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## **“ GC-EI-MS and HPLC quantification of alpha-linolenic and linoleic acid after flaxseed oil and mixed fish-vegetable oil acute and chronic in vivo administration”**

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$\alpha$ -Linolenic acid (ALA) is a major dietary ( $\omega$ -3) fatty acid. The essential fatty acids must be absorbed by food intake and play a very important role in the coagulation (inhibition of platelets aggregation) and in the inflammatory reaction (anti-inflammatory effects). In cardiovascular diseases, particularly in coronary diseases, studies demonstrated a decreased mortality in populations who eat an omega-3 rich diet or who take an omega-3 supplement.

We studied the bioavailability of acute supplementation of scalar doses of flaxseed oil (Organic Oils–Perugia) by analysing the level of ALA ( $\omega$  -3) and Linoleic acid ( $\omega$  -6) in serum and tissues (adipose, liver and brain) of rats tested at 2-4-8-16 h after the administration. The amount of flaxseed oil administered by oral route was 1.9, 4.7, 9.5 mL/kg corresponding to 1, 2.5, 5 g ALA/kg. Two techniques of lipid extraction were investigated to achieve maximal free fatty acids recovery in a reasonably short time. The corresponding fatty acid methyl esters obtained with direct methylation with MeOH/HCl, were quantified by gas chromatography/mass spectrometry (GC/MS) technique. GC-MS analyses were performed on a Gas-Chromatograph Varian 3400 on a HP-INNOWAX column (30 m x 0.25 mm; 0.25  $\mu$ m film thickness). Mass spectra were acquired on a Finnigan MAT SSQ 710A mass spectrometer in the electron impact (EI) mode with an ionization energy of 70 eV; the ion source temperature was 250°C, the filament current was 200  $\mu$ A, the conversion dynode power was -15.0 kV and electron multiplier voltage was 1500 V.

Serum ALA levels at 1 g/kg after 2h in the flaxseed oil group (n=25) increased by 70% from  $0.067 \pm 0.007$  to  $0.096 \pm 0.008$  mg/mL ( $P < 0.001$  Anova) whereas no significant increase occurred in the flaxseed oil group at 2.5 g/kg ( $0.142 \pm 0.009$ ) or at 5 g/kg after 2 h ( $0.140 \pm 0.008$ ) when compared with the value obtained with 1 g/kg. ALA (1g/kg) significantly increased after 4 h in adipose tissue and in



liver but also in this case at higher doses (2.5-5 g/kg) the concentration wasn't increased. Concerning linoleic acid ( $\omega$  -6) no significant increased concentrations were found in serum at the three doses studied confirming that flaxseed oil is a source of  $\omega$  -3 fatty acids. These data suggested that there is a limiting step in the adsorption of these fatty acids and that there is no advantage to take more than 1 g/kg of ALA supplementation.

