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Novel n-3 Fatty Acid Derived N-Acyl Ethanolamides: a Potential Link Between n-3 Fatty Acids and Inflammation

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Dietary n-3 fatty acids have been linked to a reduction of inflammatory processes, but the exact mechanisms underlying this are not completely understood. It is reported that n-3 fatty acids cause a shift in the eicosanoid profile, resulting in increased levels of n-3 eicosanoids, and reduced levels of n-6 eicosanoids. However, a link with endocannabinoids/N-acyl ethanolamides (NAEs) was not established. The present work explored the relation between n-3 fatty acids, their metabolism into novel n-3 NAEs, and their biological effects.

Two series of experiments were performed that focussed on docosahexaenoylethanolamide (DHEA) and eicosapentaenoylethanolamide (EPEA), two novel n-3 fatty acid NAEs.

The first series of experiments aimed to explore the effect of n-3 fatty acids on DHEA and EPEA levels. 3T3-L1 adipocytes were exposed to 10-50 µM of DHA and EPA for 48 hrs, and medium NAEs were quantified using LC-MS/MS [1]. In a *in vivo* study, 6 week old male C57BL/6 mice were fed a control (ctrl) diet or a diet containing 1% or 3% w/w fish oil (FO) for 6 weeks, after which plasma NAEs were determined [2]. Finally, human plasma was analysed for n-3 NAE levels [1].

The second series of experiments focussed on immune-modulating properties of DHEA and EPEA. RAW264.7 macrophages were stimulated with 1 µg/ml LPS and 0.01-10 µM DHEA and EPEA for 48 hrs, after which medium nitric oxide (NO) levels were determined [3]. Finally, human peripheral blood mononuclear cell (PBMC) were stimulated with 1 ng/ml LPS and 0.01-10 µM DHEA or DHA for 24 hrs, after which medium monocyte chemoattractant protein-1 (MCP-1) concentrations were determined using ELISA.

Exposing 3T3-L1 adipocytes to DHA and EPA increased medium DHEA from 20 pg/ml to > 40 pg/ml ($p < 0.05$), and EPEA levels from 500 pg/ml to > 1700 pg/ml ($p < 0.001$), respectively. Murine plasma DHEA levels increased from 0.32 ng/ml in the ctrl group to 0.65 and 0.59 ng/ml with the 1% and 3% FO diets, respectively (both $p < 0.001$). EPEA was only detected at 0.1 and 0.17 ng/ml in the 1% or 3% FO diets, respectively. Finally, DHEA was present in human plasma at 0.17 ng/ml, whereas EPEA was not detected.

Both DHEA and EPEA reduced NO release from LPS stimulated RAW264.7 macrophages at 1 µM ($p < 0.05$ and $p < 0.001$, respectively) and 10 µM ($p < 0.001$). Finally, DHEA reduced human PBMC MCP-1 levels at 5-10 µM (all $p < 0.001$) with a maximum reduction of 55% compared to the vehicle control, whereas DHA was ineffective.

In conclusion, DHA and EPA are converted to their NAE analogues DHEA and EPEA, both *in vitro* and *in vivo*. In addition, these compounds display anti-inflammatory properties using both murine and human *in vitro* assays. Together, these data suggest that these novel n-3 NAEs are a new link between n-3 fatty acids and inflammation.

[1] Balvers MGJ et al, Biochim Biophys Acta 1801:1107, 2010

[2] Balvers MGJ et al, Metabolomics 8:1130, 2012

[3] Meijerink J et al, Br J Nutr 105:1798, 2011