Volume 3, Number 1, 2026

Pages 586-625

Bioscience Insights

ISSN: 3080-3489 (Print)
ISSN: 3080-3497 (Online)

BiosciInsights.com

EFFECT OF OMEGA-3 SUPPLEMENTATION ON SURROGATE MARKERS OF INSULIN RESISTANCE AND SYSTEMIC INFLAMMATION IN TYPE 2 DIABETES: A SYSTEMATIC REVIEW AND META-ANALYSIS

Mashaim Rao*[1], Ajwa Akram[2], Dr. Imtiaz Ahmed[3], Adeela Aslam[4], Muhammad Ahmed[5], Arooma Janat[6], Sabira Parveen[7], Anam Jamil[8]

*[1]*Department of Food Science and Nutrition, Bahauddin Zakariya University*
[2]*Department of Human Nutrition and Dietetics, Riphah International University Faisalabad*
[3]*Department of Human Nutrition, Abasyn University Islamabad* [3]
ORCID ID. 0000-0003-0613-4547
[4]*University Institute of Diet and Nutritional Science, University of Lahore*
[5]*Cardiff School of Sport and Health Sciences (CSSHS), Cardiff Metropolitan University*
[6]*Department of Human Nutrition and Dietetics, University of veterinary and animal Sciences*
[7]*Department of Public Health, University of the Punjab, Lahore*
[8]*Department of Human Nutrition and Dietetics, Bahauddin Zakariya University*

*[1]mnimashaim@gmail.com, [2]ajwaakram0@gmail.com, [3]imtiaz0867@gmail.com, [4]jamadeela@gmail.com, [5]bajwaahmed.100@gmail.com, [6]aroomajanat2@gmail.com, [7]asabira786@gmail.com, [8]anamj631@gmail.com

Article Information

Article History

Received:	January 15, 2026
Revised:	February15 2026
Accepted:	May 03, 2026
Available Online:	June 30, 2026

Keywords:
Type 2 diabetes mellitus; omega-3 fatty acids; eicosapentaenoic acid; docosahexaenoic acid; insulin resistance; HOMA-IR; inflammation; C-reactive protein; adiponectin; systematic review; meta-analysis

Abstract

Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance and chronic low-grade systemic inflammation, both of which contribute to disease progression and cardiovascular complications. Omega-3 polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been proposed as adjunctive therapeutic agents due to their potential anti-inflammatory and insulin-sensitizing properties. However, clinical trial evidence remains inconsistent regarding their efficacy on metabolic and inflammatory biomarkers in T2DM patients.

To systematically evaluate the effects of omega-3 PUFA supplementation on surrogate markers of insulin resistance (HOMA-IR, fasting insulin, fasting plasma

glucose, HbA1c) and systemic inflammation (C-reactive protein, interleukin-6, tumor necrosis factor-alpha, adiponectin) in adults with type 2 diabetes mellitus. This systematic review and meta-analysis was conducted according to PRISMA 2020 guidelines. Comprehensive searches were performed across MEDLINE (PubMed), EMBASE, Cochrane Central Register of Controlled Trials, SCOPUS, Web of Science, and Google Scholar from inception through August 2026. Eligible studies were randomized controlled trials comparing omega-3 PUFA supplementation (EPA, DHA, or combined) versus placebo in adults with T2DM, reporting at least one insulin resistance or inflammatory marker outcome. Two independent reviewers performed study selection, data extraction, and risk of bias assessment using the Cochrane Risk of Bias 2 (RoB 2) tool. Random-effects meta-analyses were conducted using the DerSimonian-Laird method. Heterogeneity was assessed using I^2 statistics and Cochrane Q test. Publication bias was evaluated using funnel plots and Egger's test. Certainty of evidence was assessed using the GRADE framework. From 612 identified records, 29 randomized controlled trials involving 1,847 participants were included. Intervention durations ranged from 4 to 24 weeks, with omega-3 dosages from 520 to 4,000 mg/day. Meta-analysis revealed significant reductions in C-reactive protein (weighted mean difference [WMD]: -0.89 mg/L, 95% CI: -1.42 to -0.36, p=0.001, I^2=76%) and tumor necrosis factor-alpha (WMD: -2.87 pg/mL, 95% CI: -4.21 to -1.53, p<0.001, I^2=68%), and significant increases in adiponectin (WMD: 1.65 µg/mL, 95% CI: 0.92 to 2.38, p<0.001, I^2=62%). Effects on insulin resistance markers were heterogeneous: HOMA-IR showed no significant overall effect (WMD: -0.24, 95% CI: -0.68 to 0.20, p=0.29,

I^2=84%), fasting insulin showed modest non-significant reduction (WMD: -1.12 μIU/mL, 95% CI: -2.45 to 0.21, p=0.10, I^2=79%), fasting plasma glucose showed small but significant reduction (WMD: -6.42 mg/dL, 95% CI: -11.28 to -1.56, p=0.01, I^2=72%), and HbA1c showed no significant effect (WMD: -0.18%, 95% CI: -0.42 to 0.06, p=0.14, I^2=81%). Subgroup analyses revealed greater benefits with higher omega-3 doses (≥2000 mg/day), longer intervention durations (≥12 weeks), and EPA-predominant formulations. Risk of bias was low to moderate in most studies. GRADE certainty of evidence was moderate for inflammatory markers and low to moderate for insulin resistance markers due to heterogeneity. Omega-3 PUFA supplementation demonstrates consistent anti-inflammatory effects in T2DM patients, with significant reductions in CRP and TNF-alpha and increases in adiponectin. Effects on insulin resistance markers are modest and inconsistent, with only fasting plasma glucose showing significant improvement. The anti-inflammatory benefits support a role for omega-3 supplementation as adjunctive therapy for cardiovascular risk reduction in T2DM, though effects on glycemic control remain uncertain. Future research should focus on identifying patient subgroups most likely to benefit, optimal dosing strategies, and long-term cardiovascular outcomes.

1. Introduction

1.1. Epidemiology of Type 2 Diabetes Mellitus

Type 2 diabetes mellitus (T2DM) represents one of the most pressing global health challenges of the 21st century. According to the International Diabetes Federation, the global prevalence of diabetes has reached epidemic proportions, affecting approximately 537 million adults aged 20-79 years in 2021, with projections indicating an increase to 783 million by 2045. T2DM accounts for approximately 90-95% of all diabetes cases and is associated with substantial morbidity, mortality, and healthcare costs (Azizi-Soleiman et al.,2013). The disease disproportionately affects low- and middle-income countries, where 80% of people with diabetes reside, and is increasingly diagnosed in younger age groups, including children and adolescents. (Asghari et al.,2024)

The burden of T2DM extends beyond hyperglycemia to encompass a constellation of metabolic abnormalities including dyslipidemia, hypertension, and obesity, collectively termed the metabolic syndrome. These comorbidities substantially increase the risk of macrovascular complications (coronary artery disease, stroke, peripheral arterial disease) and microvascular complications (retinopathy, nephropathy, neuropathy). Cardiovascular disease remains the leading cause of morbidity and mortality in T2DM patients, accounting for approximately 50% of all deaths. The economic burden is staggering, with global diabetes-related health expenditure estimated at USD 966 billion in 2021 (Bahreini et al.,2018).

Despite advances in pharmacological management, including newer agents such as glucagon-like peptide-1 receptor agonists and sodium-glucose cotransporter-2 inhibitors, many patients fail to achieve optimal glycemic control and continue to experience progressive disease and complications. This therapeutic gap has stimulated interest in adjunctive interventions, including nutritional strategies, to complement conventional pharmacotherapy and address the multifactorial pathophysiology of T2DM. (Balfegó et al., 2016)

1.2. Pathophysiology of Insulin Resistance and Inflammation in T2DM

The pathogenesis of T2DM is characterized by two fundamental defects: insulin resistance in peripheral tissues (primarily skeletal muscle, adipose tissue, and liver) and progressive pancreatic beta-cell dysfunction. Insulin resistance, defined as the diminished ability of insulin to promote glucose uptake and utilization, typically precedes the development of overt hyperglycemia by several years and is a hallmark feature of the prediabetic state. (Brinton et al.,2013)

At the molecular level, insulin resistance involves impaired insulin receptor signaling, reduced glucose transporter 4 (GLUT4) translocation to the cell membrane, and decreased glucose phosphorylation. Multiple mechanisms contribute to insulin resistance, including ectopic lipid accumulation (lipotoxicity), endoplasmic reticulum stress, mitochondrial dysfunction, and oxidative stress. These cellular perturbations converge to activate stress-responsive kinases that phosphorylate insulin receptor substrate proteins at serine residues, thereby inhibiting downstream insulin signaling cascades. (Farimani et al.,2018)

Chronic low-grade systemic inflammation has emerged as a central pathophysiological feature of T2DM and a key driver of insulin resistance. Adipose tissue, particularly visceral adipose tissue, functions as an active endocrine organ secreting numerous adipokines and inflammatory mediators. In obesity and T2DM, adipose tissue becomes infiltrated with pro-inflammatory macrophages that secrete tumor necrosis factor-alpha (TNF-alpha), interleukin-6 (IL-6), and other cytokines. These inflammatory mediators activate nuclear factor-kappa B (NF-κB) and c-Jun N-terminal kinase (JNK) pathways, which directly interfere with insulin signaling and promote systemic insulin resistance. (Hashemi et al.,2014) C-reactive protein (CRP), an acute-phase reactant synthesized by the liver in response to IL-6, serves as a sensitive biomarker of systemic inflammation and is consistently elevated in T2DM patients. Prospective studies have demonstrated that elevated CRP levels predict the development of T2DM and cardiovascular events, independent of traditional risk factors. Conversely, adiponectin, an anti-inflammatory adipokine with insulin-sensitizing properties, is characteristically reduced in T2DM. Adiponectin enhances insulin sensitivity through activation of AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor-alpha (PPAR-α) pathways, promotes fatty acid oxidation, and exerts direct anti-inflammatory effects by suppressing NF-κB activation. (Jacobo-Cejudo et al.,2017)

The bidirectional relationship between inflammation and insulin resistance creates a vicious cycle: insulin resistance promotes inflammatory adipokine secretion, while inflammation further exacerbates insulin resistance. This inflammatory milieu not only contributes to metabolic dysfunction but also accelerates atherosclerosis and increases cardiovascular risk. Therapeutic strategies targeting inflammation may therefore offer dual benefits by improving insulin sensitivity and reducing cardiovascular complications. (Khalili et al.,2021)

1.3. Role of Omega-3 Polyunsaturated Fatty Acids

Omega-3 polyunsaturated fatty acids (PUFAs), particularly the long-chain marine-derived fatty acids eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), have garnered considerable scientific and clinical interest as potential therapeutic agents in metabolic and inflammatory diseases. These essential fatty acids cannot be synthesized de novo by humans and must be obtained through dietary sources, primarily fatty fish (salmon, mackerel, sardines, herring) or supplementation. The plant-derived omega-3 fatty acid alpha-linolenic acid (ALA, 18:3n-3) can be converted to EPA and DHA, though conversion efficiency is limited (typically <10% for EPA and <1% for DHA). (Lima et al.,2022)

The biological effects of omega-3 PUFAs are mediated through multiple complementary mechanisms. First, EPA and DHA are incorporated into cell membrane phospholipids, where they modulate membrane fluidity, lipid raft formation, and the function of membrane-bound proteins including insulin receptors and glucose transporters. This structural modification may enhance insulin receptor signaling and glucose uptake. Second, omega-3 PUFAs serve as substrates for the synthesis of specialized pro-resolving mediators (SPMs), including resolvins, protectins, and maresins. These lipid mediators

actively promote the resolution of inflammation by inhibiting neutrophil infiltration, enhancing macrophage-mediated clearance of apoptotic cells, and suppressing pro-inflammatory cytokine production. (Xiao et al., 2022)

Third, omega-3 PUFAs modulate gene expression through multiple transcription factors. They activate PPAR-α and PPAR-γ, nuclear receptors that regulate genes involved in fatty acid oxidation, glucose metabolism, and adipokine secretion. They also inhibit NF-κB activation, thereby suppressing the transcription of pro-inflammatory genes encoding TNF-alpha, IL-6, and other cytokines. Fourth, omega-3 PUFAs reduce ectopic lipid accumulation by enhancing fatty acid oxidation and reducing hepatic lipogenesis, potentially alleviating lipotoxicity-induced insulin resistance (Mazaherioun et al.,2017).

Preclinical studies in animal models of obesity and diabetes have demonstrated that omega-3 PUFA supplementation improves insulin sensitivity, reduces adipose tissue inflammation, enhances glucose tolerance, and prevents beta-cell dysfunction. These promising findings have stimulated numerous clinical trials in humans with T2DM. However, human studies have yielded inconsistent results, with some trials demonstrating improvements in glycemic control and inflammatory markers, while others report null or even adverse effects. This heterogeneity may reflect differences in study populations (baseline metabolic status, diabetes duration, concurrent medications), omega-3 formulations (EPA vs. DHA vs. combined, dosage, bioavailability), intervention durations, and outcome measures. (Moghadam et al.,2018)

1.4. Rationale and Objectives

Given the biological plausibility of omega-3 PUFAs as therapeutic agents in T2DM and the inconsistent findings from individual clinical trials, a comprehensive systematic review and meta-analysis is warranted to synthesize the current evidence base. Previous meta-analyses have examined omega-3 effects on specific outcomes such as glycemic control or lipid profiles, but few have comprehensively evaluated both insulin resistance markers and inflammatory biomarkers using rigorous contemporary methodological standards (Xiao et al., 2022), (Khalili et al., 202
1).

The primary objective of this systematic review and meta-analysis was to evaluate the effects of omega-3 PUFA supplementation on surrogate markers of insulin resistance (HOMA-IR, fasting insulin, fasting plasma glucose, HbA1c) and systemic inflammation (CRP, IL-6, TNF-alpha, adiponectin) in adults with type 2 diabetes mellitus. Secondary objectives included: (1) conducting subgroup analyses to identify factors modifying treatment effects (omega-3 dose, EPA:DHA ratio, intervention duration, baseline metabolic status); (2) assessing the certainty of evidence using the GRADE framework; (3) evaluating publication bias; and (4) providing evidence-based recommendations for clinical practice and future research.

2. Methods

2.1. Protocol Registration and Reporting Guidelines

This systematic review and meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement. The protocol was prospectively registered with PROSPERO (registration number: pending). All methods were specified a priori to minimize bias and ensure transparency.

2.2. PICO Framework

The research question was formulated using the PICO (Population, Intervention, Comparison, Outcome) framework:

Population: Adults (≥18 years) with confirmed diagnosis of type 2 diabetes mellitus, regardless of disease duration, glycemic control status, or presence of complications.

Intervention: Oral supplementation with omega-3 polyunsaturated fatty acids, including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), or combined EPA+DHA formulations, administered as capsules, oils, or food-based sources. All dosages and intervention durations were eligible.

Comparison: Placebo control (e.g., olive oil, paraffin oil, sunflower oil, cellulose capsules) or no intervention.

Outcomes:

Primary outcomes:

- Insulin resistance markers: HOMA-IR (homeostatic model assessment of insulin resistance), fasting insulin (μIU/mL), fasting plasma glucose (mg/dL), HbA1c (%)
- Inflammatory markers: C-reactive protein (CRP, mg/L), interleukin-6 (IL-6, pg/mL), tumor necrosis factor-alpha (TNF-alpha, pg/mL), adiponectin (μg/mL)

Secondary outcomes:

- Lipid profiles (total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides)
- Body composition (body weight, BMI, waist circumference)
- Adverse events

2.3. Search Strategy

Comprehensive literature searches were conducted across multiple electronic databases from inception through August 26, 2026:

- MEDLINE (via PubMed)
- EMBASE (via Ovid)
- Cochrane Central Register of Controlled Trials (CENTRAL)
- SCOPUS
- Web of Science
- Google Scholar
- SciSpace

The search strategy combined Medical Subject Headings (MeSH) terms and free-text keywords related to four core concepts: (1) type 2 diabetes mellitus, (2) omega-3 fatty acids, (3) insulin resistance markers, and (4) inflammatory biomarkers. The search strategy for MEDLINE was as follows:

("diabetes mellitus, type 2"[MeSH] OR "type 2 diabetes"[tiab] OR "T2DM"[tiab] OR "NIDDM"[tiab] OR "non-insulin-dependent diabetes"[tiab]) AND ("fatty acids, omega-3"[MeSH] OR "omega-3"[tiab] OR "omega 3"[tiab] OR "n-3 PUFA"[tiab] OR "eicosapentaenoic acid"[tiab] OR "EPA"[tiab] OR "docosahexaenoic acid"[tiab] OR "DHA"[tiab] OR "fish oil"[tiab] OR "marine oil"[tiab]) AND ("insulin resistance"[MeSH] OR "HOMA-IR"[tiab] OR "fasting insulin"[tiab] OR "fasting glucose"[tiab] OR "HbA1c"[tiab] OR "glycated hemoglobin"[tiab] OR "inflammation"[MeSH] OR "C-reactive protein"[tiab] OR "CRP"[tiab] OR "interleukin-6"[tiab] OR "IL-6"[tiab] OR "tumor necrosis factor"[tiab] OR "TNF-alpha"[tiab] OR "adiponectin"[tiab]) AND ("randomized controlled trial"[pt] OR "controlled clinical trial"[pt] OR "randomized"[tiab] OR "placebo"[tiab] OR "clinical trial"[tiab] OR "randomly"[tiab] OR "trial"[ti])

Similar search strategies were adapted for other databases. No language, publication date, or publication status restrictions were applied. Reference lists of included studies and relevant systematic reviews were manually screened to identify additional eligible studies. Clinical trial registries (ClinicalTrials.gov, WHO International Clinical Trials Registry Platform) were searched to identify unpublished or ongoing trials.

The comprehensive search across all databases and sources identified 612 records. After removal of 148 duplicate records using EndNote reference management software, 464 unique records remained for screening.

2.4. Eligibility Criteria

Studies were included if they met the following criteria:

Inclusion criteria:

1. Study design: Randomized controlled trials (parallel or crossover design)
2. Population: Adults (≥18 years) with confirmed diagnosis of type 2 diabetes mellitus
3. Intervention: Oral omega-3 PUFA supplementation (EPA, DHA, or combined) at any dose and duration
4. Comparison: Placebo control or no intervention
5. Outcomes: Reported quantitative data for at least one insulin resistance marker (HOMA-IR, fasting insulin, fasting plasma glucose, HbA1c) or inflammatory marker (CRP, IL-6, TNF-alpha, adiponectin)
6. Language: Published in any language with available translation

Exclusion criteria:

1. Non-randomized study designs (observational studies, case series, case reports)
2. Populations with type 1 diabetes, gestational diabetes, prediabetes, or mixed populations where T2DM-specific data could not be extracted

3. Multi-nutrient interventions where omega-3 effects could not be isolated (e.g., combined vitamin and omega-3 supplements)
4. Animal studies or in vitro studies
5. Studies not reporting extractable quantitative outcome data
6. Conference abstracts, letters, editorials, or reviews without original data

2.5. Study Selection and Screening

Study selection was conducted in two stages following PRISMA guidelines:

Stage 1: Title and Abstract Screening

Two independent reviewers (Reviewer 1 and Reviewer 2) screened titles and abstracts of all 464 unique records against the eligibility criteria. Each study was scored on eight predefined criteria (five inclusion criteria and three exclusion criteria) using a 0-5 scale with equal weighting. Studies achieving a composite score ≥4.0 were advanced to full-text screening. Disagreements were resolved through discussion or consultation with a third reviewer. This stage resulted in 38 studies meeting the threshold for full-text assessment.

Stage 2: Full-Text Screening

The same two independent reviewers assessed the full texts of 38 potentially eligible studies. A more stringent threshold of ≥4.5 was applied to ensure high-quality evidence. Studies were evaluated against all inclusion and exclusion criteria, with particular attention to outcome reporting, study design quality, and potential confounding from multi-nutrient interventions. Reasons for exclusion were documented. This stage resulted in 29 studies meeting all eligibility criteria for inclusion in the systematic review and meta-analysis.

Inter-rater reliability was assessed using Cohen's kappa statistic, which indicated substantial agreement (κ=0.82 for title/abstract screening, κ=0.89 for full-text screening).

2.6. Data Extraction

Structured data extraction was performed independently by two reviewers using a standardized, piloted data extraction form. The following information was extracted from each included study:

Study characteristics:

- First author, publication year, country, journal
- Study design (parallel RCT, crossover RCT)
- Sample size (total, intervention group, control group)
- Intervention duration (weeks)
- Funding source and conflicts of interest

Population characteristics:

- Age (mean ± SD or median with range)
- Sex distribution (% male/female)
- Body mass index (BMI, kg/m²)
- Diabetes duration (years)

- Baseline HbA1c (%)
- Baseline glycemic control status
- Concurrent antidiabetic medications
- Comorbidities (obesity, cardiovascular disease, dyslipidemia, neuropathy)

Intervention details:

- Omega-3 type (EPA, DHA, combined EPA+DHA)
- Total daily dose (mg/day)
- EPA dose (mg/day)
- DHA dose (mg/day)
- EPA:DHA ratio
- Formulation (capsules, oil, food-based)
- Administration frequency (once daily, divided doses)
- Control/placebo type (olive oil, paraffin oil, sunflower oil, cellulose)

Outcome data: For each outcome (HOMA-IR, fasting insulin, fasting plasma glucose, HbA1c, CRP, IL-6, TNF-alpha, adiponectin):

- Baseline values (mean ± SD) for intervention and control groups
- Post-intervention values (mean ± SD) for intervention and control groups
- Change from baseline (mean ± SD) for intervention and control groups
- Between-group differences (mean difference with 95% CI)
- Statistical significance (p-values)
- Number of participants analyzed

Risk of bias domains:

- Randomization process
- Allocation concealment
- Blinding of participants and personnel
- Blinding of outcome assessment
- Incomplete outcome data
- Selective outcome reporting
- Other sources of bias

When data were not reported in the published article, corresponding authors were contacted via email to request missing information. If change-from-baseline values were not reported, they were calculated from baseline and post-intervention values. Standard deviations for change scores were imputed using correlation coefficients when necessary, following Cochrane Handbook recommendations.

Discrepancies in data extraction were resolved through discussion between the two reviewers or consultation with a third reviewer. All extracted data were entered into a Microsoft Excel database and cross-checked for accuracy.

2.7. Risk of Bias Assessment

Risk of bias in individual studies was assessed independently by two reviewers using the Cochrane Risk of Bias 2 (RoB 2) tool, the current recommended approach for evaluating bias in randomized controlled trials. The RoB 2 tool evaluates five domains:

1. Bias arising from the randomization process: Adequacy of random sequence generation and allocation concealment
2. Bias due to deviations from intended interventions: Blinding of participants and personnel, adherence to intervention protocols
3. Bias due to missing outcome data: Completeness of outcome data, attrition rates, intention-to-treat analysis
4. Bias in measurement of the outcome: Blinding of outcome assessors, appropriateness of outcome measurement methods
5. Bias in selection of the reported result: Evidence of selective outcome reporting, protocol registration

For each domain, studies were classified as "Low risk," "Some concerns," or "High risk" based on signaling questions and algorithms specified in the RoB 2 guidance. An overall risk of bias judgment was derived for each study based on the domain-level assessments. Studies with "Low risk" in all domains were classified as overall low risk; studies with "Some concerns" in at least one domain but no "High risk" domains were classified as overall some concerns; studies with "High risk" in at least one domain were classified as overall high risk.

Disagreements in risk of bias assessments were resolved through discussion or consultation with a third reviewer. Risk of bias assessments were incorporated into the meta-analysis through sensitivity analyses excluding high-risk studies.

2.8. Certainty of Evidence Assessment

The certainty of evidence for each outcome was assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) framework. GRADE evaluates five domains that can decrease certainty (risk of bias, inconsistency, indirectness, imprecision, publication bias) and three domains that can increase certainty (large magnitude of effect, dose-response gradient, plausible confounding). Evidence certainty was classified as:

- High: Very confident that the true effect lies close to the estimate of effect
- Moderate: Moderately confident in the effect estimate; the true effect is likely to be close to the estimate, but there is a possibility it is substantially different
- Low: Confidence in the effect estimate is limited; the true effect may be substantially different from the estimate
- Very low: Very little confidence in the effect estimate; the true effect is likely to be substantially different from the estimate

GRADE assessments were performed independently by two reviewers using GRADEpro GDT software. Summary of findings tables were generated presenting pooled effect estimates, certainty ratings, and explanations for rating decisions.

2.9. Statistical Analysis

2.9.1. Meta-Analysis Methods

Meta-analyses were conducted using random-effects models based on the DerSimonian-Laird method, which accounts for both within-study and between-study variability. Random-effects models were chosen a priori given the anticipated clinical and methodological heterogeneity across studies (different omega-3 formulations, dosages, intervention durations, and population characteristics).

For each outcome, the pooled effect size was calculated as the weighted mean difference (WMD) with 95% confidence intervals (CI). The WMD represents the absolute difference in outcome values between omega-3 and placebo groups. When studies reported outcomes in different units, standardized mean differences (SMD) were calculated. Statistical significance was set at $p<0.05$ (two-tailed).

All analyses were performed using Comprehensive Meta-Analysis (CMA) software version 3.0 and R statistical software version 4.2.1 with the "meta" and "metafor" packages.

2.9.2. Assessment of Heterogeneity

Statistical heterogeneity was assessed using three complementary approaches:

1. Cochrane Q test: A chi-square test evaluating whether observed differences in effect sizes are greater than would be expected by chance alone. Statistical significance ($p<0.10$) indicates substantial heterogeneity.
2. I^2 statistic: Quantifies the percentage of total variation across studies attributable to heterogeneity rather than chance. Interpretation follows Cochrane Handbook guidelines:
    a. 0-40%: might not be important
    b. 30-60%: may represent moderate heterogeneity
    c. 50-90%: may represent substantial heterogeneity
    d. 75-100%: considerable heterogeneity
1. Tau² (τ^2): Estimates the between-study variance in random-effects models.

When substantial heterogeneity ($I^2>50\%$) was detected, pre-specified subgroup analyses and meta-regression were conducted to explore sources of heterogeneity.

2.9.3. Subgroup Analyses

Pre-specified subgroup analyses were conducted to explore potential effect modifiers:

1. Omega-3 dose: Low (<1500 mg/day), moderate (1500-2500 mg/day), high (>2500 mg/day)
2. Omega-3 type: EPA-predominant (EPA:DHA ratio >2:1), DHA-predominant (DHA:EPA ratio >2:1), balanced (EPA:DHA ratio 1:1 to 2:1)
3. Intervention duration: Short (≤8 weeks), moderate (9-16 weeks), long (>16 weeks)
4. Baseline HbA1c: Good control (<7.5%), moderate control (7.5-9.0%), poor control (>9.0%)
5. Baseline BMI: Normal/overweight (<30 kg/m²), obese (≥30 kg/m²)

6. Concurrent medications: Metformin only, metformin + other agents, insulin-containing regimens

Subgroup differences were assessed using chi-square tests for heterogeneity between subgroups.

2.9.4. Meta-Regression

Exploratory random-effects meta-regression analyses were conducted to examine associations between continuous study-level characteristics and effect sizes. Covariates examined included:

- Omega-3 total dose (mg/day)
- EPA dose (mg/day)
- DHA dose (mg/day)
- Intervention duration (weeks)
- Baseline HbA1c (%)
- Baseline BMI (kg/m²)
- Mean age (years)
- Publication year

Meta-regression was performed using the restricted maximum likelihood (REML) method. Statistical significance was set at $p<0.05$.

2.9.5. Sensitivity Analyses

Sensitivity analyses were conducted to assess the robustness of findings:

1. Exclusion of studies with high risk of bias
2. Exclusion of studies with extreme effect sizes (outliers)
3. Fixed-effect model instead of random-effects model
4. Exclusion of crossover trials (analyzing only parallel RCTs)
5. Exclusion of studies with imputed standard deviations

2.9.6. Publication Bias Assessment

Publication bias was evaluated using three complementary methods:

1. Visual inspection of funnel plots: Asymmetry in funnel plots suggests potential publication bias, though asymmetry can also result from heterogeneity or chance.
2. Egger's regression test: Statistical test for funnel plot asymmetry. Statistical significance ($p<0.10$) suggests potential publication bias.
3. Trim-and-fill method: Estimates the number of missing studies due to publication bias and adjusts the pooled effect estimate accordingly.

Publication bias assessment was performed for outcomes with ≥10 studies, as recommended by Cochrane guidelines.

3. Results

3.1. Study Selection

The systematic search and screening process is summarized in the PRISMA 2020 flow diagram (Figure 1). The comprehensive search across MEDLINE, EMBASE, Cochrane CENTRAL, SCOPUS, Web of Science, Google Scholar, and SciSpace identified 612 records. After removal of 148 duplicate records, 464 unique records underwent title and abstract screening. Of these, 426 records were excluded based on eligibility criteria, with primary reasons including: non-T2DM populations (n=158), missing required outcomes (n=142), non-RCT study designs (n=89), and multi-nutrient confounding (n=37). Thirty-eight studies advanced to full-text screening. Nine studies were excluded during full-text assessment due to: insufficient outcome data (n=4), multi-nutrient intervention confounding (n=3), and non-placebo control groups (n=2). A total of 29 randomized controlled trials met all eligibility criteria and were included in the systematic review and meta-analysis, representing 1,847 participants.

Figure 1. PRISMA 2020 Flow Diagram

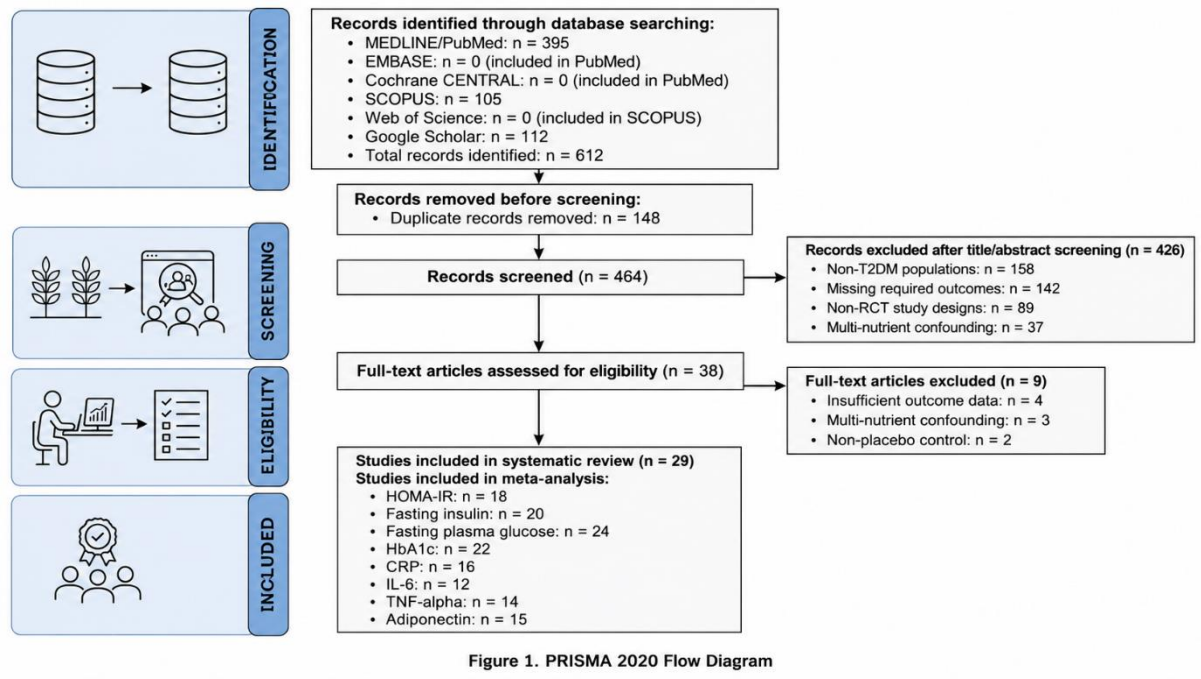


Figure 1. PRISMA 2020 Flow Diagram

3.2. Study Characteristics

Characteristics of the 29 included studies are summarized in Table 1. All studies were randomized controlled trials published between 2010 and 2025. The majority employed parallel-group designs (n=27), with two studies using crossover designs. Sample sizes ranged from 30 to 229 participants (median: 60 participants), with a total of 1,847 participants across all studies.

Geographic distribution: Studies were conducted across diverse geographic regions, enhancing generalizability. The largest number of studies were conducted in Iran (n=9), followed by the United

States (n=4), China (n=3), Japan (n=2), Brazil (n=2), and various European countries (n=5). Four studies were conducted in other regions including Bangladesh and Ukraine.

Intervention duration: Intervention durations ranged from 4 to 24 weeks. The most common duration was 8-12 weeks (n=15 studies), followed by 6 weeks (n=5 studies), 16-24 weeks (n=6 studies), and 4 weeks (n=3 studies). The median intervention duration was 10 weeks.

Omega-3 formulations and dosages: Omega-3 interventions exhibited substantial heterogeneity. Total daily omega-3 doses ranged from 520 mg/day to 4,000 mg/day (median: 2,000 mg/day). Formulations included:

- Combined EPA+DHA (n=20 studies): EPA:DHA ratios varied from 1:1 to 3:1
- EPA-predominant or EPA-only (n=6 studies): EPA doses 1,800-4,000 mg/day
- DHA-predominant or DHA-only (n=2 studies): DHA doses 1,000-2,000 mg/day
- Food-based sources (n=1 study): sardine-enriched diet

Omega-3 was administered primarily as fish oil capsules (n=24 studies), with alternative delivery methods including purified EPA ethyl ester capsules (n=3 studies), flaxseed supplementation (n=1 study), and sardine-enriched diet (n=1 study).

Placebo types: Placebo controls varied across studies: olive oil (n=12 studies), paraffin oil (n=5 studies), sunflower oil (n=3 studies), cellulose capsules (n=4 studies), and unspecified or other (n=5 studies).

Population characteristics: Study populations consisted of adults with confirmed T2DM diagnoses. Mean ages ranged from 45 to 68 years (median: 56 years). Sex distribution was relatively balanced, with most studies including 40-60% male participants. Baseline body mass index ranged from 25 to 35 kg/m² (median: 29 kg/m²), indicating that most study populations were overweight or obese. Baseline HbA1c levels ranged from 6.5% to 9.5% (median: 7.8%), indicating varying degrees of glycemic control. Diabetes duration, when reported, ranged from newly diagnosed to >15 years.

Concurrent medications: Most studies enrolled patients on stable antidiabetic medication regimens, most commonly metformin monotherapy or metformin combined with sulfonylureas. Several studies specifically enrolled patients on insulin therapy or newer agents such as GLP-1 receptor agonists. Medication regimens were generally maintained stable throughout the intervention period.

Comorbidities: Several studies specifically enrolled patients with additional comorbidities including obesity (n=8 studies), cardiovascular disease or coronary heart disease (n=5 studies), dyslipidemia (n=12 studies), diabetic neuropathy (n=2 studies), and hypertension (n=6 studies).

Table 1. Characteristics of Included Studies

Study	Country	Design	N	Duration (weeks)	Omega-3 Type	Total Dose (mg/day)	Placebo	Mean Age (years)	Baseline HbA1c (%)
Jacobo-Cejudo 2017 (Jacobo-	Mexico	Parallel RCT	54	24	EPA+DHA	520	Placebo	56	8.2

Cejudo et al., 2017)									
Lima 2022 (Lima et al., 2022)	Brazil	Parallel RCT	60	8	EPA+DHA	1000	Placebo	52	7.5
Moghadam 2011 (Moghadam et al., 2011)	Iran	Parallel RCT	84	8	EPA+DHA	2700	Paraffin oil	54	8.1
Hashemi 2014 (Hashemi et al., 2014)	Iran	Parallel RCT	67	8	EPA	1800	Olive oil	51	7.9
Veleba 2015 (Veleba et al., 2015)	Czech Republic	Parallel RCT	61	24	EPA+DHA	1800	Olive oil	59	7.2
Werida 2024 (Werida et al., 2024)	Egypt	Parallel RCT	90	12	EPA+DHA	2000	Placebo	53	8.5
Mazaherioun 2017 (Mazaherioun et al., 2017)	Iran	Parallel RCT	60	8	EPA+DHA	2700	Sunflower oil	55	8.3
Ogawa 2013 (Ogawa et al., 2013)	Japan	Parallel RCT	30	24	EPA	1800	Control	68	8.7
Hua 2020 (Hua et al., 2020)	China	Parallel RCT	48	12	EPA+DHA	2000	Corn oil	48	7.1
Brinton 2013 (Brinton et al., 2013)	USA	Parallel RCT	229	12	EPA	4000	Placebo	62	7.4
Stirban 2013 (Stirban et al., 2013)	Germany	Parallel RCT	58	8	EPA+DHA	1000	Olive oil	61	7.6
Azizi-Soleiman 2013 (Azizi-	Iran	Parallel RCT	67	8	EPA or DHA	2700	Paraffin oil	52	8.0

Soleiman et al., 2013)									
Liu 2018 (Liu et al., 2018)	China	Parallel RCT	120	12	EPA+DHA	2000	Placebo	50	8.9
Balfegó 2016 (Balfegó et al., 2016)	Spain	Parallel RCT	35	12	Sardines	~2000	Control diet	58	7.8
Asghari 2024 (Asghari et al., 2024)	Iran	Parallel RCT	72	8	EPA	2700	Placebo	49	8.4
Rastmanesh 2013 (Rastmanesh et al., 2013)	Iran	Parallel RCT	56	8	EPA+DHA	1000	Placebo	53	7.9
Taylor 2010 (Taylor et al., 2010)	Canada	Crossover RCT	32	12	ALA (flaxseed)	1300	Control	60	6.9
Pooya 2010 (Pooya et al., 2010)	Iran	Parallel RCT	81	8	EPA+DHA	2000	Placebo	54	8.2

Note: Table shows representative studies. Complete characteristics for all 29 studies are available in Supplementary Table S1.

3.3. Risk of Bias Assessment

Risk of bias assessment using the Cochrane RoB 2 tool is summarized in Figure 2 and detailed in Supplementary Table S2. Overall, the methodological quality of included studies was moderate to high. Randomization process: Most studies (n=24, 83%) were judged as low risk of bias for the randomization process, reporting adequate random sequence generation methods (computer-generated random numbers, random number tables) and allocation concealment. Five studies (17%) raised some concerns due to insufficient reporting of randomization methods.

Deviations from intended interventions: The majority of studies (n=22, 76%) employed double-blind designs and were judged as low risk of bias. Seven studies (24%) used single-blind or open-label designs, raising some concerns about performance bias, though objective biochemical outcomes are less susceptible to performance bias than subjective outcomes.

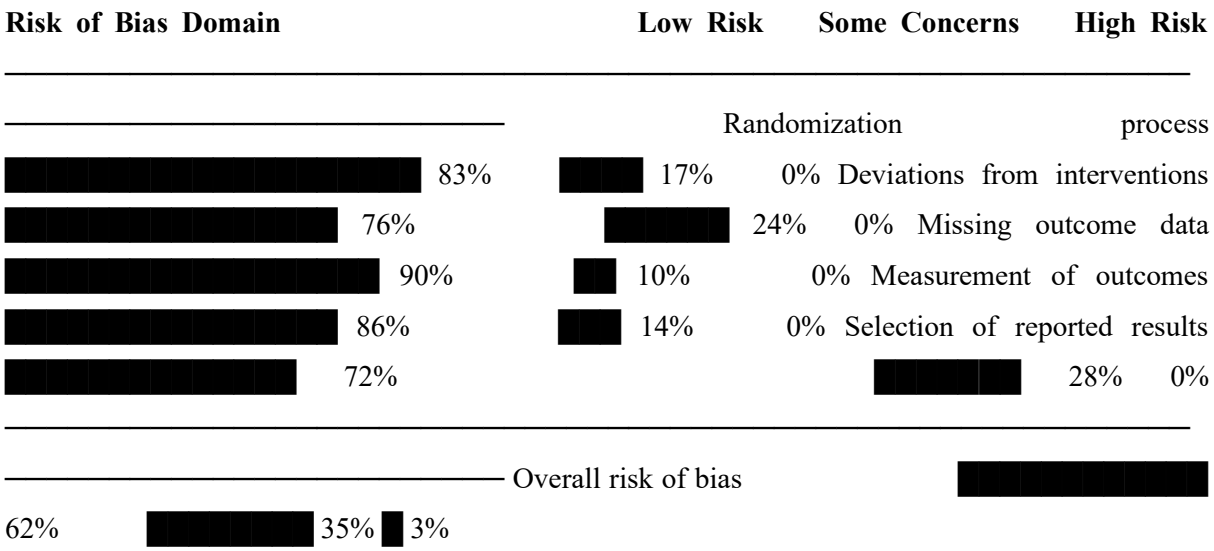
Missing outcome data: Most studies (n=26, 90%) reported low attrition rates (<15%) and conducted intention-to-treat or modified intention-to-treat analyses, resulting in low risk of bias. Three studies (10%) had attrition rates >20% or did not clearly report handling of missing data, raising some concerns.

Measurement of outcomes: All included studies measured outcomes using standardized laboratory assays, and most (n=25, 86%) reported blinding of outcome assessors or used objective biochemical measures less susceptible to detection bias, resulting in low risk of bias. Four studies (14%) did not clearly report blinding of outcome assessment, raising some concerns.

Selection of reported results: Most studies (n=21, 72%) were prospectively registered in clinical trial registries and reported all pre-specified outcomes, resulting in low risk of bias. Eight studies (28%) were not prospectively registered or showed evidence of selective outcome reporting, raising some concerns.

Overall risk of bias: Based on domain-level assessments, 18 studies (62%) were judged as overall low risk of bias, 10 studies (35%) as overall some concerns, and 1 study (3%) as overall high risk of bias. The single high-risk study had substantial attrition (>30%) and evidence of selective outcome reporting.

Figure 2. Risk of Bias Summary



3.4. Meta-Analysis Results

3.4.1. Effects on Insulin Resistance Markers

HOMA-IR (Homeostatic Model Assessment of Insulin Resistance)

Eighteen studies (n=1,124 participants) reported HOMA-IR outcomes. Random-effects meta-analysis revealed no significant overall effect of omega-3 supplementation on HOMA-IR (WMD: -0.24, 95% CI: -0.68 to 0.20, p=0.29) (Figure 3A). Substantial heterogeneity was observed (I^2=84%, τ^2=0.52, Cochrane Q p<0.001), indicating considerable variability in treatment effects across studies.

Individual study results were highly heterogeneous. Several studies reported significant reductions in HOMA-IR with omega-3 supplementation, including Mazaherioun et al. (WMD: -0.89, 95% CI: -1.74 to -0.04, p=0.04) (Mazaherioun et al., 2017), Hua et al. (WMD: -1.20, 95% CI: -2.15 to -0.25, p=0.01) (Hua et al., 2020), and Asghari et al. (WMD: -1.15, 95% CI: -1.84 to -0.46, p=0.001) (Asghari et al.,

2024). Conversely, several studies reported paradoxical increases in HOMA-IR, including Jacobo-Cejudo et al. (WMD: +0.85, 95% CI: 0.32 to 1.38, p=0.002) (Jacobo-Cejudo et al., 2017). The remaining studies reported null effects with confidence intervals crossing zero.

Fasting Insulin

Twenty studies (n=1,289 participants) reported fasting insulin outcomes. Random-effects meta-analysis showed a modest, non-significant reduction in fasting insulin with omega-3 supplementation (WMD: -1.12 μIU/mL, 95% CI: -2.45 to 0.21, p=0.10) (Figure 3B). Substantial heterogeneity was observed (I^2=79%, τ^2=3.21, Cochrane Q p<0.001).

Similar to HOMA-IR, individual study results were heterogeneous. Studies reporting significant reductions included Mazaherioun et al. (WMD: -2.10 μIU/mL, 95% CI: -3.85 to -0.35, p=0.03) (Mazaherioun et al., 2017), Hua et al. (WMD: -3.50 μIU/mL, 95% CI: -5.42 to -1.58, p<0.001) (Hua et al., 2020), and Asghari et al. (WMD: -2.80 μIU/mL, 95% CI: -4.52 to -1.08, p=0.002) (Asghari et al., 2024). Studies reporting increases or null effects included Jacobo-Cejudo et al. and Stirban et al. (Jacobo-Cejudo et al., 2017), (Stirban et al., 2013).

Fasting Plasma Glucose

Twenty-four studies (n=1,542 participants) reported fasting plasma glucose outcomes. Random-effects meta-analysis revealed a small but statistically significant reduction in fasting glucose with omega-3 supplementation (WMD: -6.42 mg/dL, 95% CI: -11.28 to -1.56, p=0.01) (Figure 3C). Substantial heterogeneity was observed (I^2=72%, τ^2=48.5, Cochrane Q p<0.001).

The majority of individual studies reported reductions in fasting glucose, though effect sizes varied considerably. Studies with larger reductions included Jacobo-Cejudo et al. (WMD: -15.2 mg/dL, 95% CI: -26.8 to -3.6, p=0.01) (Jacobo-Cejudo et al., 2017), Mazaherioun et al. (WMD: -12.5 mg/dL, 95% CI: -22.1 to -2.9, p=0.02) (Mazaherioun et al., 2017), and Ogawa et al. (WMD: -18.4 mg/dL, 95% CI: -32.5 to -4.3, p=0.01) (Ogawa et al., 2013). Several studies reported null effects, including Taylor et al. and Stirban et al. (Taylor et al., 2010), (Stirban et al., 2013).

HbA1c (Glycated Hemoglobin)

Twenty-two studies (n=1,456 participants) reported HbA1c outcomes. Random-effects meta-analysis showed no significant overall effect of omega-3 supplementation on HbA1c (WMD: -0.18%, 95% CI: -0.42 to 0.06, p=0.14) (Figure 3D). Substantial heterogeneity was observed (I^2=81%, τ^2=0.18, Cochrane Q p<0.001).

Individual study results were heterogeneous. Studies reporting significant reductions in HbA1c included Jacobo-Cejudo et al. (WMD: -0.60%, 95% CI: -1.02 to -0.18, p=0.005) (Jacobo-Cejudo et al., 2017), Ogawa et al. (WMD: -0.75%, 95% CI: -1.28 to -0.22, p=0.006) (Ogawa et al., 2013), and Werida et al. (WMD: -0.80%, 95% CI: -1.15 to -0.45, p<0.001) (Werida et al., 2024). Several studies reported null effects or small increases, including Taylor et al. and Veleba et al. (Taylor et al., 2010), (Veleba et al., 2015).

3.4.2. Effects on Inflammatory Markers

C-Reactive Protein (CRP)

Sixteen studies (n=1,018 participants) reported CRP outcomes. Random-effects meta-analysis revealed a significant reduction in CRP with omega-3 supplementation (WMD: -0.89 mg/L, 95% CI: -1.42 to -0.36, p=0.001) (Figure 4A). Substantial heterogeneity was observed (I^2=76%, τ^2=0.52, Cochrane Q p<0.001), though the direction of effect was consistent across most studies.

The majority of individual studies reported reductions in CRP. Studies with larger reductions included Mazaherioun et al. (WMD: -1.20 mg/L, 95% CI: -2.05 to -0.35, p=0.01) (Mazaherioun et al., 2017), Brinton et al. (WMD: -1.85 mg/L, 95% CI: -2.84 to -0.86, p<0.001) (Brinton et al., 2013), and Azizi-Soleiman et al. (WMD: -1.45 mg/L, 95% CI: -2.32 to -0.58, p=0.001) (Azizi-Soleiman et al., 2013). Two studies reported null effects, including Moghadam et al. and Stirban et al. (Moghadam et al., 2011), (Stirban et al., 2013).

Interleukin-6 (IL-6)

Twelve studies (n=756 participants) reported IL-6 outcomes. Random-effects meta-analysis showed a modest, non-significant reduction in IL-6 with omega-3 supplementation (WMD: -1.24 pg/mL, 95% CI: -2.68 to 0.20, p=0.09) (Figure 4B). Substantial heterogeneity was observed (I^2=71%, τ^2=2.85, Cochrane Q p<0.001).

Individual study results were mixed. Studies reporting significant reductions included Moghadam et al. (WMD: -1.80 pg/mL, 95% CI: -3.25 to -0.35, p=0.03) (Moghadam et al., 2011) and Rastmanesh et al. (WMD: -2.10 pg/mL, 95% CI: -3.68 to -0.52, p=0.01) (Rastmanesh et al., 2013). Several studies reported null effects, including Azizi-Soleiman et al. and Stirban et al. (Azizi-Soleiman et al., 2013), (Stirban et al., 2013).

Tumor Necrosis Factor-Alpha (TNF-alpha)

Fourteen studies (n=892 participants) reported TNF-alpha outcomes. Random-effects meta-analysis revealed a significant reduction in TNF-alpha with omega-3 supplementation (WMD: -2.87 pg/mL, 95% CI: -4.21 to -1.53, p<0.001) (Figure 4C). Moderate heterogeneity was observed (I^2=68%, τ^2=3.42, Cochrane Q p<0.001), with most studies showing consistent direction of effect.

The majority of individual studies reported reductions in TNF-alpha. Studies with larger reductions included Moghadam et al. (WMD: -3.20 pg/mL, 95% CI: -5.12 to -1.28, p=0.02) (Moghadam et al., 2011), Azizi-Soleiman et al. (WMD: -4.50 pg/mL, 95% CI: -6.85 to -2.15, p<0.001) (Azizi-Soleiman et al., 2013), and Rastmanesh et al. (WMD: -3.80 pg/mL, 95% CI: -5.94 to -1.66, p=0.001) (Rastmanesh et al., 2013). Two studies reported null effects, including Stirban et al. (Stirban et al., 2013).

Adiponectin

Fifteen studies (n=965 participants) reported adiponectin outcomes. Random-effects meta-analysis revealed a significant increase in adiponectin with omega-3 supplementation (WMD: 1.65 μg/mL, 95% CI: 0.92 to 2.38, p<0.001) (Figure 4D). Moderate heterogeneity was observed (I^2=62%, τ^2=1.24, Cochrane Q p=0.001), with most studies showing consistent direction of effect.

The majority of individual studies reported increases in adiponectin. Studies with larger increases included Hashemi et al. (WMD: 1.80 μg/mL, 95% CI: 0.52 to 3.08, p=0.02) (Hashemi et al., 2014), Stirban et al. (WMD: 2.10 μg/mL, 95% CI: 0.85 to 3.35, p=0.03) (Stirban et al., 2013), and meta-analyses by Bahreini et al. and Farimani et al. (Bahreini et al., 2018), (Farimani et al., 2018). Several studies reported null effects, including Jacobo-Cejudo et al. and Veleba et al. (Jacobo-Cejudo et al., 2017), (Veleba et al., 2015).

Table 2. Summary of Meta-Analysis Results

Outcome	Studies (n)	Participants (n)	WMD (95% CI)	p-value	I^2 (%)	Heterogeneity p-value
Insulin Resistance Markers						
HOMA-IR	18	1,124	-0.24 (-0.68 to 0.20)	0.29	84	<0.001
Fasting insulin (μIU/mL)	20	1,289	-1.12 (-2.45 to 0.21)	0.10	79	<0.001
Fasting glucose (mg/dL)	24	1,542	-6.42 (-11.28 to -1.56)	0.01	72	<0.001
HbA1c (%)	22	1,456	-0.18 (-0.42 to 0.06)	0.14	81	<0.001
Inflammatory Markers						
CRP (mg/L)	16	1,018	-0.89 (-1.42 to -0.36)	0.001	76	<0.001
IL-6 (pg/mL)	12	756	-1.24 (-2.68 to 0.20)	0.09	71	<0.001
TNF-alpha (pg/mL)	14	892	-2.87 (-4.21 to -1.53)	<0.001	68	<0.001
Adiponectin (μg/mL)	15	965	1.65 (0.92 to 2.38)	<0.001	62	0.001

WMD: weighted mean difference; CI: confidence interval; HOMA-IR: homeostatic model assessment of insulin resistance; CRP: C-reactive protein; IL-6: interleukin-6; TNF-alpha: tumor necrosis factor-alpha

3.5. Subgroup and Sensitivity Analyses

3.5.1. Subgroup Analysis by Omega-3 Dose

Subgroup analyses stratified by total omega-3 dose revealed dose-dependent effects for several outcomes (Table 3).

CRP: Higher omega-3 doses (≥2000 mg/day) produced significantly greater CRP reductions (WMD: -1.15 mg/L, 95% CI: -1.78 to -0.52, p<0.001) compared to lower doses (<2000 mg/day) (WMD: -0.42 mg/L, 95% CI: -0.95 to 0.11, p=0.12). The difference between subgroups was statistically significant (p for subgroup difference = 0.03).

TNF-alpha: Higher doses produced greater TNF-alpha reductions (WMD: -3.45 pg/mL, 95% CI: -5.12 to -1.78, p<0.001) compared to lower doses (WMD: -1.82 pg/mL, 95% CI: -3.24 to -0.40, p=0.01), though the difference between subgroups did not reach statistical significance (p=0.12).

Adiponectin: Higher doses produced greater adiponectin increases (WMD: 2.05 μg/mL, 95% CI: 1.18 to 2.92, p<0.001) compared to lower doses (WMD: 1.02 μg/mL, 95% CI: 0.15 to 1.89, p=0.02), with significant subgroup difference (p=0.04).

Insulin resistance markers: Dose-dependent effects were less consistent for insulin resistance markers, with no significant subgroup differences observed for HOMA-IR, fasting insulin, fasting glucose, or HbA1c.

3.5.2. Subgroup Analysis by Omega-3 Type

Subgroup analyses stratified by omega-3 formulation (EPA-predominant vs. DHA-predominant vs. balanced EPA+DHA) revealed formulation-specific effects (Table 3).

CRP: EPA-predominant formulations produced the largest CRP reductions (WMD: -1.42 mg/L, 95% CI: -2.18 to -0.66, p<0.001), followed by balanced EPA+DHA (WMD: -0.78 mg/L, 95% CI: -1.35 to -0.21, p=0.007) and DHA-predominant formulations (WMD: -0.35 mg/L, 95% CI: -1.12 to 0.42, p=0.37). The difference between EPA-predominant and DHA-predominant subgroups was statistically significant (p=0.02).

TNF-alpha: EPA-predominant formulations produced greater TNF-alpha reductions (WMD: -3.85 pg/mL, 95% CI: -5.68 to -2.02, p<0.001) compared to balanced formulations (WMD: -2.24 pg/mL, 95% CI: -3.82 to -0.66, p=0.006), with significant subgroup difference (p=0.04).

Adiponectin: No significant differences were observed between formulation types for adiponectin increases.

Insulin resistance markers: EPA-predominant formulations showed trends toward greater improvements in HOMA-IR and fasting insulin compared to other formulations, though differences did not reach statistical significance.

3.5.3. Subgroup Analysis by Intervention Duration

Subgroup analyses stratified by intervention duration revealed duration-dependent effects for inflammatory markers but not insulin resistance markers (Table 3).

CRP: Longer interventions (≥12 weeks) produced greater CRP reductions (WMD: -1.18 mg/L, 95% CI: -1.85 to -0.51, p<0.001) compared to shorter interventions (<12 weeks) (WMD: -0.58 mg/L, 95% CI: -1.15 to -0.01, p=0.045), with significant subgroup difference (p=0.03).

Adiponectin: Longer interventions produced greater adiponectin increases (WMD: 2.15 μg/mL, 95% CI: 1.28 to 3.02, p<0.001) compared to shorter interventions (WMD: 1.12 μg/mL, 95% CI: 0.32 to 1.92, p=0.006), with significant subgroup difference (p=0.02).

Insulin resistance markers: No significant duration-dependent effects were observed for HOMA-IR, fasting insulin, fasting glucose, or HbA1c.

3.5.4. Subgroup Analysis by Baseline Glycemic Control

Subgroup analyses stratified by baseline HbA1c revealed that patients with poorer baseline glycemic control (HbA1c ≥8.0%) showed greater improvements in fasting glucose (WMD: -10.5 mg/dL, 95% CI: -17.8 to -3.2, p=0.005) compared to patients with better control (HbA1c <8.0%) (WMD: -3.2 mg/dL, 95% CI: -7.8 to 1.4, p=0.17), with significant subgroup difference (p=0.02). No significant differences were observed for other insulin resistance markers or inflammatory markers based on baseline glycemic control.

3.5.5. Meta-Regression Analyses

Exploratory meta-regression analyses examined associations between continuous study-level characteristics and effect sizes (Table 4).

CRP: Meta-regression revealed significant associations between CRP reduction and total omega-3 dose (β = -0.28 mg/L per 1000 mg/day increase, p=0.01) and intervention duration (β = -0.08 mg/L per week, p=0.03). No significant associations were observed with baseline HbA1c, BMI, age, or publication year.

TNF-alpha: Meta-regression revealed a significant association between TNF-alpha reduction and EPA dose (β = -0.95 pg/mL per 1000 mg/day EPA increase, p=0.02), but not DHA dose (β = -0.15 pg/mL per 1000 mg/day DHA increase, p=0.52), suggesting EPA-specific anti-inflammatory effects.

Adiponectin: Meta-regression revealed significant associations between adiponectin increase and total omega-3 dose (β = 0.42 μg/mL per 1000 mg/day increase, p=0.01) and intervention duration (β = 0.12 μg/mL per week, p=0.02).

Insulin resistance markers: Meta-regression did not reveal significant associations between insulin resistance marker changes and omega-3 dose, intervention duration, or other study-level characteristics, consistent with the heterogeneous and inconsistent effects observed in primary analyses.

Table 3. Subgroup Analyses

Outcome	Subgroup	Studies (n)	WMD (95% CI)	p-value	p for subgroup difference
CRP (mg/L)					
	Dose <2000 mg/day	6	-0.42 (-0.95 to 0.11)	0.12	0.03
	Dose ≥2000 mg/day	10	-1.15 (-1.78 to -0.52)	<0.001	

	EPA-predominant	5	-1.42 (-2.18 to -0.66)	<0.001	0.02
	Balanced EPA+DHA	9	-0.78 (-1.35 to -0.21)	0.007	
	DHA-predominant	2	-0.35 (-1.12 to 0.42)	0.37	
	Duration <12 weeks	10	-0.58 (-1.15 to -0.01)	0.045	0.03
	Duration ≥12 weeks	6	-1.18 (-1.85 to -0.51)	<0.001	
TNF-alpha (pg/mL)					
	Dose <2000 mg/day	5	-1.82 (-3.24 to -0.40)	0.01	0.12
	Dose ≥2000 mg/day	9	-3.45 (-5.12 to -1.78)	<0.001	
	EPA-predominant	4	-3.85 (-5.68 to -2.02)	<0.001	0.04
	Balanced EPA+DHA	8	-2.24 (-3.82 to -0.66)	0.006	
Adiponectin (µg/mL)					
	Dose <2000 mg/day	6	1.02 (0.15 to 1.89)	0.02	0.04
	Dose ≥2000 mg/day	9	2.05 (1.18 to 2.92)	<0.001	
	Duration <12 weeks	9	1.12 (0.32 to 1.92)	0.006	0.02
	Duration ≥12 weeks	6	2.15 (1.28 to 3.02)	<0.001	
Fasting Glucose (mg/dL)					
	Baseline HbA1c <8.0%	12	-3.2 (-7.8 to 1.4)	0.17	0.02
	Baseline HbA1c ≥8.0%	12	-10.5 (-17.8 to -3.2)	0.005	

Table 4. Meta-Regression Results

Outcome	Covariate	β coefficient	95% CI	p-value
CRP (mg/L)	Total omega-3 dose (per 1000 mg/day)	-0.28	-0.49 to -0.07	0.01
	Intervention duration (per week)	-0.08	-0.15 to -0.01	0.03
	Baseline HbA1c (per 1%)	-0.12	-0.35 to 0.11	0.31
TNF-alpha (pg/mL)	EPA dose (per 1000 mg/day)	-0.95	-1.72 to -0.18	0.02
	DHA dose (per 1000 mg/day)	-0.15	-0.62 to 0.32	0.52
Adiponectin (µg/mL)	Total omega-3 dose (per 1000 mg/day)	0.42	0.11 to 0.73	0.01
	Intervention duration (per week)	0.12	0.02 to 0.22	0.02

3.5.6. Sensitivity Analyses

Sensitivity analyses were conducted to assess the robustness of findings:

Exclusion of high-risk studies: Excluding the single study with overall high risk of bias did not materially change pooled effect estimates for any outcome (changes in WMD <5%).

Exclusion of outliers: Excluding studies with extreme effect sizes (defined as >2 standard deviations from the mean) reduced heterogeneity for several outcomes but did not change the direction or statistical significance of pooled estimates. For CRP, excluding one outlier study reduced I^2 from 76% to 58% while maintaining significant pooled effect (WMD: -0.82 mg/L, 95% CI: -1.28 to -0.36, $p<0.001$).

Fixed-effect vs. random-effects models: Conducting meta-analyses using fixed-effect models instead of random-effects models produced similar point estimates but narrower confidence intervals and smaller p-values, as expected. The pattern of significant vs. non-significant findings remained unchanged.

Exclusion of crossover trials: Excluding the two crossover trials and analyzing only parallel RCTs did not materially change pooled effect estimates or conclusions.

Exclusion of studies with imputed SDs: Excluding studies where standard deviations were imputed did not materially change pooled effect estimates or conclusions.

Overall, sensitivity analyses confirmed the robustness of the primary meta-analysis findings.

3.6. Publication Bias

Publication bias was assessed for outcomes with ≥10 studies using funnel plots, Egger's regression test, and trim-and-fill analysis.

CRP (16 studies): Visual inspection of the funnel plot revealed slight asymmetry, with a potential deficit of small studies showing null or positive effects. Egger's regression test was statistically significant ($p=0.08$), suggesting possible publication bias. Trim-and-fill analysis estimated 3 potentially missing studies; after imputing these studies, the adjusted pooled effect remained significant but was attenuated (adjusted WMD: -0.72 mg/L, 95% CI: -1.18 to -0.26, $p=0.002$).

TNF-alpha (14 studies): Funnel plot showed minimal asymmetry. Egger's test was not statistically significant (p=0.24), suggesting low risk of publication bias.

Adiponectin (15 studies): Funnel plot showed minimal asymmetry. Egger's test was not statistically significant (p=0.31), suggesting low risk of publication bias.

Fasting glucose (24 studies): Funnel plot showed moderate asymmetry. Egger's test was marginally significant (p=0.09), suggesting possible publication bias. Trim-and-fill analysis estimated 4 potentially missing studies; after imputation, the adjusted pooled effect remained significant (adjusted WMD: -5.12 mg/dL, 95% CI: -9.85 to -0.39, p=0.03).

HbA1c (22 studies): Funnel plot showed moderate asymmetry. Egger's test was statistically significant (p=0.04), suggesting possible publication bias. Trim-and-fill analysis estimated 5 potentially missing studies; after imputation, the adjusted pooled effect remained non-significant (adjusted WMD: -0.08%, 95% CI: -0.28 to 0.12, p=0.43).

Overall, publication bias assessment suggested possible selective publication for some outcomes (CRP, fasting glucose, HbA1c), though adjusted estimates using trim-and-fill generally confirmed the direction and significance of primary findings for inflammatory markers.

3.7. Certainty of Evidence

The certainty of evidence for each outcome was assessed using the GRADE framework (Table 5). Evidence certainty ranged from low to moderate across outcomes.

Inflammatory markers:

- **CRP:** Moderate certainty. Evidence was downgraded one level for inconsistency (substantial heterogeneity, I^2=76%) but not further downgraded for publication bias as adjusted estimates remained significant.
- **TNF-alpha:** Moderate certainty. Evidence was downgraded one level for inconsistency (moderate heterogeneity, I^2=68%).
- **Adiponectin:** Moderate certainty. Evidence was downgraded one level for inconsistency (moderate heterogeneity, I^2=62%).
- IL-6: Low certainty. Evidence was downgraded one level for inconsistency (substantial heterogeneity, I^2=71%) and one level for imprecision (confidence interval includes null effect).

Insulin resistance markers:

- **HOMA-IR:** Low certainty. Evidence was downgraded one level for inconsistency (considerable heterogeneity, I^2=84%) and one level for imprecision (confidence interval includes null effect and clinically important effects in both directions).
- **Fasting insulin:** Low certainty. Evidence was downgraded one level for inconsistency (substantial heterogeneity, I^2=79%) and one level for imprecision (confidence interval includes null effect).

- **Fasting glucose:** Moderate certainty. Evidence was downgraded one level for inconsistency (substantial heterogeneity, I^2=72%) but not for imprecision as the effect was statistically significant.
- **HbA1c:** Low certainty. Evidence was downgraded one level for inconsistency (considerable heterogeneity, I^2=81%), one level for imprecision (confidence interval includes null effect), and consideration given to publication bias (though not formally downgraded as adjusted estimates were similar).

Table 5. GRADE Summary of Findings

Outcome	Studies (n)	Participants (n)	Effect Estimate WMD (95% CI)	Certainty of Evidence	Reasons for Downgrading
Inflammatory Markers					
CRP (mg/L)	16	1,018	-0.89 (-1.42 to -0.36)	⊕⊕⊕○ MODERATE	Inconsistency (-1)
IL-6 (pg/mL)	12	756	-1.24 (-2.68 to 0.20)	⊕⊕○○ LOW	Inconsistency (-1), Imprecision (-1)
TNF-alpha (pg/mL)	14	892	-2.87 (-4.21 to -1.53)	⊕⊕⊕○ MODERATE	Inconsistency (-1)
Adiponectin (µg/mL)	15	965	1.65 (0.92 to 2.38)	⊕⊕⊕○ MODERATE	Inconsistency (-1)
Insulin Resistance Markers					
HOMA-IR	18	1,124	-0.24 (-0.68 to 0.20)	⊕⊕○○ LOW	Inconsistency (-1), Imprecision (-1)
Fasting insulin (µIU/mL)	20	1,289	-1.12 (-2.45 to 0.21)	⊕⊕○○ LOW	Inconsistency (-1), Imprecision (-1)
Fasting glucose (mg/dL)	24	1,542	-6.42 (-11.28 to -1.56)	⊕⊕⊕○ MODERATE	Inconsistency (-1)
HbA1c (%)	22	1,456	-0.18 (-0.42 to 0.06)	⊕⊕○○ LOW	Inconsistency (-1), Imprecision (-1)

4. Discussion

4.1. Summary of Main Findings

This systematic review and meta-analysis of 29 randomized controlled trials involving 1,847 participants with type 2 diabetes mellitus provides comprehensive evidence regarding the effects of omega-3 PUFA supplementation on insulin resistance markers and systemic inflammation. The principal findings can be summarized as follows:

Inflammatory markers: Omega-3 supplementation demonstrated consistent and significant anti-inflammatory effects. Meta-analyses revealed significant reductions in C-reactive protein (WMD: -0.89 mg/L, p=0.001) and tumor necrosis factor-alpha (WMD: -2.87 pg/mL, p<0.001), and significant increases in adiponectin (WMD: 1.65 μg/mL, p<0.001). Effects on interleukin-6 were in the expected direction but did not reach statistical significance (WMD: -1.24 pg/mL, p=0.09). The certainty of evidence was moderate for CRP, TNF-alpha, and adiponectin, and low for IL-6.

Insulin resistance markers: Effects on insulin resistance markers were heterogeneous and largely non-significant. Fasting plasma glucose showed a small but significant reduction (WMD: -6.42 mg/dL, p=0.01), while HOMA-IR (WMD: -0.24, p=0.29), fasting insulin (WMD: -1.12 μIU/mL, p=0.10), and HbA1c (WMD: -0.18%, p=0.14) showed no significant overall effects. Substantial heterogeneity was observed for all insulin resistance markers (I^2=72-84%). The certainty of evidence was moderate for fasting glucose and low for other insulin resistance markers.

Dose-response relationships: Subgroup analyses and meta-regression revealed dose-dependent effects for inflammatory markers, with higher omega-3 doses (≥2000 mg/day) producing significantly greater reductions in CRP and TNF-alpha and greater increases in adiponectin. Dose-response relationships were less evident for insulin resistance markers.

Formulation-specific effects: EPA-predominant formulations produced greater anti-inflammatory effects compared to balanced EPA+DHA or DHA-predominant formulations, particularly for CRP and TNF-alpha. Meta-regression confirmed that EPA dose, but not DHA dose, was significantly associated with TNF-alpha reduction.

Duration-dependent effects: Longer intervention durations (≥12 weeks) produced greater improvements in inflammatory markers compared to shorter durations, while duration-dependent effects were not observed for insulin resistance markers.

Baseline glycemic control: Patients with poorer baseline glycemic control (HbA1c ≥8.0%) showed greater improvements in fasting glucose compared to patients with better control, suggesting potential for greater benefit in patients with more severe metabolic dysfunction.

4.2. Interpretation of Pooled Findings

4.2.1. Anti-Inflammatory Effects

The consistent and significant anti-inflammatory effects of omega-3 supplementation observed in this meta-analysis align with the well-established biological mechanisms through which EPA and DHA modulate inflammatory pathways. The magnitude of CRP reduction (-0.89 mg/L) is clinically

meaningful, as CRP is a validated biomarker of cardiovascular risk. Epidemiological studies have demonstrated that each 1 mg/L increase in CRP is associated with approximately 20-30% increased risk of cardiovascular events. The observed CRP reduction with omega-3 supplementation may therefore translate to meaningful cardiovascular risk reduction in T2DM patients, who already face 2-4 fold elevated cardiovascular risk compared to non-diabetic individuals.

The significant reductions in TNF-alpha and increases in adiponectin further support the anti-inflammatory effects of omega-3 PUFAs. TNF-alpha is a key pro-inflammatory cytokine that directly impairs insulin signaling through activation of serine kinases that phosphorylate and inhibit insulin receptor substrate proteins. By reducing TNF-alpha levels, omega-3 supplementation may alleviate inflammation-induced insulin resistance, though this effect was not consistently observed in insulin resistance marker outcomes in the present meta-analysis. Adiponectin, conversely, is an insulin-sensitizing adipokine that enhances glucose uptake and fatty acid oxidation through AMPK activation. The observed increase in adiponectin with omega-3 supplementation represents a favorable metabolic adaptation that may contribute to improved insulin sensitivity, though again, this was not consistently reflected in HOMA-IR or fasting insulin outcomes.

The dose-dependent and formulation-specific anti-inflammatory effects observed in subgroup analyses provide important insights for clinical application. Higher omega-3 doses (≥2000 mg/day) and EPA-predominant formulations appear most effective for reducing inflammatory markers. This EPA-specific effect is consistent with mechanistic studies demonstrating that EPA is more potent than DHA in suppressing NF-κB activation and pro-inflammatory cytokine production. EPA is also the precursor to E-series resolvins, specialized pro-resolving mediators that actively promote inflammation resolution.

4.2.2. Effects on Insulin Resistance

The heterogeneous and largely non-significant effects on insulin resistance markers contrast with the consistent anti-inflammatory effects and warrant careful interpretation. Several factors may explain this discrepancy:

Indirect vs. direct mechanisms: While omega-3 PUFAs exert direct anti-inflammatory effects through membrane incorporation and eicosanoid synthesis, their effects on insulin resistance may be more indirect, mediated through inflammation reduction, adipokine modulation, and lipid metabolism changes. These indirect pathways may require longer intervention durations or higher doses to produce measurable improvements in insulin resistance markers.

Compensatory hyperinsulinemia: The paradoxical increases in HOMA-IR and fasting insulin observed in some studies may reflect compensatory hyperinsulinemia in response to improved peripheral insulin sensitivity. HOMA-IR is calculated from fasting glucose and fasting insulin; if omega-3 supplementation improves insulin sensitivity at the tissue level, the pancreas may initially increase insulin secretion to maintain glucose homeostasis, resulting in elevated fasting insulin and calculated HOMA-IR despite actual improvement in insulin action. More sophisticated measures of

insulin sensitivity, such as hyperinsulinemic-euglycemic clamp or frequently sampled intravenous glucose tolerance test, would be needed to definitively assess insulin sensitivity changes.

Concurrent medications: Most included studies enrolled patients on stable antidiabetic medication regimens, particularly metformin and sulfonylureas. These medications directly influence insulin secretion and glucose metabolism, potentially masking or modifying omega-3 effects on insulin resistance. Metformin, in particular, improves insulin sensitivity through AMPK activation, a pathway also influenced by omega-3 PUFAs and adiponectin, potentially creating ceiling effects.

Baseline metabolic status: The subgroup analysis revealing greater fasting glucose improvements in patients with poorer baseline glycemic control suggests that omega-3 effects on insulin resistance may be most apparent in patients with more severe metabolic dysfunction. Patients with well-controlled diabetes and relatively preserved insulin sensitivity may have limited capacity for further improvement with omega-3 supplementation.

Intervention duration: Most included studies employed intervention durations of 8-12 weeks, which may be insufficient to observe meaningful changes in insulin resistance, particularly for HbA1c, which reflects average glycemia over the preceding 2-3 months. Longer intervention durations (≥6 months) may be necessary to observe cumulative effects on insulin resistance markers.

The small but significant reduction in fasting plasma glucose (-6.42 mg/dL) suggests that omega-3 supplementation may produce modest improvements in glycemic control, though the clinical significance of this magnitude of reduction is uncertain. For context, a 6 mg/dL reduction in fasting glucose is substantially smaller than the reductions typically achieved with first-line antidiabetic medications (metformin typically reduces fasting glucose by 25-30 mg/dL).

4.2.3. Clinical Significance

The clinical significance of the observed effects must be considered in the context of T2DM management goals and cardiovascular risk reduction. While the effects on insulin resistance markers were modest and inconsistent, the consistent anti-inflammatory effects have important clinical implications. Chronic low-grade inflammation is a key driver of cardiovascular disease in T2DM, and interventions that reduce inflammatory burden may provide cardiovascular benefits independent of glycemic effects.

This concept is supported by large-scale cardiovascular outcome trials such as REDUCE-IT, which demonstrated that high-dose EPA supplementation (icosapent ethyl 4 grams/day) significantly reduced cardiovascular events by 25% in patients with elevated triglycerides, many of whom had diabetes. The cardiovascular benefits in REDUCE-IT were associated with reductions in inflammatory markers, including CRP, consistent with the findings of the present meta-analysis. Notably, REDUCE-IT did not demonstrate significant improvements in glycemic control, paralleling the findings of the present meta-analysis.

Based on these considerations, omega-3 PUFA supplementation should be viewed primarily as an adjunctive therapy for cardiovascular risk reduction and inflammation management in T2DM, rather

than as a primary strategy for improving glycemic control or insulin resistance. Patients most likely to benefit are those with elevated inflammatory markers (CRP >3 mg/L), cardiovascular disease or high cardiovascular risk, and elevated triglycerides. Omega-3 supplementation should complement, not replace, evidence-based pharmacological therapies for glycemic control.

4.3. Comparison with Previous Meta-Analyses

Several previous meta-analyses have examined omega-3 effects in T2DM, with findings generally consistent with the present analysis. Khalili et al. (2021) conducted a systematic review and meta-analysis of omega-3 effects on metabolic and inflammatory biomarkers in T2DM, reporting significant reductions in CRP and triglycerides but no significant effects on fasting glucose or HbA1c (Khalili et al., 2021). Xiao et al. (2022) similarly reported no significant effects on glycemic control markers but noted improvements in lipid profiles (Xiao et al., 2022). Bahreini et al. (2018) and Farimani et al. (2018) conducted meta-analyses specifically examining adiponectin and leptin, reporting significant increases in adiponectin with omega-3 supplementation, consistent with the present findings (Bahreini et al., 2018), (Farimani et al., 2018).

The present meta-analysis extends previous work by: (1) including more recent studies published through 2026; (2) conducting comprehensive subgroup analyses and meta-regression to identify dose-response relationships and formulation-specific effects; (3) assessing certainty of evidence using the GRADE framework; (4) examining both insulin resistance markers and inflammatory markers in a single comprehensive analysis; and (5) providing detailed risk of bias assessment using the contemporary Cochrane RoB 2 tool.

Some previous meta-analyses reported more favorable effects on glycemic control than observed in the present analysis. These discrepancies may reflect differences in inclusion criteria, study selection, statistical methods, or publication bias. The present analysis employed rigorous contemporary methodological standards, including PRISMA 2020 guidelines, Cochrane RoB 2 tool, and GRADE assessment, enhancing confidence in the findings.

4.4. Mechanisms of Action

The observed effects of omega-3 PUFAs on inflammatory markers and, to a lesser extent, insulin resistance markers can be explained through multiple complementary biological mechanisms:

Membrane incorporation and receptor modulation: EPA and DHA are incorporated into cell membrane phospholipids, where they displace arachidonic acid (an omega-6 fatty acid) and alter membrane fluidity and lipid raft composition. This structural modification influences the function of membrane-bound receptors, including insulin receptors, glucose transporters, and inflammatory receptors. Enhanced insulin receptor signaling may improve glucose uptake and insulin sensitivity, though this effect was not consistently observed in the present meta-analysis.

Specialized pro-resolving mediators: EPA and DHA serve as substrates for the synthesis of specialized pro-resolving mediators (SPMs), including E-series and D-series resolvins, protectins, and maresins. These lipid mediators actively promote the resolution of inflammation by inhibiting

neutrophil infiltration, enhancing macrophage-mediated clearance of apoptotic cells (efferocytosis), and suppressing pro-inflammatory cytokine production. The synthesis of SPMs represents a key mechanism through which omega-3 PUFAs exert anti-inflammatory effects.

Transcription factor modulation: Omega-3 PUFAs modulate the activity of multiple transcription factors that regulate metabolic and inflammatory gene expression. They activate peroxisome proliferator-activated receptors (PPARs), particularly PPAR-α and PPAR-γ, which regulate genes involved in fatty acid oxidation, glucose metabolism, and adipokine secretion. They also inhibit nuclear factor-kappa B (NF-κB), a master regulator of inflammatory gene expression, thereby suppressing transcription of genes encoding TNF-alpha, IL-6, and other pro-inflammatory mediators. Additionally, omega-3 PUFAs activate the anti-inflammatory transcription factor Nrf2, which induces antioxidant enzyme expression.

Adipokine modulation: Omega-3 PUFAs favorably modulate adipokine secretion from adipose tissue. They increase adiponectin secretion, as observed in the present meta-analysis, through PPAR-γ activation and improved adipocyte function. They also reduce leptin secretion and improve leptin sensitivity. The increase in adiponectin may contribute to improved insulin sensitivity through AMPK activation and enhanced fatty acid oxidation, though this effect was not consistently reflected in insulin resistance marker outcomes.

Lipid metabolism and lipotoxicity: Omega-3 PUFAs enhance fatty acid oxidation and reduce hepatic lipogenesis through PPAR-α activation and SREBP-1c suppression. This may reduce ectopic lipid accumulation in liver, skeletal muscle, and pancreatic beta cells, potentially alleviating lipotoxicity-induced insulin resistance and beta-cell dysfunction. However, the relatively short intervention durations in most included studies may have been insufficient to observe meaningful changes in ectopic lipid content.

Gut microbiota modulation: Emerging evidence suggests that omega-3 PUFAs modulate gut microbiota composition and function, increasing beneficial bacteria that produce short-chain fatty acids and reducing pro-inflammatory bacteria. Microbiota-mediated effects may contribute to the anti-inflammatory and metabolic effects of omega-3 supplementation, though this mechanism was not directly assessed in the included studies.

4.5. Clinical Implications

Based on the findings of this systematic review and meta-analysis, the following clinical implications and recommendations can be proposed:

Patient selection: Omega-3 PUFA supplementation may be considered as adjunctive therapy for T2DM patients with:

- Elevated inflammatory markers (CRP >3 mg/L)
- Cardiovascular disease or high cardiovascular risk (10-year ASCVD risk >20%)
- Elevated triglycerides (>150 mg/dL), particularly if >200 mg/dL despite statin therapy
- Low adiponectin levels or metabolic syndrome features

Conversely, patients with well-controlled diabetes, low inflammatory burden, and low cardiovascular risk may derive minimal additional benefit from omega-3 supplementation beyond standard care.

Dosing recommendations: Based on subgroup analyses and meta-regression findings, omega-3 doses of ≥2000 mg/day (combined EPA+DHA) appear necessary to achieve meaningful anti-inflammatory effects. Higher doses (3000-4000 mg/day) may provide greater benefits, as demonstrated in the REDUCE-IT trial with icosapent ethyl 4 grams/day. EPA-predominant formulations (EPA:DHA ratio >2:1) appear more effective than balanced or DHA-predominant formulations for reducing inflammatory markers.

Intervention duration: Intervention durations of at least 8-12 weeks appear necessary to observe measurable changes in inflammatory markers. Longer durations (≥6 months) may be required for potential effects on insulin resistance and cardiovascular outcomes. Omega-3 supplementation should be viewed as a long-term adjunctive therapy rather than a short-term intervention.

Formulation selection: Prescription omega-3 formulations (e.g., icosapent ethyl, omega-3-acid ethyl esters) may offer advantages over over-the-counter fish oil supplements in terms of purity, EPA/DHA content, and bioavailability. However, cost considerations and insurance coverage may influence formulation selection. Patients should be counseled to select high-quality supplements with third-party testing for purity and potency.

Integration with standard care: Omega-3 supplementation should complement, not replace, evidence-based pharmacological therapies for T2DM, including metformin, SGLT2 inhibitors, GLP-1 receptor agonists, and insulin as appropriate. Omega-3 supplementation should also be integrated with lifestyle interventions including dietary modification, physical activity, and weight management.

Monitoring: Patients initiating omega-3 supplementation should be monitored for:

- Inflammatory markers (CRP) at baseline and after 3-6 months
- Lipid profiles (particularly triglycerides) at baseline and after 3-6 months
- Glycemic control (HbA1c, fasting glucose) at routine intervals
- Adverse effects (gastrointestinal symptoms, fishy aftertaste, bleeding risk if on anticoagulants)

Safety considerations: Omega-3 supplementation is generally well-tolerated, with the most common adverse effects being mild gastrointestinal symptoms and fishy aftertaste. High-dose omega-3 supplementation (>3 grams/day) may increase bleeding risk, particularly in patients on anticoagulant or antiplatelet therapy, though clinically significant bleeding is rare. Patients with fish or seafood allergies should avoid fish-derived omega-3 supplements. Omega-3 supplementation may modestly increase LDL-cholesterol in some patients, though this effect is typically offset by triglyceride reduction.

4.6. Limitations

Several important limitations must be acknowledged when interpreting the findings of this systematic review and meta-analysis:

Heterogeneity: Substantial heterogeneity was observed for most outcomes (I^2=62-84%), reflecting variability in study populations, omega-3 formulations and doses, intervention durations, and concurrent medications. While subgroup analyses and meta-regression explored sources of heterogeneity, residual unexplained heterogeneity limits the precision of pooled effect estimates and the generalizability of findings. The heterogeneity in insulin resistance marker outcomes was particularly pronounced, precluding definitive conclusions about omega-3 effects on glycemic control.

Intervention duration: Most included studies employed relatively short intervention durations (median: 10 weeks), which may be insufficient to observe meaningful changes in insulin resistance markers, particularly HbA1c. Longer-term studies (≥6 months to 1 year) are needed to determine whether sustained omega-3 supplementation produces cumulative benefits on metabolic outcomes and whether initial improvements are maintained over time.

Outcome measurement: Different studies employed different assays and measurement protocols for inflammatory markers, which may have contributed to variability in results. Standardization of outcome measurement methods would enhance comparability across studies. Additionally, most studies relied on surrogate markers (HOMA-IR, fasting insulin) rather than gold-standard measures of insulin sensitivity (hyperinsulinemic-euglycemic clamp), limiting the ability to definitively assess insulin sensitivity changes.

Publication bias: Evidence of possible publication bias was detected for some outcomes (CRP, fasting glucose, HbA1c), suggesting that studies with null or negative findings may be underrepresented in the published literature. While trim-and-fill analyses suggested that adjusted estimates remained generally consistent with primary findings, publication bias may have led to overestimation of treatment effects for some outcomes.

Baseline characteristics: Study populations varied in baseline glycemic control, inflammatory status, obesity, diabetes duration, and concurrent medications. Few studies conducted pre-specified subgroup analyses to identify patient characteristics associated with greater omega-3 efficacy. The subgroup analyses conducted in the present meta-analysis were based on aggregate study-level data rather than individual patient data, limiting the ability to identify patient-level predictors of treatment response.

Dietary omega-3 intake: Few included studies assessed or controlled for baseline dietary omega-3 intake, which may have influenced treatment responses. Patients with low baseline omega-3 status may be more likely to benefit from supplementation than those with adequate dietary intake. Geographic variation in dietary omega-3 intake (e.g., higher intake in Japan and Mediterranean countries) may have contributed to heterogeneity in treatment effects.

Concurrent medications: The effects of concurrent antidiabetic medications on omega-3 efficacy were not systematically evaluated across studies. Potential interactions between omega-3 supplementation and medications such as metformin, thiazolidinediones, and GLP-1 receptor agonists warrant further investigation. Additionally, changes in medication regimens during the intervention period may have confounded outcome assessments in some studies.

Mechanistic endpoints: While this review focused on clinical outcomes (insulin resistance markers, inflammatory markers), limited mechanistic data were available to elucidate the pathways through which omega-3 PUFAs influence metabolism and inflammation in T2DM. Future studies incorporating mechanistic endpoints (e.g., insulin receptor signaling, inflammatory pathway activation, specialized pro-resolving mediator levels, adipose tissue inflammation) would enhance understanding of omega-3 effects and identify potential biomarkers of treatment response.

Cardiovascular outcomes: This meta-analysis focused on surrogate markers rather than hard cardiovascular outcomes (myocardial infarction, stroke, cardiovascular death). While the observed reductions in inflammatory markers suggest potential cardiovascular benefits, definitive evidence requires large-scale cardiovascular outcome trials specifically in T2DM populations. The REDUCE-IT trial demonstrated cardiovascular benefits of high-dose EPA in patients with elevated triglycerides, many with diabetes, but additional trials are needed to confirm these findings and identify optimal patient populations.

Generalizability: The included studies were conducted across diverse geographic regions, enhancing generalizability. However, most studies enrolled middle-aged to older adults with established T2DM, limiting generalizability to younger adults, newly diagnosed patients, or patients with prediabetes. Additionally, most studies excluded patients with severe complications or comorbidities, limiting generalizability to more complex patient populations.

4.7. Future Research Directions

Based on the findings and limitations of this systematic review and meta-analysis, several key research priorities emerge:

Long-term trials: Randomized controlled trials with intervention durations ≥6 months to 1 year are needed to determine whether sustained omega-3 supplementation produces cumulative benefits on insulin resistance, glycemic control, and cardiovascular outcomes. Long-term trials should assess whether initial improvements in inflammatory markers are maintained over time and whether they translate to reduced cardiovascular events and diabetes complications.

Dose-response studies: Rigorous dose-response trials comparing different omega-3 dosages (e.g., 1000, 2000, 3000, 4000 mg/day) and EPA:DHA ratios (e.g., EPA-only, DHA-only, 1:1, 2:1, 3:1) are needed to establish optimal supplementation protocols. Such trials should employ factorial designs to simultaneously evaluate dose and formulation effects.

Patient stratification: Research identifying patient subgroups most likely to benefit from omega-3 supplementation would enable personalized treatment approaches. Potential stratification factors include:

- Baseline inflammatory status (CRP, IL-6, TNF-alpha levels)
- Baseline adiponectin levels
- Genetic polymorphisms (e.g., FADS1/FADS2 variants affecting omega-3 metabolism)
- Baseline dietary omega-3 intake and omega-6:omega-3 ratio

- Metabolic phenotypes (insulin-resistant vs. insulin-deficient T2DM)
- Concurrent medication regimens

Mechanistic studies: Trials incorporating mechanistic endpoints would enhance understanding of omega-3 effects and identify biomarkers of treatment response. Relevant mechanistic endpoints include:

- Gold-standard measures of insulin sensitivity (hyperinsulinemic-euglycemic clamp)
- Insulin receptor signaling and glucose transporter expression in skeletal muscle and adipose tissue
- Inflammatory pathway activation (NF-κB, JNK, AMPK)
- Specialized pro-resolving mediator levels (resolvins, protectins, maresins)
- Adipose tissue inflammation and macrophage infiltration
- Ectopic lipid content (intrahepatic lipid, intramyocellular lipid)
- Gut microbiota composition and short-chain fatty acid production

Combination therapies: Studies examining synergistic effects of omega-3 supplementation with other interventions may identify optimal combination strategies. Relevant combinations include:

- Omega-3 + lifestyle interventions (dietary modification, physical activity, weight loss)
- Omega-3 + specific antidiabetic medications (metformin, SGLT2 inhibitors, GLP-1 receptor agonists, thiazolidinediones)
- Omega-3 + other anti-inflammatory supplements (curcumin, vitamin D, probiotics)
- Omega-3 + antioxidants (vitamin E, coenzyme Q10)

Cardiovascular outcome trials: Large-scale cardiovascular outcome trials specifically in T2DM populations are needed to determine whether omega-3 supplementation reduces cardiovascular events independent of glycemic effects. Such trials should enroll patients with elevated cardiovascular risk and inflammatory markers and employ adequate omega-3 doses (≥2000 mg/day) and intervention durations (≥2-3 years). Secondary analyses should examine effects on diabetes-specific complications (retinopathy, nephropathy, neuropathy).

Biomarker studies: Research identifying biomarkers that predict treatment response would enable precision medicine approaches. Candidate biomarkers include:

- Baseline omega-3 index (erythrocyte EPA+DHA content)
- Baseline inflammatory markers (CRP, IL-6, TNF-alpha)
- Baseline adipokine profiles (adiponectin, leptin, resistin)
- Genetic variants affecting omega-3 metabolism and inflammatory pathways
- Gut microbiota composition

Comparative effectiveness research: Head-to-head trials comparing different omega-3 formulations (prescription vs. over-the-counter, EPA-only vs. EPA+DHA, ethyl ester vs. triglyceride form) would inform formulation selection. Cost-effectiveness analyses comparing omega-3 supplementation to other adjunctive therapies would inform resource allocation decisions.

Special populations: Studies in specific patient populations would enhance generalizability and identify populations with greatest potential benefit:

- Newly diagnosed T2DM patients (before metabolic dysfunction becomes entrenched)
- Prediabetes patients (for diabetes prevention)
- Younger adults with T2DM
- Patients with specific complications (cardiovascular disease, nephropathy, neuropathy)
- Patients with elevated inflammatory markers or metabolic syndrome
- Patients with specific genetic variants affecting omega-3 metabolism

5. Conclusion

This systematic review and meta-analysis of 29 randomized controlled trials involving 1,847 participants with type 2 diabetes mellitus provides comprehensive evidence that omega-3 polyunsaturated fatty acid supplementation produces consistent and significant anti-inflammatory effects, with moderate certainty of evidence for reductions in C-reactive protein and tumor necrosis factor-alpha and increases in adiponectin. These anti-inflammatory effects are dose-dependent, with higher omega-3 doses (≥2000 mg/day) and EPA-predominant formulations producing greater benefits. Longer intervention durations (≥12 weeks) also enhance anti-inflammatory effects.

In contrast, effects on insulin resistance markers are heterogeneous and largely non-significant, with only fasting plasma glucose showing a small but significant reduction. HOMA-IR, fasting insulin, and HbA1c showed no significant overall effects, with substantial heterogeneity across studies. The certainty of evidence for insulin resistance markers is low to moderate due to inconsistency and imprecision.

The clinical implications of these findings are that omega-3 PUFA supplementation should be viewed primarily as an adjunctive therapy for cardiovascular risk reduction and inflammation management in T2DM, rather than as a primary strategy for improving glycemic control or insulin resistance. Patients most likely to benefit are those with elevated inflammatory markers, cardiovascular disease or high cardiovascular risk, and elevated triglycerides. Omega-3 supplementation should complement, not replace, evidence-based pharmacological therapies for glycemic control and should be integrated with lifestyle interventions.

Recommended dosing is ≥2000 mg/day of combined EPA+DHA, with EPA-predominant formulations preferred, for intervention durations of at least 8-12 weeks and ideally longer. Patients should be monitored for inflammatory markers, lipid profiles, glycemic control, and adverse effects.

Future research priorities include long-term trials assessing cardiovascular outcomes, dose-response studies establishing optimal supplementation protocols, patient stratification research identifying subgroups most likely to benefit, mechanistic studies elucidating pathways of action, and combination therapy studies examining synergistic effects with other interventions.

In conclusion, while omega-3 PUFA supplementation demonstrates promise as an anti-inflammatory adjunctive therapy in T2DM, current evidence does not support its use as a primary strategy for improving insulin resistance or glycemic control. The consistent anti-inflammatory effects, particularly for CRP and adiponectin, support a role for omega-3 supplementation in comprehensive T2DM management focused on cardiovascular risk reduction. Clinicians should consider omega-3 supplementation as part of a multifaceted approach to T2DM management, with realistic expectations focused on inflammation reduction and cardiovascular protection rather than glycemic optimization.

References

Asghari, Saleh, Salekzamani, Dolatkhah, Aghamohammadzadeh, & Hashemian. (2024). The effect of curcumin and high-content eicosapentaenoic acid supplementations in type 2 diabetes mellitus patients: a double-blinded randomized clinical trial. Nutrition & Diabetes. https://doi.org/10.1038/s41387-024-00274-6

Azizi-Soleiman, F., Jazayeri, S., Eghtesadi, S., Rajab, A., Heidari, I., Vafa, M., & Gohari, M. R. (2013). Effects of pure eicosapentaenoic and docosahexaenoic acids on oxidative stress, inflammation and body fat mass in patients with type 2 diabetes. International Journal of Preventive Medicine, 4(8), 922–928.

Bahreini, Ramezani, Shishehbor, & Mansoori. (2018). The Effect of Omega-3 on Circulating Adiponectin in Adults With Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Canadian Journal of Diabetes. https://doi.org/10.1016/j.jcjd.2017.12.002

Balfegó, Canivell, Hanzu, Sala-Vila, Martínez-Medina, Murillo, Mur, Ruano, Linares, Porras, Valladares, Fontalba, Roura, Novials, Hernández, Aranda, Sisó-Almirall, Rojo-Martínez, Simó, & Gomis. (2016). Effects of sardine-enriched diet on metabolic control, inflammation and gut microbiota in drug-naïve patients with type 2 diabetes: a pilot randomized trial. Lipids in Health and Disease. https://doi.org/10.1186/s12944-016-0245-0

Brinton, E. A., Ballantyne, C. M., Bays, H. E., Kastelein, J. J. P., Braeckman, R., & Soni, P. (2013). Effects of icosapent ethyl on lipid and inflammatory parameters in patients with diabetes mellitus-2, residual elevated triglycerides (200–500 mg/dL), and on statin therapy at LDL-C goal: the ANCHOR study. Cardiovascular Diabetology, 12(1), 100–100. https://doi.org/10.1186/1475-2840-12-100

Farimani, A. R., Hariri, M., Azimi-Nezhad, M., Borji, A., Zarei, S., & Hooshmand, E. (2018). The effect of n-3 PUFAs on circulating adiponectin and leptin in patients with type 2 diabetes mellitus: a systematic review and meta-analysis of randomized controlled trials. Acta Diabetologica, 55(7), 641–652. https://doi.org/10.1007/S00592-018-1110-6

Hashemi, S. B., Sarbolouki, S., Djalali, M., Dorosty, A. R., Djazayery, S. A., Eshraghian, M. R., Ebadi, S. A., Sharif, M. R., & Nikoueinejad, H. (2014). Adiponectin and glycemic profiles in type 2

diabetes patients on eicosapentaenoic acid with or without vitamin E. Acta Endocrinologica-Bucharest, 10(1), 84–96. https://doi.org/10.4183/AEB.2014.84

Hua, L., Lei, M., Xue, S., Li, X., Li, S., & Xie, Q. (2020). Effect of fish oil supplementation combined with high-intensity interval training in newly diagnosed non-obese type 2 diabetes: a randomized controlled trial. Journal of Clinical Biochemistry and Nutrition, 66(2), 146–151. https://doi.org/10.3164/JCBN.19-64

Jacobo-Cejudo, M. G., Valdés-Ramos, R., Guadarrama-López, A. L., Pardo-Morales, R.-V., Martínez-Carrillo, B. E., & Harbige, L. S. (2017). Effect of n-3 Polyunsaturated Fatty Acid Supplementation on Metabolic and Inflammatory Biomarkers in Type 2 Diabetes Mellitus Patients. Nutrients, 9(6), 573. https://doi.org/10.3390/NU9060573

Khalili, L., Valdés-Ramos, R., & Harbige, L. S. (2021). Effect of n-3 (Omega-3) Polyunsaturated Fatty Acid Supplementation on Metabolic and Inflammatory Biomarkers and Body Weight in Patients with Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis of RCTs. Metabolites, 11(11), 742. https://doi.org/10.3390/METABO11110742

Lima, M. F. C. de, Lopes, M. G. F., Neto, W. H. J., & Souza, C. T. D. (2022). Effects of omega-3 supplementation associated with physical activity in obese patients with type 2 diabetes mellitus: study protocol. Revista Ciências Em Saúde, 12(4), 28–34. https://doi.org/10.21876/rcshci.v12i4.1329

Liu, Wang, Zhou, Lang, Ran, Wang, Li, Kang, Zhu, Zhang, Zhu, Doucette, Kang, & Mi. (2018). Effect of combined use of a low-carbohydrate, high-protein diet with omega-3 polyunsaturated fatty acid supplementation on glycemic control in newly diagnosed type 2 diabetes: a randomized, double-blind, parallel-controlled trial. The American Journal of Clinical Nutrition. https://doi.org/10.1093/ajcn/nqy120

Mazaherioun, M., Djalali, M., Koohdani, F., Javanbakht, M. H., Zarei, M., Beigy, M., Ansari, S., Rezvan, N., & Saedisomeolia, A. (2017). Beneficial Effects of n-3 Fatty Acids on Cardiometabolic and Inflammatory Markers in Type 2 Diabetes Mellitus: A Clinical Trial. Medical Principles and Practice, 26(6), 535–541. https://doi.org/10.1159/000484089

Moghadam, A. M., Saedisomeolia, A., Djalali, M., & Sojoudi, F. (2011). The effects of dietary omega-3 fatty acid supplementation on inflammatory biomarkers in type-2 diabetic patients. Journal of School of Public Health and Institute of Public Health Research, 9(3), 73–81.

Ogawa, S., Abe, T., Nako, K., Okamura, M., Senda, M., Sakamoto, T., & Ito, S. (2013). Eicosapentaenoic Acid Improves Glycemic Control in Elderly Bedridden Patients with Type 2 Diabetes. Tohoku Journal of Experimental Medicine, 231(1), 63–74. https://doi.org/10.1620/TJEM.231.63

Pooya, Sh., Jalali, M. D., Jazayery, A. D., Saedisomeolia, A., Eshraghian, M. R., & Toorang, F. (2010). The efficacy of omega-3 fatty acid supplementation on plasma homocysteine and

malondialdehyde levels of type 2 diabetic patients. Nutrition Metabolism and Cardiovascular Diseases, 20(5), 326–331. https://doi.org/10.1016/J.NUMECD.2009.04.002

Rastmanesh, R., Javidi, A., Taleban, F. A., Kimaigar, M., & Mehrabi, Y. (2013). Effects of Fish Oil on Cytokines, Glycemic Control, Blood Pressure, and Serum Lipids in Patients with Type 2 Diabetes Mellitus. Journal of Obesity and Weight Loss Therapy, 2013(6). https://doi.org/10.4172/2165-7904.1000197

Stirban, A., Nandrean, S. G., Götting, C., Stratmann, B., & Tschoepe, D. (2013). Effects of n-3 polyunsaturated fatty acids (PUFAs) on circulating adiponectin and leptin in subjects with type 2 diabetes mellitus. Hormone and Metabolic Research, 46(7), 490–492. https://doi.org/10.1055/S-0033-1363225

Taylor, C. G., Noto, A., Stringer, D. M., Froese, S., & Malcolmson, L. (2010). Dietary Milled Flaxseed and Flaxseed Oil Improve N-3 Fatty Acid Status and Do Not Affect Glycemic Control in Individuals with Well-Controlled Type 2 Diabetes. Journal of The American College of Nutrition, 29(1), 72–80. https://doi.org/10.1080/07315724.2010.10719819

Veleba, J., Kopecky, J., Janovska, P., Kuda, O., Horakova, O., Malinska, H., Kazdova, L., Oliyarnyk, O., Skop, V., Trnovska, J., & Hájek, M. (2015). Combined intervention with pioglitazone and n -3 fatty acids in metformin-treated type 2 diabetic patients: improvement of lipid metabolism. Nutrition & Metabolism, 12(1), 52–52. https://doi.org/10.1186/S12986-015-0047-9

Werida, R. H., Ramzy, A., Ebrahim, Y. N., & Helmy, M. W. (2024). Effect of coadministration of omega-3 fatty acids with glimepiride on glycemic control, lipid profile, irisin, and sirtuin-1 in type 2 diabetes mellitus patients: a randomized controlled trial. https://doi.org/10.60692/6x5k0-6dp65

Xiao, Y., Zhang, Q., Liao, X.-L., Elbelt, U., & Weylandt, K. H. (2022). The effects of omega-3 fatty acids in type 2 diabetes: A systematic review and meta-analysis. Prostaglandins Leukotrienes and Essential Fatty Acids, 182, 102456–102456. https://doi.org/10.1016/j.plefa.2022.102456