Responses to reviewer comments

We appreciate the reviewer comments and suggestions to improve the manuscript. The text changes we made are highlighted in yellow. We also restructured and somewhat text-edited the appendix tables, which changes are not highlighted to avoid variegation.

Reviewer 2

1. The authors should discuss how the duration of the interventions might influences the reported results (the longest reported intervention was 7 days – diabetes lasts for years). Limitations in this regard and how to overcome them.

Response

In fact, this is the major point throughout the review, which we discuss all over. We are convinced that it is the absence of some essential natural components in the commonly used endothelial cell growth media that makes the cells vulnerable to stringent 'metabolic' conditions. Artificially high rate of endothelial apoptosis reported in the multiple studies especially in the presence of FFA simply prevented long-term studies of hyperlipidemia on vascular endothelial cells in vitro and shaped erroneous opinion in literature regarding acute lipotoxic effects of FFA and palmitate, in particular, on endothelium. Our recent study (Samsonov et al., 2021) appeared among a few to demonstrate that under biochemically proper culture conditions palmitate interferes with insulin signaling in endothelial cells but does not bring cells to death for several weeks of experiments. We believe that it is more adequate in vitro representation of in vivo situation in diabetic vasculature but admit that it is still a limited cell-based in vitro model that, however, has potential for further improvement. We offer to interested researches our experimentally supported recommendations for setting up a long-term endothelial cell culture in diabetic conditions and suggest that many previous studies should be re-evaluated based on this methodology. We added the brief summing message as the last para in the Conclusions section where we also noted that those missed essentials are likely to work in cooperation with AMPK. We also hint at the hemodynamic forces as the likely natural stimulus keeping AMPK active in vascular endothelium even in the presence of diabetes.

2. The authors nicely collected relevant interventions but might also include recommendations for best laboratory practice (please specify your conclusions).

Response

Perhaps, the previous response relates to this comment as well. The introductory “recipe” for setting up a long-term human vascular endothelial cell culture in hyperlipidemic conditions may be found in (Samsonov et al., 2021). This paper is open to public. The importance of the proper preparation of FFA:albumin complex is discussed in the review. Currently, we are not ready to present the complete list of SOPs to support diabetic / metabolic studies in long-term endothelial cell-based culture systems. It is under development by us and hopefully by others and requires further research.

3. Since acute vascular complications (e.g. atherothrombotic events) are clinically important, the authors might include a section in which they discuss relevant literature.

Response

We located the suitable place for such a section in the Introduction. We added the phrase describing association of clinical diabetes and atherosclerosis and very briefly outlined pathologic processes that lead to vascular damage, atherothrombosis and increased cardiovascular mortality typical for this comorbidity. For the detailed description of these issues, we provided references to published review articles that highlight various aspects of clinical / experimental studies of vascular dysfunction in the context of diabetes and atherosclerosis. Alterations made are marked in yellow.