REGULATION OF STEAROYL-COA DESATURASE BY HYDROXYOCTADECADIENOIC ACIDS

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Introduction: Beneficial effects of polyunsaturated fatty acids (PUFAs) include improved lipid profile and insulin sensitivity, leading to decreased cardiovascular risk. PUFAs regulate cell growth, differentiation and alter gene expression including stearoyl-CoA desaturase (SCD), a rate-limiting enzyme in formation of monounsaturated fatty acids (MUFA). Increased SCD levels have been correlated with decreased fatty acid oxidation, increased triglyceride synthesis and thus obesity and insulin resistance. The mechanisms by which PUFAs regulate SCD are not understood. The PUFAs 9 and 13 hydroxyoctadecadienoic acids (HODEs), components of oxLDL, are oxidized derivatives of linoleic acid. Oxidized lipids are known to modulate lipid metabolism in adipocytes and macrophages. Macrophages are involved in pathogenesis of atherosclerosis, obesity and type 2 diabetes. We investigated effects of HODEs, linoleic acid (LA, n-6) and linolenic acid (ALA, n-3) on monocytes and macrophages – in particular, their effect on modulation of SCD.

Methods: THP-1 cells, a human monocyte-macrophage cell line was used. Cells were cultured in RPMI medium with 10% FBS, L-glutamine and antibiotics. To obtain macrophages, cells were treated with phorbol myristate acetate (PMA) for 36 hrs and rested for 24hrs in low serum medium before PUFA treatment. For experiments 1 million cells were treated with 30uM of LA, ALA, 9- and 13- HODEs. For differentiation experiments, THP-1 cells with or without 1nM PMA +/- PUFA were incubated for 24 hrs. Cells were harvested after 24 - 48 hrs and RNA extracted. Expression of SCD was determined by real-time RT PCR.

Results: In monocytes, expression of SCD was not altered significantly by ALA, LA, or 9-HODE but 13-HODE specifically decreased SCD to 40 – 50% of control values. By contrast, in macrophages 13-HODE was without effect (as were ALA and LA), but SCD expression was increased specifically by 9-HODE. The latter also specifically up-regulated expression of its putative receptor G protein coupled receptor 132 (GPR 132) in macrophages. Exposure of monocytes to PMA led to macrophage differentiation in a dose-dependent manner – confirmed by morphology, lipid accumulation and macrophage markers CD 11b and scavenger receptors as well as increased SCD (greater than 2-fold). Cells were fully differentiated with 100 nM PMA and this was not affected by any of the four PUFAs. With 1 nM PMA cells were partially differentiated and SCD expression was inhibited by ALA, LA or 13-HODE but not by 9-HODE.

Significance: Increased SCD expression during differentiation of monocytes into macrophages may contribute to insulin resistance and increased cardiovascular risk. HODEs appear to modulate
expression of this key enzyme. HODEs are produced by non-enzymatic oxidation of LA in the vessel wall, or in macrophages by the action of the enzyme 15-lipoxygenase. The structural differences between the two HODEs and with the parent LA are relatively subtle, but their effects distinct. Evidence is emerging that 13-HODE (an n-7 fatty acid) has protective and anti-inflammatory actions while 9-HODE (an n-6 fatty acid) is pro-atherogenic and pro-inflammatory. In this study, 13-HODE decreased SCD in monocytes and during macrophage differentiation. This latter effect was not found with 9-HODE which increased macrophage expression of SCD and GPR 132. The mechanism of these distinctive effects of HODEs on mediators of insulin resistance and atherogenesis require further study.
**LAY ABSTRACT**

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**Introduction:** We investigated the effects of four polyunsaturated fatty acids (PUFAs) on expression of the enzyme stearoyl CoA desaturase (SCD). Increased levels of SCD have been associated with risk of diabetes and atherosclerosis. We studied linoleic acid (ALA, omega-3), linoleic acid (LA, omega-6) and two derivatives of LA, 9- and 13-hydroxyoctadecadienoic acid (9-HODE and 13-HODE). HODEs are formed in the vessel wall and increased levels have been documented in atherosclerosis.

**Methods:** We used THP-1 cells, a human monocyte line which can be differentiated into macrophages by exposure to phorbol myristate acetate (PMA). Cells were exposed to the four PUFAs for 24 – 48 hours before RNA was harvested to measure expression of SCD and other genes.

**Results:** In monocytes, SCD expression was not altered by ALA, LA, or 9-HODE but 13-HODE decreased SCD to 40 – 50% of control. By contrast, in macrophages 13-HODE was without effect (as were ALA and LA), but SCD expression was increased by 9-HODE, which also increased its putative receptor GPR 132. Exposure of monocytes to PMA led to macrophage differentiation – confirmed by morphology, lipid accumulation and macrophage markers as well as increased SCD. With 1 nM PMA cells were partially differentiated. SCD expression was inhibited by ALA, LA or 13-HODE but not by 9-HODE.

**Significance:** Increased SCD during macrophage differentiation may contribute to cardiovascular risk. HODEs modulate expression of this enzyme. The structural differences between the two HODEs and with the parent LA are subtle, but their effects distinct. Evidence is emerging that 13-HODE has protective actions while 9-HODE is pro-atherogenic. The mechanism of these distinctive effects of HODEs on mediators of insulin resistance and atherogenesis require further study.