

S005 Quantitation of markers of protein glycation, oxidation and nitration in cellular and extracellular proteins and body fluids.

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Cellular and extracellular proteins suffer significant damage in vivo by glycation, oxidation and nitration. These processes form glycation, oxidation and nitration adduct residues in proteins. Glycated, oxidized and nitrated proteins undergo cellular proteolysis and release glycation, oxidation and nitration free adducts. These adducts are quantified using liquid chromatography with tandem mass spectrometric detection by stable isotopic dilution analysis. Free adducts are determined by analysis of ultrafiltrates of plasma, urine and other physiological fluids. Protein adduct residues are determined by assay of enzymatic hydrolysates of protein extracts prepared using cocktails of proteases. Protein damage markers (14 glycation adducts, 3 oxidation adducts and 3-nitrotyrosine) are quantified concurrently using 25 µg protein or 25 µl physiological fluid. Levels of markers of protein damage increase in tissue and blood cell protein extracts, plasma, urine and other body fluids in ageing and disease. Examples of marker profile changes in diabetes, renal failure, cirrhosis, Alzheimer's disease and aging will be described. Increased protein glycation – particularly dicarbonyl glycation of arginine residues – is often found in oxidative stress. Protein glycation may be a consequence or cause of oxidative stress. Protein damage may reflect the state of disease development and therapeutic intervention and hence is under investigation for clinical diagnostic and biomarker applications.