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Response to letter regarding article by Patel et al: A Novel Biomarker of Oxidative Stress is Associated with Risk of Death in Patients with Coronary Artery Disease

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Dear Sirs,

We thank Drs Giral and colleagues for their interest in our work.[1] They raise the important query of whether our findings would still persist after adjustment for Gamma Glutamyltransferase (GGT), given that GGT activity hydrolyzes GSH to produce glutamate + cysteinyl glycine. This point however is not relevant to our description of GSH/CySS as a useful biomarker of cardiovascular disease because our samples were all collected with a preservation solution containing a GGT inhibitor to prevent the very problem that they outline. Their letter is useful, however, in providing an opportunity to re-emphasize the point, that there is need for a GSH preservation solution for this assay. In our preservation solution, we use serine-borate, a well-known inhibitor of GGT that has been widely used to prevent this artifact since its discovery in 1959.[2] In our original development of methods, the efficacy of the preservation solution to prevent artifactual degradation was validated by showing that it completely prevented loss of GSH when known concentrations of GSH were added to plasma.[3] Earlier studies by CV Smith et al showed that another commonly used GGT inhibitor, acivicin, was not as effective because it required enzyme turnover to obtain complete inhibition.[4] In use of the serine-borate-containing preservation solution to study aminothiols we found that the distribution of GSH, Cys-Gly and CySS in freshly collected human plasma showed metabolite correlations indicating that GSH metabolism in vivo in

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plasma occurs by two mechanisms, hydrolysis by GGT to Glu + Cys-Gly and reaction of GSH with cystine to produce Cys + cysteine-GSH disulfide. We reiterated the need to inhibit GGT in our most recent description of this method.[5]

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