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## Gene expression profiles in rat mesenteric lymph nodes upon supplementation with conjugated linoleic acid during gestation and suckling

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The cis9,trans11 (c9,t11) and trans10,cis12 (t10,c12) isomers of the conjugated linoleic acid (CLA) have been suggested to be responsible for modulating immune responses. Previous studies in our group have found a marked immunoenhancement by this isomer mixture on early life rats. However, the mechanism of action of CLA has not been yet elucidated at the molecular level. Our study evaluates the effect of CLA on mesenteric lymph nodes (MLN) gene expression from 21 days suckling rats. The animals were fed by a 80:20 mixture of c9,t11 and t10,c12 CLA isomers (kindly supplied by Loders Croklaan, Lipid Nutrition, Wormerveer, The Netherlands) during gestation and suckling periods. Pups received dietary CLA from dams through the placental barrier and during suckling by breast milk (group A) or by oral gavage (group B). Pups from group C only received CLA during suckling by oral gavage. Group D constituted the reference group. MLN gene expression was determined by hybridisation to the GeneChip® Rat Genome 230 2.0 (Affymetrix, Santa Clara, CA). Quantification was carried out with GeneSpring GX v.10.0.2 software (Agilent Technologies, Santa Clara, CA, USA), which allows multifilter comparisons using data from different experiments to perform the normalisation, generation of restriction lists and the functional classification of the differentially expressed genes. Lists of differentially expressed genes in common between both groups A and B and/or C (for intersections, see Fig. 1) revealed several genes involved in immune response which were modulated by CLA. Moreover, Biological Association Networks (BANs) generation led to the identification of the following gene nodes in response to CLA supplementation: galanin, tissue inhibitor of metalloproteinase 1, connective tissue growth factor, synaptotagmin 1, growth factor receptor bound protein 2, actin gamma 2 and smooth muscle alpha actin. Changes induced by CLA on the mRNA levels of these gene nodes were confirmed by RT-real time PCR, using Taqman<sup>®</sup> specific probes and primers.

These observations on Wistar rats at the end of suckling suggest that  $80:20 \ c_{9,t11:t10,c12}$  CLA mix supplementation is able to modulate MLN gene expression patterns which may be responsible for its immunoenhancing effect.

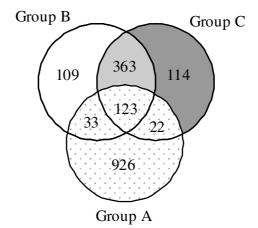


Fig. 1. Venn diagram of all genes in the three groups of 21 days suckling rats analysed: group A (dot-shaped), group B (white) and group C (grey). The intersections between circles represent the common genes using unpaired *t* test (P>0.05) and fold-change  $\ge 2.0$ .