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To Determine the Concentration and Enrichment of Glutathione in Human Plasma using Liquid Chromatography-Mass Spectrometry

Glutathione (GSH) is an antioxidant that protects the cell from reactive oxygen species (ROS) by scavenging free radicals and xenobiotics. GSH is synthesized in almost all cells of the body, particularly in liver and red blood cells (RBC). Studies have shown that the rate of GSH synthesis declines during different disease conditions, such as in diabetes and in cancer, due to elevation in ROS levels. Amino acids labeled with non-radioactive stable isotopes were administered during these studies as GSH precursors to determine enrichment, and thus to calculate the synthesis rate of GSH in RBC. Due to far lower concentration of GSH in plasma compared to RBC (μM verses mM), plasma tracer enrichments have never been reported for GSH. In the present study, a liquid chromatography-mass spectrometry (LCMS) method was developed to determine both the concentration and isotopic enrichments of GSH in human plasma. Stable isotope tracer of glycine was infused intravenously to human subjects, and blood was withdrawn at different intervals of time. GSH from plasma and from RBC was derivatized after adding homoglutathione as an internal standard. Both the concentration and enrichment of GSH were measured simultaneously. We found that the enrichments were the same in RBC and in plasma, which suggests that RBC represents a significant source of GSH in extracellular fluid. The advantage of this LCMS method is its high sensitivity and small sample size requirement (100 μl of plasma or 20 μl of RBC). This method greatly extends our range of measurement of GSH synthesis in human subjects.