DETECTION AND CHARACTERIZATION OF NITRO-FATTY ACIDS IN LDL

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INTRODUCTION
Nitro-fatty acids (nitroalkenes, NFA) exert anti-inflammatory signaling actions in human cells and tissues. These compounds have been detected in vivo and are likely to be transported by plasma lipoproteins. Lipid oxidation as well as protein oxidation and nitration in low density lipoprotein (LDL) have been used as biomarkers of proatherogenic LDL. However, there are no reports about the presence of NFA in LDL that could contribute to reduce the pro-inflammatory role of oxidized-LDL.

OBJECTIVES
The aim of this project is to 1) evaluate the presence of endogenous NFA in LDL, 2) determine experimental conditions in which NFA formation could modulate LDL lipid oxidation as well as protein nitration and oxidation and 3) chemically characterize NFA in LDL.

MATERIALS AND METHODS
Human LDL was purified from fresh plasma of normolipidemic volunteers and exposed to different nitrating agents including peroxynitrite and acidic nitrite (pH = 3 or pH = 6). Under these experimental conditions, 3-nitrotyrosine and NFA levels were compared to carbonyl, TBARS and lipid hydroperoxides. NFA levels (nitrooleic acid, nitro-conjugated linoleic acid) in LDL were determined by HPLC-MS/MS analysis following MRM transitions characteristic of nitroalkene compounds.

DISCUSSION AND RESULTS
LDL (3 µM) exposed to peroxynitrite flux (20 µM/min) or 300 µM nitrite at acidic pH, 60 min at 25 °C, exhibited nitro-oleic acid (m/z 326.2/46.1) and nitro-conjugated linoleic acid formation (m/z 324.2/46.1), concomitant with 3-nitrotyrosine formation. NFA reached picomolar levels and were not detected in non-oxidized LDL. When looking for oxidation-derived products, peroxynitrite produced greater levels of carbonyls and TBARS in LDL than nitrite. Nitrite exhibited greater levels of NFA at lower pH.

CONCLUSIONS
This work clearly demonstrates for a first time the formation of NFA in LDL exposed to biologically-relevant nitrating agents. Current experiments involve exposure of macrophages to lipid nitrated-LDL in order to probe its ability to counterpart the pro-inflammatory actions of oxidized LDL.

Keywords: Low-Density-Lipoprotein, nitroalkenes, atherosclerosis