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Journal Name:	International Journal of Biochemistry Research & Review
Manuscript Number:	2014_IJBcRR_14058
Title of the Manuscript:	Phosphatidylcholine-mediated aqueous diffusion of cellular cholesterol down-regulates the ABCA1 transporter in human skin fibroblasts
Type of the Article	Short Research Article

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	1# The authors need check the β-actin primers. The forward and reverse primer there are some gaps, and there are two gaps in 3' and 5' side. This gaps can be a limitation for the Taq polymerase, mainly because the authors evaluate the expression with a end point PCR. Probably will be better evaluate the expression with a quantitative PCR. 2# The other problem with the β-actin primer, is that there are not specific for β-actin. The primers can amplifying ACTG2 variant 1 too, forward primer link 970 to 995 and reverse primer link 1362 to 1368 with the same gaps that for β-actin. The amplicon size is different for β-actin and ACTG2, but reduce the sensibility of the technique, especially in a end point PCR. The authors need revise the primer design and repeat the PCR for β-actin using the same cDNA.	We disagree with these comments. We have used the exact same PCR primer set for β-actin in our last 5 publications, with no problems or no adverse reviews or comments. The NIH has funded 3 grants with the use of these primers. It is not practical to change the primers because these are the control primers, and all data would have to be repeated. The list of our publications using the same primers are shown below: 1. Kawashima, R.L. and <u>Medh, J.D.</u> (2014) Down-regulation of lipoprotein lipase increases ABCA1-mediated cholesterol efflux in THP-1 macrophages. <i>Biochem Biophys Res Comm.</i> 450, 1416-1421. (PMID: 25017912) 2. Dahabreh, D. F. and <u>Medh, J.D.</u> (2012) Activation of Peroxisome Proliferator Activated Receptor-gamma Results in an Atheroprotective Apolipoprotein Profile in HepG2 Cells. <i>Advances in Biological Chemistry.</i> 2, 218-225. (PMID: 23616933) 3. Lopez, V., Saraff, K., and <u>Medh, J.D.</u> (2009) Down-regulation of lipoprotein lipase increases glucose uptake in L6 muscle cells. <i>Biochem Biophys Res Comm.</i> 389, 34-39.



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		(PMID: 19698699) 4. Akopian, D., and <u>Medh, J.D.</u> (2006) Simultaneous isolation of total cellular lipids and RNA from cultured cells. <i>Biotechniques</i>. 41, 426-430. (PMID: 17068958) 5. Chen, Y., Jimenez, A., and <u>Medh, J.D.</u> (2006) Identification and regulation of novel peroxisome proliferator activated receptor-α splice variants in human THP-1 macrophages. <i>Biochem Biophys Acta</i>. 1759, 32-43. (PMID: 16542739)
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Minor REVISION comments	1# The authors don't treat the RNA samples with a specific DNase previously to the reverse transcription. By other side, the primers design is specific for amplifying only cDNA, and some DNA contaminants don't affect to the quantification of the genes evaluated. Indicate it in methods. 2# The authors need indicate 25 cycles in each PCR reaction in methods. This date appear only in the figure 2. 3# The authors need indicate in the lipid treatments if add the same concentration of ethanol absolute in the control group. Small amounts of ethanol will be toxic for so much cell culture. 4# Probably, the authors base the cholesterol efflux assay in some article. Indicate it. In this point, need indicate the numbers of repetitions for each condition and assay (RNA expression, protein, cholesterol efflux,...). The date appear in some figure, but is better indicate it in methods too. 5# The authors need indicate with more detail the lipid extraction. Is interesting know the time with the hexanes:isopropanol solvent, if require shaking, volume for a 12-well plate (probably the end volume is 500 µl/well), number of washes, centrifugation conditions for eliminate the cellular rest, ... complete this section. 6# In the figure 2 B, the authors indicate that the data are representative of at least tree experiments with similar results. Indicate the standard deviation in the graph. 7# The authors study the dose of PC that induce maximum effect after 24 hours. This information will be interesting for others researchers, and can be included in supplementary data. If there any ethical issue, please clarify. If there any competing interest issue, please clarify.	1. The reviewer means DNase (not DNaseA). We have included a statement in the Methods section to indicate that the amplicon size will be obtained only with cDNA and not with DNA. Line 106 to 108, highlighted. 2. The reviewer means 'data' not 'date'. Only figure 2 shows PCR data, so only the legend for figure 2 has that information. We have included information about 25 cycles in the 'Methods' section. Line 105, highlighted. 3. A statement is included to indicate addition of ethanol to control wells. Lines 137-138. 4. A reference is included for the efflux assay (ref 17) and line 141, highlighted. A section on statistical analysis is included in the 'Methods' section: Lines 169-176, highlighted. 5. The lipid extraction protocol is standard and has been described earlier. A reference is included (ref 18). 6. As stated in the legend to Figure 2, the graph in 2B is directly from the image in 2A. Hence there are no error bars shown. 7. The dose-dependent effect of PC is included in the text, and there is no need for a figure, since the information would be redundant.
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		There are not ethical or competing issues to clarify.
<u>Optional/General</u> comments	The authors made a good study in cell culture with PC. Will be interesting study the sphingomyelin, the other important phospholipid in HDL. The cell culture requires small amount of reactive and the authors can obtain results in not much time with a low cost.	We may pursue the effect of sphingomyelin in another study.