influence of microbubble (MB) dose on acute inflammatory response (AIR) following focused ultrasound (FUS)-induced increases in blood-brain barrier (BBB) permeability. We wish to address key points of disparity in interpretations of the data presented by us and in a previous report from Kovacs *et al* [2].

All data published to date indicate that an AIR follows increased BBB permeability induced by FUS + MBs [1–5]; however, there are discrepancies in the reported magnitude and duration of this response. To address this, we approximated sonication parameters shown by Kovacs *et al.* in *PNAS* to induce a substantial AIR [2] and compared this to parameters that better reflect those used in ongoing clinical trials (ClinicalTrials.gov identifiers: NCT02343991, NCT02986932, NCT03119961) and previous preclinical research [6–10]. Results indicated that MB dose and acoustic pressure impacts the degree of AIR, as measured by changes in the expression of NFκB pathway-related genes. We demonstrated a high degree of correlation between gene expression changes reported by Kovacs *et al.* and those measured in the sonication scheme designed to approximate this work ($r^2 = 0.84$; $p = 0.00001$). Importantly, when MB dose was reduced, and peak negative pressure was calibrated to avoid inertial cavitation, a substantial reduction in the magnitude of AIR was observed. We believe this demonstrates that the permeability of the BBB can be transiently increased using FUS + MBs with a minimal AIR, as well as highlights the need for both careful attention to sonication parameters and the use of acoustic feedback control.

In their editorial, Kovacs *et al.* discuss differences in sonication parameters between the two studies [1,2] and conclude that these differences preclude a direct comparison of results. The first major difference is in MB dose. To approximate equivalent doses, necessitated by a disparity in MB type between the two studies, we used clinical imaging dose as a normalizing factor. Optison was administered at ~500 µL/kg versus Definity administered at 100 µL/kg, in Kovacs and McMahon, respectively. Both doses equate to 10 times the clinical imaging dose of their respective MB type. Kovacs *et al.* correctly highlight differences in MB number/kg between studies. However, using MB number as a method of comparing doses of different MB types necessarily considers all cavitation nuclei as equivalent; the assumption that each Definity MB will respond like an Optison MB, or vice versa, is somewhat over simplistic since differences in their shell properties and size alter their response to ultrasound [11,12]. Additionally, McDannold *et al.* have previously demonstrated that the probability of increased blood-brain barrier (BBB) permeability following FUS is approximately equivalent for Definity and Optison at their respective clinical imaging doses over a range of pressures [6].

Kovacs *et al.* also point out differences in anesthesia carrier gases and infusion rates between studies, both of which affect the number of MBs present in circulation while FUS is delivered. Using this as a metric to compare experimental conditions, they conclude that the number of MBs/kg present in circulation is lower in Kovacs *et al.* than in McMahon and Hynynen (scheme 2; designed to approximate conditions in Kovacs *et al.*). Again, this analysis considers every cavitation nuclei as equivalent; however, the smaller mean diameter of Definity MBs would contribute to a reduced impact on BBB permeability at this frequency relative to Optison MBs [12,13].

While it is important to note that there are several differences in experimental parameters, ultimately, the high degree of correlation in differential gene expression measured in the two studies suggests that the biological responses were very similar. We believe that the work presented in both Kovacs *et al.* [2] and McMahon and Hynynen [1] highlight the importance of optimizing sonication parameters for the desired effect with careful analysis of AIR especially for repeated treatments, as well as the necessity of using acoustic emissions to calibrate and control the applied ultrasound pressure. The continued development, refinement, and careful study of FUS + MBs for increasing BBB permeability is an important endeavor for advancing this technique into clinical implementation and for assessing the spectrum of its safety profile.

Abbreviations

AIR: acute inflammatory response; BBB: blood-brain barrier; FUS: focused ultrasound; MB: microbubbles

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Competing Interests

KH is the founder of FUS Instruments, from which he receives non-study related support. DM declares no competing financial interests.

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