We thank Silburt et al. (1) for their comments on our article (2). The authors provide us with an opportunity to expand on sterile inflammation in the brain induced by pulsed focused ultrasound (pFUS) (3). pFUS with microbubbles (MB) resulting in blood–brain barrier disruption (BBBD) is accompanied by plasma protein extravasation into the extracellular space. Fundamentally, any BBBD is a nonhomeostatic condition and leakage of albumin activates microglia and astrocytes (4).

To appropriately contextualize our biological data (2), we must make a few technical notes. Silburt et al. (1) accurately point out that many techniques in the FUS field are plagued by lack of rigorous optimization. Accordingly, our study sought to use a relatively benign but clinically relevant treatment strategy. The FUS peak negative pressure was 0.3 MPa (in water), below limits for inertial cavitation and microhemorrhages (5, 6). Silburt et al. (1) incorrectly claim we infused 5- to 10-fold more MB than McMahon et al. (7), who used $2.4 \times 10^8$ MB/kg of Definity, compared with the $2.0 \times 10^8$–$3.2 \times 10^8$ MB/kg of Optison used in our study (2). Furthermore, Silburt et al. (1) ignored subtleties relating to MB type, animal oxygenation status, and number of treatment targets. Definity’s dispersity is more uniform than Optison’s, translating to a greater fraction of Definity’s population cavitating under similar sonication parameters. McDannold et al. (8) reported that BBBD is further hampered by inspiring 100% O$_2$, like our study, resulting from decreased MB lifetimes in circulation (9). Our experimental parameters essentially reduced acoustic cavitation effects relative to several other studies in the literature, but resulted in BBBD accompanied by sterile inflammation nonetheless. Finally, we sonicated nine regions in the frontal cortex, compared with four treatment regions in McMahon et al. (7).

Many studies only sonicate a single target point, which limits detection of parenchymal abnormalities. The number of sonication points is an uninvestigated area of paramount importance if pFUS is to be used in pathologies like Alzheimer’s disease, which would require sonicating large brain volumes.

The molecular changes we (2) report and the transcriptomics reported in McMahon et al. (7) do not substantially overlap. Transcriptomic analyses focused on the endothelium without considering shockwave effects on cells comprising the neurovascular unit and subsequent molecular changes. Proinflammatory interleukins and IFN-γ were not investigated in McMahon et al. (7). The increased intercellular adhesion molecule-1 (ICAM1) expression reported by us could be explained by additional target foci and the associated rapid cascade of cytokines (2).

Vascular endothelial growth factor and erythropoietin are associated with increased ICAM (10) and BBBD, and are not strictly dependent on hypoxia, although previous studies demonstrate vasospasm during BBBD by pFUS (11). Our molecular profiling was not encyclopedic and unmeasured molecular factors could increase erythropoietin following FUS. Our study (2) did not contain long-term follow-up, so we cannot comment on Silburt et al.’s (1) claim of a lack of damage or regeneration in response to the BBBD and induced sterile inflammation.

Our study (2) raises important complicating issues regarding FUS bioeffects. Substantial and rigorous evaluation of molecular and inflammatory changes from multiple sonications is required in other models (3) before the technique can be confidently moved into clinical trials.


*Frank Laboratory, Radiology and Imaging Sciences, Clinical Center, National Institutes of Health, Bethesda, MD 20892; and National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, Bethesda, MD 20892

Author contributions: Z.I.K., S.R.B., and J.A.F. wrote the paper.

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1To whom correspondence may be addressed. Email: zsofia.kovacs@nih.gov or jfrank@helix.nih.gov.

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