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## pH-Dependent 15-Lipoxygenase Catalyzed Peroxidation of Linoleic Acid: HPLC/ESR/MS Study

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Poster Presentation P54

**PH-DEPENDENT 15-LIPOXYGENASE CATALYZED PEROXIDATION OF  
LINOLEIC ACID: HPLC/ESR/MS STUDY**

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Linoleic acid (LA), abundant in plant and vegetable oils, is the most common type of omega-6 polyunsaturated fatty acid consumed in western diets and the preferred substrate for 15-Lipoxygenase-1 (15-LOX-1). Oxidative lipid peroxidation of LA by 15-LOX-1 to produce bioactive metabolites, 9- and 13-HODE, has been shown to influence progressions of cancers. Of the two metabolites, 13-HODE has been the focus of lipid peroxidation research because it is the dominate product from 15-LOX-1 catalyzed oxidative metabolism of LA. However, most of the controversial in-vitro research was performed under normal physiological (pH 7.4) overlooking the strong evidence for acidic microenvironments of tumor tissues (pH 6.8). Our purpose is to determine the metabolite generation patterns of LOX-mediated LA peroxidation under different pH conditions. A combination of LC/ESR/MS was used to detect metabolic adducts generated under pH 6.5 to 8.0 in the presence of spin trap  $\alpha$ -[4-pyridyl-1-oxide]-N-tert-butyl nitron (POBN). In-vitro studies used 15-LOX-1 isolated from soybeans while cell culture studies used human parental HCT-116 colon cancer cell line, and transfected HCT-116 with overexpressed 15-LOX-1. Our in Vitro results show the activity and specificity of 15-LOX-1 varies under different pH. A decrease in total radical adducts with decreasing pH. In addition, the specificity of soybean 15-LOX-1 oxygenation of LA shifted from preferred C-13 to the C-9 position under acidic conditions producing 9-HODE octanoic adduct, but no detectable amounts of 13-HODE pentyl adduct at pH 6.5. Interestingly, cell culture results show no detection of 13-HODE adduct, but the presence of 9-HODE adduct at tumor pH suggests further investigation of the 9-HODE metabolite is needed to determine the role of 15-LOX-1 catalyzed peroxidation of LA in colon cancer.