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Comparison of docosahexaenoic acid uptake in murine cardiomyocyte culture and tissue: Significance to physiologically relevant studies

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ABSTRACT

Long-chain $n-3$ (or omega 3) fatty acids, namely docosahexaenoic acid (DHA, 22:6$n-3$) and eicosapentaenoic acid (EPA, 20:5$n-3$) have been attributed cardioprotective properties.

In this study, we evaluated the incorporation of DHA into cardiomyocytes and the shift in the omega 3/omega 6 ratio after supplementation of primary cardiomyocyte culture. Results are compared with atrial tissue concentrations attained after prolonged feeding of rats. The major difference between in vitro vs. in vivo supplementation is the paradoxical accumulation of arachidonic acid in cultured cardiomyocyte. However, this increase does not give rise to a higher PGE_2 production after cellular stimulation, as compared with controls, possibly because of the associated inhibition of $sPLA_2$ by DHA. Notably, in vitro supplementations with DHA 10 to 25 μM approximate in vivo pharmacological treatments.

1. Introduction

Long-chain $n-3$ (or omega 3) fatty acids, namely docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5$n-3$) have been attributed cardioprotective properties, even though cumulative evidence is challenging such belief [1,2]. Nonetheless, international scientific bodies such as the ISSFAL have issued ad-hoc guidelines to increase their dietary intakes (www.issfal.org). EPA and DHA might exert their cardioprotective activities via several mechanisms, some of which are still under investigation [3,4]. In brief, omega 3 fatty acids are apparently endowed with metabolic, anti-inflammatory, cell signal-modulating, and anti-arrhythmic activities which theoretically counteract those of arachidonic acid (ARA, 20:4$n-6$) (reviewed in [5]).

It is noteworthy that DHA is more abundant in the membranes of excitable cells such as retinal and cardiac cells, as well as in synapses [6]. Also, its absolute concentrations depend on dietary intakes, as omega 3 – as well as omega 6 – fatty acids are essential and cannot be synthesized by mammals. The relative abundance of DHA and ARA in phospholipids of human tissues is difficult to evaluate, because of obvious ethical and technical limitations. Exceptions are red blood cells, where they are routinely measured in proportions of around 3.5% and 16% of total fatty acids, respectively [7]. Hence, the assumption of in vitro experiments is that they represent estimates of tissutal concentrations attainable after consumption, assimilation, and liver interconversion of omega 3 fatty acids.

In this study, we evaluated the incorporation of DHA into cardiomyocytes and the shift in the omega 3/omega 6 ratio after supplementation of primary cardiomyocyte culture, with a lipidomic approach [8,9]. Results are compared with atrial tissue concentrations reached after prolonged feeding of rats.

2. Materials and methods

2.1. In vitro experiments

The protocol was identical to that followed by Lamaziere et al. [8,10]. Male Wistar rats were purchased from Janvier Europe (St Berthevin, France) and were housed in a temperature- and humidity-controlled room with a 12 h-light/-dark cycle. Food (ssniff Spezialdiäten GmbH, Soest, Germany) and water were provided ad libitum. Background diets were devoid of EPA and DHA and contained linoleic and linolenic acids (2.45% and 0.47%, respectively).

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Rats were anesthetized with ketamine and xylazine and atrial rat cardiomyocytes (ARC) were enzymatically isolated. The atria were removed, minced, and washed in Ca^{2+}-free Krebs–Ringer solution containing (mM): 35 NaCl, 4.75 KCl, 1.19 KH_2PO_4, 16 Na_2HPO_4, 10 Hepes, 10 glucose, 25 $NaHCO_3$, 134 sucrose, and 30 2,3-butanedione 2-monoxime (BDM) (pH was adjusted to 7.4 with NaOH), gassed with 95% O_2–5% CO_2, and maintained at 37 °C. Atria were re-incubated in this solution without BDM and containing bovine serum albumin (BSA) (5 mg ml^{-1}, Hoechst-Behring), 200 U ml^{-1} collagenase (type IV, Sigma Chemical Co.), and 6 U ml^{-1} protease (type XXIV, Sigma). After 30 min of digestion, the enzyme solution was replaced by the same solution containing only collagenase (400 U ml^{-1}). Isolated myocytes were resuspended in a bicarbonate-buffered Tyrode solution containing 2 mM Ca^{2+} and incubated at 37 °C with continuous gassing with 95% O_2–5% CO_2 for at least 1 h before use.

DHA was added to fetal calf serum (Promocell, Heidelberg) before its addition to cell cultures. ARC were preincubated with DHA for 48 h. At the end of the incubation periods, cells were collected and were added with di-*t*-butyl-hydroxytoluene (BHT) 10 μM (to prevent lipid peroxidation).

2.2. Feeding experiments

Rats were fed, by oral gavage, 100 mg/kg/d of a proprietary oil (which contains ≥ 60% of long-chain omega 3 fatty acids, of which ≥ 55% of DHA and ≤ 15% of EPA), or paraffin oil as the control. This dose corresponds to 4.2 g/d total omega 3 in humans, which is compatible with therapeutic regimens (even more so after correction for normalization for body surface area [11]). This supplementation was continued for 60 days.

At the end of the supplementation period, rats were anesthetized with with ketamine and xylazine and ARC were isolated as described above.

2.3. $sPLA_2$ activity assay

$sPLA_2$ activity was determined in ARC's culture supernatants by using the $sPLA_2$ assay kit (SPIbio for Cayman Chemical, Montigny le Bretonneux, France), according to manufacturer's instructions [12]. Briefly, supernatants were incubated in an assay buffer containing DTNB and substrate solution (diheptanoylThio-PC). The absorbance was read every minute at 405 nm in a plate reader, for 20 min. The

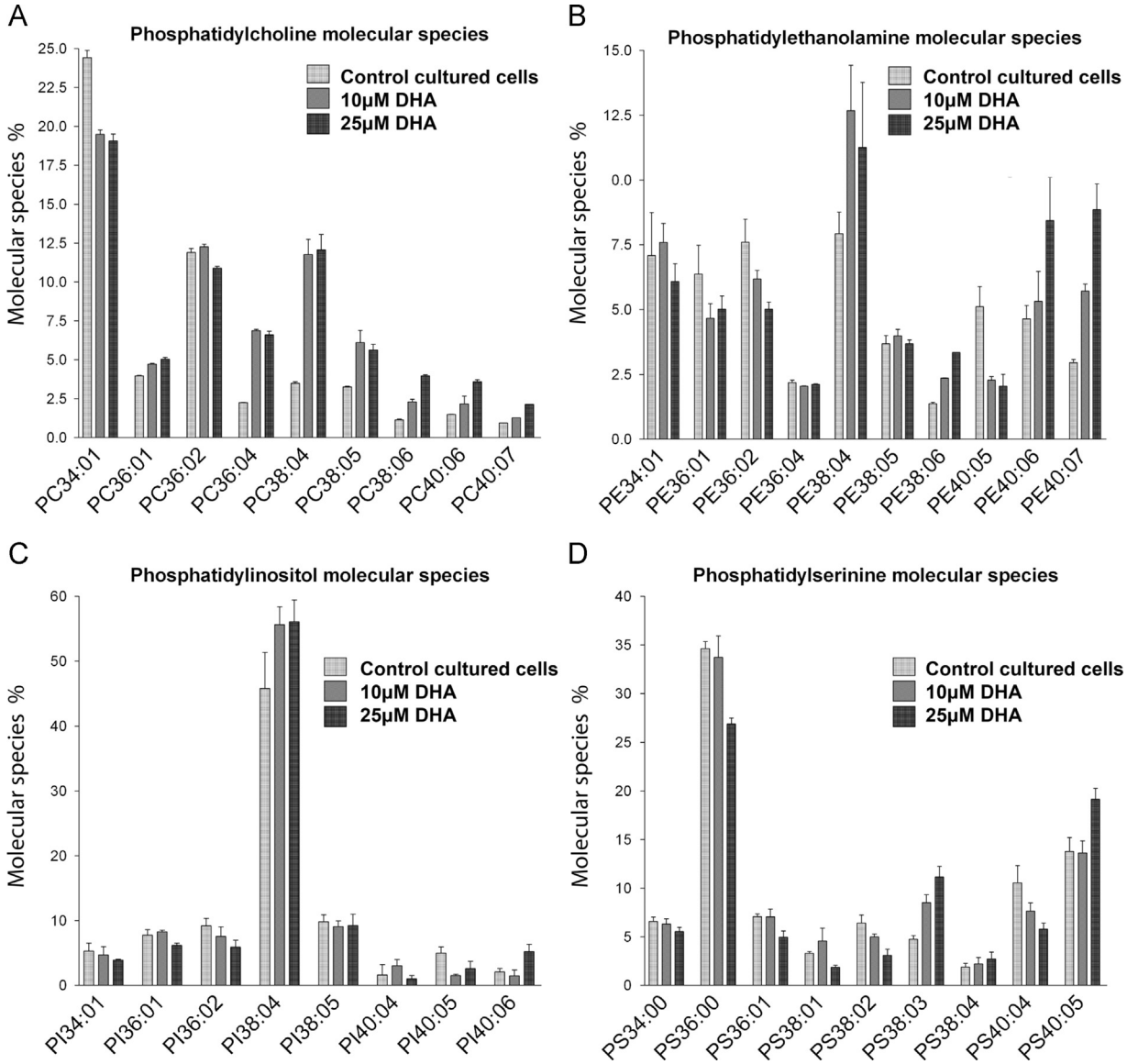

Fig. 1. Molecular species profiles of four phospholipid classes, i.e. phosphatidylcholine (A), phosphatidylethanolamine (B), phosphatidylinositol (C), phosphatidylserine (D) in cultured cardiomyocytes in the absence or in the presence of docosahexaenoic acid (DHA) at 10 and 25 μM. Profiles are given by tandem mass spectrometry (ESI-MS2) as the percentage of the total ion current ($n=3$).