

P046 Investigating post-translational modifications of Apo B-100 by Oxidative Stress using Mass Spectrometry
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Atherosclerosis is one of the biggest killers in the western world, and oxidative damage to Apo B-100, the protein moiety of LDL (low density lipoprotein) is implicated in the disease. Hence, the detection of oxidative modifications of Apo B-100 would be useful as biomarkers for atherosclerosis, as well as other diseases with an oxidative aetiology. Chlorotyrosine (ClTyr) and hydroxytryptophan (HOTrp) are two such modifications which are produced by the action of hypochlorous acid (HOCl) generated *in vivo* by myeloperoxidase (MPO) during inflammatory conditions. The detection of free chlorotyrosine in the bloodstream has previously been linked to atherosclerosis in patients. The aim of this work is to develop tools to allow the sensitive detection of ClTyr and HOTrp modified peptides, as well as other modifications in Apo B-100. LDL was treated with HOCl *in vitro* to generate HOCl-modified Apo B-100. Proteolytic digest of Apo B-100 followed by LC-MS analysis is being used to detect these oxidations, and sensitive MS² and MS³ methods, along with a standard proteomic approach, are being used to map the sites of oxidation. The susceptibility of these sites to different oxidative modifications and the extent of oxidation is also being determined. ClTyr and HOTrp have been identified in several tryptic peptides of the modified protein. Identification of a number of peptides that are more or less susceptible, coupled to MRM based quantitative methods, can then be used in biological samples to monitor the oxidative load on the system.