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Note to readers

Correspondence regarding "Long term treatment with ACE inhibitor enalapril decreases body weight gain and increases life span in rats"

In an article published by Joan Bosco Pesquero and his colleagues entitled, "Long term treatment with ACE inhibitor enalapril decreases body weight gain and increases life span in rats" *Biochemical Pharmacology* 78 (2009) pp. 951–958 [1], the effects of an ACE inhibitor "Enalapril" has been assessed by the real-time PCR method. The expression of several genes including a transcription factor, Peroxisome Proliferative Activated Receptor gamma (PPARγ), under drug induced compared with controls, has been determined. As described in the Materials and Methods under the Analyses of gene expression by quantitative real time PCR [1], each sample was quantified by determining the cycle threshold (Ct) and then normalized based on the Ct values of the *β-actin* gene. The authors have concluded that expression of eight genes (tested in this study) has been upregulated [1].

The real-time (RT)-PCR technique is a very sensitive and popular technique and expression of a given gene can be detected from very little precious clinical specimen. To determine relative gene expression of a gene of interest under two different conditions, drug induced and control, the mean of delta Ct value [differences between the mean Ct value of a gene of the interest and the mean of Ct value of a housekeeping gene (HKG) of the same sample] can be obtained from this equation: the mean of delta Ct value = [average Ct(target $gene_{unstimulated}$) − average Ct($HKG_{unstimulated}$)] [2,3]. For instance; if Ct value of the gene "A" is 28 and, the Ct value of the Beta-actin gene for the same sample is 23 (under the same condition, *e.g.*, unstimulated (subscript)), thus, delta Ct value of gene A (untreated) is: 28 − 23 = 5. On the other hand, if Ct value of the same gene (gene A) after drug treatment is 30 and Ct value of the Beta-actin gene under the same condition is 23; therefore, delta Ct value of the gene A (drug induced) would be: 30 − 23 = 7. After plotting these values, the column correspondence to number 7 is bigger than the column correspondence to number 5; however, the expression of the bigger column is lower. The bigger Ct value represents the lower gene expression and the mean of a Ct value of an undetectable gene expected to be large (see Fig. 1A, first plot on the second row [4]). Therefore, in the Pesquero study [1] expression of the eight markers shown in Figs. 5 and 6 are indeed down regulated; however, the author's conclusion is the opposite.

Currently, we are investigating the effects of several antihypertensive drugs including an ACE inhibitor on adipocytes. We have measured gene expression of several markers including the *PPARγ* gene by real-time PCR. In none of our experiments (more than 5 independent experiments), we have not observed upregulation of *PPARγ* after drug treatment (Davoodi-Semiromi and Cooper-DeHoff manuscript in preparation). The real-time PCR is still a very popular and powerful technique; thus, correction of this error would prevent ambiguity in the field.

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