Effect of ALA and DHA on rat adipocyte differentiation process. Incorporation and metabolism of ALA in differentiated rat adipocytes

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BACKGROUND AND OBJECTIVE

The ability of adipose tissue to increase its mass during adulthood through the differentiation of preadipocytes to adipocytes has stimulated scientific interest (1). Free fatty acids (FFA) have been found to affect the differentiation of preadipocytes into adipocytes (2). Docosahexaenoic acid (DHA, 22:6 n-3) is an important omega-3 long-chain polyunsaturated fatty acid (LC-PUFA), which can be synthesized by the body from alpha-linolenic acid (ALA) (3). Anti-obesity effect of DHA has been investigated and evaluated in animal models. DHA has been reported to inhibit 3T3-L1 differentiation and lead to lower triglycerides accumulation in differentiated salmon adipocytes than compared to cells incubated with a media enriched with oleic acid (4,5). On the contrary, ALA has similar effects as PPARγ agonist in differentiation of 3T3-L1 adipocytes (6). In this study we investigated the effect of DHA and ALA on the differentiation process in primary rat cultures.

METHODS

Confluent primary preadipocyte cultures from epididymal adipose tissue of male Wistar rats were incubated in the presence or absence of DHA or ALA -as fatty acid/BSA complexes at 4:1 molar ratio- and the differentiation process was studied by flow cytometry and by semi-quantitative reverse transcription-PCR (RT-PCR) of PPARg2 gene expression. The primers used for RT-PCR were: for PPARγ2, forward 5'-GGT GAA ACT CTG GGA GAT CC-3', reverse 5'-TGA GGG AGT TTG AAG ACT CTT C-3', for adiponectin forward 5'- AGG ATC CAT GCT ACT GTT GCA AGC GCT C -3', reverse 5'-GAA GCT TGT TGG TAT CAT GGT AGA GAA GG -3'.
Additionally the fatty acids in the lipids of differentiated adipocytes were determined –in the form of their methyl esters- by gas chromatography.

RESULTS

According to our results both DHA and ALA induced the differentiation of adipocytes in a dose-dependent manner, but DHA influenced the differentiation process to a lesser extent than ALA under identical conditions (Figure 1).
ALA increased the PPARγ2 and adiponectin gene expression to a higher extent than DHA, under identical conditions (Figure 2).
Both DHA and ALA are incorporated in differentiated rat adipocytes (Figure 3), while ALA is elongated and desaturated to higher ω3 LC-PUFA (Figure 3C).
Evidence of DHA retroconversion in EPA in differentiated rat adipocytes was obtained (Table 1).

ABBREVIATIONS

ALA: a-linolenic acid (18:3n-3); DHA: docosahexaenoic acid (22:6n-3), DPA: docosapentaenoic acid (22:5n-3); EPA: eicosapentaenoic acid (20:5n-3).

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Figure 1. Dose-dependent effect of (A) DHA or (B) ALA on adipocyte differentiation. Seven days after induction, cells were analyzed for their scatter profile using flow cytometry. The granularity value for each gated region at each concentration of *αLA or DHA* was the mean of two samples, and the gated region values for the same concentration are displayed in a stacked column.

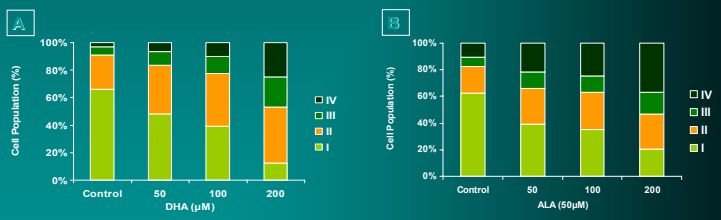

Figure 2. A. Dose- dependent effect of DHA on *PPARγ2* gene expression
B. Effect of DHA and ALA on *PPARγ2* gene expression
C. Effect of DHA and ALA on *adiponectin* gene expression.

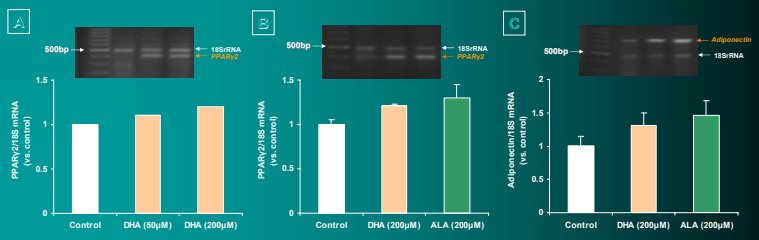

Figure 3. Fatty acids in lipids of differentiated rat adipocytes. A: control; B: after incubation with DHA; C: after incubation with ALA

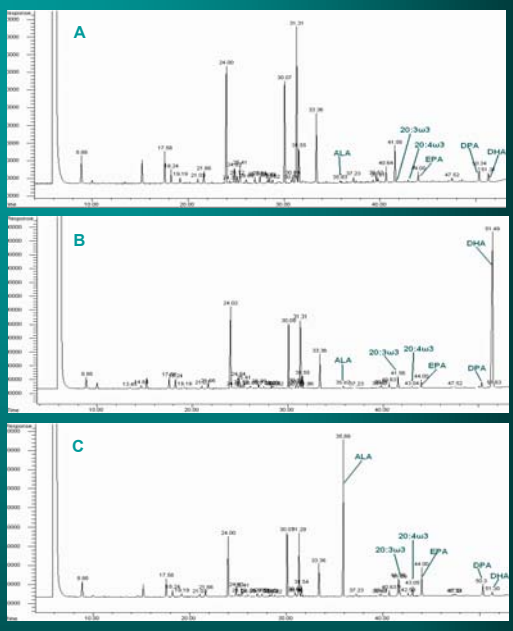

Table 1. Selected fatty acid concentrations (μmol/g of lipids) in rat preadipocytes and adipocytes treated or untreated with DHA or ALA

Sample	18:3ω3 (ALA)	20:3ω3	20:4ω3	20:5ω3 (EPA)	22:5ω3 (DPA)	22:6ω3 (DHA)
Confluent preadipocytes	0.93	0.47	0.66	3.64	6.89	8.81
Differentiated adipocytes	1.19±0.40	0.42±0.30	0.85±0.40	4.31 ± 1.77	9.03±3.50	5.47±1.88
Differentiated adipocytes + DHA	2.66±0.47	0.59±0.18	0.28±0.18	10.0 ± 1.68	10.5±2.94	339.8±42.0
Differentiated adipocytes + ALA	156.8±1.7	18.4±6.58	3.56±1.18	23.3 ± 0.23	19.0±6.39	7.27±2.68