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Original article

Effects of docosahexanoic acid on metabolic and fat parameters in HIV-infected patients on cART: A randomized, double-blind, placebo-controlled study

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SUMMARY

Background: Hypertriglyceridemia is common in HIV-infected patients. Polyunsaturated fatty acids reduce fasting serum triglyceride (TG) levels in HIV-infected patients. It is not known whether docosahexanoic acid (DHA) supplementation can reduce hypertriglyceridemia and modify fat distribution in HIV-infected patients.

Methods: We conducted a randomized, double-blind, placebo-controlled trial with 84 antiretroviral-treated patients who had fasting TG levels from 2.26 to 5.65 mmol/l and were randomized to receive DHA or placebo for 48 weeks. TG levels were assessed at baseline, week 4 and every 12 weeks. Body composition was assessed at baseline and at week 48. Registered under ClinicalTrials.gov Identifier no. NCT02005900.

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 ($P < 0.0001$). DHA levels and decrease in TG at week 4 in the DHA arm correlated significantly ($r = 0.7110$, $P < 0.0001$). The median reduction in TG levels in the DHA arm was −43.7% (−32.4% to −57.5%), and in the placebo arm +2.9% (−21.3% to +30.1%) at week 12. The difference remained statistically significant at week 48 ($P = 0.0253$). LDL cholesterol levels significantly increased at week 4 by 7.1% (IQR: −4.8% to +35.3%) in the DHA arm but not in the placebo group. No significant changes were observed in HDL cholesterol, insulin, and HOMA-IR during the study. Limb fat significantly increased in both arms, without statistically significant differences between groups ($P = 0.3889$). DHA was well tolerated; only 3 patients experienced treatment-limiting toxicity.

Conclusions: Supplementation with DHA reduced fasting TG levels in antiretroviral-treated HIV-infected patients with mild hypertriglyceridemia. DHA was well tolerated with minor GI symptoms. Peripheral fat significantly increased in the DHA group but did not increase significantly compared with placebo.

1. Introduction

Among lipid disorders associated with HIV infection and cART, the usual phenotype is that of increased triglycerides (TG), decreased HDL-cholesterol and increased LDL-cholesterol, sometimes accompanied by increased fasting glucose levels [1]. These abnormalities are even more frequent and quantitatively more important in patients with HIV/HAART-associated lipodystrophy syndromes (HALS) [2]. They are partly related to HIV infection itself and mostly to certain HIV protease inhibitors (PI) [3]. In particular, hypertriglyceridemia is often due to ritonavir boosting of most PI, with thymidine analogues also contributing to the increase in TG levels [4]. Lipid abnormalities whether or not coupled with body fat

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redistribution, especially visceral adipose tissue hypertrophy, depict a scenario of high cardiovascular risk for these patients [1].

Polyunsaturated fatty acids (PUFA), the so-called omega-3 fatty acids, are extremely variable molecules with a range of claimed beneficial effects [5]. Among them, they decrease serum TG levels, increase 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 [5]. In the HIV setting, a number of clinical trials have demonstrated that hypertriglyceridemia can be at least partially corrected by diet supplementation with a mixture of omega-3 fatty acids of fish oil origin [6,7].

Eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) are the two principal PUFA found in marine oils. Data in humans have shown that these two fatty acids have differential effects on serum lipids and lipoproteins [8], serum glucose [8], blood pressure [9], heart rate [10], and endothelial function [11]. DHA ($C_{22}H_{32}O_2$) is primarily a structural component of many organs and tissues including brain, skin, testes and retina [12]. It is usually obtained from maternal milk or fish oil although it can also be synthesized in mild amounts from alpha-linolenic acid. Dietary DHA may reduce the risk of heart disease in humans and below-normal levels have been associated with Alzheimer's disease and retinitis pigmentosa [13].

Because of its lipid effects, its claimed anti-inflammatory properties, and low levels of DHA found in HIV-infected patients [14], we implemented a randomized, double-blind, placebo-controlled clinical trial to assess DHA supplementation in cART-treated HIV-infected patients with mild hypertriglyceridemia. DHA effects on cholesterol fractions and body fat distribution measured by dual X-ray absorptiometry (DEXA) after 48 weeks were considered as secondary endpoints.

2. Patients and methods

2.1. Study population

All patients were recruited through the *Hospital de la Santa Creu i Sant Pau* HIV-1 infection clinic between July 2010 and June 2011. This clinic serves a population of 1570 adult HIV-1-infected patients on active follow-up. Inclusion criteria for screening were having 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 on two consecutive determinations within a 15-day interval. Since pharmacological therapy was indicated for patients with a TG level >5.65 mmol/l, these were excluded [15]. These limits were chosen because they are the upper limit of normality and the threshold for pharmacological intervention, respectively.

Exclusion criteria included known hypersensitivity to the active compound or product excipients, BMI >30 kg/m^2, pregnancy, breastfeeding, anticoagulant treatment, oral antidiabetics and hormonal treatments. Discontinuation of lipid-lowering drugs for more than 3 months before the selection visit was required to be screened for the study. This was done to dissect the actual effect of DHA on lipid fractions. Consumption of high levels of alcohol (>20 g/d), diabetes or an abnormal fasting blood glucose level (glycemia >6.6 mmol/l) were exclusion criteria. Additional exclusion criteria were: serum creatinine >150 μmol/l and alanine aminotransferase or aspartate aminotransferase >5 × upper limit of normal, anemia, >10% loss in body weight in the preceding 6 months, and any active AIDS-defining disease. There were 3 protocol violations, two in the DHA arm (positive pregnancy test and start of lipid-lowering therapy) and one in the placebo arm (fasting glycemia >6.6 mmol/l). The diagnosis of AIDS was based on the 1993 revised case definition of the Centers for Disease Control and Prevention CDC [16]. All participants were instructed not to make any changes to their lifestyle throughout the intervention period to assess the effect of DHA on circumstances close to "real-world" conditions. 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* on March 23, 2010, and its amended version on April 22, 2010. Registered under ClinicalTrials.gov Identifier no. NCT02005900. The first patient was randomized on July 14, 2010 and the last one on June 7, 2011 (Fig. 1). The last follow-up visit for a randomized patient was on July 7, 2011. The authors confirm that all ongoing and related trials for this drug are registered.

2.2. Study design

A double-blind, phase 4, randomized, 2-arm, placebo-controlled 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. The AHA recommends a total dose of EPA and DHA of 2–4 g per day for patients who need to lower TG levels [17]. Placebo ochre vials containing 7 g of olive oil were similar to DHA ones. 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).

Strategies to lower lipid levels have demonstrated a maximum effect within the first 4–6 weeks, and cholesterol guidelines suggest that response to lipid-lowering therapy be assessed after 4 weeks, too [18]. Consequently, the primary efficacy endpoint of the intervention was chosen to be the percent change in TG levels at week 4 after randomization. An additional 44 weeks of study follow-up was included to permit fuller characterization of the tolerability and safety of DHA, as well as the collection of fat data. Adverse clinical and laboratory events were graded according to the National Institutes of Health Division of AIDS toxicity grading table [19].

HIV infection history and demographic data were recorded, and anthropometric, blood pressure, viro-immunological, and metabolic parameters were measured at study entry. 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 percent change in TG level at 12, 24, 36, and 48 weeks and change in limb fat mass measured by DEXA from baseline to 48 weeks were secondary endpoints.

2.3. Randomization

The randomization process was centrally managed. A randomization list was generated by means of the PROC PLAN of the SAS software with a 1:1 ratio of assignment in blocks of 4 elements for two arms, using an "A" or "B" blinded codes format. The list was sent to the medication manufacturer, Brudy Technologies® (Barcelona, Spain), which was in charge of assigning the arm codes to either DHA or placebo, the medication conditioning and the blinded delivery to the hospital pharmacy. The files and programs used for randomization were deleted from the computer system of the statistical team once the list was generated whereas a sealed copy of the list and individual, numbered, opaque, sealed and stapled envelopes were centrally retained, and kept closed until the end of the study. After informed consent, patients were screened, and only once eligibility was confirmed were patients strictly assigned using

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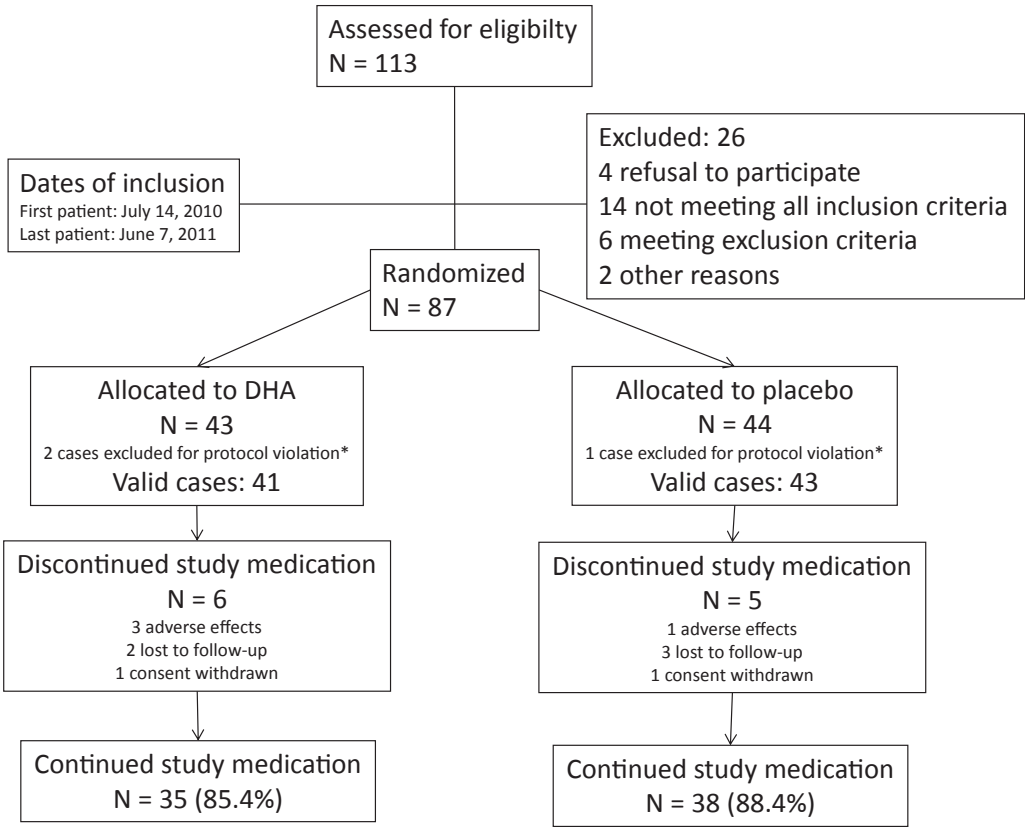

Fig. 1. Subject disposition. *3 cases excluded for protocol violation: positive pregnancy test (1), lipid-lowering therapy started (1), and fasting glycemia >6.6 mmol/l.

the sequentially random number ordering which identified them throughout the study, patients being given the medication identified by the random number.

2.4. Laboratory measurements

These measurements were performed at baseline and at week 4, 12, 24, 36 and 48. All laboratory investigations were performed as previously described [20].

2.5. Measurement of plasma fatty acid concentrations

The composition of fatty acids was determined using the method by Lepage and Roy [21], and has also been described elsewhere [14].

2.6. Body composition measurements, HALS and metabolic syndrome

Body composition measurements were performed as previously described [20,22].

HALS, measures of fat symmetry, and metabolic syndrome have been defined elsewhere [20,22].

2.7. Statistical analyses

The primary endpoint of this trial was to compare the median change (defined as the week 4 value minus the entry value, divided by the entry value) in fasting TG levels from baseline to week 4 within and between the study arms. Secondary outcomes included comparison of the 2 study arms with respect to the median TG levels from baseline to week, 12, 24, 36, and 48, and fat distribution parameters to week 48. In addition, change from study entry to the same time points in the levels of total, HDL and LDL cholesterol and lipoprotein B was also assessed. The safety and tolerability of the study medication were also secondary outcomes. Using the percentage change from baseline of the TG level as the primary endpoint, a type I error rate (α) of 0.05 (2-sided), a power (1-β) of 80%, and a SD of 40%, 33 patients per group were necessary to identify a 20% difference between treatment groups. Because this was a superiority trial, the primary analysis was performed on the intent-to-treat (ITT) population (i.e. all randomized patients who took at least 1 dose of study medication. 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.

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 the week 4 study evaluation (for primary outcome) and 77 (88.5%) completed the week 12 study evaluation (Fig. 1). Two patients in the DHA arm and one in the placebo arm were prematurely excluded (before taking study medication) because of protocol violation and were excluded from further analyses (Fig. 1).

Patient characteristics at baseline are summarized in Table 1. Treatment groups were similar regarding age, gender and history of HIV infection. In both groups, nearly 90% of patients were male; mean HIV infection duration was 14.9 ± 5.8 years; and mean

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Table 1
Characteristics of the population studied.

Parameter	DHA (n = 41)	Placebo (n = 43)
Age, yrs.	44.0 (39.7–50.2)	45.0 (41.5–50.0)
Males, n (%)	36 (87.8)	39 (90.7)
Means of HIV infection		
MsM, n (%)	20 (48.8)	26 (60.5)
Htsx, n (%)	15 (36.6)	12 (27.9)
IDU, n (%)	6 (14.6)	5 (11.6)
Weight, kg	72.4 (67.7–83.8)	74.2 (65.0–80.8)
BMI, kg/m^2	25.4 (23.7–28.2)	25.4 (22.4–26.7)
Waist circumference, cm	92.0 (88.7–97.5)	91.0 (86.5–96.5)
WHR	0.96 (0.92–1.00)	0.94 (0.92–1.02)
Duration of HIV infection, yrs.	14.0 (10.5–17.2)	15.5 (11.0–19.5)
AIDS, n (%)	13 (31.7)	17 (39.5)
Smokers, n (%)	23 (56.1)	21 (48.8)
Alcohol consumption, g/d	0.0 (0.0–6.7)	0.0 (0.0–9.0)
HBV co-infection, %	2 (4.8)	1 (2.3)
HCV co-infection, %	13 (31.7)	15 (34.9)
Baseline CD4 cell count/mm^3	191 (109–332)	179 (27–294)
Nadir CD4 cell count/mm^3	162 (95–318)	156 (26–278)
Nadir CD4 cell count < 200/mm^3	22 (53.6)	22 (51.2)
Nadir CD4 cell count < 100/mm^3	9 (21.9)	12 (27.9)
CD4 increase at study entry	699 (575–935)	677 (486–906)
Baseline HIV-1 RNA ($\log_{10}$ copies/ml)	1.28 (1.28–1.31)	1.28 (1.28–1.35)
Baseline HIV-1 RNA ≥ 5 $\log_{10}$ copies/ml, n (%)	25 (60.1)	29 (67.4)
Undetectable HIV-1 RNA at study entry, n (%)	32 (78.0)	32 (74.4)
Decrease HIV-1 RNA baseline-study entry, $\log_{10}$	3.75 (3.36–4.25)	3.89 (3.18–4.62)

All parameters expressed as median (interquartile range) unless otherwise indicated. DHA = docosahexanoic acid, MsM = Men who have sex with men, Htsx = Heterosexual, 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.

Table 2
Antiretroviral drug exposure in the population studied.

Parameter	DHA (n = 41)	Placebo (n = 43)	P value
Current ART composition			0.31
PI-based, n (%)	18 (43.9)	21 (48.8)	
NNRTI-based, n (%)	23 (56.1)	18 (41.9)	
INSTi-based, n (%)	0 (0.0)	3 (7.0)	
PI + NNRTI, n (%)	0 (0)	1 (2.3)	
NRTI backbone			
TDF/FTC, n (%)	16 (40.0)	19 (44.2)	0.66
ABC/3TC, n (%)	18 (43.9)	14 (32.5)	
ABC + TDF, n (%)	2 (4.9)	1 (2.3)	
ABC + ddI, n (%)	0 (0.0)	2 (4.6)	
3TC alone, n (%)	3 (7.3)	2 (4.6)	
TDF alone, n (%)	2 (4.9)	4 (9.3)	
NRTI-sparing, n (%)	0 (0.0)	1 (2.3)	
cART duration, years	13.3 (9.1–17.1)	13.7 (9.6–17.7)	0.67
Individual drug exposure			
AZT exposure, m	3.0 (0.0–43.7)	12.5 (0.0–47.5)	0.54
d4T exposure, m	0.0 (0.0–57.5)	12.5 (0.0–59.0)	0.73
3TC/FTC exposure, m	76.0 (46.0–105.5)	89.5 (56.0–109.5)	0.30
ddI exposure, m	0.0 (0.0–45.2)	0.0 (0.0–19.5)	0.30
ABC exposure, m	14.0 (0.0–45.7)	0.0 (0.0–44.0)	0.32
TDF exposure, m	26.0 (0.0–46.0)	31.5 (0.30–48.5)	0.73
EFV exposure, m	26.0 (0.0–72.2)	0.0 (0.0–61.5)	0.34
NVP exposure, m	0.0 (0.0–21.0)	0.0 (0.0–25.0)	0.70
Ritonavir exposure, m	32.0 (0.0–82.0)	43.0 (00–66.5)	0.84
PI exposure, m	42.0 (0.0–84.5)	42.5 (24.5–93.5)	0.63
NRTI exposure, m	236.0 (131.0–289.0)	241.0 (129.0–302.5)	0.94

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, ddI = didanosine, NRTI = nucleoside reverse transcriptase inhibitor, cART = combination antiretroviral therapy, AZT = zidovudine, d4T = stavudine, EFV = efavirenz, NVP = nevirapine.

duration of cART was 13.5 ± 4.8 years (Table 2). Thirty patients (35.7%) had presented an AIDS-defining condition. PI-exposed patients had significantly higher TG levels at baseline than NNRTI-exposed patients (4.10 [3.59–5.36] vs. 3.73 [2.83–4.29] mmol/l, respectively, P = 0.02). There were not differences between both groups in terms of total, LDL, HDL, VLDL cholesterol, glucose and insulin. PI- and NNRTI-exposed patients were evenly distributed among randomized arms (Table 2). Eleven randomized patients discontinued the study before its completion: 6 (14.6%) in the DHA arm and 5 (11.6%) in the placebo group (Fig. 1).

3.2. Antiretroviral exposure and immuno-virological status

The cumulated antiretroviral exposure for patients in both arms and the current antiretroviral regimes are shown in Table 2. NRTI plus NNRTI was the most common cART regime (48.8%), followed by PI-based regimes (46.4%). There were no statistically significant differences between arms in terms of antiretroviral exposure (Table 2). There were no discontinuations or modifications of HIV therapy by any subject. The CD4, CD8 cell count, and viral load remained stable throughout the study.

3.3. Assessment of lipid parameters over time

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, −31% vs. −2.9% IQR: 18.6, −16.5%, respectively, P < 0.0001) was observed (Fig. 2). The difference between arms at study week 12, 24, 36 and 48 continued to be statistically significant (Fig. 2). 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). The percentage of subjects who

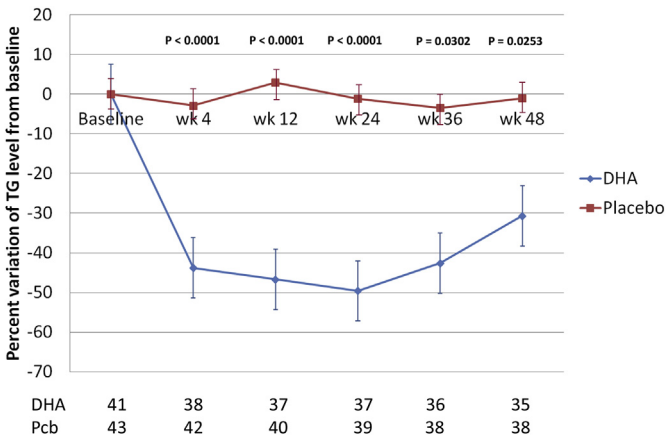

Fig. 2. Evolving triglyceride levels over time in DHA and placebo groups. DHA = docosahexanoic acid; TG = triglyceride.

achieved a TG level of <2.26 mmol/l was 63.4% and 4.6% in the DHA and placebo arm, respectively (P < 0.0001).

Total and HDL cholesterol experienced slight increases in the DHA arm, without statistical significance in either arm (data not shown). The same was true for LDL cholesterol except for week 4 point at which the increase was statistically significant (4.13 [3.34–4.69] mmol/l) (P = 0.0327). None of the cholesterol fractions were significantly different at the end of the study except for LDL and HDL cholesterol, which were significantly higher in the placebo arm (Table 3), which may be caused by the oil olive content of the placebo vials. No significant changes from baseline in either arm

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Table 3
Change in anthropometric and metabolic parameters over 48 weeks in patients randomized to DHA or placebo.

	DHA (n = 41)			Placebo (n = 43)				
	Baseline	Week 48	Within group P value	Baseline	Week 48	Within group P value	Between groups P value baseline	Between groups P value wk. 48
Weight, kg	72.4 (67.7–83.8)	72.7 (66.3–81.4)	0.29	74.2 (65.0–80.8)	75.3 (67.2–83.0)	0.30	0.57	0.66
BMI	25.4 (23.7–28.2)	25.4 (23.5–27.9)	0.40	25.4 (22.4–26.7)	25.0 (23.8–28.2)	0.44	0.35	0.98
Waist circumference, cm	92.0 (88.7–97.5)	92.0 (89.0–99.5)	0.66	91.0 (86.5–96.5)	92.0 (86.2–97.5)	0.69	0.17	0.61
Systolic BP, mm Hg	110 (110–120)	120 (110–120)	0.20	120 (110–127)	120 (120.128)	0.68	0.34	0.37
Diastolic BP, mm Hg	70 (60–70)	70 (65–75)	0.63	75 (70–80)	75 (70–80)	0.90	0.16	0.14
Total cholesterol, mmol/l	5.93 (5.38–6.78)	6.16 (5.35–7.02)	0.84	5.82 (5.18–6.32)	6.45 (5.69–7.07)	0.12	0.26	0.58
HDL cholesterol, mmol/l	0.96 (0.79–1.15)	0.98 (0.88–1.21)	0.54	1.00 (0.89–1.14)	1.06 (0.85–1.28)	0.05	0.86	0.42
Total cholesterol/HDL ratio	6.6 (5.2–7.6)	6.8 (4.6–7.6)	0.91	5.7 (5.0–6.9)	5.7 (4.1–6.6)	0.66	0.11	0.23
LDL cholesterol, mmol/l	3.60 (2.79–4.45)	4.04 (3.28–4.66)	0.15	3.41 (2.48–4.01)	3.75 (3.11–4.17)	0.01	0.34	0.25
Triglycerides, mmol/l	3.85 (3.11–4.85)	2.14 (1.39–3.16)	0.0001	3.80 (3.05–4.56)	2.99 (2.20–4.53)	0.37	0.68	0.01
Apolipoprotein B, mmol/l	1.11 (1.03–1.28)	1.15 (1.04–1.44)	0.29	1.10 (0.98–1.27)	1.19 (1.06–1.32)	0.24	0.35	0.86
Fasting glucose, mmol/l	5.1 (4.8–5.6)	5.3 (4.7–5.7)	0.96	5.0 (4.9–5.8)	5.1 (4.9–5.5)	0.33	0.73	0.75
Fasting insulin, pmol/l	44.0 (32.0–57.0)	27.7 (24.0–63.3)	0.50	33.0 (26.7–79.7)	32.0 (22.0–56.5)	0.64	0.51	0.64
Fasting C peptide, mmol/l	854 (575–1191)	865 (715–1170)	0.98	783 (605–1134)	914 (762–1067)	0.58	0.36	0.90
HbA1C, %	5.6 (5.4–5.8)	5.4 (5.3–5.6)	0.14	5.5 (5.3–5.7)	5.5 (5.3–5.7)	0.30	0.64	0.58
HOMA-IR	1.57 (10.3–2.07)	0.96 (0.76–1.89)	0.62	1.07 (0.84–2.87)	1.11 (0.72–2.05)	0.61	0.62	0.69

All parameters expressed as median (interquartile range) unless otherwise specified. BMI = body mass index, BP = blood pressure, HDL = high density lipoprotein, LDL = low density lipoprotein, mmol = millimols, pmol = picomols, HOMA-IR = homeostasis model assessment method for insulin resistance.

were seen for apolipoprotein B levels, total cholesterol/HDL ratio and cardiovascular risk assessed by the Framingham risk score. Not a single subject started non-study lipid-lowering therapy.

3.4. Evolution of glucose metabolism over time

Fasting glucose, insulin, peptide C, HOMA-IR, and glycosylated hemoglobin were comparable at the time of randomization and remained without significant changes within and between groups through 48 weeks of the study (Table 3).

3.5. Assessment of body composition over time

Fifty-eight patients (69%) had fat redistribution; 28 (68.2%) in the DHA and 30 (69.7%) in the placebo arm had lipoatrophy (P = 0.9283) at baseline. Of them, 21 (51.2%) and 17 (39.5%) respectively (P = 0.3918), had also lipohypertrophy depicting a mixed phenotype. After 48 weeks patients with lipoatrophy were 26 (63.4%) and 28 (65.1%) in DHA and placebo arms, respectively (P = 0.9481). The figures for patients with lipohypertrophy remained unaltered.

Whole body and trunk fat mass did not vary significantly during the study in either arm (Table 4). Appendicular fat mass significantly increased in both arms without significant differences between arms at week 48 (Table 4). 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 (−243, 1009) grams (P = 0.5221). The indexes of fat symmetry, bone mineral content and bone mineral density did not vary significantly during the study period.

3.6. DHA levels throughout the study

DHA levels were measured at baseline and at weeks 4, 24, and 48. Median DHA levels at baseline (measured as percent of total fatty acids) were 1.27% (IQR: 0.89–1.74%) in the DHA group and 1.38% (1.27–1.60%) in the placebo group (P = 0.6067). At 4 weeks, the DHA levels were 5.01% (4.2–5.8%) in the DHA group (P < 0.0001 vs. baseline) and 1.2% (1.0–1.5%) in the placebo group (P = 0.1136 vs. baseline) (P < 0.0001 between groups). 24-week were similar to week 4 data. At 48 weeks, the respective values were 3.38% (2.22–4.58%) and 1.16% (0.97–1.88%) (P < 0.0001). There was a significant correlation between DHA levels at week 4 and decrease in TG level (r = 0.7110, P < 0.0001), but there was no correlation between DHA levels at week 48 and increase in appendicular fat (r = 0.038, P = 0.8290).

3.7. Safety and tolerability

The safety profile and tolerability of the study is shown in Table 5. Treatment-emergent adverse events incidence during the study was not more frequent in the DHA than in the placebo group (P = 0.46). Minor gastrointestinal disorders with fishy smell belching, nausea without vomiting, and bloating were most common adverse events. They did not lead to treatment discontinuation, except for 3 patients in the DHA group (7.3%) and one patient (2.3%) in the placebo group (P = 0.57). One patient experienced a severe adverse event while on DHA, which was grade III jaundice (considered to be related to atazanavir), and 2 patients in the placebo group experienced serious adverse events, acute hepatitis C in one case and bacterial pneumonia in the other. They were considered to be unrelated to the study therapies. Laboratory parameters including transaminases, except for the patient with acute hepatitis, have no relevant variations in either arm.

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. Patients randomized to DHA had experienced a 43.9% reduction in TG levels at week 4. The TG drop observed in this trial was durable, although with smaller magnitudes, through 48 weeks, the difference between the two arms being statistically significant throughout the study period. A significant TG response among the DHA recipients was found, whereas no such response in the patients on the placebo arm was seen. In addition, TG decrease was closely related to increasing DHA levels. 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 [6,7,23–27]. This heterogeneity in trial results may be explained, in part, by the different trial designs together with

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Table 4
Change in fat parameters over 48 weeks in patients randomized to DHA or placebo.

Parameter	DHA (n = 41)			Placebo (n = 43)				
	Baseline	Week 48	Within group P value	Baseline	Week 48	Within group P value	Between groups P value baseline	Between groups P value (wk. 48)
Whole body fat, kg	18.3 (12.5–22.9)	17.2 (13.9–20.7)	0.29	15.3 (12.5–19.7)	14.9 (13.7–18.4)	0.3533	0.27	0.31
Trunk fat, kg	10.0 (8.5–14.3)	11.3 (7.9–13.1)	0.24	9.6 (7.2–11.9)	9.9 (8.2–11.7)	0.1078	0.34	0.30
Left leg fat, g	1652 (1189–2343)	2005 (1339–2588)	0.09	1569 (1033–2455)	1709 (1065–2448)	0.2295	0.66	0.59
Lower limb fat, g	3376 (2472–5646)	3969 (2820–6525)	0.09	3118 (2083–4941)	3967 (2240–5611)	0.0433	0.46	0.26
Appendicular fat mass, g	5485 (3614–7890)	5782 (3871–8987)	0.03	4393 (3424–7066)	5149 (3505–7906)	0.0264	0.42	0.39
Trunk/appendicular fat ratio	1.98 (1.48–2.37)	1.76 (1.47–2.16)	0.24	2.07 (1.41–2.70)	1.88 (1.36–2.64)	0.4244	0.72	0.84
Fat mass ratio	1.05 (0.81–1.34)	0.94 (0.84–1.15)	0.17	1.12 (0.81–1.48)	1.03 (0.84–1.46)	0.3269	0.66	0.52
Fat mass index, kg/m^2	10.5 (7.2–13.6)	10.2 (7.9–12.1)	0.24	8.8 (7.1–11.3)	8.9 (7.8–10.7)	0.2295	0.22	0.29
Leg fat % normalized to BMI	0.67 (0.52–0.91)	0.82 (0.57–0.96)	0.33	0.61 (0.41–0.88)	0.68 (0.51–1.02)	0.0146	0.48	0.73
Metabolic syndrome, n (%)	21 (51.2)	22 (53.7)	0.83	17 (39.5)	21 (48.9)	0.5147	0.39	0.82
BMC total, g	2342 (2115–2540)	2402 (2123–2600)	0.24	2279 (2019–2572)	2247 (2007–2532)	0.6900	0.84	0.35
BMD, g/cm^2	1.14 (1.06–1.21)	1.15 (1.07–1.21)	0.69	1.10 (1.03–1.20)	1.09 (1.01–1.21)	0.8683	0.60	0.51

All parameters expressed as median (interquartile range) unless otherwise specified. BMI = body mass index, BMC = bone mineral content, BMD = bone mineral density.

Table 5
Safety profile and tolerability.

	DHA (n = 41) (%)	Placebo (n = 43) (%)	P value
Adverse effects	13 (31.7)	18 (41.8)	0.46
Most frequent adverse effects			
Belching	4 (7.3)	4 (9.3)	0.76
Diarrhea	3 (7.3)	4 (9.3)	0.95
Nausea	2 (4.9)	3 (6.9)	0.95
Flatulence	2 (4.9)	3 (6.9)	0.95
Upper respiratory tract infection	1 (2.4)	2 (4.6)	0.97
Severe adverse effects	1[a] (2.4)	2[b] (4.6)	0.97
Discontinuation because of adverse effects[c]	3 (7.3)	1 (2.3)	0.57

[a] A patient with grade III hyperbilirubinemia due to atazanavir.
[b] A patient with acute HCV hepatitis and another with bacterial pneumonia.
[c] Patients who discontinued DHA or placebo experienced belching, nausea, and diarrhea.

the lipoprotein phenotype of the patients as well as by variations in the amount of omega-3 fatty acids consumed and the manner in which they are presented (fish, fish oils, or purified oils). Although patients in the DHA arm had an increase in appendicular fat, those assigned to placebo also presented a significant increase. Therefore, a beneficial effect of DHA supplementation on fat distribution could not be demonstrated in the present trial.

DHA lacks any effect on HIV disease in patients without changes in cART during the study as shown by stable viral load measurements, and CD4 and CD8 cell counts throughout the study. DHA was well tolerated in our study, adverse effects usually being minor and referring to gastrointestinal tract, so that discontinuations due to adverse effects were not quantitatively different to those which appeared in the placebo arm.

There were negligible effects of DHA supplementation on other lipid fractions, with the transient exception of an LDL cholesterol increase at week 4. In other studies in HIV population treated with fish oil, increases in LDL cholesterol levels have been observed which have ranged from 5% to 20% [7,8]. Although the increase in LDL-cholesterol has been attributed to the DHA component of fish oil mixtures [8], it has been observed that DHA supplementation increases the particle size, a result that might contribute to a reduction in atherogenic risk [28]. The increase in LDL cholesterol as a result of DHA supplementation is thought to be caused by an increased rate of conversion of VLDL to LDL particles and a reduction of TG for cholesteryl esters in plasma [29,30]. Olive oil, which was the basis of our placebo vial, may had not neutral effects for the lipid profile of our patients. In fact, olive oil supplementation has been associated with decreases in TG and LDL cholesterol levels and with increases in HDL cholesterol [31,32]. In our trial, LDL and HDL cholesterol increased after 48 weeks, whereas TG levels decreased in a non-significant way.

Another common metabolic consequence reported with the use of omega-3 fatty acids is an increase in insulin levels with fish oil [7]. Therefore, DHA supplementation has to be used cautiously, particularly in patients with type 2 diabetes [33], although increase in insulin levels has not been seen in healthy volunteers, hypertensive or dyslipidemic patients [34]. However, glycemic control can deteriorate in patients with type 2 diabetes supplemented with omega-3 fatty acids [35]. We did not observe any disturbance in glucose homeostasis, assessed with glucose, insulin, HOMA-IR and peptide C levels, among our patients exposed to DHA.

Inflammatory mechanisms, together with HIV/cART-induced disturbances in adipocyte gene transcription, play a role in the pathogenesis of HALS [2]. Therefore, the theoretical basis for an intended beneficial effect of DHA on fat distribution was its anti-inflammatory properties and its effect on gene transcription [36,37]. However, we could not see any differential effect attributable to DHA in peripheral fat. Data regarding the effects of PUFA on inflammation are discordant with studies showing a beneficial effect by decreasing interlukin-6 and tumor necrosis factor circulating levels [24], while others did not show any change [38]. It has been shown that PUFAs are able to bind and activate all peroxisome proliferator-activated receptor (PPAR) isoforms, including PPAR gamma [39], which are major regulators of adipocyte differentiation and whole-body insulin sensitivity [40]. However, EPA up-regulated PPARγ mRNA expression whereas DHA had no effect on human freshly isolated adipocytes [41].

The recommended strategies for the management of hypertriglyceridemia always include changing diet and physical activity as a first step. This strategy decreased TG level between 5% and 15% of baseline levels [7,8,42], but is difficult to adhere to. On the other hand, supplementation with DHA may offer additional benefits in terms of cardiovascular health, especially in patients with prior coronary artery disease [43], and to date there is no evidence of DHA interference with cART or deleterious action on the immune recovery [26]. We instructed our patients to stick to their lifestyle habits prior to study entry, to better assess the individual effects of DHA supplementation, although in routine clinical practice we usually combine diet and lifestyle changes along with DHA supplementation.

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Our study has limitations. First, 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, DHA dose was somewhat high but did not exceed doses recommended for omega-3 fatty acids [17]. Third, although patients were instructed not to change their diet and exercise habits, the lack of close monitoring is a clear limitation of the study. Fourth, adherence was monitored through DHA serum level measurements but it cannot be completely ascertained during the inter-measurement periods. Fifth, the use of olive oil in placebo vials may have been not neutral for the lipid profile of patients assigned to this arm, a circumstance that can distort the results. Notwithstanding these limitations, the strength of this study is the double-blind, randomized, placebo-controlled design and the ability to test compliance by measuring DHA serum levels.

In summary, our study shows that DHA supplementation is able to decrease TG levels in HIV-infected patients with mild hypertriglyceridemia. However, it did not increase appendicular fat significantly compared with placebo.

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Conflict of interest

All authors no conflict.

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