Effects of docosahexanoic acid supplementation on inflammatory and subcutaneous adipose tissue gene expression in HIV-infected patients on combination antiretroviral therapy (cART). A sub-study of a randomized, double-blind, placebo-controlled study

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ABSTRACT

Background: Omega-3 fatty acids have the potential to decrease inflammation and modify gene transcription. Whether docosahexanoic acid (DHA) supplementation can modify systemic inflammatory and subcutaneous adipose tissue (SAT) gene expression in HIV-infected patients is unknown.

Methods: A randomized, double-blind, placebo-controlled trial that enrolled 84 antiretroviral-treated patients who had fasting TG levels from 2.26 to 5.65 mmol/l and received DHA or placebo for 48 weeks was performed (ClinicalTrials.gov, NCT02005900). Systemic inflammatory and SAT gene expression was assessed at baseline and at week 48 in 39 patients.

Results: Patients receiving DHA had a 43.9% median decline in fasting TG levels at week 4 (IQR: −31% to −56%), compared with −2.9% (−18.6% to 16.5%) in the placebo group (P < 0.0001). High sensitivity C reactive protein (hsCRP) and arachidonic acid levels significantly decreased in the DHA group. Adipogenesis-related and mitochondrial-related gene expression did not experience significant changes. Mitochondrial DNA (mtDNA) significantly decreased in the placebo group. SAT inflammation-related gene expression (Tumor necrosis factor alpha [TNF-α], and monocyte chemoattractant protein-1 [MCP-1]) significantly decreased in the DHA group.

Conclusions: DHA supplementation down-regulated inflammatory gene expression in SAT. DHA impact on markers of systemic inflammation was restricted to hsCRP and arachidonic acid.

1. Introduction

HIV-1 infection is associated with chronic inflammation that most likely is a pathogenic mechanism underlying the increased appearance of co-morbid conditions not directly related to HIV infection itself, such as cardiovascular disease or non-AIDS cancers, in otherwise virologically-controlled patients [1]. This inflammatory phenotype has also often been described in patients with HIV/HAART-associated lipodystrophy syndromes (HALS) in whom it is thought to be involved, together with some antiretroviral drug toxic effects, in their pathogenesis [2].

Polyunsaturated fatty acids (PUFA), the so-called omega-3 fatty acids, are pleiotropic molecules with a wide range of claimed beneficial effects [3]. Among them, they decrease serum triglyceride levels, increase high-density lipoprotein (HDL)-cholesterol, decrease blood pressure, have anti-inflammatory effects, and in patients with a past myocardial infarction, have been associated with prevention of sudden death due to arrhythmias [3]. In the HIV setting, a number of clinical trials have demonstrated that hypertriglyceridemia can at least be partially corrected by diet supplementation with a mixture of omega-3
fatty acids of fish oil origin [4,5].

Docosahexanoic acid (DHA) competes with arachidonic acid (ARA) as a substrate for the synthesis of pro-inflammatory factors such as leukotrienes, prostaglandins, and cytokines, and this is the basis for its anti-inflammatory properties [6]. A number of clinical trials describe the effects of PUFA on the circulating levels of inflammatory mediators. A decrease in C reactive protein (CRP) and tumor necrosis alpha (TNF-α) serum levels was observed in athletic men after DHA administration [7], whereas DHA administered to patients with dyslipidemia caused a decrease in plasminogen activator-inhibitor-1, fibrinogen, and CRP serum levels [8]. Notwithstanding this, data regarding the effects of PUFA on inflammation in HIV-infected patients are discordant, some studies showing a beneficial effect by decreasing interleukin-6 (IL-6) and TNF-α circulating levels [9] while others do not show any change [10].

PUFAs have also been claimed to be gene regulators since it has been shown that they are able to bind and activate all peroxisome proliferator-activated receptor (PPAR) isoforms, including PPAR gamma [11], which are major regulators of adipocyte differentiation, whole-body insulin sensitivity, and are involved in the control of local inflammation in adipose tissue [2].

With all this information in mind, we performed a nested sub-study within a clinical, double-blind, placebo-controlled, randomized trial of DHA supplementation in virologically-controlled HIV-infected patients with mild hypertriglyceridemia [12]. Our working hypothesis was that DHA supplementation might decrease inflammation and improve adipocyte biology functional markers at subcutaneous adipose tissue level.

2. Patients and methods

2.1. Study population

Patients’ recruitment was done between July 2010 and June 2011 at the Hospital de la Santa Creu i Sant Pau HIV Infection clinic, which serves a population of 1700 adult patients on active follow-up. Candidates need to have an established diagnosis of HIV-1 infection, under stable cART for the prior 6 months and throughout the study period, and having TG levels between 2.26 and 5.65 mmol/l in two consecutive determinations within a 15-day interval. Exclusion criteria included a TG level > 5.65 mmol/l, since then pharmacological therapy was indicated [13]. Additional exclusion criteria were hypersensitivity to the active compound or product excipients, BMI > 30 kg/m2, pregnancy, breastfeeding, anticoagulant treatment, oral antidiabetics and hormonal treatments. Lipid-lowering drugs were not allowed except if stopped more than 3 months before the selection visit. Consumption of high levels of alcohol (> 20 g/d), diabetes or fasting blood glucose level (glycemia > 6.6 mmol/l) were excluded as well. Other exclusion criteria were: an active AIDS-defining disease, serum creatinine > 150 µmol/l and alanine aminotransferase or aspartate aminotransferase > 5 × upper limit of normal, anemia, and > 10% loss in body weight in the preceding 6 months. The 1993 revised case definition of the Centers for Disease Control and Prevention (CDC) was used to diagnose AIDS [14]. All participants were instructed to maintain their usual diets, alcohol intakes, and physical activities, and not to make any changes to their lifestyle during the intervention period. Written informed consent was obtained from the patients at study entry. The study was approved by the Ethics Committee of the Hospital de la Santa Creu i Sant Pau and was registered under ClinicalTrials.gov Identifier No. NCT02005900. Thirty-nine randomized patients volunteered for the inflammatory and molecular subcutaneous adipose tissue (SAT) sub-study (18 from the DHA and 21 from the placebo groups).

2.2. Study design

A placebo-controlled, double-blind, phase 4, randomized, 2-arm study was performed. After a 4-week screening, eligible patients were randomized to DHA 4 g a day (in ochre single-serving drinkable vials containing 7 g of DHA oil) or placebo, during a 48-week period. This total dose is within the 2–4 g of EPA and DHA per day recommended by the AHA for patients who need to lower TG levels [14]. Both DHA and placebo were presented as ochre vials similar to each other. Placebo vials contained 7 g of olive oil. The formulations were liquid vials but, since DHA is not tasty and has a heavy fish smell, both interventional and placebo oils were masked with lemon flavor. DHA was obtained by enzymatic synthesis and incorporated in the TG form at a 70% concentration of total fatty acid content and was provided by Brudy Technologies* (Barcelona, Spain). The National Institutes of Health Division of AIDS toxicity grading table was used to grade adverse clinical and laboratory events [15].

At recruitment for the study, HIV infection history and demographic data were recorded and anthropometric, blood pressure, viro-immunological, and metabolic parameters were measured for each patient. They were randomized 1:1 to receive DHA 4 g/day or a placebo of olive oil daily. The primary endpoint of the study was the percent change in TG level at 4 weeks, whereas change in TG level at 12, 24, 36, and 48 weeks and change in limb fat mass, and in inflammatory and fat molecular parameters were secondary endpoints.

2.3. Laboratory measurements and plasma fatty acid concentrations

These measurements were performed at baseline and at weeks 4, 12, 24, 36 and 48. Laboratory methods used have been previously described [16]. The fatty acid composition of was determined through the Lepage and Roy method [17], which has also been described elsewhere [18].

2.4. Systemic inflammatory markers

Serum levels of interleukin-1 (IL-1), IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1), nerve growth factor (NGF), TNF-α, and hepatocyte growth factor (HGF) were measured at baseline and at week 48 through an antibody-linked, fluorescently labeled microsphere bead-based multiplex analysis system (Linco Research/Millipore, Billerica, MA) and quantified by Luminex100ISv2 equipment. The intra and inter-assay coefficient of variation for each cytokine was: IL-1: 7% and 12%; IL-6: 2% and 10%; IL-8: 3% and 14%; MCP-1: 2% and 11%; NGF: 4% and 11%, TNFα: 3% and 19% and HGF: 3% and 11%, respectively.

2.5. Gene expression profiling in subcutaneous adipose tissue

A subcutaneous adipose tissue biopsy was performed on each patient at baseline and at 48 weeks. First of all, local anesthesia was applied in periumbilical zone and biopsy was undertaken with a number 8 punch. We extracted an approximated 2 cm3 sample. Fat samples were immediately frozen in liquid nitrogen and kept at ~80°C until processing. DNA was isolated, after homogenization in RLT (Qiagen, Hilden, Germany) buffer, using a phenol/chloroform extraction methodology. RNA was isolated using a column affinity-based methodology, including on-column DNA digestion (RNeasy; Qiagen). One microgram of RNA was transcribed into cDNA using MultiScribe reverse transcriptase and random-hexamer primers (TaqMan Reverse Transcription Reagents; Applied Biosystems, Foster City, California, USA). For quantitative mRNA expression analysis, TaqMan reverse transcriptase (RT)-polymerase chain reaction (PCR) was performed on the ABI PRISM 7700HT sequence detection system (Applied Biosystems). The TaqMan RT-PCR reaction was performed in a final volume of 25 µl using TaqMan Universal PCR Master Mix, No AmpErase UNG reagent and the following specific primer pair probes: PPARγ (Hs00234592_s1), Adiponectin (Hs00605917_s1), mitochondrial cytochrome b, cytochrome b (Cyt b) (Hs00259686_s1), MCP-1 (Hs00234140_s1) and TNFα (Hs00174128_s1). Quantification of miRNA was performed using Cyt band referred to nuclear DNA, as determined by the amplification of the e

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of the intronless gene CCAAT/enhancer-binding protein-α (Hs00269972_s1). In each set of experiments, controls with no cDNA, primers, or RT were included. Since each sample was run in duplicate, the mean value of the duplicate was used to calculate the mRNA expression of the genes of interest. They were normalized to that of the reference control. Expression levels were considered undetectable when the detection cycle threshold was greater than 40, under RT-PCR conditions. Values were normalized by a reference control (18S rRNA) using the comparative method \( \Delta CT \). Parallel calculations using the HPRT reference gene were performed and results were essentially the same.

### 2.6. Statistical analyses

Descriptive data are frequencies (%) and median (percentile 25–percentile 75, interquartile range [IQR]), as appropriate, or as otherwise specified. The Fisher’s exact test was used for categorical variables, and non-parametric tests for continuous variables (Mann-Whitney or Wilcoxon tests for independent and dependent data, respectively). All analyses were performed using SAS 9.3 software (SAS Institute Inc., Cary, NC, USA) and a level of significance was established at the two-sided 5% level. Due to the exploratory nature of this sub-study, no multiplicity adjustment procedure was applied.

### 3. Results

#### 3.1. Baseline characteristics and subject disposition

One hundred and thirteen patients were screened, of whom 87 subjects were randomized and 80 (95%) completed at least the week 4 study evaluation (for primary outcome) and 77 (88.5%) completed the week 12 study evaluation. Two patients in the DHA arm and one in the placebo arm were prematurely excluded (before taking study medication) because of protocol violation [12]. Thirty-nine patients were included in the inflammatory and molecular SAT sub-study. Patient characteristics including age, gender, history of HIV infection, viro-immunological status and antiretroviral exposure were not different between sub-study patients and those of the whole group. Their demographics and antiretroviral drug exposure are shown in Tables 1 and 2, respectively.

#### 3.2. Assessment of lipid, glucose homeostasis, and fat parameters over time

Baseline metabolic parameters are shown in Table 3. There was a statistically significant median percent decrease in fasting TG levels in the DHA arm compared with the placebo arm at week 4 (−43.9%, IQR: −56 to −31% vs. −2.9% IQR: 18.6 to −16.5%, respectively, \( P < 0.0001 \)). The median absolute change in TG levels at week 4 was −1.7 mmol/l in the DHA arm, whereas it was −0.1 mmol/l in the placebo arm (\( P = 0.0001 \)). HDL, LDL cholesterol and glucose parameters did not experience significant changes during the study in either arm.

Appendicular fat mass significantly increased in both arms without significant differences between arms at week 48. The median net gain in appendicular fat in the DHA group was 558 (95%CI: −19, 1926) grams, whereas in the placebo group it was 553 (95%CI: −243, 1009) grams (\( P = 0.5221 \)).

#### 3.3. DHA and ARA levels throughout the study

Median DHA levels at baseline (measured as percent of total fatty acids) were comparable between groups (\( P = 0.6067 \)) (Table 3), whereas at week 4, they were 5.01% (4.2–5.8%) (\( P < 0.0001 \) vs. baseline) in the DHA group and 1.2% (1.0–1.5%) (\( P = 0.1136 \) vs. baseline) in the placebo group (\( P < 0.0001 \) between groups). At 48 weeks, the respective values were 3.38% (2.22–4.58%) and 1.16% (0.97–1.88%) (\( P < 0.0001 \)). ARA levels at baseline were comparable between groups (\( P = 0.6067 \)). There was no significant change in the placebo group (\( P = 0.3945 \)) (Table 4). At week 48, patients in the DHA-treated group had a significantly lower ARA level than those of the placebo group (4.2% [3.4–5.0%] vs. 6.0% [5.4–6.6%], \( P = 0.0003 \)) (Table 4). There was correlation of DHA and ARA serum levels (\( r = 0.5260, P = 0.0010 \)).

#### 3.4. Systemic inflammatory markers

There were no statistically significant differences between patients assigned to DHA or placebo with respect to metabolic and inflammatory parameters at baseline (Table 3). There was no difference between inflammatory parameters over 48 weeks in the DHA group, except for a significant decrease in hsCRP (Table 4). Inflammatory serum parameters remained without statistically significant differences in the placebo group (Table 4). There were no between-group differences at week 48 (Table 4).

#### 3.5. Mitochondrial DNA (mtDNA) and gene expression in SAT

Mitochondrial DNA and mitochondrial-related genes. MtDNA content decreased over 48 weeks in the placebo group (\( P = 0.0391 \)) while

### Table 1

Characteristics of the population studied.

| Parameter                  | DHA (n = 18) | Placebo (n = 21) | \( P \) value
|----------------------------|-------------|-----------------|----------------
| Age, yrs.                  | 44.0 (40.0–49.7) | 45.0 (41.5–50.0) | 0.6112
| Males, n (%)               | 15 (83.3) | 20 (95.2) | 0.3330
| Means of HIV infection     | 9 (50.0) | 13 (61.9) | 0.6327
| MmL, n (%)                 | 6 (33.3) | 6 (28.6) | 0.6286
| IDU, n (%)                 | 3 (26.7) | 2 (9.5) | 0.3682
| Weight, kg                 | 73.9 (70.0–83.3) | 73.8 (66.3–79.2) | 0.5658
| BMI, kg/m²                 | 26.2 (22.4–29.9) | 25.1 (21.5–28.7) | 0.3520
| Waist circumference, cm    | 94.0 (90.0–101.2) | 92.0 (86.0–96.7) | 0.1714
| WHR                        | 0.96 (0.92–1.00) | 0.96 (0.88–1.04) | 0.9500
| Duration of HIV infection, yrs. | 14.0 (10.5–17.2) | 15.5 (11.0–19.5) | 0.3575
| AIDS, n (%)                | 5 (27.8) | 9 (42.9) | 0.5710
| Smokers, n (%)             | 10 (55.5) | 10 (47.6) | 0.8626
| Alcohol consumption, g/day | 0.0 (0.0–6.5) | 0.0 (0.0–3.0) | 0.8451
| HBV co-infection, n (%)    | 2 (4.8) | 1 (2.3) | 0.8893
| HCV co-infection, n (%)    | 6 (33.3) | 7 (33.3) | 0.9786
| Baseline CD4 cell count/mm³ | 450 (103–775) | 347.6 (3–1397) | 0.3020
| Nadir CD4 cell count/mm³³ | 162 (95–318) | 156 (26–278) | 0.3682
| Nadir CD4 cell count < 200/mm³, n (%) | 13 (72.2) | 12 (57.1) | 0.8050
| CD4 increase at entry study | 4 (22.2) | 7 (33.3) | 0.5560
| Baseline HIV-1 RNA (log_{10} copies/ml) | 699 (575–935) | 677 (486–906) | 0.6449
| Baseline HIV-1 RNA ≥ 5 log_{10} copies/ml, n (%) | 12.8 (1.28–1.31) | 1.28 (1.28–1.35) | 0.9190
| Undetectable HIV-1 RNA at study entry, n (%) | 11 (55.5) | 14 (66.6) | 0.7994
| Decrease HIV-1 RNA baseline-study entry, log_{10} | 18 (100) | 21 (100) | 1.0
|                         | 3.75 (3.36–4.25) | 3.89 (3.18–4.62) | 0.2848

All parameters expressed as median (interquartile range) unless otherwise indicated. DHA = docosahexaenoic acid, MmL = Men who have sex with men, IDU = Intravenous drug users, BMI = Body mass index, WHR = Waist-to-hip ratio, AIDS = Acquired immune deficiency syndrome, HBV = Hepatitis B virus, HCV = Hepatitis C virus.
there was no change in DHA-treated patients (P = 0.7340) (Fig. 1). Cyt b mRNA did not change in either group over 48 weeks (Fig. 1). Between-group comparison at week 48 did not show differences for any of these transcripts (P = 0.2370; and P = 0.4440, respectively). However, expression of genes related to SAT in DHA group but not in the placebo group. In addition, mtDNA content significantly decreased in the DHA group compared to the placebo group (P = 0.7340). Cyt b mRNA did not change in either group over 48 weeks (Fig. 1).

3.5.1. Genes involved in adipocyte differentiation and function

Transcript levels for adipogenesis-related genes were not significantly different at baseline (P = 0.4270; and P = 0.7430, respectively). The expression of PPAR-γ and adiponectin genes did not change significantly during the study in either group (Fig. 1). Between-group comparison at week 48 did not show differences for any of those transcripts (P = 0.2370; and P = 0.4440, respectively).

3.5.2. Genes involved in inflammation

MCP-1 and TNF-α transcripts significantly decreased over 48 weeks in the DHA-treated group, whereas they showed a non-significant decrease in the placebo-treated group (Fig. 1). MCP-1 and TNF-α transcript levels were comparable between groups at baseline (P = 0.6860 and P = 0.4010, respectively), but there were significant decreases for MCP-1 and TNF-α transcript levels at week 48 (P = 0.0334 and P = 0.0243, respectively) (Fig. 1).

4. Discussion

Combination antiretroviral-treated, HIV-infected patients, with mild hypertriglyceridemia who received supplementation with DHA 4 g daily had a significant decrease in fasting TG levels. However, we were unable to demonstrate any significant benefit of DHA supplementation in terms of decreasing the circulating levels of inflammatory cytokines, although we did see a significant decrease in hsCRP and ARA levels.

Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DHA (n = 18)</th>
<th>Placebo (n = 21)</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>Current ART composition</td>
<td>0.3130</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI-based, n (%)</td>
<td>9 (50.0)</td>
<td>11 (57.4)</td>
<td></td>
</tr>
<tr>
<td>NNRTI-based, n (%)</td>
<td>7 (38.9)</td>
<td>8 (38.1)</td>
<td></td>
</tr>
<tr>
<td>INSTI-based, n (%)</td>
<td>2 (11.1)</td>
<td>1 (4.8)</td>
<td></td>
</tr>
<tr>
<td>IP + NNRTI, n (%)</td>
<td>0 (0)</td>
<td>1 (4.8)</td>
<td></td>
</tr>
<tr>
<td>TDF backbone, n (%)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>TDF/FTC, n (%)</td>
<td>7 (38.9)</td>
<td>10 (47.6)</td>
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</tr>
<tr>
<td>ABC/3TC, n (%)</td>
<td>7 (38.9)</td>
<td>6 (28.6)</td>
<td></td>
</tr>
<tr>
<td>ABC + TDF, n (%)</td>
<td>1 (5.5)</td>
<td>1 (4.8)</td>
<td></td>
</tr>
<tr>
<td>ABC + ddl, n (%)</td>
<td>0 (0.0)</td>
<td>1 (4.8)</td>
<td></td>
</tr>
<tr>
<td>3TC alone, n (%)</td>
<td>2 (1.1)</td>
<td>1 (9.5)</td>
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<td>1 (9.5)</td>
<td></td>
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<tr>
<td>NRRTI-sparing, n (%)</td>
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<td>1 (4.8)</td>
<td></td>
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<tr>
<td>cART duration, m</td>
<td>160.0 (109.5–205.0)</td>
<td>164.0 (115.5–212.5)</td>
<td>0.6689</td>
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<td>Individual drug exposure</td>
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<td>AZT exposure, m</td>
<td>3.0 (0.0–4.37)</td>
<td>12.5 (0.0–47.5)</td>
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<td>d4T exposure, m</td>
<td>0.0 (0.0–57.5)</td>
<td>12.5 (0.0–59.0)</td>
<td>0.7275</td>
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<td>3TC/FTC exposure, m</td>
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<td>89.5 (56.0–109.5)</td>
<td>0.3013</td>
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<td>ddl exposure, m</td>
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<td>0.0 (0.0–19.5)</td>
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<td>ABC exposure, m</td>
<td>14.0 (0.0–45.7)</td>
<td>0.0 (0.0–44.0)</td>
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<tr>
<td>TDF exposure, m</td>
<td>26.0 (0.0–46.0)</td>
<td>31.5 (0.30–48.5)</td>
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<td>EVF exposure, m</td>
<td>26.0 (0.0–72.0)</td>
<td>0.0 (0.0–61.5)</td>
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<tr>
<td>NVP exposure, m</td>
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<td>0.0 (0.0–25.0)</td>
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<td>Ritonavir exposure, m</td>
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<td>43.0 (0.0–66.5)</td>
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<td>PI exposure, m</td>
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<td>NRRTI exposure, m</td>
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<td>241.0 (129.0–302.5)</td>
<td>0.9432</td>
</tr>
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</table>

All parameters expressed as median and (interquartile range). PI = protease inhibitor, NNRTI = non-nucleoside reverse transcriptase inhibitor, INSTI = integrase inhibitor, TDF = tenofovir, FTC = emtricitabine, ABC = abacavir, 3TC = lamivudine, ddl = didanosine, NRRTI = nucleoside reverse transcriptase inhibitor, cART = combination antiretroviral therapy, AZT = zidovudine, d4T = stavudine, EVF = efavirenz, NVP = nevirapine, m = months.

there was no change in DHA-treated patients (P = 0.7340) (Fig. 1). Cyt b mRNA did not change in either group over 48 weeks (Fig. 1). MtDNA content and Cyt b gene expression levels in the DHA and placebo groups were comparable at baseline (P = 0.7830 and P = 0.4630, respectively) and were not different at week 48 either (P = 0.3120 and P = 0.4610, respectively).

Furthermore, we did not observe any significant improvement in SAT molecular markers of adipogenesis, and mitochondrial function. However, expression of genes related to SAT inflammation significantly decreased in the DHA group but not in the placebo group. In addition, mtDNA content significantly decreased in the placebo group, whereas it remained practically unmodified in the DHA group.

Randomized clinical trials with the use of PUFA for the treatment of hypertriglyceridemia in HIV-infected patients have shown a beneficial effect in decreasing TG levels from 10% to 56% of the baseline values [4,5,9,19–22]. However, to the best of our knowledge, this is the first trial trying to demonstrate a beneficial effect of DHA supplementation on systemic inflammation and SAT molecular markers in HIV-infected patients.

Inflammatory mechanisms, together with HIV/cART-induced disturbances in adipocyte gene transcription, play a role in the pathogenesis of fat disturbances in HIV-infected patients [2]. The anti-inflammatory actions of PUFA are connected with their ability to decrease production of pro-inflammatory eicosanoids and cytokines [8]. In addition, PUFA are substrates for the synthesis of other lipid mediators such as anti-inflammatory protectins and resolvins [23]. Moreover, PUFA are ligands for GPR120, a G protein coupled receptor expressed in adipose tissue that potently inhibits inflammatory-signaling pathways [24]. The potential anti-inflammatory effect of PUFA, particularly DHA, has been demonstrated by mechanistic research [8] and by studies in HIV-negative population in which DHA intake was associated with lower levels of inflammation [25,26]. Furthermore, studies in rodent models and with adipocytes cultured in vitro have consistently shown decreases in pro-inflammatory mediators (IL-6 and TNF-α) with controversial effects on adiponectin secretion [27,28]. However, randomized trials with PUFA supplements have yielded mixed results and, in...
a recent meta-analysis summarizing the evidence from 26 trials, it was concluded that long-chain PUFA have no effect on systemic inflammation in healthy subjects but a modest anti-inflammatory effect in patients with cardiovascular or other chronic diseases [29]. Data regarding the effects of PUFA on inflammation are also discordant in patients with cardiovascular or other chronic diseases [29]. Data regarding the effects of PUFA on hsCRP are conflicting with some studies showing no significant change in hsCRP circulating levels [32]. hsCRP and ARA levels were the only significant changes found in our study, whereas other systemic inflammatory markers remained unchanged.

It has been shown that PUFA are able to bind and activate all PPAR isoforms, including PPAR-γ [11], which are major regulators of adipocyte differentiation and whole-body insulin sensitivity [33]. In fact, DHA is a potent PPAR-γ agonist [34], and there is evidence of the involvement of PPAR-γ in DHA-mediated effects on adiponectin secretion [35,36]. However, we did not find any significant change in adipocyte adiponectin and PPAR-γ gene expression in patients supplemented with DHA, findings consistent with those of others [37]. In vitro studies with adipocytes and adipocytes co-cultured with macrophages suggest that the pro-inflammatory adipokine profile generated in SAT is in part mediated through adipocyte-macrophage crosstalk. Macrophage infiltration and a pro-inflammatory state have been consistently demonstrated in SAT of HIV-infected patients, especially in those who develop fat redistribution [38,39].

In rodent models, fish oil attenuates the adipose tissue inflammation induced by high-fat diet [40]. Evidence from human studies is controversial, and the positive effect of PUFA seems to be dependent on the baseline inflammatory state of the patients, i.e. the greater it is the bigger the benefit [29]. The most constant finding is a decrease in MCP-1 and IL-6 gene expression, together with a decrease in the secretion of both cytokines [27,28,32]. This is in line with our results and consistent with DHA-induced down-regulation of pro-inflammatory cytokine gene expression. In fact, it is known that DHA’s most potent anti-inflammatory action is exerted on MCP-1 [28]. Taken together, this evidence suggests that DHA supplementation is able to down-regulate the vicious inflammatory feedback established between adipocytes and macrophages.

There is a well-known interplay between mitochondria and inflammation [41]. Inflammation courses with reactive oxygen species (ROS) generation that may affect mitochondrial proteins, lipids, and DNA, and through the action of inducible nitric oxide synthase and induction of nitric oxide may cause inhibition of mitochondrial respiratory chain, ATP reduction, and mtDNA damage [42]. In these conditions, mitochondria produce ROS that in turn decrease mitochondrial bioenergetics and further promote inflammation. To what extent persistent, chronic SAT inflammation in the placebo group may explain the observed decrease in mtDNA while its abundance was preserved in the DHA group can only be speculative at present. Furthermore, it has been observed in rodents that a DHA-rich diet is able to decrease ARA from the mitochondrial membrane [43], and it is known that the increase of ARA in cells may result in necrosis or apoptosis [44]. Moreover, ARA has the potential for modulation of inflammation and induces thromboxane production [45]. Therefore, DHA supplementation and the consequent ARA decrease observed in our study may have contributed not only to decreasing the SAT pro-inflammatory state but also to protecting mitochondria and their functionality.

Our study has limitations. The primary limitation is that our results can only be applied to populations similar to ours, i.e. non-obese, non-insulin resistant, HIV-infected patients, with virologically-controlled HIV infection and mild hypertriglyceridemia. Second, our study was originally designed to assess the effects of DHA in circulating TG levels. Third, it is possible that the study was not sufficiently powered to detect a clinically significant difference in plasma or tissue markers of inflammation between groups. However, it is unlikely that we missed an effect of DHA due to lack of power because there was not even a trend in other plasma markers together with the significant effects on hsCRP and ARA serum levels, and SAT inflammation markers. Finally, the levels of systemic inflammatory markers may be modified by unavoidable stimuli in daily life of research participants, such as unreported acute illness or psychosocial stress [46]. Notwithstanding these limitations, the strengths of this study are the double-blind, randomized, placebo-controlled design, the ability to test compliance by measuring DHA serum levels, the assessment of ARA levels, and the relatively long duration of DHA supplementation.

In summary, our study shows that DHA supplementation is able to decrease the expression of SAT inflammatory genes in otherwise well-controlled HIV-infected patients without any effect on adipogenesis and mitochondrial-related genes. Its impact on markers of systemic inflammation was restricted to a decrease in hsCRP and ARA levels.

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Possible conflicts of interest

All authors no conflict.
PD, MG, FV, FV, and JCD designed the study. PD, IF, MGM, GMM, and FV enrolled participants into the study. PD, MG, and FV contributed to the coordination and oversight of the study. FT did the statistical analysis. All authors participated in data interpretation. The manuscript was drafted by PD, FV, and JCD. All authors provided input to the report and approved the final version of the manuscript.

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