Effect of Omega-3 Polyunsaturated Fatty Acids Treatment on Lipid Pattern of HIV Patients: A Meta-Analysis of Randomized Clinical Trials

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Abstract: Even though omega-3 polyunsaturated fatty acids (PUFAs) seem to be effective in the treatment of human immunodeficiency virus (HIV)-associated dyslipidemia, their impact is still debated. For this reason, our aim was to perform a meta-analysis of the clinical evidence available to date. A systematic literature search was conducted in order to identify published clinical trials assessing the effect of PUFAs treatment on serum lipoproteins, and its safety profile. The effect sizes for lipid changes were expressed as mean difference (MD) and 95% confidence interval (CI). For safety analysis, odd ratios and the 95% CI were calculated with the Mantel–Haenszel method. Data were pooled from nine clinical studies comprising overall 578 HIV-affected subjects. Meta-analysis of the data suggested that omega-3 PUFAs significantly reduced triglycerides (TG) (MD = −1.04, 95% CI: −1.5, −0.58 mmol/L, \( p < 0.001 \)), while increasing high-density lipoprotein cholesterol (MD = 0.36, 95% CI: 0.12, 0.61 mmol/L, \( p = 0.004 \)), without affecting serum levels of total cholesterol, very-low- and low-density lipoprotein cholesterol, and apolipoprotein B and A1. Change in TG was significantly associated with eicosapentaenoic acid administered via daily dose. PUFAs treatment did not lead to an increased risk of adverse events. In conclusion, PUFAs are safe and exert a significant plasma lipid improving effect in HIV-positive patients.

Keywords: HIV; Omega-3 polyunsaturated fatty acids; triglycerides; high-density lipoprotein cholesterol; meta-analysis

1. Introduction

Among patients with chronic infection by human immunodeficiency virus (HIV), dyslipidemia is the most prevalent cardiovascular disease risk factor, being present in around 40% of the affected subjects [1]. The most common lipid alteration is hypertriglyceridemia, due both to HIV infection and the prevalence of several conditions (e.g., insulin resistance, hepatic steatosis and diabetes mellitus) leading to increased triglycerides (TG) [2]. Furthermore, elevated TG is a frequent side effect of antiretroviral treatment (ART) [3].

For the management of dyslipidemia in HIV-positive patients, the Infectious Disease Society of America (IDSA) and the Adult AIDS Clinical Trial Group (ACTG) refer to the updated recommendations from the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III) [4,5].
A number of clinical trials have shown that in patients with chronic HIV infection, hypertriglyceridemia can be at least partially corrected by treatment with omega-3 polyunsaturated fatty acids (PUFAs) [6], with a lower risk of drug–drug interaction in comparison with fibrates [7,8].

Natural sources of omega-3 are found in both animal (fish, krill, egg, squid) and plant (algae, flaxseed, walnut, edible seeds, clary sage, seed) sources, in the form of docosahexaenoic (DHA) and eicosapentaenoic acid (EPA) or as alpha-linolenic acid (ALA) respectively [9].

The European Food Safety Agency (EFSA) established a health claim indicating that the intake of at least 2 g/day of DHA and EPA is able to maintain normal blood TG levels in the general population [9,10]. The American Heart Association (AHA) indicates doses ranging from 2 to 4 g/day of EPA and DHA to reduce TG levels by 25–30% [11]. However, the metabolic effect of PUFAs treatment in HIV-affected patients treated with ART is not yet clear. Consequently, we aimed to perform a meta-analysis on the clinical evidence available to date to better define its efficacy and tolerability profile.

2. Results

2.1. Flow and Characteristics of the Included Studies

After database searches performed strictly according to inclusion and exclusion criteria, 147 published articles were identified, and their abstracts reviewed. Of these, 118 were excluded because they were non-original articles. Another 17 were eliminated because they did not meet the inclusion criteria. Thus, 12 articles were carefully assessed and reviewed. An additional three studies were excluded because they reported incomplete data. Finally, nine studies were eligible and included in the meta-analysis [12–20]. The study selection process is shown in Figure 1.

Data were pooled from nine clinical trials comprising 18 treatment arms, which included 578 subjects, with 308 in the active-treated arm and 270 in the control one.

The eligible studies were published between 2006 and 2016. Follow-up periods ranged between 8 weeks and 6 months and different treatment regimens were tested. All selected trials were designed with parallel groups [12–19] or were crossover [20], and all were multicenter [13,15,17] or single-center [12,14,16,18–20] clinical studies. The enrolled subjects were adult patients living with chronic HIV infection and iatrogenic dyslipidemia. The baseline characteristics of the evaluated studies are summarized in Table 1.
Table 1. Baseline characteristics of the included studies.

| First Author, Year | Study Design | Follow-Up | Main Inclusion Criteria | Study Group | Patients (n) | Male (n (%)) | Age (Years; Mean ± SD) | Years Since HIV Diagnosis (Mean ± SD) | Years Since ART Therapy Started (Mean ± SD) | CD4+ T Cell Count (cell/mL) |
|-----------------|--------------|-----------|-------------------------|-------------|-------------|-------------|-------------------------|---------------------------------|---------------------------------|---------------------------|
| Amador-Licona, 2016 [12] | Randomized, double-blind, parallel-group, clinical study | 6 months | HIV infection treated with stable HAART regimen for ≥ 3 months; TG ≥ 2.26 mmol/L and ≤ 5.65 mmol/L; LDL-C ≥ 3.36 mmol/L and ≤ 4.13 mmol/L; CD4+ T cell count ≥ 300 cells/mL | 2.4 g/day omega-3 PUFA (EPA/DHA 1200/600 mg/day) | 35 | 28 (80) | 39.9 ± 9.5 | 5.6 ± 2 | 4.5 ± 1.7 | 525.7 ± 129.6 |
| Baril, 2007 [13] | Multicenter, randomized, open-label, placebo-controlled, parallel-group, clinical study | 12 weeks | HIV infection treated with stable ARV regimen for ≥ 6 months; TG ≥ 6 mmol/L and ≤ 6 mmol/L | Placebo | 30 | 23 (76.7) | 39.9 ± 8 | 6.8 ± 2.2 | 5.4 ± 2 | 663.7 ± 180 |
| Capili, 2013 [14] | Randomized, double-blind, parallel-group, clinical study | 8 weeks | HIV infection treated with stable PI-ART regimen for ≥ 6 months; TG ≥ 1.69 mmol/L and ≤ 6 mmol/L; LDL-C < 3.36 mmol/L; CD4+ T cell count ≥ 300 cells/mL | Placebo | 10 | 6 (60) | 45.6 ± 6.5 | 12.6 ± 4.9 | NA | 525 ± 182 |
| De Truchis, 2006 [15] | Multicenter, randomized, double-blind, parallel-group, clinical study | 8 weeks | HIV infection treated with stable HAART regimen for ≥ 2 months; TG ≥ 3.43 mmol/L | Placebo | 62 | 55 (88.7) | 47.1 ± 8.4 | 11.6 ± 4.2 | 7.7 ± 3.1 | NA |
| Oliveira, 2013 [16] | Randomized, double-blind, parallel-group, clinical study | 24 weeks | HIV infection treated with stable ART regimen for ≥ 3 months; TG > 1.3 mmol/L; LDL-C < 4.14 mmol/L; FPG < 7 mmol/L | Placebo | 63 | 33 (76.7) | 43.1 ± 7.4 | 10.3 ± 5.7 | 8.3 ± 4.1 | 591.8 ± 259.6 |
| Paranandi, 2014 [20] | Randomized, double-blind, placebo controlled, crossover, clinical study | 12 weeks | HIV infection treated with stable HAART regimen for ≥ 3 months; TG ≥ 1.69 mmol/L | Placebo | 41 | 35 (85) | 51.7 ± 9.6 | 16.7 ± 5.2 | NA | 621.3 ± 277 |
| First Author, Year | Study Design | Follow-Up | Main Inclusion Criteria | Study Group | Patients (n) | Male (n (%)) | Age (Years; Mean ± SD) | Years Since HIV Diagnosis (Mean ± SD) | Years Since ART Therapy Started (Mean ± SD) | CD4+ T Cell Count (cell/ml) |
|-------------------|--------------|-----------|--------------------------|-------------|--------------|--------------|------------------------|----------------------------------------|------------------------------------------|----------------------------|
| Peters, 2012 [17] | Multicenter, randomized, double-blind, placebo-controlled, parallel-group, pilot clinical study | 12 weeks | HIV infection treated with stable HAART regimen for ≥ 3 months; TG ≥ 3.39 mmol/L and ≤ 11.3 mmol/L; lipid-lowering treatment with fibrate or niacin | 4 g/day omega-3 PUFA (EPA/DHA 1840/1520 mg/day) Placebo | 23 25 | 23 (100) 24 (96) | 46.1 ± 2.9 43.6 ± 8.9 | NA NA | NA | 633 ± 217 546 ± 257 |
| Thusgaard, 2009 [18] | Randomized, double-blind, placebo-controlled, parallel-group, clinical study | 12 weeks | HIV infection treated with stable ART regimen for ≥ 3 months | 3.6 g/day omega-3 PUFA (EPA/DHA 1840/1520 mg/day) Placebo | 26 25 | 19 (73) 21 (84) | 43 ± 10 47 ± 11 | NA NA | 8 | 503 ± 306 483 ± 267 |
| Woods, 2009 [19] | Randomized, open label, diet-controlled, parallel-group, clinical study | 10 weeks | HIV infection; TG > 1.69 mmol/L and/or QUICKI score < 0.35 or ≥ 0.30 | 3 g/day omega-3 PUFA (EPA/DHA 2000/1000 mg/day) Control diet | 28 26 | 24 (86) 19 (73) | 46.2 ± 8.2 46.3 ± 5 | NA NA | NA | 527.3 ± 225.2 489.7 ± 228.1 |

ART = antiretroviral treatment; ARV = antiretroviral; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; FPG = fasting plasma glucose; HAART = highly active antiretroviral therapy; HIV = human immunodeficiency virus; LDL-C = low-density lipoprotein cholesterol; NA = not available; PI-ART = Protease inhibitor based antiretroviral therapy; PUFA = polyunsaturated fatty acids; QUICKI = quantitative insulin sensitivity check index; SD = standard deviation; TG = triglycerides.
2.2. Risk of Bias Assessment

According to the Cochrane criteria, almost all of the included studies were characterized by sufficient information regarding sequence generation, allocation concealment, and personal and outcome assessments. Some trials had a high risk of bias for incomplete outcome data and selective outcome reporting. Details of the quality of bias assessment are reported in Table 2.

| Author, Year       | Sequence Generation | Allocation Concealment | Blinding of Participants, Personnel and Outcome Assessment | Incomplete Outcome Data | Selective Outcome Reporting | Other Potential Threats to Validity |
|--------------------|---------------------|------------------------|----------------------------------------------------------|-------------------------|----------------------------|----------------------------------|
| Amador-Licona, 2016 [12] | L                   | L                      | L                                                        | L                       | L                          | L                                |
| Baril, 2007 [13]     | L                   | L                      | L                                                        | H                       | H                          | L                                |
| Capili, 2013 [14]    | L                   | L                      | L                                                        | H                       | H                          | U                                |
| De Truchis, 2006 [15]| L                   | L                      | L                                                        | H                       | H                          | H                                |
| Oliveira, 2013 [16]  | L                   | L                      | U                                                        | L                       | L                          | L                                |
| Paranjadi, 2014 [20]| U                   | U                      | L                                                        | L                       | L                          | L                                |
| Peters, 2012 [17]    | L                   | L                      | L                                                        | L                       | L                          | L                                |
| Thisted, 2009 [18]   | U                   | U                      | H                                                        | L                       | L                          | U                                |
| Woods, 2009 [19]     | U                   | U                      | H                                                        | L                       | L                          | U                                |

L = low risk of bias; H = high risk of bias; U = unclear risk of bias.

2.3. Lipid-Lowering Effect of Omega-3 Polyunsaturated Fatty Acids

Meta-analysis of the data suggested that omega-3 PUFAs significantly reduced TG (mean difference (MD) = −1.04, 95% CI: −1.5, −0.58 mmol/L, \( p < 0.001; \ I^2 = 69.3\% \)) and high-density lipoprotein cholesterol (HDL-C) serum levels (MD = 0.36, 95% CI: 0.12, 0.61 mmol/L, \( p = 0.004; \ I^2 = 96.9\% \)) (Figure 2).

**Triglycerides**

| Study name          | Difference in means (MD) | Standard error | Variance | Lower limit | Upper limit | Z-Value | p-Value |
|---------------------|--------------------------|----------------|----------|-------------|-------------|---------|---------|
| Amador-Licona, 2016  | −0.900                   | 0.205          | 0.042    | −1.361      | −0.480      | -4.293  | 0.000   |
| Baril, 2007  [13]    | −1.400                   | 0.121          | 0.375    | −2.599      | −0.361      | -2.985  | 0.002   |
| Capilli, 2013 [14]   | −1.650                   | 0.099          | 0.370    | −2.823      | −0.477      | -5.758  | 0.000   |
| Da Truchis, 2006 [15]| −1.100                   | 0.242          | 0.585    | −1.852      | −0.258      | -2.952  | 0.001   |
| Oliveira, 2012 [16]  | −0.770                   | 0.149          | 0.230    | −1.163      | −0.378      | -2.952  | 0.001   |
| Paranjadi, 2014 [20] | −1.200                   | 0.195          | 0.369    | −2.560      | −0.843      | -3.616  | 0.000   |
| Peters, 2012 [17]    | −0.220                   | 0.711          | 0.510    | −0.537      | 0.096       | 0.106   | 0.002   |
| Thisted, 2009 [18]   | −0.610                   | 0.259          | 0.652    | −1.520      | 0.300       | -2.474  | 0.014   |
| Woods, 2009 [19]     | −1.041                   | 0.205          | 0.055    | −1.501      | −0.581      | -4.293  | 0.000   |

**HDL-Cholesterol**

| Study name          | Difference in means (MD) | Standard error | Variance | Lower limit | Upper limit | Z-Value | p-Value |
|---------------------|--------------------------|----------------|----------|-------------|-------------|---------|---------|
| Amador-Licona, 2016  | 0.000                    | 0.023          | 0.011    | 0.006       | 0.006       | 0.050   | 1.000   |
| Baril, 2007 [13]     | 0.000                    | 0.010          | 0.016    | 0.000       | 0.000       | 0.050   | 1.000   |
| De Truchis, 2006 [15]| 0.000                    | 0.022          | 0.052    | 0.000       | 0.000       | 0.050   | 1.000   |
| Oliveira, 2012 [16]  | 0.000                    | 0.017          | 0.032    | 0.000       | 0.000       | 0.050   | 1.000   |
| Paranjadi, 2014 [20] | 0.000                    | 0.017          | 0.032    | 0.000       | 0.000       | 0.050   | 1.000   |
| Peters, 2012 [17]    | 0.000                    | 0.017          | 0.032    | 0.000       | 0.000       | 0.050   | 1.000   |
| Thisted, 2009 [18]   | 0.000                    | 0.017          | 0.032    | 0.000       | 0.000       | 0.050   | 1.000   |
| Woods, 2009 [19]     | 0.000                    | 0.017          | 0.032    | 0.000       | 0.000       | 0.050   | 1.000   |

Figure 2. Forest plot displaying mean differences and 95% confidence intervals for the effect of treatment with omega-3 PUFA on plasma TG and HDL-C concentrations.

However, the treatment did not exert any significant effect on total cholesterol (TC) (MD = −0.06, 95% CI: −0.19, 0.07 mmol/L, \( p = 0.401; \ I^2 = 5.4\% \)), very-low-density lipoprotein cholesterol (VLDL-C)
(MD = −0.16, 95% CI: −0.48, 0.15 mmol/L, \( p = 0.311; I^2 = 76.5\%\)), low-density lipoprotein cholesterol (LDL-C) (MD = 0.1, 95% CI: −0.12, 0.33 mmol/L, \( p = 0.375; I^2 = 58.7\%\)), apolipoprotein B (Apo B) (MD = 0.02, 95% CI: −0.07, 0.12 mmol/L, \( p = 0.616; I^2 = 51.8\%\)) and apolipoprotein A-1 (Apo A-1) (MD = −0.004, 95% CI: −0.08, 0.07 mmol/L, \( p = 0.914; I^2 = 0\%\)) concentrations (Figure 3).

**Figure 3.** Forest plot displaying mean differences and 95% confidence intervals for the impact of treatment with omega-3 fatty acids on plasma TC, HDL-C and Apo-A1 concentrations.
The effect sizes were robust in the leave-one-out sensitivity analysis and not mainly driven by a single study.

Visual inspection of Begg’s funnel plots suggested potential publication biases for the effect of treatment with omega-3 PUFAs on serum TG and HDL-C concentrations. These observations were fully confirmed by Begg’s rank correlation ($p = 0.01$ in both cases) and partially confirmed by Egger’s regression asymmetry test (TG: $p = 0.02$; HDL-C: $p > 0.5$). The asymmetries were imputed to two potentially missing studies on the right side of the funnel plot which increased the estimated effect size on TG to $-0.89$ (95% CI: $-1.31, -0.46$) and three potentially missing studies on the same side of the funnel plot which increased the estimated effect size on HDL-C to 0.74 (95% CI: 0.23, 1.25) (Figure 4).

![Figure 4](image_url)  
*Figure 4. Funnel plots detailing publication biases in the studies included in the meta-analysis for the impact of treatment with omega-3 PUFAs on plasma TG and HDL-C concentrations.*

The funnel plots of standard error by effect size (MD) for TC, LDL-C and Apo-A1 were symmetric, suggesting no publication bias for the outcomes (Figure 5). These observations were confirmed by Begg’s rank correlation and Egger’s regression test. However, visual inspection of Begg’s funnel plots suggested potential publication bias for Apo B concentrations. The asymmetry was imputed to one potentially missing study on the left side of the funnel plot reducing the estimated effect size to $-0.01$ (95% CI: $-0.11, -0.09$) (Figure 5). This observation was not confirmed by Begg’s rank-correlation method or Begg’s rank-correlation test ($p > 0.5$ in both cases).

Due to the inadequate number of studies on VLDL-C, publication bias tests were not applicable.
2.4. Differential Effectiveness of EPA and DHA on Lipids

Change in TG was significantly associated with EPA daily dose (slope = −0.0008, 95% CI: −0.0012, −0.0004, $p < 0.001$), although not with DHA daily dose (slope = −0.0007, 95% CI: −0.0014, 0.0001, $p = 0.08$) (Figure 6).

Treatment-dependent change in HDL-C was neither associated with EPA daily dose (slope = −0.0003, 95% CI: −0.0018, 0.0011, $p = 0.64$) nor with DHA daily dose (slope = −0.0004, 95% CI: −0.0021, 0.0013, $p = 0.66$) (Figure 7).
The findings are robust in the leave-one-out sensitivity analyses.

2.5. Safety Analysis for Omega-3 Fatty Acids Administration

The safety analysis included all the studies considered for the efficacy analysis, except for that by Peters et al. [17], which selectively reported the adverse events that occurred during the trial.

According to our analysis, the incidence of adverse events did not differ between groups (Table 3). The findings are robust in the leave-one-out sensitivity analyses.

Table 3. Adverse events that occurred in at least two clinical trials.

| Adverse Event                          | Number of Studies | Odd Ratio | 95% Confidence Interval | Z-Value | p-Value | I² |
|----------------------------------------|-------------------|-----------|-------------------------|---------|---------|----|
|                                        |                   |           | Lower Limit | Upper Limit |       |     |
| Renal colic and urinary stones         | 2                 | 5.34      | 0.61 | 46.59 | 1.517 | 0.129 | 0% |
| Nausea                                 | 2                 | 4.33      | 0.47 | 40.4 | 1.287 | 0.198 | 0% |
| Flatulence                             | 4                 | 3.47      | 0.88 | 13.63 | 1.781 | 0.075 | 0% |
| Diarrhea                               | 5                 | 2.3       | 0.79 | 6.72 | 1.528 | 0.127 | 0% |
| Generic gastrointestinal disorders     | 3                 | 1.25      | 0.55 | 2.82 | 0.534 | 0.593 | 0% |
| Cholelithiasis                         | 2                 | 1.04      | 0.11 | 10.33 | 0.036 | 0.971 | 1% |
| Skin rash                              | 2                 | 1.02      | 0.1 | 10.2 | 0.015 | 0.988 | 0% |
| Heartburn                              | 2                 | 1         | 0.14 | 7.02 | 0.001 | 0.999 | 0% |
| Generic infections                     | 2                 | 0.67      | 0.3 | 1.48 | -0.989 | 0.322 | 0% |

3. Discussion

By analyzing data from nine clinical studies including 578 patients, this meta-analysis shows that omega-3 PUFAs significantly improve TG and HDL-C in patients with HIV chronic infection, with a favorable safety profile. The findings strengthen those previously reported by Oliveira and Rondò in a smaller sample of population [21], and emphasize the safety of PUFAs treatment in people with HIV with hypertriglyceridemia. This is of particular interest, since the pharmacological management of dyslipidemia associated with standard antiretroviral therapy (ART) or highly active retroviral treatment (HAART) is often complex for the risk of drug-drug interactions [22].

Even though lifestyle changes might improve the cardiometabolic risk of people with HIV, their efficacy is frequently limited and varies across settings [23]. Furthermore, iatrogenic hypertriglyceridemia increases the risk of acute pancreatitis in HIV-positive patients as well as in the general population [24]. In this event, the severity of hypertriglyceridemia varies, depending on the specific regimen (e.g., in stavudine-based HAART regimens TG were found to be higher than tenofovir) [25]. In such circumstances, TG-lowering pharmacological treatment should be considered [26].

Omega-3 PUFAs reduce TG synthesis through several mechanisms: reducing the amount of plasma fatty acids; increasing the synthesis of phospholipids; and finally decreasing the activity of
TG-synthesizing enzymes (diacylglycerol acyltransferase and phosphatidic acid phosphohydrolase) [27]. A recent meta-analysis of 86 randomized clinical trials (RCTs) including 162,796 participants showed that increasing PUFAs intake reduces plasma TG levels by 15% and slightly decreases the risk of coronary heart disease mortality (relative risk (RR) = 0.90, 95% CI: 0.81, 1.00) and coronary heart disease events (RR = 0.91, 95% CI: 0.85, 0.97) [28]. This is of particular interest in HIV-positive subjects, since the infection is associated with an increased risk of myocardial infarction compared with uninfected individuals (RR = 1.73; 95% CI: 1.44, 2.08), with HAART seeming to be a significant determinant of this risk [29]. Moreover, PUFAs could also have a positive impact on diseases other than cardiovascular ones [30], so our data support their use in HIV-positive patients. The effect of PUFAs on HDL-C is more debatable because it is strongly conditioned by the reduction in TG levels, which is dose-dependent [30]. From our data, it is also clear that the single study [15] where HDL-C plasma level was more significantly affected was also the one where an unusually high daily dose of PUFAs was tested.

The main limitation of this meta-analysis is related to the relatively small number of subjects involved in the trials, which were often short- or medium-term. The degree of heterogeneity for lipids change is another important limitation of the analysis. This could be partly related to another limitation of the included trials, where different formulations of PUFAs were tested. In fact, different EPA/DHA ratios and different pharmaceutical forms could be associated with variable effects on lipid pattern [31], because of the different impact of EPA and DHA on lipid fractions. Notwithstanding these limitations, our data clearly indicate that treatment with omega-3 PUFAs in patients with chronic HIV infection is safe and effective in lowering TG and improving HDL-C serum levels.

4. Materials and Methods

The study was designed according to guidelines of the 2009 preferred reporting items for systematic reviews and meta-analysis (PRISMA) statement [32]. Due to the study design, neither Institutional Review Board (IRB) approval nor patient informed consent were required.

4.1. Search Strategy

PubMed, SCOPUS, Google Scholar and ISI Web of Science by Clarivate databases were searched, with no language restriction, using the following search terms: (“HIV” OR “Human Immunodeficiency Virus” OR “HIV+” OR “HIV-positive” OR “AIDS” OR “Acquired Immunodeficiency Syndrome”) AND (“Omega-3” OR “Omega 3” OR “PUFA” OR “Polyunsaturated fatty acids”) AND (“Lipid” OR “Lipid-lowering” OR “Total cholesterol” OR “TC” OR “Low-density lipoprotein cholesterol” OR “LDL-C” OR “LDL” OR “High-density lipoprotein cholesterol” OR “HDL-C” OR “HDL” OR “Triglycerides” OR “Triglyceride” OR “TG” OR “Apolipoprotein” OR “Apolipoprotein-B” OR “Apo-B” OR “Apo B” OR “Apolipoprotein-A1” OR “Apo-A1” OR “Apo A1”) AND (“Clinical trial” OR “Clinical study” OR “Pilot study”). The wild-card term “*” was used to increase the sensitivity of the search strategy, which was limited to studies on humans. The reference list of identified papers was manually checked for additional relevant articles. In particular, additional searches for potential trials included the references of review articles on the issue, and the abstracts from selected congresses on the subject of the meta-analysis. The literature was searched from inception to 25 April 2020.

All paper abstracts were screened by two reviewers (F.F. and E.S.) in an initial process to remove ineligible articles. The remaining articles were obtained in full-text and assessed again by the same two researchers, who evaluated each article independently and carried out data extraction and quality assessment. Disagreements were resolved by discussion with a third party (A.F.G.C.).

4.2. Study Selection Criteria

Original studies were included if they met the following criteria: (i) enrolling patients with HIV, (ii) being a clinical trial with either multicenter or single-center design, (iii) having an appropriate
controlled design for treatment with omega-3 PUFAs and (iv) investigating the effect of omega-3 PUFAs on plasma lipids.

The exclusion criteria were: (i) lack of a control group for the intervention and (ii) lack of sufficient information about plasma lipids at baseline or follow-up. Studies were also excluded if they contained subjects that overlapped with other studies.

4.3. Data Extraction

The data abstracted from the eligible studies were: (i) first author’s name; (ii) year of publication; (iii) study design; (iv) follow-up; (v) main inclusion criteria; (vi) study groups; (vii) number of enrolled patients; (viii) sex and age of study participants; (ix) years since initial HIV diagnosis; (x) years since the start of ART therapy; and (xi) CD4+ T cell count at baseline. All data extraction and database typing were reviewed by the principal investigator (A.F.G.C.) before the final analysis, and doubts were resolved by mutual agreement among the authors.

4.4. Quality Assessment

A systematic assessment of the risk of bias in the included studies was performed using the Cochrane criteria [33]. The following items were used: adequacy of sequence generation, allocation concealment, blinding addressing of dropouts (incomplete outcome data), selective outcome reporting and other probable sources of bias [35]. Two reviewers (F.F. and E.S.) performed the risk-of-bias assessment independently and disagreements were resolved by a consensus-based discussion.

4.5. Data Synthesis

The meta-analysis was entirely conducted using Comprehensive Meta-Analysis (CMA) V3 software (Biostat, NJ) [34].

Net changes in the investigated parameters (change scores) were calculated by subtracting the value at baseline from the one after intervention, in the active-treated group and in the control group. Standard deviations (SDs) of the mean differences were obtained as follows, reported by Follman and colleagues: \( SD = \sqrt{\text{SD}_{\text{pre}}^2 + \text{SD}_{\text{post}}^2 - (2R \times \text{SD}_{\text{pre}} \times \text{SD}_{\text{post}})} \), assuming a correlation coefficient (R) = 0.5 [35]. If the outcome measures were reported as median and range (or 95% CI), the mean and SD values were estimated using the method described by Wan et al. [36]. The studies’ findings were combined using a fixed-effect model or a random-effect model (using the DerSimonian–Laird method) and the generic inverse variance method based on the level of inter-study heterogeneity, which was quantitatively assessed using the Higgins index (I^2) [37]. Effect sizes for changes in lipids were expressed as MD and 95% CI. For safety analysis, OR and 95% CI intervals were calculated using the Mantel–Haenszel method [38]. Safety analysis was performed by excluding studies with zero events in both arms. If one or more outcomes could not be extracted from a study, the study was removed from the analysis involving those outcomes only. Adverse events were considered in the analysis only if occurring in at least two of the included clinical trials.

Sensitivity analysis was conducted using the leave-one-out method (i.e., removing one study at a time and repeating the analysis) in order to evaluate the influence of each single study on the overall observed effect size [39].

The EPA and DHA daily administered doses were sequentially entered into a random-effect meta-regression model to explore their association with the estimated effect sizes.

Two-sided p-values ≤ 0.05 were considered as statistically significant for all tests.

4.6. Publication Biases

Potential publication biases were explored using visual inspection of Begg’s funnel plot asymmetry, Begg’s rank correlation test, and Egger’s weighted regression test [40,41]. The Duval and Tweedie “trim and fill” method was used to adjust the analysis for the effects of publication biases [42]. Two-sided p-values ≤ 0.05 were considered statistically significant.
5. Conclusions

In conclusion, based on the results of this meta-analysis of randomized clinical studies, treatment with omega-3 PUFAs seems to exert a favorable effect on TG and HDL-C serum levels, being suggestive of a positive prognostic effect. Further clinical trials are expected to investigate the long-term safety of the treatment.

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References

1. Grand, M.; Bia, D.; Diaz, A. Cardiovascular Risk Assessment in People Living With HIV: A Systematic Review and Meta-Analysis of Real-Life Data. Curr. HIV Res. 2020, 18, 5–18. [CrossRef] [PubMed]
2. Worm, S.W.; Kamara, D.A.; Reiss, P.; Kirk, O.; El-Sadr, W.; Fux, C.; Fontas, E.; Phillips, A.; D’Arminio Monforte, A.; De Wit, S.; et al. Elevated triglycerides and risk of myocardial infarction in HIV-positive persons. AIDS 2011, 25, 1497–1504. [CrossRef] [PubMed]
3. Fontas, E.; van Leth, F.; Sabin, C.A.; Friis-Moller, N.; Rickenbach, M.; D’Arminio Monforte, A.; Kirk, O.; Dupon, M.; Morfeldt, L.; Mateu, S.; et al. Lipid profiles in HIV-infected patients receiving combination antiretroviral therapy: Are different antiretroviral drugs associated with different lipid profiles? J. Infect. Dis. 2004, 189, 1056–1074. [CrossRef] [PubMed]
4. Dubé, M.P.; Stein, J.H.; Aberg, J.A.; Fichtenbaum, C.J.; Gerber, J.G.; Tashima, K.T.; Henry, W.K.; Currier, J.S.; Sprecher, D.; Glesby, M.J. Guidelines for the evaluation and management of dyslipidemia in human immunodeficiency virus (HIV)-infected adults receiving antiretroviral therapy: Recommendations of the HIV Medical Association of the Infectious Disease Society of America and the Adult AIDS Clinical Trials Group. Clin. Infect. Dis. 2003, 37, 613–627.
5. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001, 285, 2486–2497. [CrossRef] [PubMed]
6. Domingo, P.; Gallego-Escuredo, J.M.; Fernández, I.; Villarroya, J.; Torres, F.; Del Mar Gutierrez, M.; Mateo, M.G.; Villarroya, F.; Vidal, F.; Giralt, M.; et al. 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. Cytokine 2018, 105, 73–79. [CrossRef]
7. Gebhardt, A.; Fichtenbaum, C.J. Current pharmacotherapy for the treatment of dyslipidemia associated with HIV infection. Expert Opin. Pharmacother. 2019, 20, 1719–1729. [CrossRef]
8. Muñoz, M.A.; Liu, W.; Delaney, J.A.; Brown, E.; Mugavero, M.J.; Mathews, W.C.; Napravnik, S.; Willig, J.H.; Eron, J.J.; Hunt, P.W.; et al. Comparative effectiveness of fish oil versus fenofibrate, gemfibrozil, and atorvastatin in lowering triglyceride levels among HIV-infected patients in routine clinical care. J. Acquir. Immune Defic. Syndr. 2013, 64, 254–260. [CrossRef]
9. EFSA Panel on Dietetic Products NaAN. Scientific Opinion on the substantiation of health claims related to EPA, DHA, DPA and maintenance of normal blood pressure (ID 502), maintenance of normal HDL-cholesterol concentrations (ID 515), maintenance of normal (fasting) blood concentrations of triglycerides (ID 517), maintenance of normal LDL-cholesterol concentrations (ID 528, 698) and maintenance of joints (ID 503, 505, 507, 511, 518, 524, 526, 535, 537) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA J. 2009, 7, 1263–1289.
10. Howe, P.; Mori, T.; Buckley, J. Long Chain Omega-3 Fatty Acids and Cardiovascular Disease-FSANZ Consideration of a Commissioned Review. FSANZ 2013; 1–8. Available online: http://www.foodstandards.gov.au (accessed on 25 May 2020).

11. Miller, M.; Stone, N.J.; Ballantyne, C.; Bittner, V.; Criqui, M.H.; Ginsberg, H.N.; Goldberg, A.C.; Howard, W.J.; Jacobson, M.S.; Kris-Etherton, P.M.; et al. American Heart Association Clinical Lipidology, Thrombosis, and Prevention Committee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Arteriosclerosis, Thrombosis and Vascular Biology; Council on Cardiovascular Nursing; Council on the Kidney in Cardiovascular Disease. Triglycerides and cardiovascular disease: A scientific statement from the American Heart Association. Circulation 2011, 123, 2292–2333.

12. Amador-Licona, N.; Díaz-Murillo, T.A.; Gabriel-Ortiz, G.; Pacheco-Moises, F.P.; Pereyra-Nobara, T.A.; Guizar-Mendoza, J.M.; Barbosa-Sabanero, G.; Orozco-Aviña, G.; Moreno-Martínez, S.C.; Luna-Montalbán, R.; et al. Omega 3 Fatty Acids Supplementation and Oxidative Stress in HIV-Seropositive Patients. A Clinical Trial. PLoS ONE 2016, 11, e0151637. [CrossRef]

13. Baril, J.G.; Kovacs, C.M.; Trottier, S.; Reederer, G.; Martel, A.Y.; Ackad, N.; Kouliis, T.; Sampalis, J.S. Effectiveness and tolerability of oral administration of low-dose salmon oil to HIV patients with HAART-associated dyslipidemia. HIV Clin. Trials 2007, 8, 400–411. [CrossRef]

14. Capili, B.; Anastasi, J.K. Exploratory study: Evaluating the effects of fish oil and controlled diet reduce triglyceride levels in HIV. J. Assoc. Nurses AIDS Care 2013, 24, 276–282. [CrossRef]

15. De Truchis, P.; Kirstetter, M.; Perrier, A.; Meunier, C.; Zucman, D.; Force, G.; Doll, J.; Katlama, C.; Rozenbaum, W.; Masson, H.; et al. Reduction in triglyceride level with N-3 polyunsaturated fatty acids in HIV-infected patients taking potent antiretroviral therapy: A randomized prospective study. J. Acquir. Immune Defic. Syndr. 2007, 44, 278–285. [CrossRef] [PubMed]

16. Oliveira, J.M.; Rondó, P.H.; Yudkin, J.S.; Souza, J.M.; Pereira, T.N.; Catalani, A.W.; Picone, C.M.; Segurado, A.A. Effects of fish oil on lipid profile and other metabolic outcomes in HIV-infected patients on antiretroviral therapy: A randomized placebo-controlled trial. Int. J. STD AIDS 2014, 25, 96–104. [CrossRef] [PubMed]

17. Peters, B.S.; Wierzbicki, A.S.; Moyle, G.; Nair, D.; Brockmeyer, N. The effect of a 12-week course of omega-3 polyunsaturated fatty acids on lipid parameters in hypertriglyceridemic adult HIV-infected patients undergoing HAART: A randomized, placebo-controlled pilot trial. Clin. Ther. 2012, 34, 67–76. [CrossRef]

18. Thussgaard, M.; Christensen, J.H.; Mørm, B.; Andersen, T.S.; Vige, R.; Arildsen, H.; Schmidt, E.B.; Nielsen, H. Effect of fish oil (n-3 polyunsaturated fatty acids) on plasma lipids, lipoproteins and inflammatory markers in HIV-infected patients treated with antiretroviral therapy: A randomized, double-blind, placebo-controlled study. Scand. J. Infect. Dis. 2009, 41, 760–766. [CrossRef] [PubMed]

19. Woods, M.N.; Wanke, C.A.; Ling, P.R.; Hendricks, K.M.; Tang, A.M.; Knox, T.A.; Andersson, C.E.; Dong, K.R.; Skinner, S.C.; Bistrian, B.R. Effect of a dietary intervention and n-3 fatty acid supplementation on measures of serum lipid and insulin sensitivity in persons with HIV. Am. J. Clin. Nutr. 2009, 90, 1566–1578. [CrossRef]

20. Pararnandi, A.; Asztalos, B.F.; Mangili, A.; Kuvin, J.;Gerrior, J.; Sheehan, H.; Skinner, S.C.; Tang, A.M.; Wanke, C.A. Short communication: Effects of omega-3 fatty acids on triglycerides and high-density lipoprotein subprofiles in HIV-infected persons with hypertriglyceridemia. AIDS Res. Hum. Retrovir. 2014, 30, 800–805. [CrossRef]

21. Oliveira, J.M.; Rondó, P.H. Omega-3 fatty acids and hypertriglyceridemia in HIV-infected subjects on antiretroviral therapy: Systematic review and meta-analysis. HIV Clin. Trials 2011, 12, 268–274. [CrossRef]

22. Gutierrez, M.D.M.; Mateo, M.G.; Corbacho, N.; Vidal, F.; Domingo, P. Drug-drug interactions when treating HIV-related metabolic disorders. Expert Opin. Drug Metab. Toxicol. 2019, 15, 787–802. [CrossRef]

23. Fitch, K.V. Contemporary Lifestyle Modification Interventions to Improve Metabolic Comorbidities in HIV. Curr. HIV/AIDS Rep. 2019, 16, 482–491. [CrossRef]

24. Sun, H.Y.; Chang, S.Y.; Sheng, W.H.; Chen, M.Y.; Hsieh, S.M.; Tseng, Y.T.; Lu, C.L.; Yang, C.J.; Wu, H.; Liu, W.C.; et al. Incidence of acute pancreatitis in human immunodeficiency virus-positive patients with hypertriglyceridemia: Is it really high? Pancreas 2012, 41, 283–289. [CrossRef]

25. Madruga, J.R.; Cassetti, I.; Suleiman, J.M.; Etzel, A.; Zhong, L.; Holmes, C.B.; Cheng, A.K.; Enejosa, J.; Study 903E Team. The safety and efficacy of switching stavudine to tenofovir df in combination with lamivudine and efavirenz in hiv-1-infected patients: Three-year follow-up after switching therapy. HIV Clin. Trials 2007, 8, 381–390. [CrossRef]
26. Laufs, U.; Parhofer, K.G.; Ginsberg, H.N.; Hegele, R.A. Clinical review on triglycerides. *Eur. Heart J.* 2020, 41, 99–109c. [CrossRef]

27. Harris, W.S.; Bulchandani, D. Why do omega-3 fatty acids lower serum triglycerides? *Curr. Opin. Lipidol.* 2006, 17, 387–393. [CrossRef] [PubMed]

28. Abdelhamid, A.S.; Brown, T.J.; Brainard, J.S.; Biswas, P.; Thorpe, G.C.; Moore, H.J.; Deane, K.H.; Summerbell, C.D.; Worthington, H.V.; Song, F.; et al. Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease. *Cochrane Database Syst. Rev.* 2020, 3, CD003177. [CrossRef] [PubMed]

29. Eyawo, O.; Brockman, G.; Goldsmith, C.H.; Hull, M.W.; Lear, S.A.; Bennett, M.; Guillemi, S.; Franco-Villalobos, C.; Adam, A.; Mills, E.J.; et al. Risk of myocardial infarction among people living with HIV: An updated systematic review and meta-analysis. *BMJ Open* 2019, 9, e025874. [CrossRef] [PubMed]

30. Cicero, A.F.; Reggi, A.; Parini, A.; Borghi, C. Application of polyunsaturated fatty acids in internal medicine: Beyond the established cardiovascular effects. *Arch. Med. Sci.* 2012, 8, 784–793. [CrossRef] [PubMed]

31. Cicero, A.F.; Morbini, M.; Borghi, C. Do we need ‘new’ omega-3 polyunsaturated fatty acids formulations? *Expert Opin. Pharmacother.* 2015, 16, 285–288. [CrossRef]

32. Moher, D.; Liberati, A.; Tetzlauff, J.; Altman, D.G.; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *BMJ* 2009, 339, b2535. [CrossRef] [PubMed]

33. Higgins, J.; Green, S. *Cochrane Handbook for Systematic Reviews of Interventions*; Version 5.0. 2. 2009; Ref Type: Report; John Wiley and Sons Ltd.: Chichester, UK, 2010.

34. Borenstein, M.; Hedges, L.; Higgins, J.; Rothstein, H. *Comprehensive Meta-Analysis Version 3*; Biostatistics: Englewood, NJ, USA, 2005; Volume 104.

35. Follmann, D.; Elliott, P.; Suh, I.; Cutler, J. Variance imputation for overviews of clinical trials with continuous response. *J. Clin. Epidemiol.* 1992, 45, 769–773. [CrossRef]

36. Wan, X.; Wang, W.; Liu, J.; Tong, T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. *BMC Med. Res. Methodol.* 2014, 14, 135. [CrossRef]

37. Melsen, W.G.; Bootsma, M.C.; Rovers, M.M.; Bonten, M.J. The effects of clinical and statistical heterogeneity on the predictive values of results from meta-analyses. *Clin. Microbiol. Infect.* 2014, 20, 123–129. [CrossRef]

38. Haenszel, W.; Hon, N.B. Statistical approaches to the study of cancer with particular reference to case registers. *J. Clin. Epidemiol.* 1956, 4, 589–599. [CrossRef]

39. Bown, M.J.; Sutton, A.J. Quality control in systematic reviews and meta-analyses. *Eur. J. Vasc. Endovasc. Surg.* 2010, 40, 669–677. [CrossRef]

40. Begg, C.B.; Mazumdar, M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 1994, 50, 1088–1101. [CrossRef]

41. Sterne, J.A.; Gavaghan, D.; Egger, M. Publication and related bias in meta-analysis: Power of statistical tests and prevalence in the literature. *J. Clin. Epidemiol.* 2000, 53, 1119–1129. [CrossRef]

42. Duval, S.; Tweedie, R. Trim and fill: A simple funnel plot–based method of testing and adjusting for publication bias in meta-analysis. *Biometrics* 2000, 56, 455–463. [CrossRef]