

Research Support

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Competing Interests

The authors declare no competing interests.

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In Reply:

We have carefully read the letters from Fettiplace *et al.* and Zaugg *et al.* regarding our paper¹ and would like to respond to their comments.

In response to Fettiplace *et al.*, we disagree that the study lacks appropriate controls for both the models used. For the bupivacaine model, we believe that we have all the controls needed, given that we investigated the possible effects of 30-min pretreatment of GW1100 on heart function before bupivacaine administration. For the ischemia-reperfusion injury model, we have two control groups: the ischemia-reperfusion group without lipid emulsion, and the GW1100 perfusion group without ischemia-reperfusion injury. We agree with both Fettiplace *et al.* and Zaugg *et al.* that having an additional control group of ischemia-reperfusion injury with GW1100 pretreatment would have shown potential effects of GW1100 pretreatment on post-ischemia-reperfusion cardiac function, if any.

In addition, in our study we did not aim to identify the role of GPR40 inhibition in normal cardiac function. We did, however, perform transthoracic echocardiography to measure left ventricular function before and 30 min after an intravenous bolus of GPR40 inhibitor GW1100. GW1100 is a specific antagonist of GPR40 and does not block GPR120 at the dose used.² Contrary to their comment on GW1100 having physiologic effects in this model, we observed that pretreatment with GW1100 had no significant effect on the heart rate and left ventricular ejection fraction after 30 min (heart rate: 302 \pm 7 *vs.* 312 \pm 14, *P* = 0.36; ejection fraction: 69 \pm 1% *vs.* 71 \pm 1%, *P* = 0.11) excluding any acute adverse effects of GW1100 on the heart rate and left ventricular ejection fraction.¹ Hence, the possibility of GW1100 causing cardiotoxicity on its own in this time frame, as suggested by the authors, is unlikely.

Furthermore, we have used two different animal models in our study. The fact that the ischemia-reperfusion injury model is an *ex vivo* Langendorff perfused mouse heart model leaves little possibility of pancreatic insulin influencing the results. The results in this model, therefore, suggest a direct effect of GPR40 inhibition

independent of pancreatic insulin release. In the bupivacaine-induced cardiotoxicity model, however, we did not measure insulin or glucose levels. We agree that measuring insulin or glucose levels before and 30 min after GW1100 bolus could demonstrate the possible effect of that dose of GW1100 on the levels of insulin and glucose, if any. We agree that the use of cardiac specific GPR40 knockout mice for assessing the effect of lipid emulsion in ischemia-reperfusion injury model is a promising avenue for future research. To the best of our knowledge, however, the bupivacaine-induced cardiotoxicity model has not yet been established in mice. Most of the pharmacologic studies in the bupivacaine cardiotoxicity model done by different groups, including Dr. Weinberg's group, have been done using the rat model.^{3–5}

In response to Zaugg *et al.*, first we did not aim to investigate the functional role of GPR40 (FFAR1) in cardiomyocytes in this study. Instead, we demonstrated the presence and localization of GPR40 using two methods; Western immunoblotting in mouse and rat hearts and immunofluorescence staining in isolated mouse cardiomyocytes. The presence of GPR40 in rodent hearts is in agreement with the previous studies showing its messenger RNA detection in murine⁶ and human hearts.⁷

Because we did not measure fatty acid concentrations in this study, the comment on fatty acid concentrations in this study is purely speculative. In addition, the comment of Zaugg *et al.* regarding missing links to previously reported signaling pathways is inaccurate. We did refer to studies reporting signaling pathways involved in lipid emulsion-induced cardioprotection, namely activation of Akt, GSK, ERK, and STAT^{8–11} (Refer to paragraph 1 of the Introduction and paragraph 4 of the Discussion). Our group was the first to discover the activation of intracellular signaling pathways (such as GSK and Akt) in the heart in response to lipid emulsion-induced cardioprotection in ischemia-reperfusion injury and bupivacaine cardiotoxicity models.^{5,8–10}

Regarding the author's suggestion of an alternate mechanism for intralipid-mediated cardioprotection, we already have cited their previous work in the Introduction section of our paper (see reference 4 in our paper). The mention of their alternate mechanism in this letter seems purely out of context.

In summary, we appreciate the constructive comments from Fettiplace *et al.* and Zaugg *et al.* on our work. Our work highlights the effects of inhibition of GPR40 (FFAR1) using a specific antagonist in abolishing the cardioprotective effects of the lipid emulsion in cardiac ischemia-reperfusion injury and bupivacaine-induced cardiotoxicity. Future studies are needed to (1) investigate the possible effect of GPR40 inhibition on insulin and glucose levels in the context of lipid emulsion-induced rescue of bupivacaine cardiotoxicity and (2) investigate lipid rescue of cardiac ischemia-reperfusion injury in GPR40 knockout mice.

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Competing Interests

The authors declare no competing interests.

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