P044 Protein glycation, oxidation and nitration adducts in human low density lipoprotein from normal healthy human subjects and effect of glycation and oxidation *in vitro*.

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Damage to apolipoprotein B100 (apoB100) of low density lipoprotein (LDL) by glycation and oxidation may change functionality and increase atherogenicity in vivo. LDL was isolated from normal healthy human subjects by density gradient ultracentrifugation using Iodixanal density gradient medium. Protein glycation, oxidation and nitration adducts were quantified by LC-MS/MS with stable isotopic dilution analysed of de-lipidated exhaustive enzymatic hydrolysates of apoB100. The major markers of protein damage (mol/mol apoB100) were: glycation adducts - $N\varepsilon$-fructosyl-lysine FL 2.2 ± 0.2, $N\varepsilon$-carboxymethyl-lysine CML 0.031 ± 0.001, methylglyoxal-derived hydroimidazolone MG-H1 0.15 ± 0.01, 3-deoxyglucosone-derived hydroimidazolone 3DG-H 0.08 ± 0.01; oxidation adducts – methionine sulfoxide 1.22 ± 0.016, N-formylkynurenine 0.028 ± 0.006 and dityrosine 0.00084 ± 0.00017; and 3-nitrotyrosine 3-NT 0.0010 ± 0.0002. Glycation of LDL by glucose and by methylglyoxal in vitro to similar minimal extents of glycation for in diabetes in vivo produced mainly increased in FL and MG-H1 glycation adducts, respectively. Oxidation of LDL with 5 μM copper (II) ions for 24 h at 37 ^{o}C increased protein oxidation adduct content by only 9 – 32% and 3-NT residue content by 38%. Early glycation adduct (FL), advanced glycation endproducts (MG-H1) and methionine and tryptophan oxidation adducts are major types of lipoprotein damage *in vivo*. The effects of these modifications on functionality are under investigation.