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ω-3 supplementation in HIV-1-infected individuals with unsuppressed viral load: cause for caution?

Dietary n-3 (ω-3) fatty acids, mainly eicosapentaenoic (C20 : 5n-3, EPA) and docosahexaenoic acids (C22 : 6n-3, DHA) are useful to decrease hypertriglyceridemia in HIV-1-infected patients [1]. These fatty acids are readily incorporated in cell membranes, changing the properties of the phospholipid bilayer [2]. Whether this triggers HIV-1 replication and infectivity remains unexplored, as data are limited to clinical trials conducted in patients under antiretroviral therapy (ART) [3–5], thus precluding the evaluation of any potential effect on viral load. To address this issue, we set up an ex-vivo experiment to test HIV-1 infectivity and replication after inducing a range of ω-3 content in $CD4^+$ T cell membranes resembling to those obtained after dietary supplementation with ω-3 fatty acids. Fatty fish and most fish oil capsules contain both EPA and DHA species at different doses, with DHA usually being the most abundant. The issue of whether all ω-3 species are equal regarding their effects remains elusive. To avoid this caveat, we used DHA as supplemental fatty acid because it is the most abundant ω-3 in cell membranes [6].

$CD4^+$ T cells purified from healthy donors were infected per triplicate with NL4-3 HIV-1 viruses and treated for 7 days with different concentrations of unesterified, albumin-bound DHA (1.25, 2.5, 5, and 10 μmol/l). At the end of the challenge, cell membrane composition was determined by gas chromatography. DHA incorporated into cell membranes in a dose-dependent manner (data not shown), concurring with previous data [7]. Based on previous studies in Western populations, the cardioprotective target level for the ω-3 index (i.e. sum of the proportions of EPA and DHA in red blood cell membranes) has been tentatively set at 8% [8]. In a trial conducted in patients with well controlled HIV-1 infection and hypertriglyceridemia, after 8 weeks of supplementation with 3.4 g/day of ω-3, 60% of patients allocated in the ω-3 arm reached this optimal value [3]. Being aware of some existing methodological differences (mostly regarding to the considered cell type [7] and in-vivo/ex-vivo approaches), in our experiments, such a value was only reached at DHA exposures at least 5 μmol/l ($9.6 \pm 0.8\%$ in DHA-treated cells versus $3.8 \pm 0.2\%$ in untreated condition, $P < 0.0001$, $n = 8$; Fig. 1a). We therefore selected this concentration to investigate whether DHA enrichment influences HIV-1 infectivity and viral particle production, assessed by 50% tissue culture infective doses on TZM-bI cells and by p24 ELISA, respectively.

We found that DHA induced a significant increase in 50% tissue culture infective doses of the harvested medium (1683 ± 355 in the untreated condition versus 3985 ± 1083 in DHA-treated, $P = 0.02$, $n = 8$; Fig. 1b). We ruled out the possibility that the infectivity rise was explained by an overproduction of viral particles, as the HIV-1 particle amount remained essentially unaffected (p24 ELISA, 689 ± 73 ng/ml in the untreated condition versus 717 ± 108 ng/ml in DHA-treated, $P = 0.83$, $n = 9$; Fig. 1c). Finally, we explored whether DHA proportion in $CD4^+$ T cell membranes was related to the infectivity of the HIV-1 particles produced by those cells. To that aim, we pooled data from all experiments (three experiments per triplicate adding 5 μmol/l of DHA, and a fourth experiment with all range of tested DHA concentrations). We found a moderate but significant direct correlation between DHA membrane content and HIV-1 infectivity (Pearson's correlation coefficient = 0.39; $P = 0.03$; $n = 30$).

The mechanisms underlying our findings remain elusive, but studies in experimental models of infection diseases revealed that DHA incorporation can disrupt the physical clustering of lipid rafts and associated proteins, modulating immunity [9,10]. Future research is warranted, in particular studies that focus on the lipid composition of the virus envelope and receptor distribution in cell membranes. Recognizing that our hypothesis can only be directly tested in a randomized clinical trial, our unprecedented data argue against the use of dietary supplements of ω-3 in patients with unsuppressed viral load. This phenomenon could have an impact, but not exclusively, on HIV-1-infected individuals with difficult access to ART, HIV-ART patients with nonresponding ART, or HIV-1-infected undiagnosed individuals.

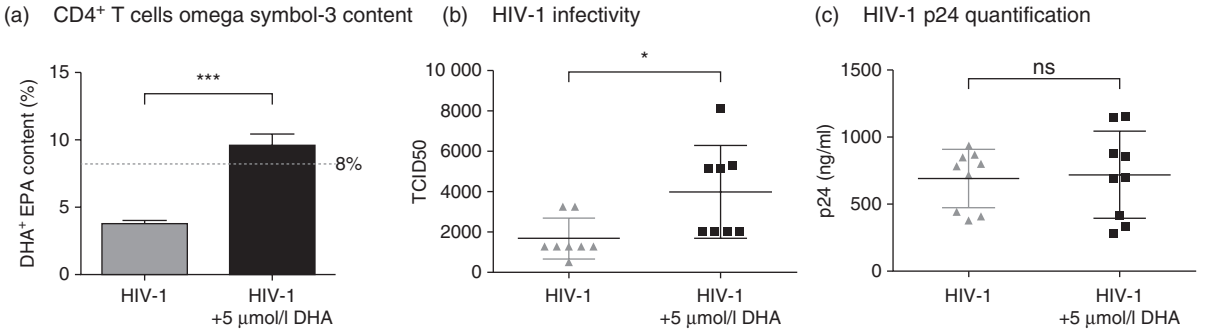

Fig. 1. Infectivity and viral replication in medium from HIV-1-infected CD4$^+$ T cells, by absence or presence of DHA in medium for 7 days. (a) DHA + EPA proportion in membrane of CD4$^+$ T cells treated with 5 μmol/l DHA (black) or untreated (grey). Discontinuous line at 8% indicate proposed low-risk cutoff in red blood cells for cardiovascular protection. (b) Infectivity of supernatant after culture in presence or absence of 5 μmol/l DHA (black or grey, respectively), expressed as 50% tissue culture infective dose determined in TZM-bl cells. (c) Number of viral particles (HIV-1 p24 protein quantification) in supernatant after culture in presence or absence of 5 μmol/l DHA (black or grey, respectively). Data expressed as mean ± SD, obtained from three independent biological experiments performed per triplicate. Student's *t*-test was used to determine significance levels (*$P < 0.05$; ***$P < 0.001$). DHA, docosahexaenoic acids; EPA, eicosapentaenoic; TCID50, 50% tissue culture infective dose.

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Conflicts of interest

There are no conflicts of interest.

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