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Nutrient-sensitive interactions between NF-κB and PPARγ

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Dendritic cells (DC) play a key role in the initiation of the inflammatory response, directing the adaptive immune response and determining the nature of the T-helper cell response to inflammatory stimuli. Long-chain *n*-3 PUFA, EPA and DHA have the potential to modulate the immune response[(1)]. However, little is known about the ability of EPA and DHA to modify the interaction between PPARγ and NF-κB.

The present study has investigated the effects of EPA and DHA on expression of PPARγ and on components of the NF-κB pathway in DC isolated from BALB/c mice. Bone marrow-derived immature DC were cultured in RPMI 1640 medium supplemented with fetal calf serum and granulocyte-macrophage colony-stimulating factor for 7 d. DC were treated with either dimethyl suphoxide (vehicle control), EPA (25 μM) or DHA (25 μM) during the length of the culture period. On day 7 DC were stimulated with lipopolysaccharide (LPS; 100 ng/ml) for either 2 or 5 h. At the end of the incubation period, nuclear and cytosolic protein was extracted using the TransAm Nuclear Extraction Kit (Active Motif, Rixensart, Belgium) and levels of NF-κB, PPARγ, inhibitory subunit of NF-κB (IκB) kinaseα and IκBα were measured by Western blot. PPARγ and NF-κB TransAm™ kits (Active Motif) were used to assess the ability of PPARγ and NF-κB to bind target DNA sequences. In order to understand the interaction between PPARγ and NF-κB within the cell, DC cultured under similar conditions were immunostained for PPARγ and NF-κB, and their nuclear and cytoplasmic expression were quantified using a confocal microscope (Improvision Software; Improvision, Coventry, UK).

Western blot and TransAm™ analysis showed that EPA and DHA supplementation was associated with significant down-regulation of components of the NF-κB pathway and decreased affinity to bind target DNA. Treatment with EPA and DHA also lead to an increase in PPARγ levels and an increase in PPARγ:DNA binding affinity.

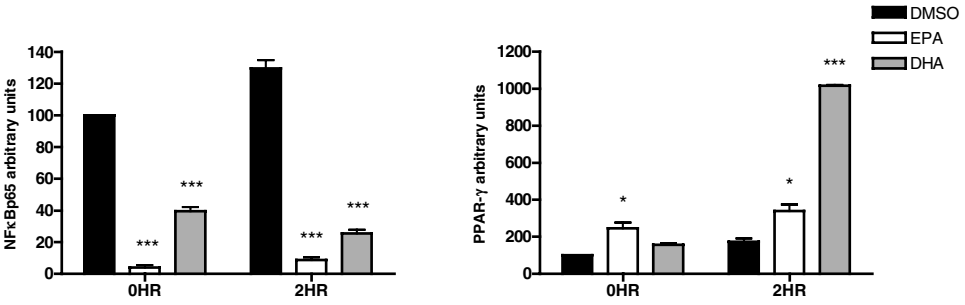

Figure. EPA and DHA modulate PPARγ and NFκBp65.

Confocal microscopy confirmed that pre-LPS stimulation, nuclear and cytoplasmic NF-κB expression was down regulated in both DHA- and EPA-treated cells. In LPS-stimulated DC DHA markedly reduced nuclear NF-κB localisation.

The study shows important nutrient-sensitive interactions between NF-κB and PPARγ in DC, wherein DHA and EPA mediate their anti-inflammatory effects in PPARγ-dependent and -independent mechanisms. Given the important role of fatty acids and DC in innate immunity this observation requires further investigation.

1. Wang, Hao Q, Li QR, Yan XW, Ye S, Li YS, Li N & Li JS (2007) *Nutrition* **23**, 474–482.