Metabolite Palmitoylcarnitine Mediates Intralipid Cardioprotection Rather Than Membrane Receptors

To the Editor:

We read with interest the article by Umar *et al.*[1] on Intralipid-induced cardioprotection and the potential mechanistic involvement of the G-protein-coupled receptor 40 (GPCR40), now officially named Free Fatty Acid Receptor 1 (FFA1).[2] Based on the abolished Intralipid-induced postischemic functional recovery in Langendorff-perfused mouse hearts subjected to ischemia-reperfusion and treated with Intralipid postconditioning in the presence of GW1100 (a noncompetitive FFA1 antagonist), the authors concluded that activation of FFA1 mediates Intralipid-induced cardioprotection against ischemia-reperfusion injury. We have concerns with their conclusions because of several limitations in their experimental design and methodologic approach.

First, immunoblots and immunostaining as presented do not show positive and negative controls. Although FFA1 messenger RNA has been previously detected in both murine[3] and human[4] cardiac tissue, its expression levels are very low. Thus, positive and negative controls are required to ultimately prove the presence of the FFA1 protein in cardiomyocytes. Moreover, no data demonstrate that FFA1 is functional in cardiomyocytes and activated by agonists.

Second, an essential control group with GW1100 alone in the absence of Intralipid is missing. This is required to demonstrate that GW1100 itself (at the concentration used) does not affect postischemic functional recovery. Without this control, the observed GW1100-induced reduction in recovery in Intralipid-treated hearts could be simply due to direct adverse effects of the antagonist itself. The authors present data on GW1100 alone under aerobic but not reperfusion conditions showing a decrease in heart rate, which could worsen Ca^{2+} handling and deteriorate functional recovery during reperfusion. Lack of data on heart rates during reperfusion makes a proper interpretation of the results impossible.

Third, heart perfusions in the study by Umar *et al.*[1] were conducted in the absence of physiologic fatty acid concentrations. Hence, improved cardiac function in the Intralipid group could be due to provision of supplementary fatty acids, the preferred energy substrates of the heart, which we have shown previously boost recovery of postischemic mechanical function.[5]

Fourth, appropriate links to the previously reported signaling pathways involved in Intralipid cardioprotection, namely activation of Akt, ERK, and/or STAT[6–8] are absent. Specifically, there are no data showing that GW1100 blocks any of these pathways.

Although only limited data are available on the pharmacology of FFA1, previous studies report that several fatty acids act as agonists at FFA1 *in vitro*.[9] Even in the absence of exogenous fatty acids, such ligands may be produced by surrounding cells and released to act as agonists, contributing to receptor activity in a tissue-dependent manner. Given that Intralipid-induced cardioprotection occurs in the presence of physiologic fatty acid concentrations, it appears unlikely that these receptors would be further activated upon Intralipid administration. In light of these limitations, we do not see evidence that the membrane receptor FFA1 is involved in Intralipid cardioprotection.

An alternate mechanism of action, one that is independent of FFA1, has been proposed previously to explain Intralipid-mediated cardioprotection. This involves inhibition of mitochondrial complex IV by an active metabolite of Intralipid, palmitoylcarnitine, which results in the generation of mitochondrial reactive oxygen species (ROS) that then activates reperfusion injury salvage kinases (RISK) during early reperfusion.[6] Support for such a mechanism is derived from a number of studies. First, the key role of ROS was confirmed by the demonstration that a ROS scavenger, N-(2-mercaptopropionyl)-glycine, prevents RISK activation and abolishes cardioprotection. Second, examination of the role of individual acylcarnitines generated from each of the principal constituents of Intralipid (oleic acid, linoleic acid, and palmitic acid) revealed that palmitoylcarnitine is the active cardioprotective stimulant, whereas other major metabolites such as oleoylcarnitine or linoleoylcarnitine are ineffective. Third, administration of Intralipid for only 2 min at the onset of reperfusion is sufficient to trigger the same degree of protection as if Intralipid were present during the entire duration of reperfusion.[8] This observation, together with data indicating that enhanced recovery of postischemic mechanical function by Intralipid is demonstrable in hearts perfused with levels of fatty acids in the perfusate mimicking *in vivo* conditions, implies that Intralipid does not act as a source of supplementary energy substrates, and there is no evidence of a "cardiotonic" effect as reported by Rahman *et al.*,[7] using a perfusate devoid of fatty acids.

In conclusion, in the face of rather limited evidence that Intralipid cardioprotection against ischemia-reperfusion is mediated by FFA1, we suggest that Intralipid cardioprotection against ischemia-reperfusion is a classic postconditioning phenomenon triggered by the production of mitochondrial ROS at the onset of reperfusion that activates cardioprotective signaling.

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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 paper[1] 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 ± 7 *vs.* 312 ± 14, $P = 0.36$; ejection fraction: 69 ± 1% *vs.* 71 ± 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