Metabolic aspects of n-3 PUFAs supplementation to rat cardiomyocytes: a HR-MAS NMR and GC/MS study

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Introduction– Cardiovascular diseases (CVD) are responsible for significant morbidity and mortality throughout the world. It is well recognized that dietary intake of polyunsaturated fatty acids (PUFAs) has profound benefits on normal health and prevention of chronic disease states, although the mechanisms responsible remain partially unclear (1,2). Essential fatty acids (EFAs), whether of the omega-3 (n-3) or the omega-6 (n-6) classes, are not synthesized in vivo and thus must be consumed in the diet (3). In this study we present a first investigation using High Resolution Magic Angle Spinning (HR-MAS) NMR Spectroscopy and Gas Chromatography (GC/MS) on neonatal rat cardiomyocytes supplemented with two different polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), in order to understand the metabolic change occurring in these cells following the increase of their n-3 PUFA content. HR-MAS information on the metabolomic profiles in living system is at the beginning, and investigations involving the cardiomyocytes cells are still virtually absent.

Materials and Methods- *Cardiomyocytes cell cultures* Primary cultures of neonatal rat cardiomyocytes were obtained from the ventricles of 2-4 day-old Wistar rats (4). The study protocol was approved by The Animal Care Committee of the University of Bologna. *Magnetic Resonance Spectroscopy.* Before MRS analysis cells were were washed scraped off in deuterated water (D_2O) using a cell scraper. Fifty µl of the cell suspension were introduced in a MAS zirconia rotor (4 mm OD), and transferred into the probe cooled to 4 °C. ^1H and ^{13}C HR-MAS MRS spectra were recorded with a Bruker Avance400 spectrometer operating at 400.13 and 100.61 MHz, respectively. The samples were spun at 4000 Hz and one and two dimensional spectra were acquired using a standard pulse sequence (mlevphpr).*Determination of cardiomyocytes fatty acids composition* On day 8 after seeding cardiomyocytes were washed three times with ice-cold PBS, scraped off and total cellular lipids were extracted according to Folch . Fatty acid composition (as methyl esters) was determined by gas chromatography (GC 8000, Fisons, Milano, Italy) using a capillary column (SP 2340, 0.2 µm film thickness) at a programmed temperature gradient (160–210°C, 8°C/min). Gas chromatographic traces and quantitative evaluations were obtained using a Chrom Card Softwer (Thermo Electron Scientific, Milan, Italy) computing integrator.

Results– First, we characterized the metabolic profile of control cardiomyocytes (figure 1). Control cardiomyocytes spectra were used for the comparison with spectra obtained from cells supplemented with EPA and DHA (figure 2). Quantification of the fatty acid content in each lipid fraction (PL, TG CE) from control, EPA and DHA supplemented cardiomyocytes, derived fron GC/MS analysis, was reported in figure 3.

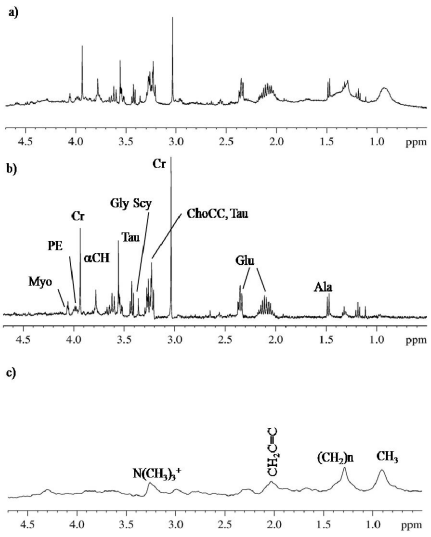

Figure 1. Representative *ex vivo* HR-MAS ^1H NMR spectra of neonatal rat cardiomyocytes**:** a) water-presaturated pulse sequence with composite pulse; b) CPMG spectrum and c) diffusion-edited spectrum.

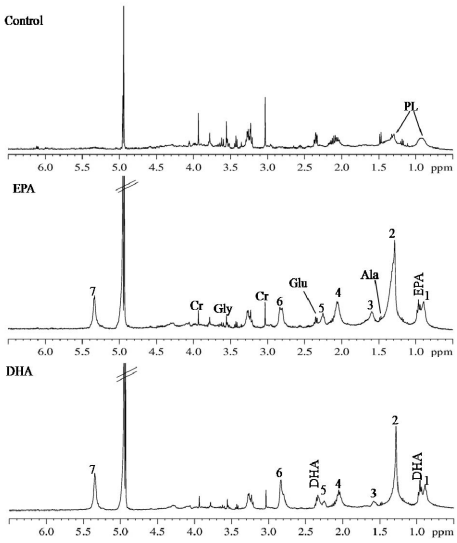

Figure 2. Water-presaturated *ex vivo* HR-MAS ^1H spectra of control, EPA, and DHA supplemented cardiomyocytes.

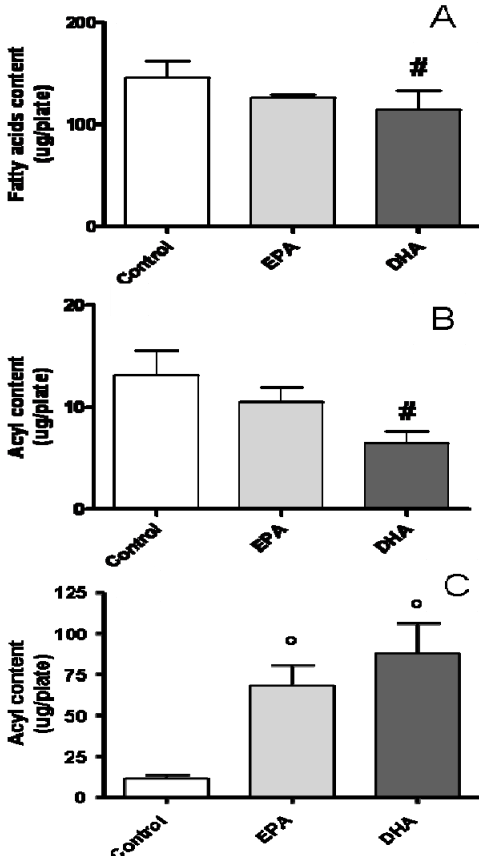

Figure 3. Fatty acids composition (A) in PL , (B) in TG and (C) in CE fraction derived from control, EPA and DHA supplemented cells.

Discussion– In neonatal rat cardiomyocytes, EPA and DHA supplementation caused a wide modification of FA composition in PL fraction suggesting that EPA and DHA represent a "preferential choise" in spite of the others FAs contained in HS and FCS added to cell media. Once inside the cell, EPA and DHA appear to be preferentially incorporated in the PL fraction. Interestingly, the total amount of fatty acid esterified in PL was decreased in supplemented cardiomyocytes, particularly in DHA supplemented ones. These data was confirmed by HR-MAS NMR study.We hypothesize that the larger packing free volume associated with EPA or DHA-rich membranes could explain in part the reduction of the acyl content in phospholipid fraction. Supplemented EPA and DHA were not incorporated in TG fraction, in agreement with de Vries (5). As evidenced by both GC and HR-MAS TG content was lower in n-3 PUFA supplemented cells. In this study an exploratory work on the metabolic cardiomyocytes profile has been performed to characterize the metabolome of these cells. Different small metabolites like Lys, GSH, Gln, Ala, Tau, PE, Myo, PC, GPC, Cr, were detect from one and 2D experiments. The same analysis has been performed on the supplemented cells, in order to see if the PUFA's supplementation induces any change in the metabolic cardiomyocytes profile.

References.

1. Jump DB. J Biol Chem. 2002. 277(11), 8755-8, 2002. **2**. W. Stillwell W, Jenski LJ, Zerouga M *et al.* Chem. Phys. Lipids 104**,** 113–132, 2002.
3. Kris-Etherton PM, Harris WS, Appel LJ. Circulation 106, 2747–57, 2002. **4**. Bordoni A, Astolfi A, Morandi L , *et al*. FEBS Letters 581, 923–929, 2007.
5.de Vries JE, Vork MM, Roemen TH, *et al.* J Lipid Res.38(7):1384-94, 1997.