UNIVERSITÀ DEGLI STUDI DI MILANO

CORSO DI DOTTORATO INDUSTRIALE
IN INTERSECTORAL INNOVATION – DOTTORATO INTERSETTORIALE PER L'INNOVAZIONE

XXXVIII ciclo

Scienze mediche

DIPARTIMENTO DI MEDICINA VETERINARIA E SCIENZE ANIMALI

Fatty acids in physiology and pathology: focus on alpha-linolenic acid (ALA) and PUFAs

Camilla Bertoni
Matr. R14045
ORCID n.0009-0002-5310-6274

TUTOR
Prof. Carlo Virginio Agostoni

CO-TUTOR
Prof. Gregorio Paolo Milani

COORDINATORE DEL CORSO DI DOTTORATO
Prof. Ernesto Damiani

A.A. 2024/2025

INDEX

INTRODUCTION

CHAPTER 1 – Fatty Acids: Classification, Structure, and Functions

---

CHAPTER 2 – Alpha-Linolenic Acid: Biochemistry, Metabolism, and Clinical Potential

---

CHAPTER 3 – Polyunsaturated Fatty Acids in Physiology and Chronic Disorders

---

CHAPTER 4 – Clinical Study: The DANTE Project

CHAPTER 5 – Clinical Study II: Salivary Fatty Acids as Emerging Biomarkers

---

CHAPTER 6 – Nutraceutical Approaches and Translational Applications

INTRODUCTION

Clinical and scientific relevance of fatty acids: physiological and pathological aspects

Fatty acids (FAs) constitute a fundamental class of biomolecules that serve multiple roles in human biology, functioning both as a primary source of energy and as indispensable components of cellular membranes. Their structural variability, determined by chain length, saturation degree, and stereochemical configuration, underlies a broad spectrum of biological effects and physiological functions. On the basis of their chemical characteristics, FAs are typically categorized into saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs), the latter encompassing the omega-6 and the omega-3 series.

From a physiological perspective, FAs exert critical influence over membrane fluidity, intracellular signaling pathways, and the regulation of gene expression. They are also precursors of a wide variety of bioactive lipid mediators, including eicosanoids, prostaglandins and specialized pro-resolving mediators which orchestrate processes such as immune responses, vascular homeostasis, and metabolic regulation (1).

On the pathological side, unbalanced dietary intake and dysregulated FA metabolism have been consistently linked with a wide array of chronic conditions, spanning cardiovascular diseases, obesity, insulin resistance, neurodegenerative disorders, and cancer (2). For instance, excessive consumption of SFAs has long been associated with atherogenic processes, whereas a dietary profile richer in MUFAs and PUFAs is often correlated with improved cardiometabolic outcomes (3).

Despite extensive research, significant areas of uncertainty persist. The optimal ratio of dietary omega-3 to omega-6 FAs remains debated, as do sex-specific differences in FA metabolism and the context-dependent roles of these molecules in modulating inflammation and oxidative stress. These open questions highlight the importance of further investigation, especially in physiologically sensitive contexts such as reproduction, pregnancy, aging, and neurodevelopment (4).

Focus on essential fatty acids: Alpha-linolenic acid and linoleic acid

Within the PUFA family, alpha-linolenic acid (ALA) and linoleic acid (LA) occupy a particularly important position as they are considered essential fatty acids (EFAs). Since the human body lacks the enzymatic machinery to synthesize them *de novo*, both ALA and LA must be obtained through dietary sources (5).

LA is the most abundant omega-6 PUFA in Western diets, primarily derived from common vegetable oils. A substantial body of evidence associates dietary LA with reductions in LDL cholesterol and lower risk of coronary heart disease (6). Nonetheless, there is growing concern that excessive LA intake may promote adverse biological processes, including oxidative stress, adipose tissue expansion, and chronic low-grade inflammation (7).

Conversely, ALA represents the main plant-derived omega-3 PUFA and has been associated with a wide range of protective effects. Evidence supports its potential in reducing cardiovascular risk, modulating inflammatory processes, and even exerting neuroprotective actions (8). Interestingly, emerging research indicates sex-specific differences in ALA metabolism, with women demonstrating higher

conversion rates to Docosaesaenoic acid (DHA), likely an evolutionary adaptation linked to reproductive demands (9).

Given their central role at the interface of diet, metabolism, and signaling, both ALA and LA are gaining increasing attention in new clinical contexts. Preliminary evidence suggests potential involvement in fertility and assisted reproductive technologies (ART), IN pregnancy outcomes and fetal development, in the prevention of sarcopenia, and in neurodevelopmental conditions such as ADHD (10-12). However, mechanistic insights are still scarce, and translational evidence remains limited, leaving a significant gap that this doctoral thesis aims to address.

Aims

The overarching aim of this research is to deepen understanding of PUFAS, focusing on ALA and LA, withing emerging clinical domains: ART, pregnancy, sarcopenia, and ADHD.

The work is structured around several objectives: i) to elucidate the physiological roles of PUFAs across these contexts, clarifying beneficial versus potentially harmful effects depending on intake levels, metabolic state, and timing; ii) to investigate the efficiency of ALA conversion into long-chain omega-3 PUFAs across sex and life stages, with particular attention to pregnancy and ART, thereby informing dietary strategies; iii) to explore the neuroprotective potential of PUFAs during perinatal brain development and in relation to ADHD pathophysiology; iv) to evaluate the involvement of PUFAs in oxidative and metabolic processes relevant to sarcopenia and reproductive health, particularly through their interaction with lipid signaling pathways, and redox balance; v) to bridge translational gaps by integrating findings from experimental, epidemiological, and clinical studies, with the aim of shaping future therapeutic and nutritional interventions.

Through this approach, the thesis intends not only to strengthen the scientific basis concerning ALA and LA but also to provide novel insights for procession nutrition and targeted therapeutic strategies, with a strong emphasis on clinical applicability and innovation.

CHAPTER 1:

Fatty acids: classification, structure and functions

1.0 Definition and classification

FAs are among the most extensively studied biomolecules in human biochemistry, owing to their indispensable role in energy metabolism, the preservation of cellular membrane architecture, and the regulation of signaling pathways that influence inflammation, immune responses, gene expression, and neurodevelopment. Structurally, they are carboxylic acids with an aliphatic chain of variable length that may be either saturated or contain one or more double bonds, features that determine their classification and biological function (13).

From a biochemical perspective, FAs are classified into three main groups:

- SFAs: containing no double bonds
- MUFAs: containing a single double bond
- PUFAs: containing two or more double bonds.

A key distinction lies in whether FAs can be synthesized endogenously or must be obtained from the diet. The latter are referred to as EFAs. The two major EFAs are ALA (18:3 n-3) and LA (8:2 n-6), which act as precursors for the biosynthesis of long-chain omega-3 and omega-6 FAs, such as Eicosapentaenoic Acid (EPA), DHA, and Arachidonic Acid (AA) (14).

Physiologically, SFAs are associated with energy storage and membrane rigidity, while MUFAs, like Oleic Acid (OA), are linked to improved lipid profiles and a reduction in cardiovascular risk. PUFAs exert more complex effects depending on their series. Omega-3 PUFAs, particularly EPA and DHA, exhibit anti-inflammatory, antithrombotic, cardioprotective, neuroprotective, and immunomodulatory properties (15). Omega-6 PUFAs, like AA, are crucial for immune defense and cellular signaling, although an excess relative to omega-3 intake may promote pro-inflammatory states.

1.1 Focus on precursors: Alpha-Linolenic Acid and Linoleic Acid

Within the PUFAs category, ALA and LA are of particular significance, acting as indispensable precursors for the synthesis of long-chain derivatives involved in cellular physiology, development, and disease modulation.

On the one hand, LA is the primary omega-6 fatty acid, abundant in vegetable oils such as sunflower, corn, soybean, and safflower. LA is metabolized into AA, which becomes incorporated into cell membranes. Upon activation, aa is released and converted into eicosanoids, including prostaglandins, thromboxanes, and leukotrienes, potent signaling molecules that regulated vascular tone, inflammation, and immune responses.

On the other hand, ALA is the parent compound of the omega-3 PUFA family. It is predominantly found in plant sources such as flaxseed, chia seeds, walnuts, and canola oil. Through a series of desaturation and elongation reactions, ALA can be converted into EPA and subsequently DHA, two different long-chain omega-3 fatty acids associated with anti-inflammatory, cardioprotective, neuroprotective, and immunomodulatory effects.

However, the efficiency of this metabolic conversion from ALA to EPA and DHA in humans is relatively low and highly variable. Typically, less than 10% of dietary ALA is converted into EPA, and less than 0.5-1% is converted to DHA, with wide interindividual variation depending on sex, hormonal status, age, genetic polymorphisms (e.g., FADS1/FADS2), and the ratio of omega-6/omega-3 intake in the diet (16). For example, women of reproductive age trend to have higher conversion rates, potentially due to estrogen-mediated upregulation of desaturase activity.

Because both LA and ALA compete for the Δ6-desaturase enzyme, a high dietary intake of LA can inhibit ALA conversion, limiting the endogenous availability of EPA and DHA. Thus, an appropriate dietary balance between omega-6 and omega-3 FAs is essential to prevent inflammatory dominance.

In addition to their precursor role, both ALA and ALA may have independent biological activities. For instance, ALA has been reported to directly modulate cardiovascular health, inflammation, and oxidative stress, independently of its conversion to EPA and DHA (17). Similarly, LA contributes to skin barrier integrity and immune function, though excessive intake has been linked to pro-inflammatory states in some populations. (18).

Thus, understanding the dual role of ALA and LA, both as essential precursors of bioactive long-chain PUFAs and as independent modulators of physiological processes, is fundamental in nutritional biochemistry.

1.2 Metabolic and structural role: cell membranes and biochemical functions

FAs fulfill a dual role in human physiology, acting both as structural components of cell membranes and as metabolic substrates for numerous biochemical pathways. Their structural features, as chain length, saturation degree and double bond position, determine their physical properties and functional outcomes.

Structurally, FAs are esterified into phospholipids and incorporated into cell membranes, influencing membrane fluidity, permeability, and the activity of embedded proteins such as receptors, ion channels, and transporters. SFAs, with straight chains, tend to pack tightly and confer rigidity, while MUFAs and PUFAs introduce links via cis double bonds, enhancing fluidity and adaptability. (19).

PUFAs, especially EPA, DHA, and AA, are highly enriched in neural and immune cells, where they contribute to synaptic plasticity, neuronal signaling, immune cell activation, and intracellular communication. For example, DHA is a predominant component of the phospholipids in the brain and retina, essential for normal visual and cognitive development (19, 20).

Metabolically, FAs are precursors of bioactive lipid mediators. Through enzymatic oxidation via cyclooxygenases (COX), lipoxygenases (LOX), and cytochrome P450 monooxygenases, they give rise to eicosanoids, which regulate inflammation, hemostasis, and immunity (21) and specialized pro-resolving mediators (SPMS) such as resolvins, protectins, and maresins, derived from omega-3 PUFAs, which promote the resolution of inflammation and tissue repair. (22).

Moreover, FAs modulate gene expression by acting as ligands for transcription factors, for instance retinoid X receptors (RXRs). These nuclear receptors are involved in regulating lipid metabolism, glucose homeostasis, adipogenesis, and inflammation. In energy metabolism, FAs undergo β-oxidation in mitochondria, providing a major source of ATP during fasting or prolonged exercise. Their availability and

oxidation are regulated and influence metabolic flexibility, insulin sensitivity, and body weight homeostasis (20).

Ultimately, dietary FA composition profoundly affects tissue FA profiles, with downstream consequences on membrane properties, inflammatory tone, and the risk of chronic diseases such as cardiovascular, neurodegenerative, and autoimmune disorders (20,23).

1.3 Metabolic pathways and biosynthesis: elongation and desaturation

Long-chain PUFAs are biosynthesized from the EFAs precursors ALA and LA through sequential enzymatic reactions of desaturation and elongation, primarily in the endoplasmic reticulum (24) (*Figure 1).* The first-rate limiting step involves Δ6-desaturase, which introduces a double bond into ALA and LA to introduce stearidonic acid (18:4 n-3) and γ-linolenic acid (18:3 n-6), respectively. This is followed by elongation, catalyzed by other enzymes that extend the carbon chain by two units. The subsequent Δ5-desaturation step leads to the formation of EPA and AA (25). Further, elongation and peroxisomal β-oxidation allow the final conversion to DHA (26).

Recent findings have also shown that high levels of long-chain PUFAs such as DHA and AA can exert feedback inhibition on Δ6-desaturase, further modulating the pathway (27). Moreover, emerging research has identified alternative biosynthetic contributors, such as the mitochondrial enzyme AACS, which appears to support PUFA synthesis under ketogenic conditions. Understanding these metabolic pathways is essential not only for nutritional biochemistry, but also for clinical applications, particularly in conditions where long-chain PUFAs biosynthesis is impaired or insufficient to meet physiological demands.

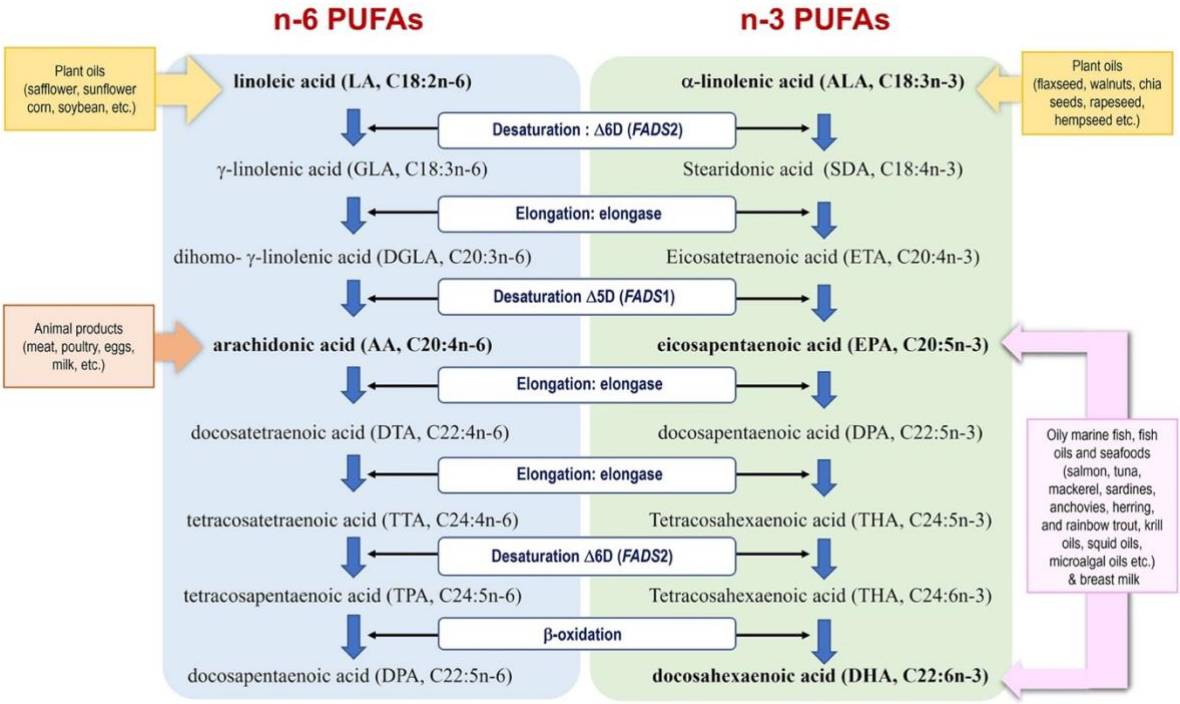


Figure 1. Biosynthetic pathways of essential fatty acids. Alpha-linolenic acid (ALA; 18:3 n-3, blue) is converted via Δ6- and Δ5-desaturases and elongases to eicosapentaenoic acid (EPA; 20:5 n-3), docosapentaenoic acid (DPA; 22:5 n-3), and docosahexaenoic acid (DHA; 22:6 n-3). Linoleic acid (LA; 18:2 n-6, red) undergoes analogous reactions to yield γ-linolenic acid (GLA; 18:3 n-6), dihomo-γ-linolenic acid (DGLA; 20:3 n-6), and arachidonic acid (AA; 20:4 n-6). Both pathways compete for Δ6-desaturase, influencing the balance between omega-3 and omega-6 long-chain PUFA synthesis.

1.4 Bioavailability and adsorption

The bioavailability of dietary FAs refers to the proportion that is digested, adsorbed, and used for physiological functions. FAs are primarily ingested as triacylglycerols (TAGs), phospholipids, or cholesteryl esters, which require enzymatic hydrolysis before adsorption. This process begins in the stomach through the action of gastric lipase but is predominantly carried out in the small intestine by pancreatic lipase, which acts in the presence of bile salts to emulsify fats and facilitate the formation of mixed micelles. These micelles enable the diffusion of free FAs and other lipophilic molecules across the unstirred water layer to the enterocyte membrane.

Once inside enterocytes, FAs are re-esterified and incorporated into chylomicrons, secreted into the lymphatic system before entering the bloodstream. Chain length and degree of unsaturation significantly affect adsorption efficiency. Short-chain and medium-chain fatty acids are adsorbed more rapidly and directly into the portal circulation, whereas long-chain PUFAs follow the chylomicron route (28).

Moreover, PUFAs, such as ALA, EPA, and DHA exhibit lower and more variable bioavailability due to their structural complexity and greater susceptibility to oxidation (29).

Food matrix, type of formulation, and co-ingested nutrients also influence bioavailability. For example, FAs in phospholipid form (e.g. krill oil) may have superior adsorption compared to those in triglyceride or ethyl ester forms (30). Additionally, individual variability, including genetic polymorphisms in lipid transporters, ages, sex, and gut microbiota composition, can modulate adsorption kinetics. Understanding these dynamics is essential for optimizing FAs delivery through dietary or supplemental means, particularly in populations with increased needs or impaired lipid digestion and adsorption.

CHAPTER 2

ALA: biochemistry, metabolism and clinical potential

2.0 Structural characteristics and dietary sources

ALA (18:3 n-3) is an essential PUFA, with an 18-carbon chain with three cis double bonds, the first of which is located at the third carbon from the methyl (omega) end. This structural feature classifies ALA as a member of the omega-3 family of FAs. In the human physiology, ALA serves multiple roles: it provides energy through β-oxidation, contributes to adipose tissue stores, and is incorporated in significant amounts into phospholipid bilayers, thereby influencing membrane fluidity and cellular signaling processes.

Dietary ALA is primarily derived from plant sources, especially oils and seeds; among these, flaxseed oil contains the highest concentration of ALA, accounting for approximately 50-60% of its total FAs content (31). Other notable contributors include chia seeds, walnuts, canola oil, and perilla oil, whereas smaller but relevant amounts are also present in green leafy vegetables and soy-derived products. Nuts and seeds not only provide ALA but also deliver complementary bioactive nutrients such as plant sterols, dietary fibers, and polyphenolic compounds, which together exert protective effects against cardiovascular, inflammatory, and chronic degenerative conditions (32-35).

Despite its wide availability, the efficiency of ALA bioconversion into EPA and DHA in humans is relatively limited. Therefore, ensuring adequate dietary intake is critical. Current recommendations indicate an intake of approximately 1.1 g/day for women and 1.6 g/day for men (36). The European Food Safety Authority (EFSA) further specifies reference values of 2–3 g/day of ALA, corresponding to about 1% of total energy intake in an average diet of 1800–2700 kcal/day (37,38).

Importantly, the high prevalence of omega-6 FAs, particularly LA, in modern Western diets may further hinder the conversion of ALA into longer-chain derivatives by competing for the same enzymatic machinery. This emphasizes not only the absolute intake but also the balance between omega-6 and omega-3 FAs as a crucial determinant of health outcomes.

2.1 Metabolism and factors influencing ALA conversion and its limitations

Once ingested, ALA undergoes a sequence of enzymatic transformations in the liver and other peripheral tissues, leading to the biosynthesis of long-chain omega-3 PUFAs EPA and DHA. These reactions involve alternating steps of desaturation and elongation, catalyzed predominantly by Δ6-desaturase, elongase, and Δ5-desaturase enzymes. Although this pathway is biochemically well defined, the overall efficiency of conversion in humans remains low, with estimates suggesting that only a small fraction of dietary ALA is converted into EPA, and an even smaller amount into DHA.

Conversion efficiency is influenced by both intrinsic and extrinsic factors. One of the primary intrinsic determinants is sex: women, particularly during the reproductive years, exhibit higher conversion rates than men. This phenomenon is attributed to the estrogen-mediated upregulation of Δ6-desaturase activity, enhancing the metabolic flux toward long-chain PUFA synthesis (39). Age also plays a role, with aging associated with a decline in enzymatic efficiency (40).

Genetic variability also plays a crucial role. Polymorphisms in the *FADS1* and *FADS2* genes, which encode the desaturase enzymes central to the pathway, are associated with substantial interindividual differences in conversion capacity. Carriers of minor allelic variants may show markedly reduced ability to synthesize long-chain PUFAs from dietary ALA, with potential implications for developmental and metabolic health (41,42).

Among extrinsic factors, dietary composition is particularly important. A high intake of LA, the predominant omega-6 fatty acid in Western diet, directly competes with ALA for Δ6-desaturase. This competition results in decreased flux toward omega-3 long-chain PUFA synthesis and contributes to the unfavorable omega-6/omega-3 ratio characteristic of many modern dietary patterns (39). Consequently, even when ALA intake is sufficient, excessive consumption of omega-6 fatty acids can substantially blunt its metabolic conversion.

In addition, metabolic conditions such as obesity, insulin resistance, and chronic low-grade inflammation are associated with impaired desaturase activity and reduced conversion efficiency. These conditions may alter enzymatic regulation and lipid metabolism, thereby limiting the synthesis of DHA and EPA from ALA and reducing the bioavailability of their anti-inflammatory and cardioprotective benefits (43).

Taken together, these intrinsic and extrinsic modulators underscore the metabolic limitations of ALA and highlight the clinical relevance of dietary strategies that ensure an adequate balance of omega-3 and omega-6 FAs, particularly in populations at risk of reduced conversion.

2.2 Physiological effects

ALA exerts multiple physiological effects through both its direct biological activities and its role as a precursor to long-chain PUFAs (*Figure 2*). One of its most widely recognized properties is its anti-inflammatory action. Mechanistically, ALA modulates the activity of nuclear transcription factors such as NF-kB, thereby reducing the expression of pro-inflammatory cytokines including TNF- α, IL-1β, and IL-6 (39,44). Furthermore, ALA competes with AA for COX and LOX enzymes, leading to the formation of less pro-inflammatory eicosanoids and specialized pro-resolving mediators that contribute to inflammation resolution (15).

In lipid metabolism, ALA exhibits hypocholesterolemic effects, with evidence supporting its ability to lower total cholesterol (TC) and LDL-C, while raising high-density lipoprotein cholesterol (HDL-C). These effects may be mediated by activation of peroxisome proliferator-activated receptors (PPARs) and upregulation of hepatic LDL receptor expression, thereby enhancing clearance of circulating lipoproteins (45).

Neuroprotective actions of ALA are attributed both to its conversion into DHA, a critical structural component of neuronal membranes that supports synaptic plasticity and neurogenesis, and to its intrinsic antioxidant capacity. ALA supplementation has been shown to reduce oxidative stress markers, attenuate neuroinflammation, and improve cognitive performance in both preclinical and clinical studies (46). Supplementation with ALA has been associated with reduced oxidative stress markers, improved cognitive performance, and attenuation of neuroinflammation in both preclinical and clinical models.

In addition, ALA plays a hepatoprotective role. Diets enriched with ALA have been associated with reductions in hepatic lipid accumulation and improvements in insulin sensitivity. These effects are

thought to result from modulation of transcription factors involved in lipid metabolism, such as sterol regulatory element-binding protein-1c (SREBP-1c) and PPAR-α, as well as a reduction in hepatic inflammatory processes (47).

Moreover, ALA contributes to redox homeostasis. Its antioxidant activity includes enhancing endogenous defense systems and reducing lipid peroxidation, thereby protecting membrane phospholipids rich in PUFAs from oxidative damage (15).

Collectively, these pleiotropic effects underline the significance of ALA as a dietary component with cardiometabolic, neuroprotective, hepatoprotective, and anti-inflammatory properties. This aspect makes ALA a key focus in the design of preventive and therapeutic nutritional strategies aimed at reducing the burden of chronic diseases.

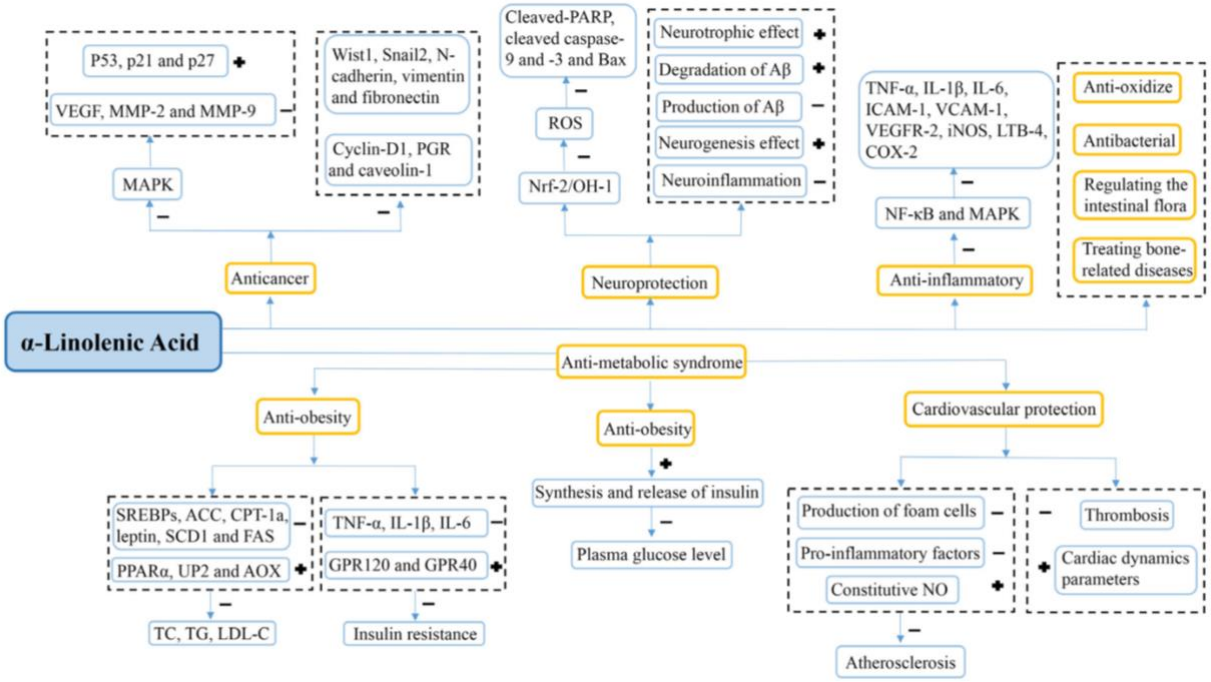


Figure 2. Major physiological effects of ALA. This schematic summarizes the primary biological effects of ALA, including the regulation of lipid metabolism, anti-inflammatory and antioxidant actions, cardiovascular protection through endothelial function improvement, and neuroprotective effects supporting cognitive function. The figure emphasizes ALA's multifaceted role in maintaining systemic homeostasis. Yuan Q. et al. The review of alpha-linolenic acid: sources, metabolism, and pharmacology. Phytother Res. 2022;36(1):164-188. doi: 10.1002/ptr.7295.

2.3 Clinical and experimental evidence: preventive and therapeutic roles of ALA

An increasing body of evidence from epidemiological studies, randomized controlled trials (RCTs), and experimental research supports the preventive and therapeutic potential of ALA across multiple chronic disease domains (*Table 1*). In the cardiovascular field, higher dietary intake of ALA has consistently been associated with a reduced incidence of cardiovascular diseases (CVDs), particularly coronary heart disease (CHD) and fatal arrhythmias. Meta-analyses and RCTs demonstrate that ALA supplementation contributes to improved lipid profiles, blood pressure reduction, preservation of endothelial function, and attenuation of systemic inflammation (48-52).

Beyond lipid modulation, ALA intake has been linked to reductions in C-reactive protein levels and improvements in arterial compliance, reinforcing its role in cardiovascular prevention (53,54).

In the context of metabolic health, both clinical and preclinical studies indicate that ALA improves insulin sensitivity and reduces markers of metabolic syndrome. These effects are thought to be mediated by modulation of adipokine secretion and activation of AMP-activated protein kinase (AMPK) signaling, which promotes lipid oxidation and glucose uptake (47,55). These findings position ALA as a promising dietary factor in the prevention and management of obesity-related disorders and non-alcoholic fatty liver disease (NAFLD).

In the neurological domain, ALA demonstrates neuroprotective properties. Observational and interventional studies suggest that ALA preserves neuronal membrane fluidity, reduces neuroinflammation, and supports synaptic plasticity by serving as a precursor to DHA. Supplementation has been linked to improvements in memory performance and cognitive outcomes, as well as attenuation of Alzheimer's disease-related pathology, including reduced amyloid-β deposition (43,56,57). These results highlight ALA's therapeutic potential in age-related cognitive decline and neurodegenerative disorders.

Evidence also supports a potential anti-cancer role for ALA. In vitro and in vivo models of colorectal, breast, and prostate cancer suggest that ALA may exert anti-proliferative effects, induce apoptosis, and suppress pro-tumorigenic pathways such as NF-κB signaling. Epidemiological data further indicate that higher ALA intake is associated with reduced all-cause, cardiovascular, and cancer-related mortality (58,59).

From a hepatological perspective, both experimental and clinical data suggest that ALA supplementation reduces hepatic steatosis, enhances fatty acid oxidation, and attenuates inflammatory responses in NAFLD and related metabolic disorders. These benefits may extend to alcohol-related liver injury, where omega-3 fatty acids, including ALA, contribute to improved liver function and reduced progression of disease (60).

Taken together, the convergence of mechanistic, clinical, and epidemiological evidence supports ALA as a multifunctional nutrient with broad preventive and therapeutic applications. While its limited bioconversion to EPA and DHA remains a constraint, direct physiological actions of ALA, combined with its widespread availability in plant-based foods, highlight its relevance in both population-level dietary guidelines and targeted interventions for chronic disease prevention and management.

Health Domain	***Physiological/Molecular effects***	***Evidence***	***References***
Cardiovascular	○ Improved lipid profile ○ Reduced BP ○ Preserved endothelial function ○ Reduced inflammation	Epidemiological studies, RCTs, meta-analyses	○ Yin et al., 2023 ○ Jaca et al. 2020 ○ Kromhout et al., 2011 ○ Simopoulos et al. 2002
Metabolic/Liver	○ Enhanced insulin sensitivity ○ AMPK activation ○ Increased lipid oxidation ○ Reduced hepatic steatosis	Clinical and pre-clinical studies	○ Gonçalves et al., 2018 ○ Han et al., 2022 ○ Wang et al., 2019
Neurological	○ Neuroprotection ○ Reduced neuroinflammation ○ Improved synaptic plasticity	Observational, interventional studies and review	○ Dyall et al., 2015 ○ Bertoni et al., 2024 ○ Lee et al., 2018
Cancer	○ Anti-proliferative effects ○ Induced apoptosis ○ NF- κB pathway suppression	In vitro, in vivo, epidemiological studies	○ Naghshi et al., 2021 ○ Murff et al., 2009

Table 1. Physiological effects and health benefits of ALA.

CHAPTER 3

PUFAs in physiology and their emerging roles in chronic disorders

Beyond their structural contribution to cellular membranes, PUFAs function as precursors of eicosanoids and specialized pro-resolving mediators, thereby exerting a profound influence on inflammation, vascular physiology, and neurobiological pathways (61).

3.0 PUFAs, ALA and mental health

Omega-3 FAs are increasingly recognized as central players in mental health, neurodevelopment, and brain function. Several neurological and psychiatric disorders, including neurodegenerative diseases, are linked to altered PUFA homeostasis. Interest in long-chain PUFAs within psychiatry stems from the fact that the brain is particularly rich in omega-3 fatty acids, mainly DHA, EPA, and ALA. Multiple studies have highlighted their neuroprotective actions, with evidence supporting their role in preserving neuronal membrane integrity and synaptic plasticity (62). In addition, PUFAs modulate neurotransmission, especially monoaminergic regulation, and influence receptor properties or receptor-mediated signaling cascades. They modulate brain cell signaling, including monoamine regulation, and are involved in modifying receptor properties or activating signal transduction by receptors (63) potentially explaining their involvement in the pathophysiology of psychiatric conditions.

A comprehensive narrative review (56) examines the influence of PUFAs, with a particular focus on ALA, on mental health outcomes across different populations, including children, adolescents, adults, older adults, pregnant and lactating women, as well as in animal models such as rats and pigs. A broad spectrum of mental health domains was considered, and most studies reported a positive association between higher PUFA and ALA intake and improvements in cognitive performance, a reduced risk of neurodegenerative disease (e.g. Alzheimer's disease), and alleviation of symptoms related to psychiatric conditions such as anxiety, depression, and bipolar disorder (BD). The selected studies were grouped into 5 categories: neurodevelopment, eating disorders, affective disorders, cognition, and psychosis.

In the domain of neurodevelopment, the majority of human studies, especially those focusing on ADHD, consistently reported that increased intake or higher circulating levels of ALA and its metabolites (EPA, DHA) correlated with improvements in attention span, executive function, fluid intelligence, and psychomotor development (64-67). The only animal study (68) demonstrated that maternal PUFA supplementation was able to enhance maternal care behaviors and positively shape offspring developmental trajectories, supporting a key role for early-life exposure.

With respect to eating disorders, research investigating PUFAs and anorexia nervosa (AN) (69,70) showed altered FAs profiles in patients, with reduced total PUFA levels, although no specific deficiency in ALA or LA was identified. These findings suggest that while PUFA metabolism may be altered in AN, n-3 and n-6 supplementation alone cannot be considered a targeted therapeutic option, although it may reflect a biomarker of metabolic imbalance.

For affective disorders, some studies addressed affective disorders (71-78). Human trials consistently demonstrated that individuals with depression exhibited lower circulating levels of ALA, AA, EPA, DHA, and total PUFAs, pointing to a deficit in both n-3 and n-6 pathways. Similar patterns were observed in anxiety-related disorders. In BD, altered ratios of FAs (AA/DHA, AA/EPA, DHA/ALA) were associated with an increased suicide risk, underscoring the potential preventive role of balanced ALA intake. In

postpartum depression (PPD), reduced ALA intake was consistently linked to higher disease incidence. Interestingly, some animal studies yielded contrasting results, with certain plant-based diets high in ALA correlating with increased anxiety in pigs, highlighting species-specific or context-dependent effects.

Within the cognition domain, a number of influential contributions (79-86) investigated outcomes such as memory, verbal fluency, risk of dementia, and neurodegenerative disease progression. Higher ALA intake was strongly associated with improved cognitive function and lower risk of Alzheimer's disease and dementia. In addition, protective roles were also attributed to other fatty acids, including LA and OA, suggesting that multiple PUFAs may synergistically contribute to brain health.

In the phychosis category, studies by Amminger, Jones, Rice, and Rog (87-90) focused on schizophrenia (SCZ) and high-risk adolescents. Across all studies, patients consistently presented with reduced levels of EPA, ALA, and omega-6 FAs, supporting the hypothesis that insufficient PUFA availability may play a role in the onset of psychotic disorders. These findings reinforce the potential of ALA and its metabolites as preventive factors or early biomarkers in populations at risk.

Taken together, the evidence suggests that reduced PUFA availability, particularly low levels of ALA, DHA, and EPA compared to dietary recommendations, is a common feature in individuals with psychiatric disorders or at elevated risk. Thus, current literature substantiates a strong positive association between adequate ALA intake and the prevention or amelioration of mental health disturbances, while also pointing to potential therapeutic implications.

International Journal of Molecular Sciences

MDPI

Review

The role of alpha-linolenic acid and other polyunsaturated fatty acids in mental health: a narrative review

Camilla Bertoni[1], Cecilia Pini [2], Alessandra Mazzocchi[3], Carlo Agostoni[3,4,*] e Paolo Brambilla[5,2]

[1] Department of Veterinary Sciences for Health, Animal Production and Food Safety, University of Milan, 20122 Milan, Italy; camilla.bertoni@unimi.it (C.B.)
[2] Department of Pathophysiology and Transplantation, University of Milan, 20122 Milan, Italy; cecilia.pini@unimi.it (C.P)
[3] Department of Clinical Sciences and Community Health, University of Milan, 20122 Milan, Italy; alessandra.mazzocchi@unimi.it (A.M.)
[4] Pediatric Area, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy
[5] Department of Neurosciences and Mental Health, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy; paolo.brambilla1@unimi.it
* Correspondence: carlo.agostoni@unimi.it; Tel.: +39-02-5503-2497

1. *Introduction*

Omega-3 fatty acids, particularly alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), play critical roles in mental health, neurodevelopment, brain functions, and behavior. ALA is considered an essential fatty acid because humans lack the enzymes (Δ12 and Δ15 desaturase) needed to synthesize it, meaning it must be acquired directly from dietary sources such as walnuts, chia seeds, flax seeds, and hemp seeds and oil [1,2]. Once in the body, ALA undergoes conversion to longer-chain fatty acids with 20 or 22 carbon atoms through cellular desaturation and elongation processes. Specifically, in the endoplasmic reticulum, ALA is converted to C18:4 ω-3 by the enzyme Δ6 desaturase. Next, two carbons are added by elongase, followed by desaturation at the Δ5 position by Δ5 desaturase, resulting in C20:5 ω-3 (EPA). Further elongation produces C22:5 ω-3, and finally, peroxisomal β-oxidation completes the synthesis of DHA [3]. In addition to endogenous synthesis, EPA and DHA can also be obtained directly from the diet, especially from fatty fish and fish oils. ALA is metabolized in vivo and partially converted to EPA and DHA. Alter-natively, EPA and DHA can also be obtained by consuming fish oil [4]. ALA contributes to brain function and protection without adverse effects [5,6] and supports the proper development of the central nervous system, particularly in childhood [7]. Imbalances in polyunsaturated fatty acids (PUFAs) are implicated in various central nervous system diseases, including neurodegenerative disorders, due to the brain's reliance on PUFAs like DHA, EPA, and ALA to maintain structural integrity and function [8]. Re-search on dietary PUFA incorporation, particularly DHA, shows that PUFAs in the brain begin accumulating in utero and continue into early childhood, maintaining high concentrations throughout life to support synaptic function and neuronal resilience [9,10]. As omega-3 and omega-6 PUFAs are integral to biological processes like metabolism, neurotransmission, synaptogenesis, and inflammation, their role in mental health throughout the lifespan continues to be a subject of intensive study [11,12,13].

Considering PUFAs from a lifespan perspective and their potential role in the development of mental disorders, it is important to note that early life is marked by an increased demand for PUFAs, particularly DHA, for optimal brain development and function. Adequate levels of DHA and arachidonic acid (AA), supplied via the mother through the amniotic fluid in utero and breast milk post-birth, are crucial for the neurological and myelination processes that are necessary for fetal and infant development [14,15]. Deficiencies in omega-3 PUFAs, particularly DHA, have been associated with neurodevelopmental disorders such as attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD), which typically manifest in childhood and can lead to long-term cognitive and behavioral challenges [16,17]. Studies have shown that omega-3 supplementation, particularly DHA and EPA, may improve symptoms in children with ADHD, indicating the importance of PUFA balance in early

neurodevelopment [18]. Young adulthood sees an increased incidence of mood disorders, such as major depressive disorder (MDD), and psychotic disorders, including schizophrenia (SCZ) [19]. Deficits in omega-3 PUFAs are commonly found in patients with these conditions, with studies showing that first-episode, drug-naïve SCZ patients have significant abnormalities in the fatty acid composition of peripheral tissues com-pared to controls [18].

These abnormalities are partially normalized with antipsychotic treatment, suggesting a link between PUFA levels and the onset of psychiatric symptoms. Omega-3 PUFAs, particularly EPA, are believed to modulate brain cell signaling pathways, including monoamine regulation, receptor properties, and signal transduction, all of which may contribute to mood stabilization and symptom improvement in MDD and SCZ [8,20,21]. In middle and older adulthood, the prevalence of neurodegenerative conditions, such as Alzheimer's disease (AD) and dementia, rises significantly. DHA is a primary component of neuronal membranes in regions critical to memory and cognition, such as the hippocampus and cortex, and low levels of DHA are associated with an increased risk of cognitive decline [16,22]. Omega-3 supplementation has shown promise in delaying cognitive decline and neurodegeneration, potentially due to its anti-inflammatory and antioxidative properties, as well as its role in neurogenesis and brain-derived neurotrophic factor (BDNF) enhancement [23,24]. Furthermore, animal studies indicate that ALA influences hippocampal and cortical health by modulating synaptic transmission, inhibiting glutamatergic transmission, and activating potassium channels, which collectively support neurogenesis and neuronal preservation [9,25,26,27].

Based on these considerations, this review aims to explore the correlation between omega-3 PUFA supplementation, particularly ALA, and mental health across different stages of life. By focusing on age-specific psychiatric disorders, we seek to clarify the potential of PUFAs in preventing and managing these conditions, examining how omega-3 supplementation may impact mental health outcomes from childhood through late adulthood.

2. *Results*

In the following section, we will examine the primary clinical evidence across key mental health disorders, structured according to the likely age of onset from a lifespan perspective. This includes neurodevelopmental disorders, mood disorders, depression and psychosis, eating disorders, and cognitive disorders, with a particular emphasis on neurodegenerative disease prevention. Table 1 provides an overview of all the studies in humans included in the Results section, detailing the study population, area of investigation, dietary interventions, and conclusions.

2.1. Neurodevelopmental Disorders

Neurodevelopmental disorders are a group of conditions that manifest early in development, often before school age, and impact cognitive, motor, social, and emotional functioning [28]. These disorders, which include attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorder (ASD), intellectual disabilities, and learning disorders, are characterized by delays or abnormalities in brain development that affect daily functioning and adaptability. Genetic, environmental, and epigenetic factors interact in complex ways to influence neurodevelopment, with a critical period for brain growth occurring in utero and extending through early childhood [29]. The research has increasingly identified links between neurodevelopmental disorders and metabolic imbalances, including imbalances in

polyunsaturated fatty acids (PUFAs), particularly omega-3 and omega-6 fatty acids, which are crucial for brain development and function [30]. This review examines the association between omega-3 intake and improvements in ADHD-related symptoms, impulsivity, and motor and psychomotor development. A prospective cohort study by Gustafsson et al. [31] showed that, in a group of 68 pregnant women, the n-6/n-3 ($p = 0.04$) and AA/EPA ratios ($p = 0.007$) were significantly higher in those experiencing heightened ADHD symptoms, while the EPA levels were lower ($\beta = -0.24$, $p = 0.017$). In contrast, no reliable differences were found in the plasma concentrations of dietary ALA. The randomized intervention, two-arm, controlled trial of Pinar-Martì, Gignac, et al. [32] showed that ALA intake can improve sustained attention (score: −11.26 ms; 95% CI = −19.92, −2.60; $p = 0.011$), fluid intelligence (score: 1.78; 95% CI = 0.90, 2.67; $p < 0.0001$), and ADHD symptoms (score: −2.18; 95% CI = −3.70, −0.67; $p = 0.0050$) in 771 adolescents with an average age of 13.9 who adhered to the walnut intake intervention (30 g/day).

The association between ALA and attention in healthy adolescents has also been investigated [33]. Interestingly, in contrast to DHA, a higher intake of ALA was not associated with better attentional performance, although a higher amount of ALA in red blood cells seemed to have a positive effect on impulsivity. Nishi et al. [34] observed a direct correlation between ALA-rich walnut consumption and improved cognitive reaction time and attentional function, while another cross-sectional study on adolescents [32] found an association between increased erythrocyte ALA levels and reduced impulsivity, a key feature of many psychiatric disorders. The results of this study support the hypothesis that ALA may have a beneficial effect on cognition.

The relationship between mean omega-3 concentrations in breast milk samples and psychomotor development in exclusively breastfed infants has been investigated [35]. In this observational study, associations were found between breast milk DHA ($\beta = 0.275$; $p \leq 0.05$), ALA ($\beta = 0.432$; $p \leq 0.05$), and DHA ($\beta = 0.423$; $p \leq 0.05$) levels, and motor development. A total of 39 pregnant women were enrolled, and 39 breastfed infants were studied. No particularly significant associations were found between these nutrients and psychomotor development.

2.2. Eating Disorders

Nutritional and eating disorders (EDs), such as anorexia nervosa (AN) and bulimia nervosa (BN), commonly emerge during childhood or adolescence, with a traditional peak age of onset between 16 and 19 years. However, recent evidence indicates a downward shift in the age of onset, with cases now documented in children as young as 8–9 years (Italian National Institute of Health). The energy deficit in eating disorders, including anorexia nervosa (AN), results from a low intake of calories and PUFAs. The data in the literature are conflicting regarding deficiencies in essential fatty acids (EFAs), including ALA and LA [36,37]. It has been observed that the red blood cells of individuals with AN have greatly reduced levels of n-3 in the absence of overt ALA and LA deficiency. However, a negative correlation was found between fat mass and ALA in an observational study of 22 women [37]. On the other hand, Nomura et al. [36] found no statistically significant association between n-3 levels and the risk of AN. This Mendelian randomization study that included 72.517 participants does not support the hypothesis that PUFAs may reduce the risk of an eating disorder.

2.3. Psychosis

Psychosis is a mental health condition characterized by disruptions in perception, thought processes, and emotional responsiveness, leading to symptoms like hallucinations, delusions, and disorganized thinking. Psychotic disorders, including schizophrenia (SCZ), schizoaffective disorder, and brief psychotic disorder, have a typical onset in late adolescence or early adulthood, though symptoms can emerge across a wide age range [38]. Alterations in PUFA levels are associated with neuropsychiatric disorders, including SCZ [39]. A 12-week randomized interventional study enrolled 81 ultra-high-risk (UHR) subjects for psychosis with an average age of 16.4 years [40]. In this case, EPA and DHA from oil marine fish (220 mg of PUFAs/day) were administered for 12 weeks. The study examined the fatty acid composition of the erythrocyte membrane and found significant ALA deficits in the patients at high risk of psychosis, suggesting that PUFA supplementation may be effective in preventing psychotic onset. The concept of a PUFA imbalance in patients with psychosis was further explored by Rice et al. [41], who assessed omega-3 levels in the erythrocytes of individuals at ultra-high risk (UHR) for psychosis, comparing them with data from healthy controls. Except for DHA, significant deficits in PUFAs, including ALA, EPA, LA, and AA, were observed in the UHR patients compared to the health controls (specifically, ALA 0.19 vs. 0.29 ($p < 0.001$), EPA 0.48 vs. 0.57 ($p < 0.001$), LA 6.26 vs. 6.81 ($p < 0.001$), and AA 15.52 vs. 17.86 ($p < 0.001$)). These findings provide a basis for PUFA-based interventions for emerging psychosis. An observational study by Rog et al. [42] aimed to determine differences in nutritional status and PUFA metabolism in 80 patients aged 18 to 65 years with SCZ compared to controls. Although no significant differences in serum omega-3 concentrations (AA, DHA, EPA, and ALA) were found between the patients and healthy individuals, lower omega-3 levels were detected in the SCZ patients.

A two-sample Mendelian randomization study conducted by Jones et al. [43] sought to estimate the effects of omega-3 on SCZ risk. The analyses indicated that higher DHA concentrations were associated with a lower risk of SCZ (OR = 0.83; 95% CI: 0.75–0.92), while elevated ALA concentrations were linked to an increased risk (OR = 0.07; 95% CI: 0.98–1.18). Numerous studies in the literature have confirmed that schizophrenic patients have lower blood omega-3 levels than healthy individuals [19,44]. Given the mixed results, further research is necessary to clarify whether ome-ga-3 supplementation can effectively prevent the onset of schizophrenia and psychotic symptoms.

2.4. Affective Disorders

Depression is a major cause of disability worldwide, affecting approximately 300 million people [45]. It is becoming increasingly common in childhood and adolescence and is a risk factor for chronic and recurrent depression in adulthood [46]. Gracious et al. [47], in a randomized, placebo-controlled trial with 51 children and adolescents with an average age of 13 years, investigated whether ALA supplementation (550 mg of ALA) with flaxseed oil for 16 weeks could safely reduce the severity of symptoms associated with bipolar disorder (BD). It was observed that overall, symptom severity was negatively correlated with the final serum omega-3 composition (ALA, EPA, and AA). Therefore, the studies reported here suggest that dietary omega-3 PUFA deficiency may contribute to the development of mood disorders and that supplementation may offer a new treatment option [48]. The fatty acid composition in the red blood cells of 95 adolescents aged 13 to 17 years old was analyzed in a case–control study [49], and lower levels of EPA and DHA were found in the cases compared to the controls (EPA: 0.41 ± 0.11 vs. 0.46 ± 0.12, $p < 0.001$; DHA: 4.07 ± 1.04 vs. 4.73 ± 1.04, $p < 0.001$). Adolescents with a higher n-3 serum concentration (4.84 ± 1.16) had lower odds of de-pression (OR = 0.49 [95% CI: 0.32–0.71]), whereas a high n-6/n-3 ratio (5.51 ± 1.25 vs. 4.96 ±1.08) was associated with higher odds of

depression (OR = 1.58 [95% CI: 1.14–2.25]). There are conflicting data in the literature regarding the role of omega-3 fatty acids, especially ALA, in depression. Zeng et al. [50], using a two-sample MR analysis, claimed that only adipic acid and EPA have protective effects against the risk of de-pression, while ALA is a potential risk factor for this disorder. In contrast, the ELSA Brazil study, a multicenter prospective cohort study [51], found that the risk of devel-oping depressive episodes was reduced by increasing the intake of omega-3 fatty acids, with significant associations with ALA consumption (OR = 0.71: 95% CI [0.59–0.91]). A total of 13,879 adults aged 39 to 65 years old were enrolled in the trial. Regarding the risk of postpartum depression, the postpartum mental health of 250 primiparous women who consumed ALA from perilla and fish oil for 12 weeks was also studied. The association between fatty acids in maternal red blood cells and risk factors for mental health was examined, and it was found that abundant amounts of this fatty acid were present in the women's red blood cells (OR = 0.23, 95% CI: 0.06, 0.84, p = 0.018), suggesting that ALA during pregnancy may stabilize mental health and reduce episodes of postpartum depression [2].

Regarding anxiety symptoms, higher intakes of oleic acid (OR 0.25; 95% CI 0.09–0.67; p trend = 0.002), ALA (OR 0.07; 95% CI 0.02–0.23; trend p < 0.001), and n-3/n-6 (OR 0.56; 95% CI 0.24–1.03; p trend = 0.02) were found to correlate with a lower OR for anxiety in 300 women between 18 and 49 years old, with an average age of 33.5 years [52].

PUFAs have also been shown to be effective in the treatment of bipolar disorder (BD), although the specific role of PUFAs in the treatment of BD remains unclear. Ev-ans et al. [53] studied 27 bipolar patients in their cross-sectional study and found that those with a history of suicide had very high levels of AA/DHA, AA/EPA, and n-6/n-3. This suggests that these ratios may be considered markers of suicide risk.

2.5. Cognitive Impairment

It is estimated that over 55 million people suffer from dementia [54].

Currently, despite pharmacological progress, there are no effective therapies to treat cognitive impairment, so it is very important to find therapeutic alternatives. One example of prevention comes from adherence to a plant-based diet, such as the Medi-terranean diet, which is rich in PUFAs, fiber, and polyphenols, and is associated with a lower risk of age-related cognitive decline. Data from scientific studies have shown that walnuts, which are rich in omega-3, especially ALA, LA, and oleic and palmitic acids, are effective in preventing AD [55,56]. Furthermore, other studies have revealed that these foods increase cognitive function in elderly subjects [57-60]. Studies by Esselun et al. [61,62] showed that feeding animal models food enriched with 6% walnuts led to a significant improvement in spatial memory, due to an increase in the length and number of neurites. This leads to the conclusion that the essential fatty acids contained in walnuts, such as ALA and LA, might have beneficial properties on neuronal development and Alzheimer's disease-associated amyloid-beta in a cell model with early-stage disease. In a cross-sectional investigation study by Current et al. [63], an inverse association was found between ALA and cognitive impairment, and the oppo-site was found for LA intake, in 833 adults with an average age of 67.1 years. Indeed, ALA has been shown to improve learning, and semantic, spatial, and short-term memory in the elderly, thus preventing cognitive decline. The fatty acid profile of red blood cells in patients with mild cognitive impairment (MCI) and AD was determined; in the 78 patients with MCI, a significant increase in stearic acid (p = 0.0001), AA (p = 0.003), and tricosanoic acid (p = 0.007) levels were found. In contrast, the levels of both ALA and DHA were reduced in patients with

MCI and AD ($p = 0.0005$ and $p = 0.00003$, respectively). This suggests that fatty acid testing may prove useful as potential diagnostic biomarkers reflecting an increased risk of dementia [64]. A scientific explanation for their function is that PUFAs omega-3 (ALA, EPA, and DHA) enhance neurogenesis in the adult hippocampus, promote synaptic plasticity, and modulate synaptic protein expression. This allows PUFAs, especially ALA, to be used as a therapeutic for neuro-degenerative diseases [65]. The cognitive function of 60 healthy elderly subjects with an average age of 72 years was tested after using a diet enriched with 2.2 g ALA [66]. After 12 weeks of treatment, improvements in verbal fluency scores were observed compared to the control group (0.30 ± 0.53 vs. 0.03 ± 0.49, $p < 0.05$). This suggests that daily ALA supplementation may be effective in improving cognitive function despite age-related decline. Yamagishi et al. [67] performed an intra-cohort case–control study and found an inverse relationship between serum ALA and the risk of dementia impairment (OR = 0.57, 0.51 and 0.61; 95% CI) in 7586 Japanese individuals aged 40 to 74 years old. This result led to the identification of ALA as a predictive biomarker for future dementia. In the InCHIANTI study on 935 elderly subjects [68], using epidemiological analysis, the participants with dementia were found to have a significantly lower level of ALA (0.34% vs. 0.39%; $p < 0.05$) than the participants with normal cognitive function. This suggests that dementia is associated with low plasma ALA concentrations. Zhang [69] and Thomas [70] in their articles, were interested in the association between dementia and different dietary patterns, including the MIND Diet (Medi-terranean DASH Intervention for Neurodegenerative Delay Diet), characterized by higher intakes of fish such as salmon, herring, tuna, and nuts, as well as fruits and vegetables, in middle-aged subjects with incident dementia and abnormal brain structures. The large population-based study by Zhang et al. [69], which included 114.684 adults over the age of 40 who followed different dietary patterns, found that about 0.42% of participants developed dementia. After adjusting for age and sex, it was found that subject who followed the MIND diet, which contains fish and nuts and therefore high levels of total PUFAs, may be associated with a low risk of dementia; furthermore, the same dietary patterns were positively associated with higher brain volumes in several regions, including the parietal and temporal lobes, hippocampus, and thalamus. This is important because volume loss, particularly in the temporal lobe and hippocampus, has been suggested to be a predictive biomarker of incident dementia [71]. Here, we presented a comprehensive picture of the consistent associations of dietary patterns with dementia risk and brain health, suggesting a potential role for diet in slowing the progression of brain atrophy. Similarly, Thomas et al. [70] investigated the association between the MIND diet, adapted to the French population, and grey matter volume, white matter microstructure, and incident dementia. This longitudinal study found that better adherence to the MIND diet was associated with lower diffusivity values in the splenium of the corpus callosum, which translates into a lower risk of developing dementia. The results of both studies therefore provide further evidence for the role of a diet rich in PUFAs in the prevention of dementia.

3. Discussion

The present narrative aimed to investigate the efficacy and benefits of PUFA administration in subjects with mood, behavioral, and neurodegeneration-related disorders in different populations, from children and adolescents to the elderly, with a particular focus on depressive disorders in pregnant women.

Specifically, the following areas were analyzed: neurodevelopmental disorders, eating disorders, psychosis, affective diseases, and cognitive impairment.

In the area of neurodevelopment, we focused on ADHD, autistic spectrum disorders, intellectual disabilities, and learning disabilities. We noted that the human studies showed promising conclusions:

the consumption of both omega-3 and omega-6 was much lower in subjects presenting with ADHD symptoms [30]. A population of pregnant women was also considered, and it was found their EPA level and AA/EPA ratio were much lower than those of the controls [31]. The interventional studies by Pinar Martì and Gignac [32] are interesting. They studied the benefits of giving walnuts (30 g/day), which are particularly rich in ALA, to a population of adolescents and observed a significant improvement in attention, fluid intelligence, and ADHD symptoms, suggesting an effect of this essential fatty acid on the central nervous system. The efficacy and validity of this innovative dietary strategy is supported by another interventional trial, which once again observed the usefulness of an ALA-rich diet in reducing impulsivity, a typical symptom of this neurodevelopmental disorder, and improving both attention and cognitive reaction time in a young population. These interventional studies show that, although studies are still rather scarce and there are many more animal studies than human ones, this is an area of research worth investigating, considering that neurodevelopmental disorders are often underestimated and the con-sequences they can have in the medium to long-term (e.g., global impairment of social skills and intelligence, and deficits in personal, social, academic, or occupational functioning) are ignored [72].

Regarding eating and nutritional disorders, we focused on anorexia nervosa and bulimia, disorders that are common in the younger population. The results for these diseases are particularly heterogeneous for several reasons, including the limited number of subjects recruited and the various study designs. No interventional trials in human cohorts were found and analyzed, only observational and retrospective studies in which the absence of ALA and LA, precursors of omega-3 and omega-6, was observed in the plasma membrane of the erythrocytes of the subjects that were recruited. These findings could be significant, indicating that a supplementation strategy based on essential fatty acids could probably be useful in the prevention of these eating disorders, but the data are too scarce and supplementation of subjects with high levels of these nutrients has not yet been proven to be effective. From this point of view, we are a long way from knowing whether PUFAs could be helpful in this type of eating disorder.

A very interesting aspect of mental health is the group of psychoses, which includes schizophrenia, schizoaffective disorder, and brief psychotic disorder. These disorders affect the general population, with no particular focus on one area. Different types of studies were analyzed for this area of research, including randomized and interventional studies with young subjects, such as 81 adolescents at high risk of psychosis (UHR). The analysis of the fatty acid composition of the plasma membrane of their erythrocytes revealed a significant ALA deficiency, suggesting that supplementation with this nutrient could prevent the onset of this disorder. Other observational studies and two-sample Mendelian randomization studies [43] in humans evaluated the effects of omega-3 supplementation on schizophrenia patients. The results confirmed a strong association between elevated omega-3 (EPA) levels and a reduced risk of developing schizophrenia, whereas the results regarding ALA levels were more heterogeneous and controversial; specifically, a positive association was found between elevated ALA levels and an increased risk of developing schizophrenia. Given the amount of heterogeneous data and particularly the inconsistent results, more research is needed to confirm that PUFA supplementation is effective in preventing the onset of schizophrenia and psychotic symptoms. Given the paucity of literature on ALA and more abundant literature on EPA/DHA, the number of interventional studies conducted with ALA-rich food supplementation would need to be increased to be able to state with certainty that this nutrient is indeed effective in reducing psychotic and schizophrenia-related symptoms.

Affective disorders include depression, the world's leading cause of disability. Its onset is increasingly occurring early in childhood and adolescence, increasing the risk of developing in adults and the elderly. The number of studies considered was considerable and the type varied: there were randomized, placebo-controlled, observational, and interventional studies. In subjects with bipolar disorder, supplementation with flaxseed oil, which is particularly rich in ALA, was found to be helpful in reducing the symptoms associated with BD. The literature is also very heterogeneous on this point: although there are data clarifying the actual efficacy of EPA, especially ALA, in reducing depressive and borderline symptoms, some case–control studies and two-sample analyses have suggested that although EPA is associated with a protective effect against the risk of depression, the same cannot be said for ALA, which has been suggested to be a potential risk factor. This association was also studied in a population of pregnant women at high risk of developing postpartum depression. A significant re-duction in the frequency of episodes of postpartum depression was observed after 12 weeks of supplementation with ALA-rich fish oil and perilla, suggesting that this could be used as an adjunctive therapy to alleviate depressive symptoms in individuals who may be more susceptible to developing the condition. The heterogeneity of data from studies in scientific literature is probably due to the specific role of PUFAs in the treatment of disorders such as depression and DB still being unclear. Currently, it cannot be confirmed that PUFAs are effective in treating the symptoms associated with mood and behavioral disorders. Further research is needed to differentiate between depression and DB and to increase the number of studies that can effectively test diets containing nuts, flaxseed, or hempseed, which are known to be rich in ALA, in this population.

When we talk about cognitive impairment, we mainly mean dementia, for which there are mainly pharmacological therapies. Alternatively, there are studies confirming the efficacy of the Mediterranean diet, which is particularly rich in PUFAs, fiber, and polyphenols, in preventing cognitive decline. In this field, cross-sectional studies were performed, sometimes using animal models, to test the relationship between the consumption of ALA-rich nuts and improvements in spatial memory. The explanation for the efficacy of this diet may lie in the fact that PUFAs, especially ALA, EPA, and DHA, enhances neurogenesis in the hippocampus of adult subjects by promoting synaptic plasticity, modulating the expression of synaptic proteins and, above all, in-creasing the serum levels of neurodegeneration-related peptide hormones such as BDNF and IGF-1. Reduced levels of these peptide hormones increase neuronal atrophy, thereby increasing the risk of neurodegenerative disease and decreasing the level of neuroprotection. However, it is still too early to define the overall efficacy of this fatty acid on the human population suffering from neurodegenerative diseases. Promising results have come from interventional studies [66]. For 12 weeks, 60 elderly people were given nuts containing a total of 2.2 g/day of ALA. Improvements in verbal fluency were observed in the patient group compared with the control group. Similar results were also observed in the studies by Zhang et al. [69] and Thomas et al. [70], in which it was observed that adherence to a specific diet, a combination of the DASH diet and the Mediterranean diet, which is high in PUFAs, was directly associated with a very low risk of developing dementia. This aspect is worth highlighting as it underlines the effectiveness of PUFAs in slowing the progression of brain atrophy. The results of the studies focusing on dementia are very promising. However, there is a need to carry out case series and to focus more on interventional studies rather than retrospective and observational studies.

In addition, regarding clinical trials focusing on ALA, it is also important to note that the exact amount of ALA that is converted into EPA and DHA is still somewhat unknown. Plant organisms, for example, phytoplankton, can convert ALA into its two higher derivatives and the same applies to humans, but with lower yields. According to the AFFSA (French Food Safety Agency), due to the low yields of the

endogenous bio-synthetic pathway, an average bioequivalence factor of 10 has been used for the conversion of EPA and DHA to ALA. In other words, 1 g of ALA leads to the formation of 100 mg of EPA and DHA. This means that, with this conversion rate, the amount of ALA that needs to be administered daily for this fatty acid to be converted into its metabolites is extremely high.

The main problem with scientific literature is that the studies are too heterogeneous in terms of the nutrients administered and daily dosages, ranging from 30 g of walnuts per day [32] to 550 mg of ALA [47]. According to the LARNs, the daily requirements for PUFAs are as follows: 2.5/2.6 g of ALA per day, and from 2 to 4 g of EPA and DHA per day. The doses administered in clinical trials vary considerably. This methodological problem makes it difficult to compare data. One aspect that could be improved would be to encourage interventional studies in which foods rich in ALA from plant sources are fed in equal amounts and compared; this same protocol should be used in interventional studies evaluating the feeding of food of animal origin that is rich in EPA and DHA. One factor that affects the evaluation of the results is the duration of the study and was similar between studies with most studies evaluating a 3-month period (approx. 12 weeks).

Despite the promising results and proven efficacy of daily nuts supplementation, as seen in some of the interventional studies mentioned above, the effects of this supplementation in the areas of neurodevelopment, eating disorders, cognitive decline, and affective disorders are still very much unexplored and it may be premature to state with certainty that PUFAs could represent an innovative new integrative strategy for these types of disorders. For example, it may be useful to combine supplementation of these nutrients with conventional drug therapy to minimize the risk of cognitive decline and limit long-term neuronal damage. Many more studies still need to be conducted before significant results can be reported in scientific literature.

4. *Materials and Methods*

This narrative review was performed using the PubMed, Google Scholar, and Scopus databases, searching for all studies published in English from 2005 to 2023. The most used keywords were (alpha-linolenic acid) AND (psychiatry OR mental health OR schizophrenia OR depression OR bipolar disorder OR anorexia OR psychosis OR neurodevelopment OR dementia OR Alzheimer's disease OR adult ADHD). The inclusion criteria were studies in humans; systematic reviews; Mendelian randomization studies; case–control studies; cross-sectional studies; studies using dietary supplementation such as supplementation with PUFAs, specifically ALA; epidemiological studies; observational studies; and studies carried out on children, adolescents, and middle-aged and older adults, pregnant and lactating women, and elderly people. Studies on other effects of ALA (e.g., on metabolic syndrome, hypercholesterolemia, and hypertriglyceridemia) and studies on the relationship between mental health and PUFAs/ALA published before 2005 were not included.

5. *Conclusions*

In the present review, we looked at how PUFAs, particularly ALA, EPA, and DHA, can be helpful and supportive in the prevention of behavioral, mood, and neurodegenerative diseases. In most of the studies included, from development to old age, it was observed that the levels of PUFAs were particularly low in subjects presenting with these conditions, which suggests that supplementation with polyunsaturated fatty acids may be beneficial in preventing or treating these conditions, given their

known neuroprotective role. However, due to the paucity of human studies in this area, further research is needed to confirm the efficacy and usefulness of this supplementation strategy in the context of neurological and mental illnesses.

3.1 Impact on lipid, glycemic, and cardiovascular metabolism

The most important health claim officially approved by the EFSA regarding ALA concerns its role in maintaining normal blood cholesterol concentrations (91). Specifically, EFSA concluded that dietary ALA contributes to the maintenance of physiological cholesterol levels, distinguishing its effects from those of EPA and DHA, which are directly associated with the reduction of elevated triglycerides.

Beyond this claim, several additional beneficial actions of ALA have been documented, including anti-proliferative, anti-hypertensive, anti-atherosclerotic, and cardioprotective properties. Importantly, dietary interventions with ALA-rich oils, such as hempseed oil, have demonstrated the capacity to improve erythrocyte membrane fatty acid composition in both adults and children with hyperlipidemia (34,92).

"Alpha-linolenic acid and cardiovascular events: a narrative review" (93) highlights the multifaced cardiovascular effects of ALA in mitigating risk factors for CVDs and its potential direct cardioprotective actions. With respect to lipid-lowering properties, evidence indicates that ALA favorably influences plasma concentrations of LDL-C and triglycerides (TGs) in both adult and pediatric populations. Dietary intake of ALA has been shown to significantly modulate levels of LDL-C, TC and TGs, while also improving erythrocyte membrane composition. Notably, the extent of lipid reduction may depend on the plant source of ALA: for instance, hempseed oil (HSO) has been associated with reduction in LDL-C, TC, and red blood cell indices, whereas sesame seed intake appears to lower TGs without affecting other cholesterol fractions.

With regard to blood pressure regulation, multiple clinical and experimental studies confirmed the antihypertensive effect of ALA (94- 96). Regular consumption of ALA-rich foods, including flaxseed, was associated with reductions in both systolic (SBP) and diastolic blood pressure (DBP). These effects are thought to be mediated, at least in part, by enhanced nitric oxide (NO) bioavailability and improved endothelial function.

The potential antiarrhythmic effects of ALA have also been investigated, possibly through the production of anti-inflammatory eicosanoids. Some studies suggest that ALA intake may reduce arrhythmia incidence and subsequent myocardial infarction (MI), although further high-quality clinical trials are required to substantiate these findings.

Despite accumulating evidence, the cardioprotective role of ALA remains debated. While several cohort studies and meta-analyses reported associations between higher ALA intake and reduced risk of cardiovascular disease (CVD), ischemic heart disease (IHD), and cardiac mortality, other investigations did not confirm significant benefits. The discrepancies may reflect dose-dependency, dietary background (e.g., Mediterranean diet), or differences in study design. Current data suggest that modest increases in ALA intake, such as through flaxseed or hempseed oil consumption, may contribute to lowering CVD risk, although effects on overall cardiovascular and all-cause mortality remain inconclusive. Evidence regarding stroke prevention is particularly limited due to low-quality and heterogeneous data.

Beyond CVD, emerging findings highlight broader anti-inflammatory and anti-proliferative properties of ALA**,** suggesting potential applications in the prevention and management of chronic inflammatory conditions and cancer (97-103). Preliminary evidence indicates that omega-3 PUFAs, including ALA, may target cancer stem-like cells and modulate carcinogenesis. However, these observations are still in early phases and require validation through large-scale, randomized controlled trials.

Taken together, current evidence supports the safety and functional importance of dietary ALA within an optimal n-6/n-3 ratio. While it is well established as an essential fatty acid and a metabolic precursor of EPA and DHA, ALA itself exerts independent biological effects that position it closer to a nutraceutical compound. According to the EFSA (2009) claim, its main validated role lies in the maintenance of normal blood cholesterol levels. Nevertheless, accumulating studies point toward a broader cardioprotective and anti-inflammatory potential, which may ultimately extend to oncological and metabolic health. Further clinical investigations are warranted to clarify whether cardiovascular prevention strategies should prioritize the absolute intake of ALA, the balance with n-6 fatty acids, or a combination of both.

International Journal of
Molecular Sciences

MDPI

Review

Alpha-Linolenic Acid and Cardiovascular Events: A Narrative Review

Camilla Bertoni [1], Martina Abodi [1], Veronica D'Oria [2], Gregorio P. Milani [2,3], Carlo Agostoni [2,3,*] and Alessandra Mazzocchi [3]

[1] Department of Veterinary Sciences for Health, Animal Production and Food Safety, University of Milan, 20122 Milan, Italy; camilla.bertoni@unimi.it (C.B.); martina.abodi@unimi.it (M.A.)
[2] Pediatric Area, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy; veronica.doria@policlinico.mi.it (V.D.); gregorio.milani@unimi.it (G.P.M.)
[3] Department of Clinical Sciences and Community Health, University of Milan, 20122 Milan, Italy; alessandra.mazzocchi@unimi.it
* Correspondence: carlo.agostoni@unimi.it; Tel.: +39-02-5503-2497

1. *Introduction*

Cardiovascular diseases (CVDs) represent the primary cause of global mortality, as they are responsible for about 1,7 million deaths a year, and the major contributor to reduced quality of life [1,2].

CVDs include ischemic heart disease (IHD), stroke, heart failure, peripheral arterial disease, and several other cardiac and vascular conditions. As of 2023, the American Heart Association identifies CVDs as the leading cause of death in the United States, accounting for 928,741 deaths in 2020. Between 2018 and 2019, the direct and indirect costs of total CVD were $407.3 billion ($251.4 billion in direct costs and $155.9 billion in lost productivity/mortality) [3].

In addition to conventional pharmacological therapy (ACE inhibitors, beta-blockers, statins, fibrates and PCSK9 inhibitors), nutraceutical solutions have been proposed in recent years as contributing factors to reduce cardiovascular risk [4,5]. Accordingly, nutraceuticals may act on the reduction of lipid risk markers, including total cholesterol (TC), low-density lipoprotein (LDL-C), and triglycerides (TG), and can be divided on the basis of their mechanism of action: sterols and glucomannan may reduce LDL by decreasing the intestinal adsorption of endogenous cholesterol [6,7], while red yeast, garlic, panthetine and policosanols inhibit hepatic cholesterol synthesis [8].

Similarly, plant-derived polyunsaturated fatty acids (PUFAs) may share analogous functional effects. The human body can synthesize polyunsaturated fatty acids (PUFAs), except for linoleic acid (LA), the precursor of the omega-6fatty acids, and alpha-linolenic acid (ALA), the precursor of the omega-3 fatty acids. Since these compounds must be introduced to perform with diet, they are called essential fatty acids (EFAs).

Omega-3 and omega-6 fatty acids play a crucial role in brain function, growth, and development as well as in the prevention of heart disease. The unique biochemical structure of ALA is so important because it is involved in certain processes linked to immunity, vision and the production of hormone-like compounds.

Concerning the PUFAs, only ALA and LA are essential fatty acids. They are essential nutrients that cannot be synthesized by the human body and have to be obtained in the diet. Like all fatty acids, ALA and LA are used to provide energy and are stores in adipose tissue; significant amounts are incorporated into cell membranes as well.

ALA and LA are important structural components of cell membranes and can influence membrane properties, such as fluidity, flexibility, permeability and activity of membrane-bound enzymes [9,10].

ALA gives origin to longer-chain eicosapenthaenoic acid (EPA) and docosahexaenoic acid (DHA); EPA and DHA are PUFAs, derived from ALA. The main role of EPA is anti-inflammatory: in fact, the cascade of enzymatic re-actions to which it is subjected in certain circumstances leads to the production of signal molecules (called "good" eicosanoids) that counteract the pro-inflammatory activity of other similar molecules originating from the so-called arachidonic acid (AA) inflammatory cascade [11,12]. DHA (a 22C compound of the omega-3 series) is a semi-essential fatty acid known for its distinct metabolic activities. Specifically, DHA is attributed with hypolipidemic properties, useful in reducing blood concentrations of triglycerides and LDL; neuroprotective properties, effective in protecting the central nervous system from the damaging effects of reactive oxygen species; antioxidant properties, biologically valuable for various organs and tissues, including the reproductive system; anti-inflammatory properties, capable of shutting down the upstream inflammatory cascade; and immunomodulatory and antiallergic properties [11,12]. DHA is necessary for the development of brain functions and retinal functions associated with vision [13]. Both LA and ALA give origin to eicosanoids in their 20-C derivatives. Eicosanoids are powerful chemical messengers that intervene in the immune and inflammatory response. Increasing the intake of omega-3 fatty acids increases the EPA content of cell membranes, resulting in a higher proportion of eicosanoids derived from EPA. The omega-3- derived eicosanoids may contrast the pro-inflammatory, procoagulatory, and atherogenic effects of eicosanoids derived from the omega-6 [9].

Like ALA, LA may have relevant biologic benefits, such as maintaining healthy cell membranes and promoting cell growth. LA is a key nutrient throughout life from birth. In the first months of life, it is supplied to the infant through breast milk, in which this essential PUFA represents approximately 10–15% of total fatty acids [14]. Infant formulas must contain both omega-3 and omega-6 PUFAs for optimal growth and development, with a minimum LA content of 500 mg/100 kcal and a maximum of 1200 mg/100 kcal [15,16], according to Regulation EU 2016/2017 (Supplementing Regulation (EU) Number 609/2013 of the European Parliament and of the Council with regard to "specific compositional and information requirements for infant formulas and follow-on formulas and with regard to infant and young child feeding information requirement". UE, 2016). The role of derived eicosanoids, within physiological limits, is beneficial to support reactions against infective agents and clotting processes. Nevertheless, an excess of LA at the expense of ALA may lead to an overproduction of arachidonic acid (AA) [11,12] and derived eicosanoids, representing a risk factor for long-term chronic inflammatory disorders. Also, very high intakes of LA, in the short term, can cause significant metabolic and functional damage through the synthesis of powerful pro-oxidant agents (formation of toxic lipoperoxides, for instance).

LA and ALA compete for the enzyme delta-6-desaturation reaction. This has been suggested to be important for health, as a high intake of LA may reduce the amount of the enzyme available for ALA metabolism, thereby increasing the risk of heart disease through mechanisms mainly associated with pro-inflammatory conditions [17–19]. Although ALA is the preferred substrate of the desaturated delta-6 enzyme, an excess of LA in the diet compared to ALA brings more clearly the formation of AA [20]. While the protective effects of the precursor and metabolites of the omega-3 series (ALA and EPA/DHA) have been known for a long time, the role of omega-6, especially LA, in dis-eases of the cardiovascular system is only recently known [21]. Until recently, LA and its derivatives, AA, were known for pro-inflammatory properties; now, scientific evidence has investigated the role of LA and its benefits in CVD. The balanced intake of LA and ALA is critical in order to give omega-6 its anti-inflammatory properties, which is useful in CVDs [22,23]. For this reason, it is important to maintain a proper balance between omega-3 and omega-6 in the diet, because these two substances compete for the enzyme delta-6

desaturase, although they should work together to pro-mote health. Evidence supporting this theory shows that over the past 150 years, Omega-6 intake through today's diet has increased, while omega-3 intake has de-creased in parallel with the increase in heart disease. Thus, the concept of an "ideal" ratio of omega-6/omega-3 in the diet was developed and is between 10:1 and 5:1 [24,25].

Humans evolved on a diet with an omega-6/omega-3 ratio of about 1, while Westerners (e.g., those on Western diets) have a ratio of 15/1–16/1 [20,24]. These diets are deficient in omega-3 fatty acids and contain excessive amounts of omega-6 fatty acids. Excessive amounts of omega-6, including LA, or a very high unbalanced omega-6/omega-3 ratio, promote the pathogenesis of many diseases, including cardiovascular, cancer and inflammatory/autoimmune diseases. In contrast, high levels of ALA and omega-3 exert suppressive effects against these diseases. In the secondary prevention of CVD, a 4/1 ratio was associated with a 70% reduction in total mortality. A ratio of 2.5:1 reduced cell proliferation in colorectal cancer patients, while a lower omega-6/omega-3 ratio was associated with a significant reduction in the risk of breast cancer in women [11,24].

Within this context, while the functional effects of LA are well known [26–30], ALA, present in hempseed oil (HSO), walnuts, and olive and flaxseed oils, still has relevant and functional effects. Nuts and seeds are important sources of ALA and other micro-nutrients, such as sterols, fibers and polyphenolic compounds. These nutrients are effective in protecting against cardiovascular, inflammatory and chronic diseases [31–33]. As for the ALA content of nuts and seeds, 28 g of hempseed or walnuts exceeds the adequate intake for ALA, which is ideally set at 1.1 g/day for women and 1.6 g/day for men [34,35]. The EFSA has set reference values for of omega-3 PUFA intake and, considering cardiovascular health and neurological development, is fixed at about 2–3 g daily intake of ALA, corresponding to 1% of the total energy intake of 18,000/2700 kcal/day [24,36]. Flaxseed, hempseed and canola oil are the main sources of ALA; soy-bean oil is often considered to be a low-to-moderate source of this nutrient, as studies of its fatty acid composition have shown ALA concentrations ranging from 2.7% to 7.8% [37,38]. These dietary sources of ALA may also be relevant in either pregnant and breastfeeding women, not only for their rich nutritional composition—inclusive of either fat and non-fat compounds vitamins, f.i.—but also because of the well-founded indication to avoid complex mixtures of herbal supplements that may jeopardize the health of both mother and child. In addition, plant sources of omega-3 PUFAs could be considered an effective option for women who cannot tolerate fatty fish and for those who suffer from nausea, a common manifestation during pregnancy [39,40]. While there are numerous studies on the fatty acid composition of human milk, information regarding the types of individual fatty acids provided by the mother to the suckling infant during the first year of life is scarce. Marangoni et al. [41] measured the total fat contents and the concentrations of major fatty acids in pooled breast milk and the following was observed: that total saturated fatty acids gradually increases and the per-centage of total monounsaturated fatty acids gradually decreases. Then, it was observed that the amounts of LA and ALA progressively increase. The amounts (mg/kg) of omega-6and omega-3 increase about 2–5 times between colostrum and 1 month, maintaining a very constant ratio, and remain stable up to 3 months [41]. These data suggest that LA is as important as ALA during this period of life in the newborn. LA is critically supportive of infant growth, as are ALA and its series-3 derivatives. A supplementation with ALA to lower the omega-6/omega-3 ratio may lead to up to a 50% reduction in C-reactive protein (CRP), a risk factor for coronary heart disease [9]. Exclusively vegan diets should be evaluated carefully because of the risk of omega-3 PUFA deficiency. In addition to lower intakes of total and saturated fats, another characteristic of exclusively vegan diets is a higher proportional intake of ome-ga-6 PUFAs compared with the more diverse vegetarian diets [42,43]. For these reasons, recommendations for

vegan diets to include adequate amounts of ALA are important to keep the advantages of a plant-derived diet [44].

Therefore, the main purpose of this narrative review is to analyze the effects of ALA at the cardiovascular level and to investigate not only the relevant omega-6/omega-3 ratio, but also the absolute amount of ALA in order to maintain functionally beneficial effects [45–47].

2. Functional Effects of ALA

We selected different studies on ALA and its effects on the cardiovascular system and categorized them by effects on risk factors for CVD and direct cardio protection.

2.1 Risk Factors for Cardiovascular Disease

The most important claim declared by the EFSA concerning ALA is related to the influence on blood cholesterol concentrations (EFSA 2009; 7 (9):1252) [48]. Specifically, the claim indicates that ALA contributes to the maintenance of normal blood cholesterol levels, unlike EPA and DHA, which, being of animal origin, act directly in significantly reducing elevated triglyceride levels.

A cause-effect relationship between the dietary intake of ALA and the reduction of plasma concentrations of TC and LDL-C has been recognized. Studies issued after the EFSA statement further suggest that ALA has anti-proliferative [49], anti-hypertensive [50–52], anti-atherosclerotic [53,54] and cardioprotective effects [53–58], and it successfully improves the composition of the red cell membrane in children with hyperlipidemia [19,59], possibly reducing the risk of cardiovascular events in adulthood. Like ALA, there are numerous studies that have focused on the use of the precursor of the omega-6 series, LA, in CVDs. A 2020 study by Marangoni et al. [30] clarifies the correlation between LA intake and the risk of CVDs. This narrative review states that high tissue and blood LA concentrations are associated with lower cardiovascular risk and reduced mortality. In fact, dietary guidelines suggest that high consumption of omega-6, along with omega-3, reduce CVD risk, although more studies with longer follow-up are needed to clarify the role of LA in this field. The 2018 study by Hooper et al. [60] also confirms this aspect: significant intakes of omega-6, particularly LA, have been associated with reduced risk of heart attack, lowered TC-C, and decreased risk of not only cardiac but also cerebral events. The efficacy of this nutrient at reducing TG levels and LDL-C, increasing HDL-C and reducing BMI remain unclear. Therefore, from a cardiovascular standpoint, both ALA and LA may be effective. Based on this, a 2023 study [61] concluded that intake of trans fatty acids (palmitic acid, stearic acid, and saturated fatty acids of animal origin) was modestly associated with a higher risk of mortality and CHD, whereas intake of ALA, long-chain ome-ga-3 fatty acids, and LA was modestly associated with a lower risk. Supplementation with long-chain omega-3 fatty acids and increased consumption of ALA and LA instead of saturated fats reduced the risk of coronary events. Taken together, the available evidence provides modest support for current recommendations to replace saturated fats with PUFAs [60].

Although it has been said that LA and omega-6 are also effective in treating CVDs, it is known that they play a prominent role in CVD risk, sometimes increasing the risk of CVD. In fact, the beneficial effects of LA are dose-dependent: excess LA is considered potentially harmful to the body because it induces high production of AA, which ap-pears to alter the body's inflammatory balance. High levels of LA induce AA and the production of bad eicosanoids with pro-inflammatory activity. Increased inflammation is a

major factor that greatly increases CVD risk. Thus, excess LA is detrimental to omega-3 metabolism. Since omega-6 as LA is found in many more foods than ALA, it is easy to understand how easy it is to overdo LA intake and AA production [23].

In the study conducted by Greupner et al. [19], the aim was to compare two extreme LA to ALA ratios involving a low LA/high ALA diet with a ratio of 0.5–1:1 and a high LA/low ALA diet with an LA ratio of 20–30:1 on fatty acid concentrations in red blood cells (RBCs), with emphasis on EPA and DHA. To obtain the two LA/ALA ratios in the diet, the subject's fatty acid intake was tightly controlled by a multistep method. During the course of the low-LA/high-ALA diet, ALA concentrations increased rapidly ($p < 0.001$) from 1.44 ± 0.17 μg·mL−1 at baseline to 5.63 ± 0.45 μg·mL−1 at day 7 and to 6.34 ± 0.63 μg·mL−1 at day 14, corresponding to a mean change of 332 ± 40% and 354 ± 47%, respectively, whereas in the high-LA/low-ALA diet, ALA concentrations decreased after 7 days from 1.47 ± 0.13 μg·mL−1 to 1.09 ± 0.09 μg·mL−1 ($p = 0.011$) and increased again on day 14 to 1.41 ± 0.17 μg·mL−1, which is not significantly different from the baseline level. The results showed that the low-LA/high-ALA diet is effective in increasing ALA and EPA concentrations in red blood cell membranes. The findings that EPA concentrations in red blood cells increased significantly by 35.0 ± 13% at 7 days and by 57.6 ± 18% at 14 days after the low-LA/high-ALA diet is likely a result of in-creased conversion of ALA to EPA, since no EPA was ingested with the basal diet.

In a pilot study by Del Bo' et al. [59], an 8-week-long randomized clinical trial (RCT) dietary intervention study aimed to evaluate the impact of HSO supplementation on the lipid profile and fatty acid composition of RBCs in children and adolescents with primary hyperlipidemia. The 36 study subjects, aged 6 to 16 years, were divided into two different groups: the control group and the HSO group, receiving 3 g of HSO with 1.4 g of linoleic acid (LA) and 0.7 g/day of ALA. Both groups received specific dietary guidelines. Blood samples were kept for each subject, before and after administration with HSO, in order to analyze the lipid profile, composition of RBCs, and omega-3 index. After an eight-week supplementation with HSO, there were significant reductions in the RBC content of total saturated and monounsaturated FAs (−5.02 ± 7.94% and −2.12 ± 2.23%, respectively). Conversely, the levels of total omega-3 and omega-6 PUFAs increased (+1.57 ± 1.96% and +5.39 ± 7.18%, respectively), as did the omega-3 index (+1.18 ± 1.42%). This study confirms that diet represents the first line of therapy for primary hyperlipidemia.

In the clinical study by Yue et al. of 2021 [62], the effect of ALA intake on blood lipid profiles was examined, especially on triglycerides (TG), TC, HDL-C, LDL-C, VLDL-C and the ratio of TC to HDL-C. A total of 1305 subjects were enrolled in the ALA group and 1325 were enrolled in the control group. Compared with the control group, dietary intake of ALA significantly reduced the concentrations of TG (WMD −0.101 mmol/L; 95% CI: −0.158 to −0.044 mmol/L; $p = 0.001$), TC (WMD −0.140 mmol/L; 95% CI: −0.224 to −0.056 mmol/L; $p = 0.001$), LDL-C (WMD −0.131 mmol/L; 95% CI: −0.191 to −0.071 mmol/L; $p < 0.001$), VLDL-C (WMD −0.121 mmol/L; 95% CI: −0.170 to −0.073 mmol/L; $p < 0.001$), TC/HDL-C ratio WMD −0.165 mmol/L; 95% CI: −0.317 to −0.013 mmol/L; $p = 0.033$) and LDL-C/HDL-C ratio (WMD −0.158 mmol/L; 95% CI: −0.291 to −0.025 mmol/L; $p = 0.02$). ALA has no effect on HDL-C (WMD 0.008 mmol/L; 95% CI: −0.018 to 0.034 mmol/L; $p = 0.541$). Dose-response analysis showed that a 1 g/day increase in ALA was associated with 0.0016 mmol/L, 0.0071 mmol/L, 0.0015 and 0.0061 mmol/L reductions in TG (95% CI: −0.0029 to −0.0002 mmol/L), TC (95% CI: −0.0085 to −0.0058 mmol/L), HDL-C (95% CI: −0.0020 to −0.0011 mmol/L) and LDL-C (95% CI: −0.0073 to −0.0049 mmol/L) levels, respectively. The effects of ALA intake on TG, TC, and LDL-C concentrations were pronounced in patients with hyperlipidemia or hyperglycemia compared with healthy subjects.

Dietary ALA intervention improved blood lipid profiles by reducing levels of TG, TC, LDL-C and VLDL-C. These results show that increasing ALA intake could potentially prevent the risk of CVDs.

In a meta-analysis of controlled trials conducted by Khalesi et al. [63], the intake of ALA-rich sesame fractions is associated with a reduction in TG. As a result, the consumption of sesame did not significantly change the TC (−0.32 mmol/L, 95% CI: −0.75 to 0.11; p = 0.14, I2 = 96%), LDL-C (−0.15 mmol/L, 95% CI: −0.50 to 0.19; p = 0.39, I2 = 96%) or HDL-C levels (0.01 mmol/L, 95% CI:−0.00 to 0.02; p = 0.16, I2 = 0%). However, a significant reduction was observed in serum TG levels (−0.24 mmol/L, 95% CI: −0.32 to −0.15; p < 0.001, I2 = 84%) after consumption of sesame. Although the consumption of sesame rich in ALA seems to significantly reduce blood TG levels.

The positive effects of ALA can also be extended to non-lipid outcomes as well. Experiments show that ALA stimulates nitric oxide (NO) production [64,65], and increases action mediated by prostanoids, with effects on platelet aggregation and coagulation. Therefore, it reduces the probability of thrombotic events, regulates the heart rhythm and decreases the onset of arrhythmias and inflammation, with a direct action on prostaglandins [66]. Recent evidence agrees that the intake of ALA has a cardio-protective effect, through mild lowering of blood pressure (BP). Dietary ALA intake was reported to be epidemiologically associated with a lower prevalence of hypertension, and ALA has been indicated as a promising alternative addition to available life-style medications for the prevention of CVD [52]. The present study was designed to comparatively investigate the effects of ALA and LA supplementation against hyper-tension and the underlying molecular mechanisms. Hypertensive rats and normotensive control rats were used. They were given an LA diet (10% canola oil, 20% protein, 54% carbohydrate and 26% fat) and an ALA-supplemented diet (10% flaxseed oil, 20% protein, 54% carbohydrate and 26% fat). The animals were randomly divided into four groups with different diets for 8 weeks. After 8 weeks of dietary treatment, ALA supplementation significantly reduced systolic blood pressure, whereas LA supplementation showed no apparent effects. In addition, ALA supplementation not only reduced BP but also improved endothelial function. In conclusion, the data showed that ALA could be used in dietary interventions for the prevention and treatment of hypertension [50].

A series of studies aimed to understand how the intake of ALA in the diet was useful in tracing BP and in increasing the aortic thickness of the intima and media tunica in small-gestational-age (SGA) infants [50,51]. In a 2015 study by Skilton et al. [51], 1009 participants were recruited at 6 months and were followed until age 19 years. The purpose of this study was to evaluate a possible association between dietary ALA in-take, low BP and aortic intima-media thickness in children born with SGA. BP and food records were assessed at each visit. A total of 1009 participants had at least one blood pressure measurement and complete birth weight and gestational age data, including 115 (11%) with SGA. These children had higher systolic BP and pulse pressure (PP) from age 14 years onwards. In SGA infants, systolic blood pressure (SBP) was 2.1 mmHg lower ([95% CI 0.8–3.3]; p = 0.001) and PP 1.4 mmHg lower ([95% CI 0.3–2.4]; p = 0.01), per exponential increase in ALA intake. It can be concluded from the study that ALA supplementation during childhood improves cardiovascular health of children with SGA.

Flaxseed and HSO contain omega-3 fatty acids in the fatty components, with lignans, and fiber as non-fat components, and may be beneficial for patients with CVDs. Hypertension is often associated with peripheral artery disease. Rodriguez-Leyva and colleagues aimed to investigate the effects of daily flaxseed intake on SBP and diastolic blood pressure (DBP) in patients with peripheral artery disease. In this prospective, double-blinded, placebo-controlled, randomized trial, 110 patients consumed a variety of food containing 30 g of ALA or placebo daily for 6 months. Plasma levels of omega-3 ALA increased 2- to 50-fold in the flaxseed-fed group but did not increase significantly in the placebo group.

SBP was ≈10 mmHg lower, and DBP was ≈7 mmHg lower in the flaxseed group compared with placebo at 6 months. Enrolled patients with an SBP ≥ 140 mmHg at baseline achieved a significant reduction of 15 mmHg in SBP and 7 mmHg in DBP with flaxseed supplementation. These results confirmed that ALA is one of the most powerful antihypertensive agents achieved by dietary intervention.

The MARGARIN trial [67] examined changes in atherosclerosis markers in 110 sub-jects with a high risk of IHD. The experimental group received margarine (80% fat, of which, 60% as PUFAs) containing either 15% or 0.3% of total fat as ALA for two years. Results showed that the intake of ALA reduced C-reactive protein, a marker of inflammation, but the present study found no effect on markers of atherosclerosis.

2.2 Cardio protective Effect

The 2020–2025 American Dietary Guidelines showed that there is strong evidence to demonstrate that replacing saturated fatty acids (SFAs) with PUFAs reduces the risk of IHD events and CVD mortality [64]. In high-risk patients, ALA prevents coronary heart disease (CHD), considered one of the major causes of death worldwide [52–55,57,68–76].

The aim of the study by Vedtofte et al. [69] was to examine the association between ALA intake and the risk of CHD. Data from eight American and European prospective cohort studies including 148,675 women and 80,368 men were used. During 4–10 years of follow-up, 4,493 CHD events and 1,751 CHD deaths occurred. Among men, the re-searchers found an inverse association between ALA intake and CHD events and deaths. Each additional gram of ALA was associated with a 15% lower risk of CHD events (HR: 0.85; 95% CI: 0.72, 1.01) and a 23% lower risk of CHD deaths (HR: 0.77; 95% CI: 0.58, 1.01). The Cardiovascular Health Study [31] was designed to examine the associations of dietary ALA with the risk of mortality, CHD and stroke among older adults who participated in the study, a cohort study of 2709 adults aged ≥ 65 years. After adjustment for age, sex, race, enrollment site, education, smoking status, diabetes, body mass index (BMI), alcohol consumption, treated hypertension and total energy intake, higher dietary ALA intake was found to be associated with a lower risk of total and non-cardiovascular mortality. When the highest quintile of dietary ALA was compared with the lowest quintile, the HR for total and non-cardiovascular mortality was found to be 0.73 (95% CI: 0.61, 0.88) and 0.64 (95% CI: 0.52, 0.80), respectively. In conclusion, this study suggests that dietary ALA is associated with a lower risk of total and non-cardiovascular mortality in older adults.

In a 2012 meta-analysis of observational studies by Pan et al. [53], increasing dietary ALA was associated with a moderately lower risk of total CVD (RR: 0.90; 95% CI: 0.81, 0.99). Twenty-seven studies were analyzed, including 251,049 individuals and 15,327 CVD events. The association between ALA intake and reduced risk of CVD was significant in 13 comparisons, but 17 comparisons showed similar but non-significant trends. Therefore, considering observational studies, higher ALA intake is associated with a moderately lower risk of CVD.

In the study by Zelniker et al. of 2021 [77], after intake of omega-3 PUFAs, patients with acute coronary syndrome experienced a lower risk of cardiovascular death (OR: 0.82; 95% CI: 0.68, 0.98 per 1-SD increment), and an attenuated relation was observed after ALA administration, specifically (OR: 0.92; 95% CI: 0.74, 1.14).

In the European Prospective Investigation into Cancer and Nutrition (EPIC) study [78] which included 22,043 subjects in Greece, there were 275 deaths after 44 months. These individuals consumed a traditional Mediterranean diet rich in ALA, and there were significant reductions in both total mortality

and death from CHD of 25% and 33%, respectively. In 1302 individuals with known CHD, there were reductions in total mortality and CHD of 27% and 31%, respectively [79].

The Lyon Diet Heart Study [80] was an effective study in demonstrating the efficacy of a Mediterranean-style diet supplemented with ALA on composite measures of IHD recurrence after a first myocardial infarction (MI). Subjects in the experimental group were instructed to follow a Mediterranean diet and were given a canola-oil-based margarine containing 4.8% ALA. After 46 months of this diet, these subjects had a 50–70% lower risk of recurrent IHD [81]. Only ALA was significantly associated with improved prognosis (RR for the composite of cardiac death and nonfatal acute MI: 0.20; 95% CI: 0.05, 0.84) when plasma fatty acids were analyzed as crude estimates of dietary data.

Another systematic review and meta-analysis of cohort studies by Jingkai et al. [70] examined the overall association between ALA intake and CHD risk, assessing dose-response relationships. Fourteen studies of 13 cohorts were identified and included in the meta-analysis. The pooled results showed that higher ALA intake was associated with a modestly reduced risk of combined CHD (risk ratios (RR) = 0.91; 95% CI: 0.85, 0.97) and fatal CHD (RR = 0.85; 95% CI: 0.75, 0.96). Compared with individuals with lower ALA intake, only subjects with ALA intake <1.4 g/d showed a reduced risk of composite CHD. ALA intake was linearly associated with fatal CHD, and each 1 g/d increase in ALA intake was associated with a 12% reduction in the risk of fatal CHD (95% CI: −0.21, −0.04).

To better understand the role of ALA, Sala-Vila et al. [82] prospectively evaluated the association between dietary ALA and fatal CVD in participants of the PREvèncion with DIeta MEDiterranea (PREDIMED) study (n = 7202). These results showed that dietary ALA at > 0.7% of daily energy intake was associated with a 28% reduced risk of all-cause mortality. Participants (n = 7447) at high CVD risk in a treatment arm receiving 30 g/day of mixed nuts (15 g walnuts, 7.5 g hazelnuts, and 7.5 g almonds) had a reduced incidence of cardiovascular events (HR: 0.72; 95% IC: 0.54, 0.95) with 4.8 daily grams of ALA consumption compared to the control group. In the intervention group, dietary ALA increased by 0.43 g/day and plasma ALA increased by 0.30%–0.44% in a random subsample [79]. In addition, several studies have examined the association between ALA intake and CHD risk and evaluated a possible dose-response relationship. Results showed that higher intakes of ALA were associated with a modestly reduced risk of composite coronary disease (RR = 0.91; CI 95% 0.85, 0.97) and fatal coronary disease (RR = 0.85; 95%; CI 0.75, 0.96). Subjects who consumed ALA < 1.4 g/day had a significant risk reduction, in contrast to those who did not include ALA in their diet [70].

Regarding ALA and its association with cardiovascular risk, a working hypothesis suggests that ALA may influence cardiovascular risk through effects on arrhythmogenesis and lethal ventricular arrhythmia. Intravenous infusions of ALA reduced the risk of ventricular fibrillation during coronary artery ischemia in different animals, and dietary ALA is associated with a reduced risk of abnormal repolarization in men and women [83]. In addition, after intake, ALA can be converted to EPA and DHA, both of which have antiarrhythmic effects. Other plausible pathways by which ALA may exert beneficial effects on coronary and CVD risk include endothelial function, inflammation and thrombosis. With respect to CVD, only one large trial has evaluated ALA supplementation for cardiovascular outcomes, including arrhythmias. In the AlphaOmega trial [84], ALA was associated with a significant reduction in arrhythmia-related events compared with placebo or EPA and DHA in post hoc analyses in the subgroup of patients with diabetes, who are particularly prone to ventricular arrhythmias and sudden death after MI (HR: 0.39; 95% CI: 0.17, 0.88). In addition, the results of the study by Kromhout et al. [85] suggest an antiarrhythmic effect of ALA intake, but further clinical trials are needed to confirm this. Meta-analyses of observational studies have

shown that increasing dietary ALA is associated with a 10% lower risk of total CVD and a 20% lower risk of fatal CHD.

Dietary intake of ALA reduces atherogenesis and inhibits arterial thrombosis [54]. ALA exerts beneficial cardiovascular effects on atherosclerosis and inflammation and has been shown to induce antithrombotic and antiplatelet effects and to reduce mortality [71–76]. Dietary ALA was incorporated into adipose tissue and shifted the balance toward the anti-inflammatory class of the thromboxane/prostacyclin mediators [68]. In the study by Stivala et al. [54], eight-week-old male mice were fed a high-fat diet containing 7.3 g of ALA or a low-fat diet containing 0.03 g of ALA for 16 weeks. ALA was administered as flaxseed oil, while cocoa butter was used in the control group. The results showed that dietary ALA increased the platelet count to a biologically relevant extent (51%) without affecting bleeding times. This finding is very relevant and gives the possibility to consider ALA as a powerful and effective element potentially used to reduce thrombotic risk and, consequently, reduce the risk of CVD.

3. Discussion

This narrative review describes the functional effects of ALA in preventing risk factors for CVDs, and its direct cardioprotective effects.

Regarding the lipid-lowering preventive effects, this review shows that ALA may favorably affect the levels of LDL-C and TGs in both adult and pediatric populations [59–63]. Dietary ALA intake has a significant effect on the plasma values of LDL-C, TC, and TG levels, as well as on the improvement of erythrocyte membrane composition. However, the reduction of cholesterol and triglyceride levels may also depend on the plant source of ALA used. For example, HSO allows for a significant reduction of LDL-C, TC and RBC, while sesame seeds are effective in reducing triglyceride levels, but not other cholesterol-rich fractions.

A beneficial and protective effect against hypertension has been reported in three studies [50–52]. All of them confirm that daily consumption of ALA reduces SBP and DBP, possibly through the release of NO, a potent vasodilator. The results were also confirmed in subjects with borderline BP values, suggesting that ALA is an effective dietary antihypertensive compound.

About cardioprotective effects, the studies and meta-analyses [52–55,57,69–83], reported by us, show controversial results. While some studies confirm an important association between daily ALA intake and a significant reduction in CVD risk, others indicate that there is no strong correlation. A dose-dependent dietary intake of ALA appears to be associated with a reduced risk of CVD. Some studies confirm that adherence to the Mediterranean diet, with the addition of ALA following the correct intakes, has positive and beneficial effects on significantly reducing IHD, cardiac death and nonfatal acute MI. Although some studies are significant and vouch for the effectiveness of ALA on CVDs, further clinical trials are needed to confirm the relationship between ALA intake at different doses and CVD events. Assuming more ALA, for ex-ample, by eating more hempseed or flaxseed oil, probably makes little or no difference in all-cause or cardiovascular death or coronary events, but slightly reduces CVD, coronary mortality and heart arrhythmias. The effects of ALA on stroke are unclear, because the evidence was of low quality.

Three studies [83–85] investigated the association between ALA intake and its anti-arrhythmic effect, caused, probably, by its direct action on anti-inflammatory eicosanoids. In fact, due to the action of

these metabolites, the regulation of heart rhythm is also affected. Some of these trials verified that ALA intake was associated with a significant reduction in arrythmia, and subsequently MI, while others need further clinical evidence to confirm the efficacy.

ALA has the potential to provide novel and promising research perspectives, which are indirectly related to CVD prevention. A growing body of evidence [86–92] suggests that ALA intake may be a co-adjuvant intervention to modulate the progression of inflammatory and cancer-related conditions. The effects of omega-3 PUFAs on cancer stem-like cells (CLSCs) may be an important target for cancer therapy and will be an interesting challenge for future studies. In any case, the antitumor activity of omega-3 PUFAs, shown through multiple mechanisms, suggests that they may have an important therapeutic role in the management of cancer stem cells (CSCs). At this stage, further large observational and prospective studies are needed to confirm these effective and innovative properties of ALA and to develop RCTs.

4. Conclusions

Based on the studies and research presented herein, increasing ALA in the daily diet within the recommended omega-6/omega-3 ratio is safe. Thus, in addition to serving as an essential fatty acid and a precursor to more bioactive long-chain omega-3 fatty acid derivatives, ALA has its own significant functional properties like those associated with nutraceuticals. As stated in the 2009 EFSA report, the inclusion of dietary ALA may play a role in reducing the risk of cardiovascular events (CHD and IHD) due to its anti-hypertensive, anti-atherosclerotic, and cardioprotective effects. Given the paucity of data in the literature regarding the use of ALA in the setting of CVD, this narrative review aims to summarize the main effects not so much of the metabolites produced by omega-3 (EPA and DHA), but of ALA alone. From the re-ported trial and meta-analyses, it is still not well understood whether it is more useful and effective to dose the omega-6/omega-3 ratio or to evaluate only the absolute amount of ALA for the prevention of CVDs. More studies are needed to investigate this further. Recently, there has been promising evidence of its potential anti-inflammatory and anti-proliferative properties, which may extend beyond CVDs to include inflammatory and oncological conditions. However, further validation through high-quality studies is required to definitively establish these effects.

3.2 PUFAs and muscular disorders: sarcopenia and muscle weakness in older adults

"Omega-3 polyunsaturated fatty acids and physical performance across the lifespan: a narrative review" (104) comprehensively explores the available evidence on the role of omega-3 PUFAs in physical performance from early life to advanced age. Accumulating data suggest that long-chain n-3 FAs, particularly EPA and DHA, may provide a protective effect against age-related muscular decline. Sarcopenia and muscle weakness are highly prevalent among older adults, representing major determinants of frailty, functional impairment, and reduced quality of life.

At the mechanistic level, omega-3 PUFAs exert multiple effects on muscle homeostasis (*Figure 3*). One of the most studied pathways involves the mTORC1 complex**,** which regulates muscle protein synthesis. Both EPA and DHA have been shown to activate mTORC1, either directly or synergistically with amino acids, thereby stimulating anabolic processes. However, while transient activation supports protein accretion, chronic mTORC1 stimulation may impair autophagy and contribute to muscle atrophy. Thus, a balanced dietary intake of omega-3 fatty acids, ensuring alternating cycles of activation and inhibition, appears crucial for sustaining muscle integrity and preventing maladaptive responses.

In parallel, omega-3s enhance mitochondrial bioenergetics**,** improving respiratory efficiency, reducing reactive oxygen species (ROS) generation, and preserving ATP availability. These adaptations mitigate protein breakdown and promote muscle endurance. Furthermore, omega-3 PUFAs positively affect insulin sensitivity through remodeling of membrane phospholipids and by increasing circulating insulin-like growth factor 1 (IGF-1), which stimulates protein synthesis via both mTORC1-dependent and independent signaling cascades.

Another important axis involves their interaction with G-protein coupled receptors (GPCRs**)** and activation of PPARs**.** This regulatory cascade leads to inhibition of NF-κB signaling, reduced pro-inflammatory mediator release, suppression of muscle-specific ubiquitin ligases such as MuRF-1, and consequently decreased protein degradation. PPARγ, in particular, plays a central role in limiting intramuscular lipid accumulation and enhancing insulin responsiveness, two hallmarks of sarcopenia pathogenesis. Clinical investigations further corroborate these molecular findings. Data from athletic populations indicate that omega-3 supplementation enhances muscle recovery, metabolic efficiency, and strength. In elderly cohorts, meta-analyses and randomized controlled trials consistently demonstrate improvements in muscle function parameters, attributed to increased PPARγ expression, better mitochondrial dynamics, and enhanced neuromuscular transmission. The incorporation of omega-3 PUFAs into cellular membranes improves fluidity and synaptic signaling, which may translate into superior muscle contractility and performance throughout the lifespan.

Taken together, these findings highlight omega-3 PUFAs as promising modulators of skeletal muscle physiology, with implications extending from athletic performance in young individuals to the prevention of frailty in older adults. Nevertheless, while preliminary data are encouraging, larger and rigorously designed RCTs are needed to definitively establish the efficacy of omega-3 supplementation in preserving muscle mass and function. Importantly, future research should integrate objective measures of physical performance **(**e.g., grip strength, gait speed, endurance testing) alongside molecular outcomes, in order to clarify whether early and sustained omega-3 intake can translate into clinically meaningful improvements in physical function across the entire lifespan.

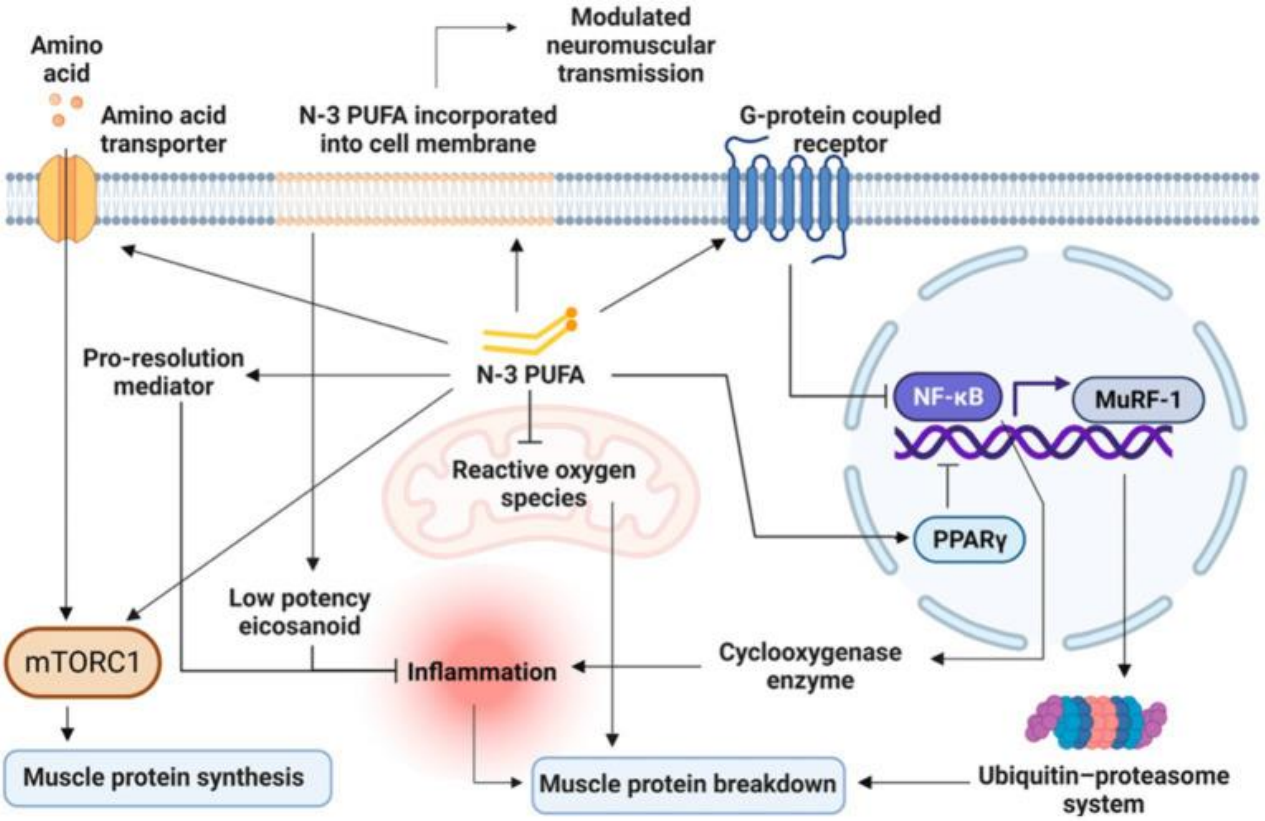


Figure 3. Overview of the main mechanisms through which omega-3 PUFAs influence muscle function**.** *By incorporating into cell membranes, omega-3 PUFAs enhance neuromuscular transmission and stimulate mTORC1, promoting protein synthesis. They also reduce inflammation and oxidative stress, activate PPARs, and inhibit NF-κB and MuRF-1 pathways, thereby limiting protein degradation and preserving muscle mass. Azzolino D. et al. Omega-3 polyunsaturated fatty acids and physical performance across the lifespan: a narrative review. Fron Nutr. 2024:11:1414132. Doi:10.3389/fnut.2024.1414132*

Front Nutr. 2024 Jun 20;11:1414132. doi: 10.3389/fnut.2024.1414132

Omega-3 polyunsatured fatty acids and physical performance across the lifespan: a narrative review

Domenico Azzolino [1,†], Camilla Bertoni [2,†], Valentina De Cosmi [3,4], Giulia Carla Immacolata Spolidoro [4], Carlo Agostoni [4,5,*], Tiziano Lucchi [1], Alessandra Mazzocchi [4]

1. *Introduction*

Advancing age is characterized by a progressive and generalized decline in muscle mass and function, the so-called "sarcopenia." However, sarcopenia can occur earlier in life (1, 2). The original definition of sarcopenia focused on muscle mass as stand-alone. Subsequently, much more emphasis has been given to muscle function such that it currently comes to the forefront of international guidelines (3, 4). Physical performance, a major contributor of mobility and independence during older life (5), has been defined as "an objectively measured whole-body function related to locomotion" (3, 6). The multidimensional concept of physical performance is not merely limited to skeletal muscle but also involves central and peripheral nervous function including balance (3, 6). Low physical performance has been formerly considered a core component of sarcopenia (3, 7) and has been widely associated with adverse outcomes including frailty, disability and subsequent death (3, 8–14). Consequently, low physical performance is used to identify the severity of sarcopenia (3). There is a large consensus on the key role that physical function, and particular mobility, plays in the determination of frailty status (15, 16), regardless of the operational definition used (17). At the same time, the relationship between frailty and physical performance may be bi-directional since frailty status can also negatively affect mobility and physical function. In fact, physical performance measures are often used as an outcome measure in most of the trials targeting frailty and sarcopenia. Frailty and sarcopenia can be considered complementary in many aspects. To date, the physical frailty phenotype proposed by Fried et al. (18) shows a remarkable overlap with sarcopenia. The Physical Frailty and Sarcopenia (PF&S) model (15) has been therefore suggested as a possible solution to combine the two entities (i.e., frailty and sarcopenia) into a unique operational definition. Furthermore, the large body of literature about physical function impairment as well as the presence of dedicated measures that are widely accepted (e.g., short physical performance battery, handgrip strength, gait speed), make the PF&S model an easy-to-implement model to capture both frailty and sarcopenia (15). It is indeed largely agreed that the physical function impairment that results from the combination of frailty and sarcopenia acquires completely different connotations toward worst outcomes (19).

Despite a progressive decline in musculoskeletal function starting from middle age, several factors acting during the life course can influence musculoskeletal functional capacities. Starting from early life, everyone rapidly acquires supporting muscle functions to reach a peak or a plateau nearly at the end of adolescence period. Subsequently, after the fourth decade of life, a progressive decline in muscle mass (i.e., ~1–2% per year) and strength (i.e., ~1.5% per year) is seen (20). Kaymak et al. (3), in a comment to the European Working Group on Sarcopenia in Older People revised consensus (EWGSOP2), suggested that measurement of muscle power (intended as the product of strength and

velocity) is more relevant than muscle strength as stand-alone in reflecting physical performance. Accordingly, muscle power has been suggested as the most relevant measure of muscle function, being more strongly correlated with functional performance than strength as stand-alone in older people. An example may be the chair stand test that requires both strength and velocity for its execution (3). In the early phases of muscle decline (i.e., initial decrease in muscle mass and strength), an individual could still have preserved physical performance and may be very far from the threshold of disability (3) (Figure 1). Hall et al. (5), in the Physical Performance Across the Life-span Study, reported that physical performance is almost stable in the first two decades of adulthood (i.e., from 30 to 50 years of age) with a progressive decline in the middle years (i.e., 50+) and late adulthood. Besides genetic and lifestyle factors operating across the life course, also some pathological conditions can accelerate this degenerative process (with a consequent progression toward functional impairment and disability). Lifestyle interventions incorporating nutrition and physical exercise can slow or reverse this process (Figure 1) (3, 21). The rate of decline in muscle mass and function is also reflected by their peaks attained during early life (2). Indeed, it is essential to maximize muscle function in early life as well as maintain this peak during adult life to minimize losses during older life (3).

Nutritional strategies to counteract muscle mass and function decline are mainly based on protein supplementation combined with adequate calorie intake while there is limited knowledge on other nutritional interventions (22). Furthermore, the efficacy of nutritional interventions is enhanced when combined with physical activity (i.e., resistance training) (23). Inflammation and oxidative stress are considered hallmarks of the aging process (24) influencing the rate of functional decline observed in aging. Therefore, supplementation with individual nutrients sharing antioxidant and anti-inflammatory properties has recently gained attention for potential effects against the age-related functional decline (1, 25, 26). Within the plethora of various dietary supplements, polyunsaturated fatty acids (PUFAs), particularly omega-3 PUFAs and derived long-chain PUFAs (LC-PUFAs), are of particular interest for their potential effects on muscle function and thus on physical performance through various mechanisms. Omega-3 PUFAs may promote muscle anabolism through activation of the mammalian target of rapamycin (mTOR) signaling and reduction of insulin resistance (27, 28). Furthermore, omega-3 PUFAs are widely acknowledged as nutrients with clear anti-inflammatory and antioxidant properties (27, 29, 30).

This narrative review aims to provide an overview of current knowledge about the role of omega-3 PUFAs on physical performance across the lifespan.

The role of PUFAs in the body

Linoleic acid (LA, 18:2) and α-linolenic acid (ALA, 18:3) are considered essential fatty acids (EFAs) because of the absence of enzymes necessary for their production in humans and other mammals. Instead, they are obtained from plants or other organisms that possess enzymatic pathways for their synthesis. They must be part of the diet and, once introduced into the body, they can be further metabolized in the liver by the enzymes Δ6 and Δ5 desaturases and elongases to generate PUFAs (31) (see Figure 2).

LA is the precursor of the omega-6 PUFA family and arachidonic acid (AA) is its derived compound, while ALA, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) belong to the omega-3 PUFA family. These two groups of fatty acids influence in different ways the property of the cell membranes in terms of fluidity and biological effects. DHA, EPA, and AA compete for the sn-2 position on membrane

phospholipids. Therefore, their relative abundance in the membrane influences their availability as substrates for the same metabolic pathway enzymes, such as cyclooxygenases and lipoxygenases. Consequently, this balance affects the production of bioactive compounds with antagonistic roles involved in various disease processes (32). Generally, AA-derived metabolites have pro-inflammatory properties, whereas EPA-derived compounds are less inflammatory. DHA-derivates have anti-inflammatory and pro-resolution activities contributing to speeding up the inflammatory response's physiological resolution (33, 34).

Omega-3 PUFAs such as ALA and DHA and omega-6 PUFAs, such as LA and AA, are important structural components of cell membranes mainly within phospholipids. DHA is necessary for the development of brain functions and retinal functions associated to vision (35, 36). The incorporation of this omega-3 metabolite takes place at uniquely high levels in the central nervous system, where omega-3 PUFA are main determinant of membrane PUFA composition and unsaturation (37). Once high levels of DHA are established in the brain, they tend to be sustained throughout later life. This maintenance likely relies on an optimal dietary supply, particularly considering the potential decrease in efficiency of precursor conversion by the enzymatic pathway among older individuals (35, 38).

The membrane PUFA composition seems to be more responsive to dietary DHA compared to intake of LA and AA, showing a high sensitivity to dietary variations in PUFA-supply (39).

DHA, derived from ALA, has relevant metabolic activities. Besides hypolipidemic properties, reducing blood concentrations of triglycerides, DHA contributes to protect the central nervous system from reactive oxygen species, together with antioxidant properties, capable of shutting down the upstream inflammatory cascade. Furthermore, DHA exhibits immunomodulatory and antiallergic activities, contributing to its overall neuroprotective effects. From intrauterine life through later ages, DHA contributes to the maintenance of cognitive abilities and the prevention of neuropsychiatric and neurodegenerative disorders (40).

DHA and EPA share anti-inflammatory and inflammation-resolving properties including the partial inhibition of leucocyte chemotaxis, adhesion molecule expression and leukocyte-endothelial adhesive interactions, production of eicosanoids such as prostaglandins and leukotrienes from the AA, as well as the production of pro-inflammatory cytokines (30). These anti-inflammatory and pro-resolving effects show to be relevant to improve clinical outcomes in different therapeutic areas, supporting the protective role of the immune system (41). Omega-3 PUFAs seem to be involved in the activation of cells from both the innate and the adaptive immune system (42). In particular, in the innate immune cells omega-3 PUFAs (1) reduce neutrophil migration and increase their phagocytosis, (2) reduce pro-inflammatory cytokine release, increase phagocytosis and M2 macrophages phenotype that promote tissue repair at macrophage level and (3) reduce presentation at dendritic cells level (42). In the adaptive immune cells, omega-3 PUFAs limit excessive B-cells responses, increase T regulatory cells differentiation and function while reduce T helper 17 differentiation, and limit the release of pro-inflammatory cytokines (42, 43). Consistent with aims of the present review, in older populations growing evidence suggests a role of omega-3 PUFAs in the maintenance of muscle mass and function and in musculoskeletal health in general (44).

Main mechanisms of omega-3 PUFAs in the muscle

Most studies on omega-3 PUFAs have been primarily focused on cellular and molecular mechanisms underlying muscle protein metabolism (45). Accordingly, the main mechanisms through which omega-

3 PUFAs could benefit muscle parameters seem to be (1) both anti-catabolic and anabolic effects on muscle protein synthesis (2) modulation of insulin sensitivity (3) amelioration of mitochondrial functioning, inflammation and muscle fiber contractile properties (4) neuroprotective and motor neuron excitability properties (46). Figure 3 presents an overview on the main mechanisms, which will be discussed in detail by each life stage (e.g., early life, adult and older life) in the following specific sections, by which omega-3 PUFAs can influence muscle parameters.

Omega-3 PUFAs EPA and DHA, both as stand-alone and synergically with amino acids ingestion, seem to directly stimulate mTORC1 thus enhancing muscle protein synthesis (Figure 3). However, it should be considered that while the acute activation of mTORC1 could promote muscle protein synthesis (48), the prolonged activation of mTORC1 has been associated with severe muscle atrophy, mainly because of decreased autophagy in the muscle (49). Therefore, it seems that alternating periods of high and low mTORC1 activation, as occurring with a healthy diet incorporating omega-3 PUFAs, is the key for an optimal muscle function (50). Omega-3 PUFAs seem also to improve mitochondrial function, mainly by reducing non-mitochondrial respiration and by augmenting the reserve respiratory capacity and bioenergetics (51). By reducing ROS production at the mitochondrial level, omega-3 PUFAs seem also to reduce muscle protein breakdown (47). Additionally, omega-3 PUFAs seem to counteract insulin resistance by improving mitochondrial function and bioenergetics as well as by modulating phospholipid membranes (52, 53), but also by increasing serum levels of insulin-like growth factor 1 (IGF-1). In turn, IGF-1 stimulates muscle protein synthesis via mTORC1-dependent and independent pathways (47, 54).

Omega-3 PUFAs could inhibit muscle protein breakdown also by acting as ligands for G-protein coupled receptors (GPCRs) and by activating the peroxisome proliferator-activated receptors (PPARs), with the consequent inhibition of nuclear factor kappa B (NFκB). In turn, the inhibition of NFκB leads to a reduced cyclooxygenase production resulting in a decreased inflammatory response (55) (Figure 3). The inhibition of NF-κB also leads to the downregulation of the muscle ring finger-1 (MuRF-1) gene counteracting the ubiquitin-proteasome system and thus reducing muscle protein breakdown (47). Furthermore, PPARs which are transcription factors activated by fatty acids and their derivatives, are involved in development, metabolism, inflammation, and many cellular processes in different tissues including the muscle (56). In particular, there are three different PPAR isotypes: (1) PPARα is highly expressed in tissues, like skeletal muscle, with effective fatty acid catabolism; (2) PPARβ/δ, which is more ubiquitously with a predominance in the skeletal muscle, is implicated in energy metabolism, mitochondrial biogenesis, and fiber-type switching; (3) PPARγ is highly expressed in adipocytes, but it is also involved in fat deposition in the muscle (56). Indeed, beyond NF-κB inhibition, PPARγ activation seems to play a relevant role in the inhibition of myosteatosis (i.e., intramuscular and intermuscular fat infiltration) and muscle fiber type switching (56) which are key features in the age-related sarcopenia (1). In particular, fat deposition in the muscle with its lipotoxic action, can exert detrimental effects on both muscle quality and strength, also negatively affecting mobility function (57–59). These effects are even more magnified when sarcopenia is accompanied by obesity (60). Additionally, PPARγ is a key regulator of glucose homeostasis and insulin sensitivity in the human skeletal muscle (61), with abnormalities in its expression being involved in skeletal muscle insulin resistance, especially in the presence of obesity and/or type II diabetes (62). The effects of omega-3 PUFAs supplementation on PPARγ activity have been demonstrated also in young athletes (e.g., age range of 20 to 30 years) who were supplemented with 2000 mg/day of omega-3 PUFAs (EPA: 360 mg, DHA: 240 mg) or placebo (2000 mg/day edible paraffin) for 3 weeks (63). The authors found that omega-3 PUFAs supplementation was significantly associated with the up-regulation of PPARγ, with an increase in resting energy

expenditure and appetite (63). Also a recent meta-analysis of randomized controlled trials (RCTs) (64), involving both young, middle-aged and older people, showed that omega-3 PUFAs supplementation at varying doses (from 2000 mg/day to 7,000 mg/day) and with varying duration (from 3 to 48 weeks) led to a significant up-regulation of PPAR-γ gene expression. Finally, the incorporation of omega-3 PUFAs in the phospholipid bilayers of the cell membrane favors membrane fluidity and modulates neuromuscular transmission (Figure 3) resulting in greater muscle strength (47, 65–67).

Physical performance measures

The assessment of physical performance in older people can be envisioned as a summary marker of functional status as well as of the underlying biology of ageing (8). Tests of physical performance are strongly associated with frailty, disability and death in older people (9–14) and are used to identify the severity of sarcopenia (3). Physical performance measures are thus intended to monitor the evolution of functional status over time or the change after an intervention (68, 69), so such tests are often used as an outcome measure in most trials (3). Much research has been conducted in large, prospective studies of older populations, assessing physical performance in several ways. The main tests of physical performance in older people are summarized in Table 1. Physical performance is usually measured in older people by the Short Physical Performance Battery (SPPB) (11, 72), the 400-meter walk test (400-MWT) (74), gait speed (70, 71), and the Timed-Up and Go (TUG) test (73), as suggested by the EWGSOP2 (3). Shortly, the SPPB test combines the assessment of gait speed, a balance test, and a chair stand test. The SPPB scores range from 0 to 12, with a score of ≤8 points indicating poor physical performance (11, 72). However, the SPPB is frequently used in research rather than in clinical practice because of its length of administration (i.e., at least 10 min) (3). The 400-MWT evaluates both walking ability and endurance and consists of completing 20 laps of 20 m as fast as possible, with up to two rest stops during the test that are allowed. Non-completion or ≥6 min for completion of this test indicates poor physical performance (74). Also in this case, the length of the 400-MWT as well as the need for a corridor at least 20 m long make it difficult to implement in routine clinical practice. However, in the geriatric context, some tests with shorter distances (e.g., 4, 7, and 10 Meter Walk Test) have been proposed as good alternatives showing high test–retest reliability and validity in measuring walking speed (76–78). However, also the 10-meter walk test (10-MWT) requires a corridor 20 m long making it difficult to implement in most clinical settings (79). Indeed, the 4-meter walk test (4-MWT), which is part of the SPPB test, is considered a valid alternative to the 10-MWT in both clinical and research settings (3, 80), with a cut-off ≤0.8 m/s that has been advised to indicate poor physical performance (i.e., severe sarcopenia) (3). Gait speed is instead considered an easy-to-implement highly reliable measure and it is advised, for its convenience, by EWGSOP2 for physical performance assessment (3). Likewise, the TUG test is widely used in the geriatric context for its simplicity and reliability. In particular, the TUG test asks the participant to rise from a chair, walk up to a marker 3 m away, turn around, walk back to the chair and sit down again, with a cut-off of ≥20 s suggested as indicative of poor physical performance (3, 73). To better capture the age-related modifications in functional status, physical performance should be assessed before reaching old age, taking into consideration the determinants that influence it over one's lifetime (8). In this way, to intervene earlier in life could slow the rate of functional decline associated with aging. There is a gap in understanding early-life factors influencing late-life physical performance (8). Furthermore, there is a paucity of measures for assessing physical performance in young people as well as the fact that physical performance is rarely assessed in young individuals. Until now, tests of physical performance such as the TUG test or the 10-MWT have been used in pediatric populations with specific pathological conditions (e.g., Down Syndrome (81, 82) or neuromuscular

disease (83–85)). These tests, typically employed in the geriatric population, could therefore be applied to younger populations (i.e., young adults) to evaluate physical performance despite different cut-offs may be needed.

Omega-3 PUFAs and physical performance in early life (childhood and adolescence)

As the global population continues to age, there is a growing need to identify modifiable factors throughout life that influence physical function in later years. These factors may influence the peak of function achieved earlier in life determining the timing and rate of subsequent decline (Figure 1). Literature about the role of omega-3 PUFAs on physical performance in early life is scarce, although beneficial effects of these compounds on sport performance of young athletes have been reported (86, 87). Notwithstanding, some potential mechanisms by which PUFAs could exert beneficial effects on physical performance starting from early life could be argued (Figure 4).

The role of the immune system and inflammatory processes throughout the lifespan, including early and in-utero life, has gained growing attention as a driver of a wide spectrum of age-related chronic conditions including metabolic syndrome, type 2 diabetes, cardiovascular disease, osteoporosis and sarcopenia (88). Systemic chronic inflammation, even during childhood and pregnancy, has been reported to influence the inflammatory trajectories in later stages of life (59, 88, 89). Conversely, inflammation, defined as the elevation of pro-inflammatory cytokines (i.e., C-reactive protein, interleukin-6), has been associated with poor physical performance in both older individuals (90, 91) and young adults (i.e., sedentary young adults aged 18–35 years) (92). Inflammation could be regarded as an early determinant of the physical performance decline seen with aging, starting from childhood and even from pregnancy. Starting from pregnancy, the so-called “maternal exposome” (i.e., diet, physical activity, psychological stress and exposure to xenobiotics) influences the immune system programming of the offspring towards a more pro-inflammatory profile in adulthood (88). Poor nutrition during early life (i.e., both undernutrition and overweight and obesity) has been associated with increased levels of inflammatory markers and with consequences during adult life including cardiometabolic disease and sarcopenia (93). The omega-3 PUFAs EPA and DHA are able to partially inhibit many aspects of inflammation, including leukocyte chemotaxis, adhesion molecule expression and leukocyte-endothelial adhesive interactions, production of eicosanoids such as prostaglandins and leukotrienes from the AA, and production of pro-inflammatory cytokines (30). In this context, omega-3 supplementation during pregnancy contributes to increase omega-3 PUFAs status of the offspring (94) and influence immunological outcomes through the modification of offspring cytokine concentrations (95, 96). See et al. (97) reported for the first time that omega-3 PUFAs supplementation during pregnancy was associated with an increase in specialized pro-resolving mediators precursors in the cord blood of the offspring at birth, suggesting a beneficial role in the alleviation of low-grade inflammatory status associated with pregnancy. However, the authors found the effects were not sustained at 12 years of age highlighting that the continuation of supplementation across life course may be also a relevant factor. This is because the effects of omega-3 fatty acid consumption might require a more prolonged and continuous intervention to observe a sustained difference in pro-resolving mediators as the half-life of EPA, DHA and the resolvins is in the hour range (98, 99). The time windows (i.e., at birth and at 12 years of age), used in the study of See et al. (97), have probably been chosen because those life stages can be envisioned as critical and sensitive periods of human development, according to Barker’s ‘developmental origins of health and disease’ hypothesis (100). This theory proposes that factors that modify physiological processes during the critical developmental period in

early life may exert a long-term influence on disease in adult life. In particular, early life nutritional exposures, especially during critical or sensitive periods, are significant determinants of both growth and development and later health (100). Specifically, omega-3 PUFAs supplementation during pregnancy seems to increase omega-3 PUFAs status of the newborn (94), influencing immune function by acting on cytokine concentrations (95, 96) and attenuating lipid peroxidation in the newborn (101).Various RCTs involving children and adolescents with specific pathological conditions (i.e., autism spectrum disorder, attention-deficit-hyperactivity disorder, and cystic fibrosis) have demonstrated the role of omega-3 PUFAs in countering pro-inflammatory mediators after birth (102–104).

On the other hand, low birth weight as well as altered physical growth and development parameters (at the age of 1, 7, and 12 years, respectively), have been associated with poor grip strength and physical performance in mid-to-later life (i.e., at the age of 53 and 56 years for mid-life and at the age of 65–70 years for later life) (8, 105–109). Muscle strength is a major determinant of the age-related decline of physical performance (106), as shown in Figure 1. Indeed, incorporating lifestyle modifications to maximize the peak of muscle functional capacities during early life is functional to preserve physical performance in mid-to-later life. Exploring the potential of omega-3 PUFAs to positively affect muscle strength from early life by influencing birth weight and parameters related to growth and development could be of significant interest. In this regard, a longer duration of breastfeeding has been associated with both greater grip strength in older life (110) and increased lower body explosive strength in adolescence (111). The beneficial effects of breastfeeding on grip strength could probably be mediated by breast milk fatty acids content. This is of particular interest since achieving a higher peak in muscle strength starting from early life is directly associated with a lower decline in muscle strength and, consequently, in physical performance being the latter strongly influenced by muscle strength (3, 106). Human milk contains essential dietary fatty acids such as LA and ALA, along with their metabolites AA and DHA, which play a supportive role in the growth and development of breastfed infants (112). The amount of omega-6 and omega-3 fatty acids secreted in the milk is reflective of the maternal dietary intake of PUFAs (112). The hypothesis that omega-3 PUFAs supplementation could prevent preterm birth and low birth weight has originated from studies conducted in the Faroe Islands (113). In these islands, the diet is characterized by a greater intake of marine foods compared with the population of Denmark. This likely accounts for the higher birth weights (approximately 200 g more at term) observed in babies born in this area. Furthermore, birth weights of infants from the Faroe Islands have been found to be higher than those of 33 other countries (113).

From a life course perspective, another factor to be considered is obesity (and adiposity). Maternal obesity has been associated with increased fetal adiposity, especially when accompanied by gestational diabetes (93). Nutritional excess during early childhood (i.e., greater gestational weight gain, higher birth weight, faster postnatal weight gain) is associated with an increased risk of obesity, central adiposity as well as insulin resistance in adult and older life (from 20 till 70 years of age) (93, 114). Subsequently, excess weight gain during childhood and adolescence period is likely to lead to persistent overweight and obesity throughout life (115).

In this context, the role of chronic inflammation should not be overlooked since increased adiposity is characterized by the abnormal secretion of a wide range of pro-inflammatory molecules including adipokines, cytokines and chemokines thus predisposing to adult adverse conditions although inflammation is a necessary biological response to various stimulus, having defense and tissue restructuring functions (88, 116). Inflammatory trajectories across the life course could be thus probably mediated by body composition alterations as both undernutrition and overweight are associated with inflammation (93, 117, 118). In turn, obesity status, either independently or in

combination with sarcopenia, has been associated with reduced physical performance in older individuals (119). Based on these considerations, omega-3 PUFAs have been suggested to play a role in the context of overweight/obesity through numerous mechanisms including the modulation of lipid metabolism and inflammation, the regulation of adipokines as well as the promotion of adipogenesis and the alteration of epigenetic mechanisms (120). A reduced red blood cell omega-3 PUFAs status has been reported in children with greater adiposity and has been associated with a suboptimal intake of omega-3 PUFAs (121). In this context, a plausible explanation may be also related to the evolution of the Western diet towards a pro-inflammatory diet rich in refined grains and ultra-processed foods and low in fruits and vegetables, thus poor in vitamins, minerals and with a suboptimal omega-3 content in favor of omega-6 PUFAs (88). However, no significant effects of omega-3 PUFAs supplementation on anthropometric parameters have been reported in children and adolescents living with overweight/obesity (122, 123).

Lower socioeconomic position (SEP) has been associated with lower serum levels of omega-3 PUFAs starting from pregnancy through adulthood (124–129). Robinson et al. (130) in a multi-cohort analysis, reported that a low SEP was independently associated with an unfavorable metabolic profile including low omega-3 status both in children (i.e., aged 7 years), adolescents (i.e., aged 15 and 17 years), adults and older adults (from 31 till 75 years of age). This is probably due to the poor quality of the diet associated with a lower socioeconomic status as reported in ethnic minorities (i.e., Latino immigrants in the U.S.), but also in urban areas of Australia (129, 131) with a reduced consumption of fruits, vegetables, whole grains, fiber, fish and seafood thus reflective of low omega-3 status (132). Additionally it should be considered that low levels and intake of omega-3 may in part be related to the limited accessibility of marine-food sources, especially in certain geographic areas (129). In a systematic review and meta-analysis, Birnie et al. (133) reported an association between lower childhood SEP and reduced physical performance in adulthood and older life (from 18 to 79 years). This connection persisted even after adjustment by adult SEP, despite the presence of heterogeneity among studies. These findings suggest that the accumulation of adverse exposures across the life course may be more predictive of the functional decline observed during aging than models considering only adult factors. It can be assumed that the association between low childhood SEP and reduced physical performance in adult and older life may be mediated, in addition to other adversities, by a poor quality of the diet, including inadequate omega-3 PUFAs intake.

It has been documented that attainment of gross motor development milestones (i.e., standing and walking) during childhood, as well as higher scores on tests measuring cognitive ability and motor coordination, are associated with enhanced physical performance in midlife, independently of other factors (8). In particular, Kuh et al. showed that the age at which an individual first walked was associated with both midlife standing balance and chair stand test, which are two out of three components of the Short Physical Performance Battery (SPPB) (8). The authors reported that better scores on cognitive ability tests at age 8 years and of motor coordination at age 15 years were associated with greater standing balance and chair standing (8). The attainment and maturation of motor and cognitive function during childhood, as well as the age-related motor and cognitive functional decline in older life, are highly integrated (134, 135), indicating that these developmental factors may be envisioned as markers of more complex cortical–subcortical neural circuits connected with higher levels of function later in life (8). This aligns with the findings of Ridler et al., who showed an anatomically related overlap between frontocerebellar system related to infant motor development and adult executive function (136). Similarly, Murray et al. reported that early development in the gross motor domain is associated with higher adult executive function (137). In this regard, omega-3 PUFAs

are widely acknowledged to play a central role in brain function and contribute to the structure of the neuronal cell membranes (138). They are crucial for myelination and vision development during the perinatal period (139). In particular, DHA represents nearly 90% of total omega-3 PUFAs in the brain and is especially concentrated in the gray matter (140, 141). Early life accumulation of omega-3 PUFAs represents a golden opportunity for their storage in neural tissues (38, 140–142). In this context, it has been demonstrated that omega-3 PUFAs supplementation during pregnancy is associated with earlier achievement of gross motor milestones and improved cognitive development in children (143). The study by Beblo et al. (144) reported that fish oil supplementation enhanced omega-3 PUFAs levels and improved motor skills in children with phenylketonuria. Additionally, Agostoni et al. (145) in a RCT, demonstrated that infants who received DHA supplementation achieved sitting without support in a shorter period. Other studies reported contrasting results. A systematic review by U.S. Departments of Agriculture nutrition evidence reported insufficient evidence to establish a relationship between omega-3 supplementation during pregnancy and lactation with motor and visual development in infants (146). Richardson and Montgomery (147) found that in children with development coordination disorder, PUFAs supplementation (i.e., 2 capsules 3 times/day providing 558 mg of EPA, 174 mg of DHA and 60 mg of LA for 3 months) did not improve motor function, while they improved reading and spelling age and symptoms of attention-deficit/hyperactivity disorder.

In summary, the role of omega-3 PUFAs starting from early life, and even in utero, is intriguing given their long-lasting effects on human health through the various mechanisms discussed (e.g., birth weight, motor development, modulation of inflammation, immune response and oxidative stress) which could probably influence adult physical performance, with potential implications for the prevention of sarcopenia, frailty and disability.

Omega-3 PUFAs and physical performance in mid to later life

Growing clinical evidence supports the positive role of omega-3 PUFAs on physical performance in mid to later life (148–150). Some studies focused on the relationship between omega-3 PUFAs supplementation and muscle strength (27, 151). As shown in Figure 1, with advancing age muscle strength tends to decline earlier and more rapidly than physical performance, especially when a lower peak in muscle strength is reached during early life. According to the latest consensus guidelines of the EWGSOP2, in the early phases of sarcopenia development, a person may result above the threshold of low physical performance despite a reduction in muscle strength (3). The reduction in muscle strength can be considered an early indicator of overt functional decline. This is evidenced by the EWGSOP2 algorithm for sarcopenia case finding where low muscle strength represents the first step in sarcopenia assessment defining probable sarcopenia and low physical performance the last step to quantify sarcopenia severity (3). Muscle strength can be thus considered as the early component, on which acting, to preserve muscle function and thus physical performance during older life. In this regard, a recent meta-analysis showed a beneficial effect of omega-3 PUFAs supplementation on lower body muscle strength while no effects were found on upper body strength (151). Bird et al. (152), in a recent meta-analysis, reported a significant relationship in favor of omega-3 PUFAs supplementation for quadriceps maximal voluntary capacity. Another meta-analysis by Rondanelli et al. (153) found no effects of omega-3 EPA plus DHA supplementation on chair rise test and handgrip strength. On the other hand, a meta-analysis of 9 RCTs showed that omega-3 PUFAs supplementation significantly increased the grip strength (154). In the Hertfordshire cohort study, Robinson et al. found a positive association between fatty fish consumption and grip strength (155). Regarding physical performance, in 2017,

Frison et al. (150) reported that higher omega-3 PUFAs plasma levels were associated with lower odds of low gait speed (i.e., <0.63 m/s) in older individuals. In a cross-sectional analysis of the Multidomain Alzheimer Preventive Trial (MAPT), recruiting older adults aged 70 years and older, Fougère et al. (156) found an association between low levels of omega-3 PUFAs in red blood cell membranes and lower physical performance measured through the SPPB. However, in a secondary analysis of the MAPT trial, Rolland et al. (157) reported no significant effects of long-term omega-3 PUFAs supplementation, either alone or in combination with a multidomain lifestyle intervention comprising physical activity counseling, on the walking speed test and SPPB.

In the InCHIANTI study, including 1,273 participants between 22 and 104 years of age living in Tuscany (Italy), Abbatecola et al. (148) found that higher levels of total PUFAs, omega-3 PUFAs, and omega-6 PUFAs were associated with high physical performance (i.e., SPPB score > 9) at baseline, after adjusting for age. However, after adjusting for potential confounders, baseline 7 m walk time was associated with total PUFAs levels. Additionally, the authors found that baseline omega-3 PUFAs levels were inversely associated to the risk of developing a decline in SPPB to scores ≤9, while the omega-6/omega-3 ratio was associated with a higher risk of SPPB decline and with a longer time to walk 7 meters.

Hutchins-Wiese et al. (158), in a RCT recruiting 126 postmenopausal women, demonstrated that omega-3 PUFAs supplementation resulted in greater physical performance, measured by change in walking speed. On contrary, Krzymińska-Siemaszko et al. (159) did not observe an improvement in physical performance after 12 weeks of omega-3 PUFAs supplementation in a sample of older people with low muscle mass. A recent meta-analysis of RCTs in older adults (153), reported that daily omega-3 PUFAs supplementation is associated with a reduction in the time of the TUG test, while no statistically significant effect was found on 4-MWT. These results are in line with another recent meta-analysis (151), focused on people aged 55 years and older, which reported a significant association between omega-3 supplementation and lower TUG but not on walking speed. However, in another systematic review and meta-analysis, it was found that there were minor benefits of omega-3 supplementation for TUG performance, while subgroup analyses showed that omega-3 PUFAs supplements at more than 2 g/day may contribute to improving walking speed, especially if the intervention is carried out for more than 6 months (160).

In summary, evidence from interventional studies and meta-analyses gave controversial results on the beneficial effect of omega-3 PUFAs on physical performance parameters. As outlined by some authors, small study size and heterogeneity in the intervention protocol, including the ratios between EPA and DHA, may have limited the applicability of these results.

2. Conclusions

Mechanisms through which omega-3 PUFAs could benefit muscle function including physical performance parameters across the life course are intriguing. However, it should be considered that few interventional studies explored the effects of omega-3 PUFAs supplementation on physical performance, with varying duration, dosage and use (i.e., alone or in combination with other interventions). This has led to controversial results reported in literature. However, it seems that omega-3 PUFAs supplementation at high doses (i.e., more than 2 g/day) and for longer periods (i.e., for more than 6 months) may contribute to improving physical performance (e.g., walking speed) in older people. Larger and high-quality RCTs are needed to fully elucidate the beneficial effects of omega-3 PUFAs supplementation on muscle function parameters. Although, a direct association in early life is not

available in literature, some mechanisms by which omega-3 PUFAs may contribute to improved physical performance could be hypothesized. The integration of physical function measures in future RCTs would be of great interest to explore whether omega-3 PUFAs could contribute to improved muscle function parameters, starting from early life and extending throughout the lifespan.

3.3 PUFAs and autoimmune disorders: immune modulation

PUFAs, particularly omega-3 derivatives, play a pivotal role in the regulation of immune function and inflammatory responses (*Table 2*). Their incorporation into cell membranes influences lipid raft organization and modulates eicosanoid biosynthesis, ultimately shifting the balance from pro-inflammatory toward anti-inflammatory mediators. In addition, omega-3 PUFAs are precursors of specialized pro-resolving mediators, such as resolvins and protectins, which actively promote the resolution of inflammation and may mitigate the persistent low-grade inflammation that characterizes autoimmune diseases.

ALA, one of the two essential plant-derived fatty acids, represents the primary dietary precursor of EPA and DHA and is itself endowed with intrinsic anti-inflammatory properties. Evidence indicates that ALA contributes to cardiovascular protection not only through lipid modulation but also by attenuating vascular inflammation. Reductions in inflammatory and endothelial activation markers, including C-reactive protein (CRP), VCAM-1, E-selectin, as well as improvements in serum lipid profiles (TC, LDL-C, triglycerides) have been documented (105). Supplementation with ALA has also been associated with decreased circulating IL-6 levels, further reinforcing its role as an anti-inflammatory nutrient. These observations are consistent with the EFSA claim acknowledging the cholesterol-lowering effect of ALA.

Another critical aspect concerns the dietary n-6/n-3 ratio, which in Western populations often exceeds 15:1 or 16:1, far above the recommended values. Such an imbalance, largely due to excessive intake of LA and other omega-6 FAs, is increasingly recognized as a contributing factor in the pathogenesis of chronic conditions, including cancer, autoimmune, and inflammatory disorders. By contrast, an increased intake of omega-3 fatty acids, including ALA, is able to counteract these detrimental effects by suppressing pro-inflammatory signaling pathways and restoring a more favorable ratio.

Importantly, autoimmune diseases characterized by an inflammatory substrate often present with lipid profile abnormalities and a markedly increased cardiovascular risk. For example, patients with Juvenile Systemic Connective Tissue Diseases frequently display elevated triglycerides, TC, LDL-C, reduced HDL-C, and a higher incidence of hypertension and atrial fibrillation. In this context, ALA supplementation has been explored as a potential nutritional strategy, with positive effects observed on inflammatory markers, lipid metabolism, and even glycemic control in subjects with dyslipidemia. A recent narrative review further emphasized the link between ALA and autoimmune disorders, underlining its potential role in modulating both inflammation and cardiometabolic risk in these patients (54).

Taken together, these findings suggest that ALA may exert a dual action in autoimmune disorders by attenuating inflammation and simultaneously improving metabolic and cardiovascular parameters, thereby offering a valuable dietary approach in the management of these conditions.

Condition	***Effects of ALA / Omega-3 PUFAs***	***References***
Cardiovascular risk in autoimmune diseases	Associated with dyslipidemia (↑ TG, TC, LDL-C; ↓ HDL-C), higher risk of hypertension and atrial fibrillation	Bertoni C., et al, 2024 Zhao G., et al., 2004
ALA supplementation in dyslipidemia / autoimmune	Reduces inflammatory parameters, fasting glucose, CRP, IL-6; improves lipid profile	Zhao G., et al, 2004 Bertoni C., et al 2024
Immune modulation	Incorporation into membranes alters lipid rafts and eicosanoid biosynthesis; promotes resolvins and protectins; attenuates chronic inflammation	Bertoni C., et al 2024
Balanced n-6/n-3 ratio	Corrects Western diet imbalance (15–16:1); reduces pro-inflammatory signaling and chronic disease risk	Bertoni C., et al 2024

Table 2. Effects of ALA and omega-3 PUFAs on autoimmune disorders, highlighting cardiovascular risk, immune modulation, and lipid profile improvements.

frontiers in Immunology

Front Immunol. 2024 Mar 28;15:1380372. doi: 10.3389/fimmu.2024.1380372

Cardiovascular risk and inflammation in a population with autoimmune diseases: a narrative review

Camilla Bertoni [1,†], Alessandra Mazzocchi [2,†], Ludovica Leone [3,†], Carlo Agostoni [2,3,*], Giovanni Filocamo [2,3]

1. *Introduction*

Juvenile Systemic Connective Tissue Diseases (JSCTD) are a heterogeneous group of juvenile-onset chronic autoimmune diseases, characterized by the presence of systemic inflammation and autoimmune-mediated multi-organ system involvement. The most common diseases in children are juvenile onset systemic lupus erythematosus (JSLE), juvenile dermatomyositis (JDM), juvenile systemic sclerosis (JSSC), juvenile localized scleroderma, juvenile mixed connective tissue disease (JMCTD) and juvenile Sjögren' syndrome.

SLE is a chronic autoimmune disease characterized by autoantibody-associated multi-organ damage, including renal, cardiovascular, neural, and primarily affects women of childbearing age [1]. Symptoms vary widely, from mild cutaneous manifestations to life-threatening organ failure [2]. Childhood-onset SLE has a more aggressive disease course with greater morbidity and mortality than adult SLE [3].

JDM is a multisystemic disease of unknown etiology characterized by nonsuppurative inflammation of striated muscle and skin [4,5]. Most common forms of idiopathic inflammatory myopathy (IIM) are associated with marked activation and damage to small blood vessels in the connective tissues of the muscle and skin [6,7]. Capillary loss precedes other pathological muscle changes, and the inflammatory infiltrate in dermatomyositis is predominantly perivascular and perimysial [8]. Vascular damage is considered integral to the disease pathogenesis of DM which also shows activated capillaries with increased adhesion molecule expression [9].

JSSC, also known as scleroderma, is a connective tissue disease [10]. The etiology mirrors that of adult-onset systemic sclerosis (SSC), with similar inflammatory mediators and autoantibodies, but with a significant population of children with uncharacterized antinuclear antibodies [11]. Several pathways in SSC vasculopathy can lead to atherosclerotic manifestations [12]. SSC-related vasculopathy combined with immune dysregulation and progressive fibrosis are implicated in the pathogenesis of large vessel disease and specific cardiac dysfunction [13,14,15].

Sjögren's syndrome is a systemic autoimmune disease that affects middle-aged women [16]. Patients with this disease are present with oral and ocular dryness, including interstitial pneumonitis, thyroiditis, central nervous system involvement and vasculitis [17]. In the pediatric population, Sjögren's syndrome is less characterized in terms of clinical presentation and long-term outcome [18].

2. Dyslipidemia, inflammation, and atherosclerosis in pediatric populations with autoimmune rheumatic diseases

Dyslipidemia has rarely been studied in pediatric populations with autoimmune rheumatic diseases. Lipid abnormalities in these diseases can occur due to various risk factors (body composition, corticosteroid therapy, chronic inflammation, etc) [19].

Dyslipidemia is diagnosed in 50-85% of children and adolescents with SLE [20,21,23], and is a major risk factor for heart failure and cardiovascular diseases (CVDs). The risk of CVD is twice as high in adults with SLE compared to the general population [22]. Lipid metabolism affects both the innate and adaptive immune systems through various mechanisms, creating a vicious cycle that promotes atherosclerosis [23].

Studies in children and adolescents with SLE using biomarkers are still scarce in the literature [24-28]. Several lipid abnormalities have been described in JSLE, including dyslipidemia with elevated triglyceride (TG) and LDL levels, decreased levels of HDL and apolipoprotein-A1 (Apo-A1), and an increased abundance of dysfunctional proinflammatory HDL, which lacks the antioxidant capacity of conventional cardioprotective HDL [22,29, 30,31].

Inflammation is a major cause of atherosclerotic disease. The process may be accelerated in JSLE patients due to the prolonged exposure to inflammation. Inflammation and atherosclerosis are causally related. A stringent lipid-lowering therapy is required to reduce the risk of adverse outcomes of atherosclerosis, and inflammation is currently considered a novel therapeutic target to counteract atherosclerotic complications [23]. Inflammation is a fundamental pathological mechanism in autoimmune diseases. The cytokines released by the activation of the immune system can affect fatty acid oxidation, and activate lipoprotein lipase in adipose and muscle tissue, together with hepatic lipase, leading to dyslipoproteinemia [32]. Dysregulation of lipid metabolism initiates an inflammatory and immune-mediated response in atherosclerosis [23].

However, the pathogenesis of dyslipidemia and accelerated atherosclerosis in SLE and JSLE has not been clearly identified [33]. Several drugs commonly used in SLE patients may have adverse effects on lipid metabolism, such as corticosteroids [34], cyclosporine A [35], and tacrolimus [36]. Metabolic disorders caused by a chronic excess of nutrients such as lipids and glucose can also accelerate the process too, and both conditions may simultaneously trigger pro-inflammatory responses [37,38]. Accordingly, an immune metabolic response may contribute to the abnormal lipid metabolism of SLE. Oxidized LDL, defective clearance of dead cells, and a pro-inflammatory T-cell profile are features of both atherosclerosis and SLE [2], underpinned by an imbalance in the immune system with lower levels of T-regulatory cells (Tregs) relevant for suppressing of autoimmune, pro-inflammatory responses against the self [39,40,41].

3. Dietary strategies on inflammatory markers

A very powerful nutrient that can help to reduce inflammatory markers is ALA, a precursor of the omega-3 series. ALA is one of the two essential fatty acids of plant origin and is the main precursor of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA, which belong to the omega-3 series). ALA is mainly found in walnut oil, flaxseed oil, hemp oil, canola oil and seeds, and other types of vegetable oils. ALA reduces the risk of cardiovascular disease risk, possibly by favorably modifying vascular inflammation, with reduced levels of CRP, vascular cell adhesion molecule-1 (VCAM-1), E-

selectin, serum total cholesterol, LDL and triglyceride levels. It may reduce CVD risk by inhibiting vascular inflammation and endothelial activation beyond its lipid-lowering effects [42]. Dietary supplementation with ALA significantly reduced IL-6 levels [43]. To support the cholesterol-lowering effect of ALA, EFSA (European Food Safety Authority) has endorsed a claim [44]. In addition, ALA supplementation is also essential to maintain the omega-6/omega-3 ratio, which is currently unbalanced around 15/1 and 16/1 in the Western diet [45,46]. An imbalance of these nutrients in the diet, such as linoleic acid (LA), and a ratio of fatty acids clearly in favor of omega-6, favors the pathogenesis of many diseases, including cancer, inflammation and autoimmune diseases. On the other hand, a high intake of omega-3, especially ALA for example, suppresses inflammation. An excess of LA is therefore detrimental to omega-3 metabolism. Since omega-6 as LA is found in many more foods than ALA, it is easy to understand how easy it is to overdo LA intake and AA production [47].

4. Results

4.1 Dyslipidemia in JSLE

In the study by Machado et al [48], adolescent females (n=33) aged 10-19 years old diagnosed with JSLE according to the American College of Rheumatology were evaluated [49]. The control group consisted of 33 healthy adolescent females. Dyslipidemia was observed in 39.4% of the patients. Multivariate analysis showed that the SLE group had a lower ApoA1 concentration (163.1 vs 96.2, p value 0.001) and a lower LDL-C/ApoB ratio (1.1 vs 0.9, p value 0.001). As for the lipid profile, values of TC-C, TG and LDL-C levels were elevated in 9.1%, in 24.2% and 21.2% of SLE patients, respectively, while HDL-C concentrations decreased in 24.2% of SLE patients.

Zhou et al. describe in the cross-sectional study of 2020 [50], the relationship between serum lipid profile and SLE disease activity in young female adults with SLE. Seventy-one female adults (20-30 years old) newly diagnosed with active SLE were included in this study. As expected, TG and VLDL levels were significantly elevated in the subjects with SLE and compared to controls (0.8 ± 0.03 vs 2.2 ± 0.06, p value < 0.01 for TG and 0.3 ± 0.02 vs 0.8 ± 0.03, p value < 0.01 for VLDL). HDL-C and ApoA1 were also significantly decreased in the SLE group compared to the control (1.6 ± 0.05 vs 0.8 ± 0.04, p value 0.02 for HDL-C and 1.3 ± 0.04 vs 0.9 ± 0.03, p value < 0.01 for Apo A). Levels of TC-C and LDL-C decreased in the SLE group compared to the control group.

As for the pediatric and adolescent populations, the study by Ortiz et al [51] evaluated the presence of dyslipidemia and cardiovascular risk factors, introducing homocysteine (Hcy) and cysteine (Cys) as additional markers of higher risk of developing CVD. A controlled cross-sectional study was conducted in 26 female adolescents with SLE and 26 with HC. Dyslipidemia was found in 46.2% of the patients. The SLE group had a lower HDL-C and higher Hcy and Cys concentrations than the HC group. In multivariate analysis only Hcy was significantly and independently associated with the presence of dyslipidemia in the JSLE group. The Cys concentration was correlated with Hcy, TC-C LDL-C, and TG concentrations. These data confirm the presence of cardiovascular risk factors in adolescents with JSLE.

A retrospective cross-sectional study of 2020 by Rodrigues et al [52], aimed to describe the prevalence of dyslipidemia in children and adolescents with autoimmune diseases (ARDs), such as JSLE. They studied 186 children and adolescents between the ages of 5 and 19 years old and found dyslipidemia in 128 patients (68.8%), the most common being low HDL-C (74 patients, 39.8%). In JSLE patients, the

cumulative corticosteroid dose was associated with an increase in LDL-C (p=0.013) and with a decrease in HDL-C (p=0.022).

4.2 Dyslipidemia in JDM

In a study by Kozu et al [53], 33 JDM patients were enrolled from the Pediatric Rheumatology Unit of University Hospital. The patients had significantly higher levels of VLDL and TG compared to controls [16 (6–68) vs. 13 (4–36) mg/dL, p=0.02; and 80 (31–340) vs. 61 (19–182) mg/dL, p=0.011 respectively]. This group had a higher frequency of low HDL levels (28% vs 4%, p=0.004). In JDM, dyslipidemia was characterized by low HDL levels in seven patients (28%), high TG in four (16%) and high LDL in one (4%). These patients then had significantly higher levels of fasting glucose insulin levels [8 (3–45) vs. 3.9 (2–63) μU/ml, p=0.01], and lower fasting glycemia [80 (63-95) vs. 86 (76–102) mg/dL, p=0.009] and glucose/insulin rate [8.8 (1.5–32) vs. 19.7 (1.6–39), p=0.004] compared to controls.

Of particular interest is high-density lipoprotein function; Bae et al [54] evaluated the antioxidant function of HDL in IIM patients in the 2020 study. Myositis patients and HC were recruited from the University of California, Los Angeles (UCLA). The cholesterol profile was measured in all patients, and subgroup analysis included assessment of oxidized fatty acids in HDL. The results showed that the antioxidant function of HDL was significantly worse in patients with IIM (n=95) compared to HC (n=41). These findings support the notion that the antioxidant function of HDL is abnormal in IIM patients and may warrant further investigation of its role in the propagation of microvascular inflammation and damage in this patient population.

4.3 Dyslipidemia in JSSC

A nationwide cohort study of 2019 by Butt et al [55] included 2778 patients (76% female) with a diagnosis of SSC and 13.890 controls with a total follow-up of almost 9 years. Patients with SSC had more established cardiovascular risk factors than their respective controls at baseline, including a higher prevalence of hypertension (31.2% vs 21.0%, p<0.0001) and dyslipidemia, despite treatment (9.8% vs 8.5%, p=0.02). SSC was associated with an increased relative risk of developing most cardiovascular diseases, including myocardial infarction (HR: 2.1; 95% CI, 1.6–2.6), peripheral vascular disease (HR: 5.7; 95% CI, 4.6–7.1), pulmonary hypertension (HR: 21.9; 95% CI, 14.7–30.5), heart failure (HR: 2.9; 95% CI, 2.4–3.4), and atrial fibrillation (HR: 1.8; 95% CI, 1.5–2.0).

A study in 2022 by Zulian et al [56] included 52 JSSC pediatric patients. Due to the lack of “pediatric” criteria for the diagnosis of this disease, the authors used adult criteria to select patients with JSSC. The results show that of these 52 patients, six had cardiac involvement, three had primary cardiomyopathy, two died after a short brief disease course and one underwent heart transplantation. JSSC patients had a significantly longer delay in diagnosis (20.1 vs 8.3 months, P=0.017), a higher frequency of cardiac involvement (85.7 vs 15.6%, P=0.001) and a worse outcome, defined as mortality or end-stage organ failure rates (42.9% vs 6.2%, P<0.001). Cardiac involvement and dyslipidemia are the main features of JSSC patients and are associated with high morbidity and mortality.

4.4 ALA: the anti-inflammatory effect

Among the nutraceuticals with anti-inflammatory effects, ALA is worth mentioning. Rallidis et al [43] investigated whether dietary supplementation with ALA affects the levels of inflammatory markers in dyslipidemic patients. 76 male adults aged 71 years were assigned to receive 15 ml of linseed oil, rich in ALA per day. The dietary intervention lasted for 3 months. Blood lipids and CRP, serum amyloid A, and IL-6 levels were measured before and after supplementation. The results show a significant reduction in CRP (1.2 vs 0.9 mg/l; P=0.0008), serum amyloid A (3.2 vs 2.4 mg/l, P=0.0001) and IL-6 levels (2.2 vs 1.7 pg/ml, P=0.01). This anti-inflammatory effect may be a possible additional mechanism for the beneficial effect of plant omega-3 PUFAs in the primary and secondary prevention of coronary heart disease.

In a pilot study by Cavina et al [57], the anti-inflammatory effect of ALA was evaluated in a group of 12 volunteers, aged 40-70 years with borderline dyslipidemia or overweight, who were treated with an ALA-based dietary supplement, according to international guidelines. The patients received 6 g/day of ALA for two months. Fasting blood glucose (FBS), CRP, transaminases (ALT, AST) were measured by routine methods. CRP levels in the supplementation group decreased from 0.8 ± 0.32 to 0.6 ± 0.19 (T=60); the HC group an increase in CRP concentrations, from 0.7 ± 1.4 to 0.7 ± 0.15. The results confirm that ALA is effective in reducing CRP and it has an anti-inflammatory activity.

Niknam et al [58] investigated associations of anti-inflammatory effects of dietary fatty acid intake. This cross-sectional study investigated the association between dietary fatty acid intake and inflammatory markers, such as IL-6, and high sensitivity C-reactive protein (hs-CRP) in patients with coronary artery disease (CAD). The study included 150 CAD male patients aged ≥ 45. Participants were asked to report how often they had eaten a certain amount of each food item on a daily, weekly, or monthly basis in the past year. The effect of fatty acid intake, including saturated fat, monounsaturated fat, LA, ALA, EPA, and DHA on plasma levels of inflammatory markers was then measured. Partial correlation analysis was performed to evaluate the association of fatty acid intake with plasma IL-6 and hs-CRP concentrations. The correlation between EPA+DHA and IL-6 was -0.3, p=0.003 and hs-CRP was -0.4, p<0.001. Monounsaturated fat was significantly inversely related to plasma levels of IL-6 and hs-CRP, whereas SFA was directly related to IL-6 and hs-CRP. There was a significant association between ALA, IL-6, and hs-CRP. No significant associations were found for LA and IL-6 and hs-CRP. The results of this study suggest an inverse relationship between omega-3 fatty acids, EPA+DHA, and MUFA (monounsaturated fatty acids) with hs-CRP concentration in CAD patients. SFA intake was directly related to IL-6 and hs-CRP levels.

5. Discussions

Cardiovascular disease is the leading cause of death in the most developed countries, accounting for about 30% of all deaths [59]. Research is trying to find ways to act directly on risk factors such as atherosclerosis, hypertension, dyslipidemia including hypercholesterolemia and hypertriglyceridemia, chronic and systemic inflammation, and insulin resistance. The first confirmed effect of omega-3 is its cholesterol-lowering action, as well as its anti-inflammatory, anti-thrombotic, anti-atherosclerotic, and anti-arrhythmogenic functions. In fact, it has been observed that a situation of progressive inflammation is closely linked to an increased cardiovascular risk, which can manifest itself as atherosclerosis or coronary heart disease [60,61].

There is a close correlation between lipid profile and plasma membrane composition in terms of fatty acids. Both omega-3 and omega-6 produce eicosanoids, molecules that regulate inflammation. Eicosanoids play an important role in eliminating systemic inflammation. To explain the existing link between cardiovascular risk and inflammation, studies have shown that an increased intake of fatty acids that are deposited at the level of the plasma membrane is an important factor in reducing systemic inflammation and, consequently, cardiovascular risk [62].

Studies conducted over the last 20 years have demonstrated the efficacy and usefulness of these nutrients significantly reducing LDL and total cholesterol levels, improving erythrocyte membrane composition in terms of PUFA, and minimizing inflammation. There are important correlations, linking increased omega-3 intake to reduced inflammation, manifested by changes in eicosanoid metabolism, and reduced cytokine secretion. Significant reductions in TNF-α, IL-6, and inflammatory markers, including hs-CRP, are seen when are administered to reduce systemic inflammation. In this context, low-grade inflammation plays a key role in the progression of atherosclerosis and consequently increases cardiovascular risk. Therefore, the use of ALA, as opposed to longer chain derived omega-3 compounds, may be critical in achieving cardiovascular risk reduction due to its antioxidant, anti-inflammatory and anti-arrhythmic effects [62].

3.4 PUFAs, women and female reproductive system

PUFAs, especially DHA, EPA, and ALA play a critical role in maternal and fetal health through anti-inflammatory, vasodilatory, and antithrombotic effects (*Table 3)* (15,106). During pregnancy, higher maternal omega-3 status has been associated with improved placental perfusion and endothelial function, potentially lowering the risk of hypertensive disorders such as preeclampsia via modulation of nitric oxide bioavailability, reduction of oxidative stress, and a shift from pro- to anti-inflammatory eicosanoids (107). DHA and EPA are also incorporated into fetal neuronal membranes, supporting neurogenesis, synaptogenesis, and retinal development, which are critical for long-term cognitive and visual outcomes. Adequate maternal intake of omega-3s has additionally been linked to more favorable gestational length, lower risk of preterm birth, and optimized birthweight in some populations, likely mediated by regulation of inflammatory cytokines and prostaglandin synthesis (108).

In the postpartum period, low maternal omega-3 status has been associated with increased incidence of postpartum depression. Supplementation trials indicate potential benefits for mood stabilization and reduced depressive symptoms, though efficacy appears to depend on baseline omega-3 index, EPA/DHA ratio, and timing of intervention (109,110). During lactation, maternal intake of omega-.3s enriches breast-milk DHA, supporting infant visual and cognitive development (111).

Overall, ensuring adequate omega-3 intake within a balanced n-6/n-3 ratio is considered safe and physiologically justified across preconception, pregnancy, and lactation. Recommended daily intakes in pregnancy often range between 200–300 mg DHA, though optimal doses may vary depending on dietary background and maternal status. Effect sizes vary across populations and study designs, highlighting the need for individualized nutritional guidance (112).

Period	***Mechanism/Action***	***Clinical effect and outcome***
Preconception	○ Anti-inflammatory ○ Vasodilatatory ○ Antithrombotic ○ Improves endothelial function	○ Supports fertility ○ Reduces oxidative stress ○ Prepares optimal environment for implantation
Pregnancy	○ Modulates NO bioavailability ○ Shifts eicosanoids ○ Incorporation of omega-3 into fetal neuronal and retinal membranes	○ Reduces risk of preeclampsia ○ Improves placental perfusion and fetal neurodevelopment ○ Optimize gestational length and birthweight
Post-partum	○ Modulates inflammatory cytokines and neurotransmitter pathways ○ Influences maternal omega-3 status	○ Lowers risk of post-partum depression ○ Supports maternal mood and well-being
Lactation	○ Enrichment of omega-3 in breast milk	○ Supports infant visual and cognitive development ○ Psychomotor function

Table 3. Effects of polyunsaturated fatty acids (PUFAs) on women's reproductive health, highlighting mechanisms and clinical outcomes across preconception, pregnancy, postpartum, and lactation.

3.5 Endometriosis, assisted reproductive technologies and clinical evidence

PUFAs, particularly omega-3s, are increasingly recognized for their role in female reproductive health, with specific relevance to endometriosis and ART (*Table 4*) (113). Their anti-inflammatory and immunomodulatory properties, mediated by the competitive inhibition of AA metabolism and the production of specialized pro-resolving mediators, may mitigate chronic inflammation and prostaglandin imbalances, key contributors to pelvic pain and lesion progression in endometriosis (114). Observational evidence suggests that higher dietary omega-3 intake may reduce symptom burden and diseases risk, though randomized controlled trial remain limited.

Recent evidence indicates that omega-3 intake may beneficially influence the biochemical milieu of follicular fluid, enhancing oocyte quality and developmental competence (115).

Clinical findings remain inconsistent: while certain trials reported improvements in fertilization, implantation, and pregnancy rates (116,117), while others find no significant associations (118). These discrepancies are likely attributable to heterogeneity in study design, including differences in baseline maternal dietary patterns, the omega-6/omega-3 ratio, supplementation protocols, and treatment duration.

Mechanistically, omega-3 PUFAs may enhance endometrial receptivity through anti-inflammatory and vasodilatory effects, as well as modulation of prostaglandin pathways, potentially favoring embryo implantation (119). Although these data support a biologically plausible role for omega-3 supplementation in ART, current evidence is insufficient for definitive clinical recommendations. High-quality randomized trials are needed to determine optimal dosage, timing, and clinical applicability of omega-3 PUFAs in reproductive medicine.

Context	***Mechanism/Action***	***Clinical effect and outcome***
Endometriosis	○ Anti-inflammatory ○ Immunomodulatory ○ Competitive inhibition of AA metabolism ○ Production of specialized pro-resolving mediators	○ May reduce chronic inflammation, pelvic pain and lesion progression
Follicular fluid in ART	○ Modulates biochemical milieu ○ Enhances oocyte membrane composition	○ Improvements in oocyte quality and developmental competence
Fertilization, implantation, pregnancy	○ Anti-inflammatory ○ Vasodilatory ○ Modulation of prostaglandin pathways	○ Improvements in fertilization, implantation and pregnancy rates
Endometrial receptivity	○ Anti-inflammatory ○ Vasodilatory ○ Prostaglandin modulation	○ May favor embryo implantation

Table 4. Summary of the effects of omega-3 PUFAs on endometriosis and assisted reproductive technologies (ART), highlighting mechanisms of action and clinical outcomes.

Nutrition
Volume 130, February 2025, 112616

Applied nutritional investigation

Peripheral fatty acids and outcome of assisted reproduction

Valentina De Cosmi [a b], Sonia Cipriani [c], Marie-Louise Syren [b], Stefano Turolo [d], Fabio Parazzini [b], Marco Reschini [g], Martina Abodi [b], Walter Vegetti [g], Francesca Chiaffarino [c], Camilla Bertoni [e], Edgardo Somigliana [b g], Carlo Agostoni [b f]

1. Introduction

Overweight and obesity can affect female reproductive function and cause subfertility [1]. Excess adipose tissue perturbs the hypothalamic–pituitary–ovarian axis and sex hormone metabolism and impairs folliculogenesis [2]. Obesity is associated with hyperinsulinemia and inflammation, which stimulate ovarian androgen production, hamper oocyte quality, and negatively affect endometrial receptivity. Industrially processed foods are high in fats, especially saturated fatty acids (SFAs) and trans fatty acids, and may affect female fertility. Oocytes from mice fed a high-fat diet show significantly increased lipid droplet volume, mitochondrial ultrastructural abnormalities [3], and reduced developmental capacity [4]. In rodents, fat intake exceeding 35% of calories can lead to reproductive dysfunction [5]. Recently, in our cohort study of 494 Italian women undergoing assisted reproductive technologies (ARTs), high intake of SFAs was associated with a lower chance of clinical pregnancy [6]. The quality of fats can also be relevant to reproduction. The energy metabolism of mammalian oocytes mostly relies on pyruvate from granulosa cells and fatty acids to perform oxidative phosphorylation [7]. The lipid composition of the extracellular medium of in vitro cultures impacts oocyte competence and cryotolerance, which in turn influences in vitro fertilization (IVF) outcomes [8,9]. In vivo, the composition of the follicular fluid is influenced by the female diet, which affects the concentration of fatty acids up to oocyte metabolism [10]. For instance, evidence suggests a positive role of ω-3 fatty acids in oocyte quality and blastocyst rate in both animals [[11], [12], [13]] and humans [14,15]. However, the evidence is not conclusive [16]. This study investigated the relationship between fatty acid levels in whole blood in women undergoing IVF and four successive ART outcomes: 1) availability of good-quality oocytes, 2) availability of embryos for transfer, 3) cumulative clinical pregnancies, and 4) cumulative successful term pregnancies.

2. Materials and methods

From September 2014 to December 2016, subfertile couples referred for evaluation to the infertility unit of the Fondazione Istituto di Ricovero e Cura a Carattere Scientifico Ca' Granda Ospedale Maggiore Policlinico (Milan, Italy) who were eligible for ARTs were invited to participate in an ongoing prospective cohort study on the role of lifestyle habits and diet in ART outcomes. The study protocol was approved (reference no. 2616, December 9, 2014) by the ethical review board of the Fondazione Istituto di Ricovero e Cura a Carattere Scientifico Ca' Granda Ospedale Maggiore Policlinico. All procedures were in accordance with the Declaration of Helsinki and all participants gave written informed consent.

The study was explained to eligible women during the preliminary phase of preparation for the ART cycle. Trained personnel collected information on age, sex, weight, and height, and body mass index (BMI) was calculated [17]. For the laboratory analysis, a 5-mL venous blood sample in ethylenediaminetetraacetic acid was collected under fasting conditions and stored at –20°C. Fatty acids were measured at baseline. Non–Italian-speaking couples were excluded from the study.

3. Stimulation protocol

A standardized clinical protocol was used to manage women [18]. The protocol of stimulation and drug dosages was applied according to clinical characteristics and biomarkers of ovarian reserve. The pickup of oocytes was performed 36 h after the ovulation trigger. In the case of hyporesponse or abnormal follicular growth, the cycle was canceled before oocyte retrieval. If more than 15 oocytes were retrieved or serum estradiol level exceeded 4000 pg mL−1, an embryo vitrification procedure was followed to reduce the risk of ovarian hyperstimulation syndrome. Conventional IVF or intracytoplasmic sperm injection was performed as clinically indicated based on sperm characteristics. The outcome of good-quality oocytes was defined by the presence of at least one oocyte suitable for fertilization for either conventional IVF or intracytoplasmic sperm injection (corresponding to mature oocytes). The embryo transfer was performed 2 to 5 d after oocyte insemination according to embryo quantity and quality. A freeze-all approach was chosen in hyperresponsive women or if serum progesterone exceeded 1500 pg mL−1 at the time of ovulation triggering. Viable non-replaced embryos were vitrified mostly at the blastocyst stage. The outcome of embryo transfer was defined as the possibility of performing the transfer of embryos in general (i.e., fresh embryos, frozen embryos, or both). Women with frozen embryos who reported regular menstrual cycles (mean length of 24 and 35 d) went through natural cycle embryo transfer. Luteinizing hormone surge was identified with urinary sticks, and embryo transfer was executed 4 to 6 d after according to embryo stage. No luteal phase support was administered. Women with irregular menstrual cycles or failure in natural cycle monitoring received hormone replacement therapy. Serum human chorionic gonadotropin was measured 14 to 16 d after the triggering of ovulation or luteinizing hormone surge or the initiation of progesterone in hormone replacement therapy cycles to determine biochemical pregnancy. If present, transvaginal ultrasound was performed 21 d later to determine the presence of an intrauterine gestational sac and fetal cardiac activity as the confirming markers of clinical pregnancy. Subsequent pregnancy outcome was determined by consulting hospital charts or with a phone call after the presumed date of delivery. The best outcome from oocytes within the same cycle was considered for the analyses. For instance, if a woman did not achieve a pregnancy with a fresh embryo transfer but a frozen embryo from the same cycle subsequently led to a clinical pregnancy, we considered the latter as the main outcome. All clinical data (including infertility diagnoses) were collected from medical records.

4. Fatty acid analysis

The blood samples were thawed at room temperature, and 50 µL was transferred into glass tubes with butylated hydroxytoluene (Fluka; Sigma-Aldrich, St. Louis, MO, USA) as an antioxidant and incubated for 1 h at 90°C with 800 µL of hydrogen chloride–methanol 3 M (Sigma-Aldrich). After methylation, the samples were refrigerated at 4°C for 10 min. Next, 2 mL of potassium chloride solution (Sigma-Aldrich) and 350 µL of hexane (Honeywell) were added. The samples were vortexed and centrifuged at 3000 rpm for 10 min. Finally, the hexane layer was collected from each tube and transferred into a gas

chromatography vial. The fatty acid profile was analyzed by gas chromatography (Nexis GC-2030; Shimadzu, Kyoto, Japan) using a 30-m fused silica capillary column (FAMEWAX; Restek, Bellefonte, PA, USA). The gas chromatography results were analyzed by applying LabSolutions software (version 5.97 SP1) (Shimadzu). Fatty acids were identified by the retention times of reference standards and expressed as percentage of total fatty acids.

5. Statistical analysis

We described the distribution of categorical and ordinal variables using frequency and percentage and continuous variables using mean and SD. Comparisons were made using Student's t test and Wilcoxon test in case of normally and non-normally distributed continuous variables, respectively. We estimated unadjusted relative risk (RR) and corresponding P values for good-quality oocytes, embryo transfer, clinical pregnancy, and live birth in quartiles of blood levels of fatty acids: palmitic acid (C16:0) and stearic acid (C18:0) of the SFAs; oleic acid (18:1 π-9) of the monounsaturated fatty acids (MUFAs); linoleic acid (LA) (18:2 ω-6), dihomo-gamma-linolenic acid (20:3 ω-6), and arachidonic acid (AA) (20:4 ω-6) of the ω-6 polyunsaturated fatty acids (PUFAs); and alpha-linolenic acid (ALA) (18:3 ω-3), eicosapentaenoic acid (EPA) (C20:5 ω-3), docosahexaenoic acid (DHA) (22:6 ω-3), and docosapentaenoic acid (22:5 ω-3) of the ω-3 PUFAs. Several indexes were calculated, including the inflammatory state with the fatty acid ratios AA/DHA, AA/EPA, ω-6/ω-3 and ω-6 derivatives/LA, ω-3 derivatives/ALA [19], and LA/ALA. We calculated the PUFA balance as follows: (%EPA + %DHA) / total PUFA × 100. To account for potential confounding factors, we computed the multivariate analysis using the backward selection method to obtain adjusted RR of the final models. The variables initially included in the multivariate analysis were those significant in univariate analysis models: serum folic acid, ferritin, vitamin B12, and anti-Müllerian hormone (AMH) for good-quality oocyte outcome; serum folic acid, ferritin, vitamin B12, vitamin A, and AMH for embryo transfer outcome; and serum folic acid, follicle-stimulating hormone, and AMH for clinical pregnancy and live birth outcomes. The cutoff values used to define the four categories are specified in the tables.

6. Results

In total, 238 women were included in the present study. Considering the four ART outcomes, 207 women (87%) obtained good-quality oocytes, 184 (77%) achieved embryo transfer, 60 (25%) achieved clinical pregnancy, and 40 (17%) had a live birth. Age, BMI category, and use of supplements are reported in Table 1 according to the considered IVF outcomes.

The mean (SD) age was 37.2 (3.5) y (range, 27–46). The mean (SD) BMI was 22.4 (4.4) kg/m2 (range, 15.2–51.1). Fourteen women (5.9%) were obese (BMI ≥30 kg/m2). Vitamin A, vitamin E, vitamin B12, serum and calculated folic acid [20], iron, homocysteine, ferritin, follicle-stimulating hormone, and AMH variables are depicted in Table 2.

Table 3 presents the blood levels of selected fatty acids according to the four ART outcomes. Differences between total ω-3–PUFA blood levels emerged when comparing women who achieved embryo transfer with those who did not (P = 0.03). Moreover, a higher PUFA balance was found in women who achieved live birth (P = 0.03). The concentration of DHA was higher in women without good-quality oocytes (P = 0.02) and women who did not achieve embryo transfer (P = 0.01). Women with good-quality oocytes had

a higher AA/DHA ratio (P = 0.01) and women who achieved embryo transfer had a higher ω-6/ω-3 ratio (P = 0.02). The PUFA balance was higher among women who had a live birth (P = 0.03).

Blood levels of LA and ALA, the parental fatty acids of the ω-6 and ω-3 series, respectively, are also shown in Table 3. No differences in blood levels of LA/ALA were found when comparing women who achieved embryo transfer with women who did not, women with good oocyte quality, women who became pregnant, and women who had a live birth.

The relationships between the quartiles of blood fatty acids and clinical results are reported in Supplementary Table 1. Statistically significant associations emerged in univariate analyses between good-quality oocytes and total ω-3 PUFAs (fourth quartile versus first, RR = 0.88, P = 0.048), total LC PUFAs (third quartile, RR = 0.86, P = 0.04), PUFA balance (third quartile, RR = 0.83, P = 0.03), oleic acid (third quartile, RR = 1.14, P = 0.04), DHA (third and fourth quartiles, RR = 0.82, P < 0.01 and RR = 0.89, P = 0.04, respectively), and AA/DHA (fourth quartile, RR = 1.16, P = 0.04).

Associations emerged between the achievement of embryo transfer and total MUFAs (third quartile, RR = 1.27, P = 0.02), total ω-3 PUFAs (second and fourth quartiles, RR = 0.80, P = 0.02 and RR = 0.76, P < 0.01, respectively), total LC PUFAs (second and third quartiles, RR = 0.79, P = 0.01 and RR = 0.76, P < 0.01, respectively), and oleic acid (third quartile, RR = 1.27, P = 0.01). The second, third and fourth quartiles of DHA levels were associated with the achievement of embryo transfer (RR = 0.83, 0.75, and 0.81 and P = 0.03, 0.003, and 0.02, respectively) and the fourth quartile of the ω-6/ω-3 ratio (RR = 1.21, P = 0.047). Regarding the achievement of clinical pregnancy, two associations emerged: the third quartile of the PUFA balance (RR = 0.35, P = 0.0154) and the second quartile of the ω-6/ω-3 ratio (RR = 0.43, P = 0.03). Finally, regarding the outcome of live birth, the following associations resulted: the third quartile of total MUFAs (RR = 3.00, P = 0.02), the third quartile of the PUFA balance (RR = 0.19, P = 0.02), the second quartile of ALA (RR = 2.75, P = 0.04), and the second quartile of the ω-6/ω-3 ratio (RR = 0.36, P = 0.04).

In multivariate analysis, none of the associations concerning good-quality oocyte, clinical pregnancy, and live birth outcomes was confirmed (data not shown). The associations between MUFAs and embryo transfer and between LC PUFAs and embryo transfer were confirmed in multivariate analyses that included ferritin levels and vitamin A: RR = 1.20, P = 0.03 (MUFA third quartile versus first); RR = 0.80, P = 0.03 (LC PUFA third quartile versus first); and RR = 0.81, P = 0.03 (LC PUFA second quartile versus first). Three associations with embryo transfer not detected in univariate analyses emerged in multivariate analyses that included ferritin levels and vitamin A: RR = 0.82, P = 0.045 for the fourth quartile of total PUFAs compared with the first; RR = 0.81, P = 0.04 for the fourth quartile of LA versus the first; and RR = 0.80, P = 0.03 for the third quartile of DHA versus the first.

7. Discussion

Our study assessed the relationships between whole blood fatty acid levels and the four main ART outcomes. Overall, our results showed that good-quality oocytes were associated with ω-3 PUFAs, total LC PUFAs, PUFA balance, 18:1 ω-9, 22:6 ω-3, and AA/DHA. Achievement of embryo transfer was associated with total MUFAs, total ω-3 PUFAs, LC PUFAs, 18:1 ω-9, 22:6 ω-3, and ω-6/ω-3 ratio. As for the achievement of clinical pregnancy, two associations emerged with regard to PUFA balance and ω-6/ω-3 ratio. As for the outcome of live birth, associations with total MUFAs, PUFA balance, 18:3 ω-3, and ω-6/ω-3 ratio emerged. SFAs did not show any association with the different ART outcomes. Finally,

on multivariate analysis, the fourth quartile of PUFAs and LA and the third quartile of DHA were associated with the probability of performing embryo transfer.

The effects of ω-3 and ω-6 on fertility are controversial. ALA intake during pregnancy has a positive role in improving embryo morphology and possibly follicular steroid hormone synthesis [16]. If ALA may act on hormone synthesis and embryo morphology, LA acts as a precursor of two series of prostaglandins [21] and stimulates protein kinase C [22], contributing to cell growth and differentiation [23]. Despite the different beneficial functions of these two fatty acids in fertility, studies in the literature demonstrate heterogeneous results. A study by Jahangirifar [24] showed that higher intake of ALA compared with LA was associated with a higher fertility rate. By contrast, Jungheim et al. [25] found that higher concentrations of ω-3 precursors compared with ω-6 were associated with a lower chance of pregnancy. A study from the same group showed that women with elevated LA/ALA ratios had a higher likelihood of clinical pregnancy and live birth compared with women with lower LA/ALA ratios [26].

Fatty acids and their metabolites are necessary for female reproductive functions and oocyte quality [10], with possible effects on embryo development and pregnancy rate [27]. Considering the procedures involved in ARTs, the availability of energy sources in culture medium during in vitro maturation is essential for optimizing embryo development. It has been shown that different lipid exposures can significantly alter the lipid profile of oocytes, with long-term effects still observed at the blastocyst stage[28]. However, there are conflicting reports about how lipid exposure during in vitro maturation impacts the gamete and further embryo development [29]. A follow-up Cochrane review analysis stated that there is not a superior culture medium and that additional and better designed studies are needed to understand the metabolic impact of supplementation [30].

In humans, serum fatty acids reflect follicular fluid concentrations, with possible implications for follicle maturation and blastocyst development [[31], [32]]. However, associations between peripheral levels of fatty acids, such as MUFAs and PUFAs, and pregnancy rates in women have not yet been established. There is biological plausibility for the effect of ω-3 and ω-6 PUFAs on oocyte development, implantation, and gestation via eicosanoid synthesis [10]. Although several studies have highlighted the beneficial role of dietary ω-3 fatty acids in female fertility [33], our results suggest that higher amounts of n-3 PUFAs may have unfavorable effects. A higher ω-6/ω-3 ratio was instead positively associated with embryo transfer, and a lower ratio was positively associated with clinical pregnancy and live birth. Similarly, a study by the same group showed that increased serum ω-6/ω-3 PUFA ratios in women undergoing IVF were associated with increased implantation and pregnancy rates [26]. The authors also showed that none of the PUFAs alone was associated with a chance of pregnancy. In our study, whole blood was used, and thus membrane-derived fatty acids contributed to the associations. We can speculate that associations with the n-6 series could be explained by a favorable effect of eicosanoids derived from AA, the main n-6 derivative, in the early stages of ART bioprocesses. However, the literature on the impact of PUFAs on fertility is conflicting. In a prospective cohort study involving 200 women trying to conceive naturally, no significant differences in ω-3 or ω-6 serum concentrations between fertile, subfertile, and infertile women and fecundability ratios were reported [34]. In animal studies, MUFAs improve embryo development, as they probably promote lipid storage and thus prevent lipid peroxidation [10]. Remarkably, in another study, EPA and DHA levels in follicular fluid seemed to correlate with worse embryo quality even though the women were younger, whereas oleic acid seemed to improve embryo development [32]. A possible explanation for these results is that elevated PUFA levels correlate with lipid peroxidation, with detrimental effects on reproductive events.

It is worth remembering that confounding effects may intervene, possibly related to dietary intake. A descriptive longitudinal study on 217 women assessed the dietary intake of total PUFAs and MUFAs, which was associated with a lower fertilization rate, whereas increased intake of LA and EPA improved the fertilization rate [24]. In another study [14], the authors analyzed both the ω-3 intake and the concentration in serum after induced ovulation. The results showed that women who consumed foods rich in DHA and EPA and who had higher blood concentrations of these molecules were more likely to have successful ART outcomes. EPA was reported to be linked to higher live birth rates when its concentration was high [14]. Once more, we point out that only heterogeneous results are available to date.

A strength of our study is its prospective design and the relatively large sample size within a monocentric survey with a high participation rate. However, some limitations exist. First, apart from folic acid, we did not collect information on the types of supplements the women were using before IVF treatment. However, only 18% of the total sample reported using other supplements and, at the time of the study, we did not advise the use of antioxidants or other supplements except folic acid. Second, we did not adjust for confounders such as ovarian reserve and indications for ART. Third, the multiple outcomes and higher number of analyses performed expose our findings to type I errors. Accordingly, it must be emphasized that the most relevant clinical outcome was live birth. The decision to include in the primary outcomes some surrogate outcomes was made to capture possible detrimental effects in specific steps of the fertilization process and to allow comparisons with other studies in the literature. Fourth, our results may not be generalizable to women without known fertility problems.

8. Conclusions

Fatty acids are likely to have an impact on the success of ART, even if, presumably, very complex. Different molecules may interact in a network that ultimately affects folliculogenesis. A reductionist approach focusing on single molecules is unlikely to provide definite and valid information. Large prospective studies followed by well-designed supplementation trials, after adjusting for baseline characteristics of couples, and tests for interactions are needed to determine the ranges of fatty acid concentrations as well as the ideal fatty acid intake through diet or pharmacological interventions that may benefit women planning to conceive.

CHAPTER 4

Clinical Study: The DANTE project

4.0 Introduction

Endometriosis is a chronic inflammatory disease affecting around 10% of individuals assigned female at birth, though its true prevalence is likely higher (120). Despite its frequency, it remains underfunded and under-researched (121) leading to diagnostic delays, limited treatments, and lack of personalized care (122,123). Alongside chronic pelvic pain, infertility is one of the most burdensome consequences, with inflammation playing a key role in both symptom generation and impaired fertility by disrupting folliculogenesis, gamete function, and fertilization (124,125). Current management of endometriosis-related infertility is challenging. Surgery may benefit superficial lesions but can compromise ovarian reserve in ovarian endometriosis, while medically assisted reproduction (MAR), including IVF, often yields suboptimal outcomes with lower oocyte numbers and reduced pregnancy rates. This has led many patients to explore lifestyle changes and complementary approaches, including dietary interventions (126).

Diet has gained particular attention as an accessible, modifiable factor influencing systemic inflammation, hormonal balance, and overall health. Evidence suggests that pro-inflammatory dietary patterns, such as the Western diet, are associated with increased endometriosis risk, while anti-inflammatory patterns like the Mediterranean diet may improve metabolic and oxidative profiles and enhance quality of life. Although no specific dietary regimen is currently recommended, focusing on overall dietary patterns rather than isolated nutrients may provide more meaningful insights into nutrition's role in endometriosis management (126,127).

A prospective cohort study of 81.997 reproductive-aged women (128) found a 27% higher risk of endometriosis among those with dietary patterns high in red and processed meats, refined grains, and sweets, consistent with a Western diet. Similarly, a cross-sectional analysis of 265 women from the NHANES survey showed that those in the highest tertile of the Dietary Inflammatory Index (DII) had a significantly increased risk of endometriosis (OR: 1.57; 95% CI: 1.14–2.17), further supporting a link between pro-inflammatory diets and the disease. While the precise mechanisms remain unclear, potential benefits of dietary interventions are thought to derive from anti-inflammatory effects and modulation of estrogen levels. In this context, the Mediterranean diet, rich in fruits, vegetables, legumes, whole grains, omega-3 rich fish, and olive oils, has shown promise: a 6-month intervention improved metabolic and oxidative profiles and enhanced quality of life in women with endometriosis.

4.1 Outcome measures

4.1.1 Primary outcomes

The DietAry intervention in ameliorating fertility parameters in subjects with Endometriosis (DANTE) randomized controlled trial aims to compare reproductive outcomes in infertile patients with endometriosis undergoing a standard IVF protocol after a 12-week intervention based on an anti-inflammatory diet versus a standard IVF protocol. Specifically, the primary endpoint is the different in the rate of poor ovarian responders despite preserved ovarian reserve (defined as patients retrieving

three or fewer oocytes following controlled ovarian stimulation, according to the POSEIDON criteria (2016) (129).

4.1.2 Secondary outcomes

- *Inflammatory markers:* changes in systemic inflammatory status, as measured by the INFLA-score, are compared between group and withing groups before and after the intervention, to evaluate the effect of dietary intervention on systemic inflammation. This outcome is assessed on biological samples collected at T0 and T2.
- *Vaginal and fecal microbiome*: composition and diversity of vaginal and fecal microbiome are compared between groups, and within groups across timepoints. Bioinformatic analyses provide detailed evaluation of bacterial abundance and diversity, with correlation analyses exploring the influence of diet on intestinal and vaginal microbiomes in relation to IVF outcomes. This outcome is assessed on samples collected at T0 and T2.
- *Patient-reported outcomes (PROMs):* PROMs are evaluated through between-group and within group comparisons in the intervention arm. Quality of life is assessed using the EHP-20, symptom severity with ne NRS, and sexual health with the FSFI. These outcomes are assessed on questionnaires collected at T0 and T2.
- *Embryological parameters:* embryological outcomes include the number of mature oocyte (MII) retrieved at T3, as well as the number of available embryos and blastocysts at follow-up (T4).
- *Steroid cascade in follicular fluid:* change in sex steroid hormone concentrations in follicular fluid are compared between groups to evaluate the impact of the dietary intervention on the hormonal milieu. This outcome is assessed at the time of oocyte retrieval (T3).
- *Pregnancy outcomes:* pregnancy outcomes include live birth, clinical pregnancy, and miscarriage rates, compared between groups at T4.

Specifically, secondary endpoint address the broader impact of the dietary intervention on several clinical, biological, and PROMs, including: i) changes in systemic inflammation as reflected by the INFLA-score (130,131), measured at baseline and study end in both groups; ii) changes in composition and diversity of the fecal and vaginal microbiome; iii) changes in quality of life assessed using the Endometriosis Health Profile-30 (EHP-30) (132); iv) changes in symptoms severity, measured through the Numeric Rating Scale (NRS) (133), specifically addressing multiple endometriosis-specific pain domains: dysmenorrhea, superficial dyspareunia, deep dyspareunia, dyschezia, dysuria; v) changes in sexual function, evaluated using the Female Sexual Function Index (FSFI) (134) vi) differences between the intervention and control groups in concentrations of steroid hormones in the follicular fluid to explore effects of the diet on the local hormonal environment (135); vii) differences between the intervention and control groups in embryological outcomes, including number of oocytes retrieved, number of cleavage stage embryos and number of blastocysts (136); viii) differences between the intervention and control groups in pregnancy outcomes, including live birth rate, clinical pregnancy rate, miscarriage rate (136).

In this specific doctoral thesis, it is important to explore the hypothetical correlation between FAs levels (16:0, 16:1, 18:0, 18:1 n-9, 18:1 n-7, 18:2 n-6, 18:3 n-3, 20:3 n-9, 20:3 n-6, 20:6 n-6, 20:5 n-3, 22:5 n-3, 24:0, 22:6 n-3, 24:1) and their ratios (SFA, PUFA, MUFA, n-3, n-6, SFA/PUFA, MUFA/PUFA, n-6/n-3, n-3/n-6, DHA/AA, LA/ALA, and AA/LA) and inflammatory biomarkers (INFLA-score), and understand how and anti-inflammatory diet could be effective in reducing this score and ameliorating the conditions of clinical pregnancy rate and live birth.

4.2 Study design

The study describes a two-arm, parallel-group, assessor-blinded, superiority RCT. The study is conducted at the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico in Milan, Italy. Randomization runs from March 2025 to January 2029. The primary endpoint is assessed at the time of oocyte retrieval, approximately 14 weeks after randomization.

The study enrolls 438 infertile subjects with endometriosis aged between 18 and 39 years. Infertility is defined according to WHO criteria, i.e., failure to conceive after 12 months or more of regular unprotected intercourse. The diagnosis of endometriosis is based either on surgical reports with histological confirmation or on imaging for endometrioma or deep endometriosis, according to international standards. All the inclusion and exclusion criteria are reported in *Table 5*.

In this doctoral thesis, however, data on baseline (T0) fatty acid levels and the INFLA-score of the 40 enrolled patients have been reported. The study is expected to be completed by 2030.

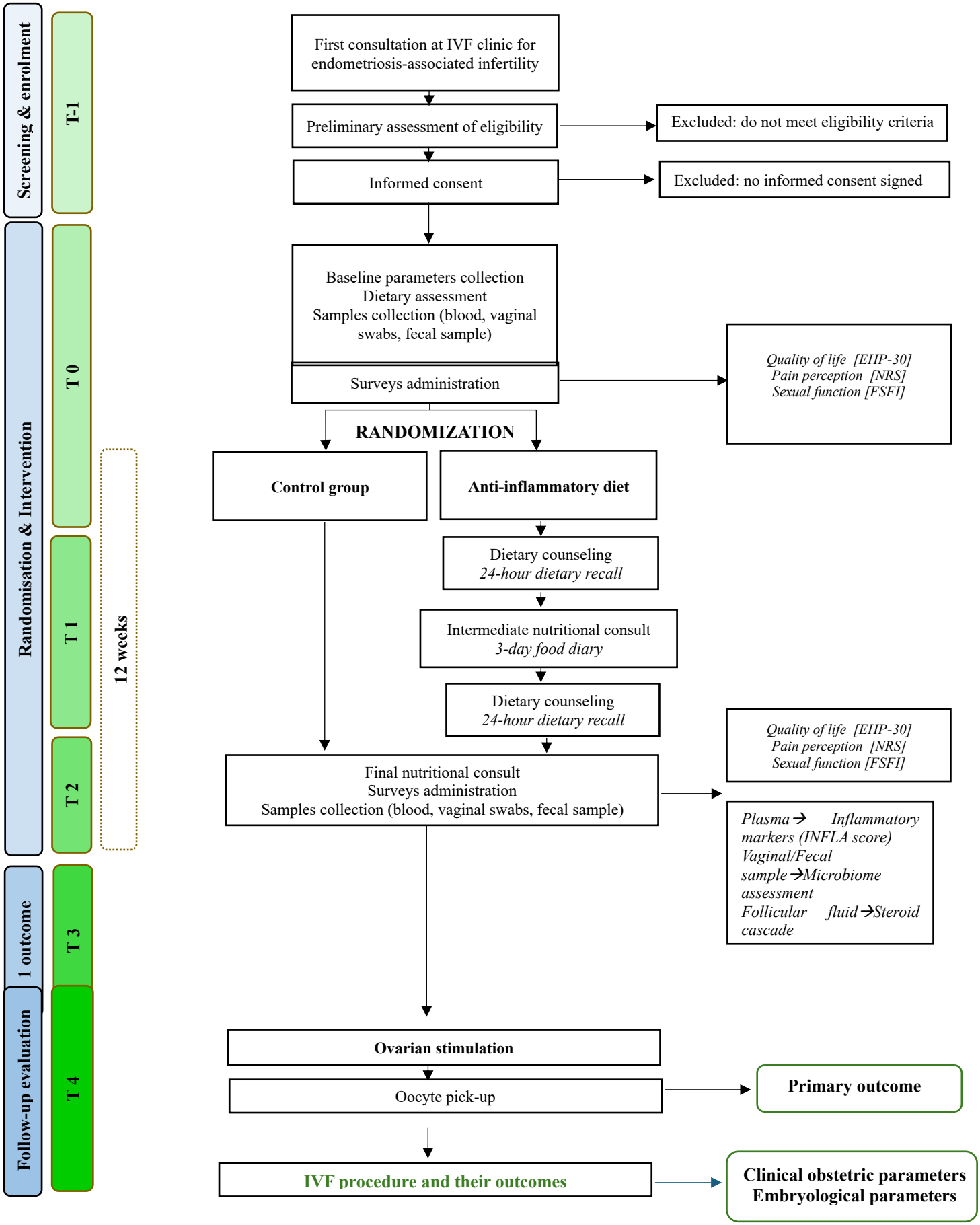
Inclusion criteria	***Exclusion criteria***
Age 18-39 years	Contraindication to pregnancy
Pregnancy seeking for more than 12 months	Hydrosalpinx
Regular menstrual cycle (21-35 days)	Endometriomas with a mean diameter > 4 cm
Signed informed consent	Severe male factor (< million sperm/ml)
Normal ovarian reserve (AFC ≥ 5)	SM fibroids or large IM/SS fibroids (≥ 5 cm)
	Doubtful US findings that do not allow to reliably rule out maligniancies
	Uteral stenosis or intestinal subocclusive symptoms

Table 5. Eligibility criteria

4.2.1 Recruitment and screening procedure

Figure 4 shows the flowchart of the RCT. At their first routine consultation in IVF Unit, individuals affected by endometriosis undergo screening which includes the comprehensive transvaginal ultrasound examination to confirm the presence of endometriosis. Eligible participants receive detailed information about the study purpose, procedures, temporal commitment, possible discomforts, and potential risks and benefits. Participation is entirely voluntary, with the right to withdraw at any time. Finally, written informed consent is obtained from all participants willing to participate.

Figure 4: *Flow of participants through the trial.*



4.2.2 Randomization procedure

The second consultation (T0) is scheduled on days 5-7 of the menstrual cycle. For participants with menstrual suppression due to continuous hormone therapies, the timing of the cycle is not considered. Baseline and clinical data are collected during a gynecological visit, and a nutritional assessment is conducted. After the initial gynecological and nutritional visits, patients are randomly assigned (1:1 ratio) to either: a control group receiving no specific dietary instructions or anti-inflammatory dietary intervention group. Randomization is conducted via the secure Research Electronic Data Capture (REDCap), using a computer-generated allocation sequence and block randomization to ensure balance and conceal allocation. Allocation is revealed automatically at the time of randomization for each individual participant. Blinding is maintained for both the clinical and lab staff performing the ovarian stimulation and IVF lab procedures, respectively. An independent external monitor oversee study progress and ensure compliance with data management protocols.

4.2.3 Gynaecological assessment

A clinical gynaecological assessment is performed according to international standards, following the evaluation criteria established by the World Endometriosis Society (WES) and the World Endometriosis Research Foundation (WERF) (137). The assessment includes a pelvic examination and structured symptom evaluation. Imaging is conducted using high-resolution transvaginal ultrasound. Pain severity is recorded using the NRS for endometriosis-specific pain domains, including dysmenorrhea, superficial and deep dyspareunia, dyschezia, and dysuria.

4.2.4 Nutritional assessment

At T0, a clinical nutritionist assesses nutritional status through the evaluation of dietary habits, gastrointestinal (GI) symptoms, physical activity, anthropometric parameters, and body composition. Anthropometric measurements are repeated at week-6 of the intervention for the intervention group and at week-12 for both intervention and control groups. Dietary habits during the 6 months preceding enrollment are investigated using a 78-item Food Frequency Questionnaire (FFQ) validated for the Italian population (138). The dietary inflammatory potential of each participant is assessed by calculating the DII score, which assigns negative values to nutrients such as magnesium, vitamins A, C, D, and E, flavonoids, fiber, MUFAs, PUFAs, tea, garlic, and ginger, and positive values to pro-inflammatory components including cholesterol. SFs, refined carbohydrates, total protein, vitamin B12, and high caloric intake (139,140).

Anthropometric measures of height, weight, and body circumferences (chest, breast, arm, wrist, waist, abdomen, hip, thigh, knee, and ankle) are obtained according to standard procedures, using appropriate instruments. Body composition is estimated through bioelectrical impedance analysis with Akern 101 BIVA pro (Akern Bioresearch, Florence, Italy), measuring resistance, reactance, and phase angle parameters, in accordance with the ESPEN (European Society of Parenteral and Enteral Nutrition) guidelines (141).

4.2.5 Interventions

The anti-inflammatory dietary intervention and standard care diet are summarized in accordance with the Template for Intervention Description and Replication (TiDier) guidelines (142) *(Table 6*).

Brief name	**Anti-inflammatory dietary programme**	**Standard care**
WHY	Anti-inflammatory diets targeting systemic inflammation assist in the prevention and management of various chronic diseases and chronic pain conditions. Inflammation is also supposed to play a pivotal role in endometriosis natural history and in infertility occurrence.	/
WHAT (MATERIALS)	Participants will receive an intervention handbook containing all study details, key anti-inflammatory eating principles, meal plans, frequently asked questions, encouraged and discouraged foods, and education (eg, common myths, tips for eating out, shopping tips); and a specific meal guide personalized for the patient according to previous consultation, allergies and preferences.	/
WHAT (PROCEDURES)	Participants are encouraged to follow an anti-inflammatory dietary intervention based on Mediterranean diet principles. The consultation will include a comprehensive explanation of the anti-inflammatory dietary principles, the rationale behind the intervention, the potential benefits and risks, and a space to address participant concerns. To monitor dietary adherence, a 24-hour dietary recall will be conducted via telephone at both three and nine weeks. At six weeks, participants will attend a second in-person consultation with the nutritionist, who will further evaluate adherence with a 3-day food diary and assess anthropometric parameters. The dietary intervention will span 12 weeks leading up to the beginning of the ovarian stimulation, when a final in-person consultation will take place and anthropometric parameters will be reassessed.	At the end of the 12-week period, participants will attend a single consultation with the nutritionist to assess anthropometric and dietary parameters.
WHO PROVIDED	A qualified nutritionist specially trained to deliver all components	A qualified nutritionist specially trained to deliver all components
HOW	Delivered with individual support for 12 weeks. Consultations are one to one in person or by phone.	/

WHERE	In-person consultations will occur at Fondazione Ca' Granda, Milan. Additional consultations will occur via telephone. Participants will integrate the diet principles into their daily consumption of foods and beverages.	In-person consultations will occur at Fondazione Ca' Granda, Milan.
WHEN AND HOW MUCH	Three in-person consultations at baseline, week 6, and week 12. Two phone follow-up consultations (~30 min) in weeks 3 and 9.	Two in-person consultations at baseline and week 12.
TAILORING	Anti-inflammatory dietary advice, education and support aligning with participant preferences and goals	/
MODIFICATIONS	Any modifications will be reported	Any modifications will be reported
HOW WELL (planned)	Clinical nutritionists receive prior training to standardize measurements and unify information delivered in the program. Participant adherence to the anti-inflammatory diet is assessed through 24 h recalls, and 3-day food diaries.	Clinical nutritionists receive prior training to standardize measurements and unify information delivered in the program.
HOW WELL (actual)	Reported in the primary paper	

Table 6. Overview of intervention delivery described according to the TIDIeR guidelines.

Control group

After randomization, participants allocated to the control group do not receive any specific dietary guidance or recommendations and maintain their habitual eating patterns. They begin the standard IVF treatment protocol no earlier than 12-weekdn after the initial visits.

Anti-inflammatory dietary intervention group

Participants are encouraged to follow an anti-inflammatory dietary intervention based on Mediterranea diet principles. The anti-inflammatory potential of the diet is assessed using the DII score. The nutritional consultation includes a detailed explanation of the dietary principles, the rationale for the intervention, potential benefits and risks, and an opportunity to address participant concerns. Participants receive a general guide and a personalized 3-week meal plan to be repeated throughout the intervention period.

Nutritional advice emphasized the use of extra virgin olive oil as the main cooking fat (≥4 tablespoons/day), consumption of whole grains, nuts, at least 2 daily servings of fresh seasonal fruit and vegetables, at least 2 weekly servings of fish (especially omega-3 rich varieties), and no more than 2 monthly servings of red meat. Additional recommendations include the consumption of organic foods, the use of anti-inflammatory spices (e.g., ginger, garlic, curcumin), and the avoidance of ultra-processed foods such as pre-cooked meals, breakfast cereals, biscuits, pre-sliced bread, rusks, sweetened beverages, salt, refined oils, trans fats, and food additives. All recommendations are tailored to energy requirements, intestinal symptoms, body composition, dietary preferences, and allergies.

To monitor adherence, a 24-hour dietary recall is conducted via telephone at both 3 and 9 weeks after the baseline visit (T0). At 6 weeks, participants attend a second in-person consultation with the nutritionist, during which adherence is further assessed with a 3-day food diary and anthropometric parameters are measured. Participants in the control group undergo the same final nutritional evaluation with a 3-day food diary to confirm the absence of significant changes in dietary habits or inflammatory potential.

The dietary intervention spans the 12-weekend preceding the start of ovarian stimulation (between T0 and T2), with a final in-person consultation conducted at T2. Participants are encouraged to maintain adherence to the dietary recommendations until at least the time of oocyte retrieval (T3) (*Figure 5*).

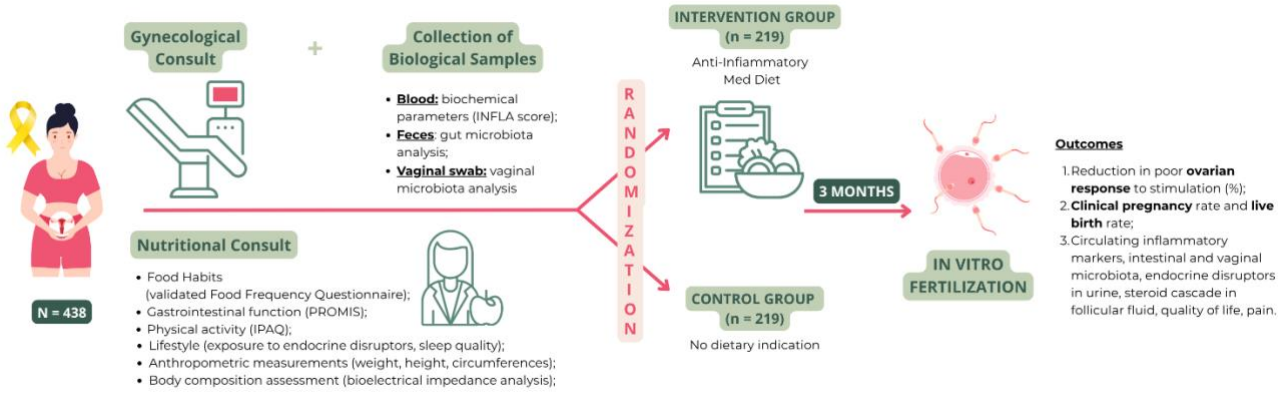


Figure 5. Workflow of the clinical trial from recruitment to the randomization in two different groups: intervention or control ones.

4.2.6 Data and specimen collection

Clinical, biological, and nutritional data are collected at multiple timepoint using the REDCap platform. At baseline, all participants provide biological samples, including peripheral blood, vaginal swabs, and feces. The same collection is repeated at the end of the 12-week intervention period, prior to the initiation of ovarian stimulation (T2). To minimize variability related to the menstrual cycle, samples are collected between days 5-7 of the cycle. For participants with menstrual suppression due to continuous hormone therapy, cycle timing is not considered.

4.2.7 Data management

Data are collected and managed electronically using REDCap. Each subject is assigned a unique identification code to de-identify data, ensuring that only local investigators can re-link information to individual identities. REDCap provides: i) user-level access control with role specific permissions; ii) real-time data validation and integrity checks; iii) automatic de-identification of patient information prior to data export; and iv) centralized data storage on a secure server with daily backups to guarantee data safety.

4.2.8 Sample size calculation

This trial is powered to detect a clinically significant difference between groups for the primary outcome. A total of 438 women with endometriosis are randomized to either the control or the anti-inflammatory dietary intervention groups. The sample size calculation is based on the following assumptions: i) type I and type II errors of 0.05 and 0.20, respectively; ii) an expected rate of poor ovarian response of 20% in the untreated group (136); iii) an absolute reduction of 10% in poor ovarian response in the dietary intervention group; iv) an estimated drop-out rate of 10% from the dietary intervention group, which ensures sufficient power for both ITT and per-protocol analyses; v) the use of a two-tailed test for proportions in two independent groups with binomial approximation to the Gaussian distribution.

4.3 Statistical analyses

Statistical analyses are performed using IBM SPSS Statistics (Armonk, NY, IBM Corp). Participants drop-out from the dietary intervention group is anticipated; however, if ovarian stimulation starts after the intervention period, these participants remain included in the analysis. The primary analysis follows the intention-to-treat (ITT) principle, with an additional per-protocol (PP) analysis conducted to test the robustness of findings. The primary outcome is expressed as a percentage in each group, with relative risks (RRs) and 95% confidence intervals (CIs). Between-group differences are assessed using Chi-square tests. Secondary outcomes are analyzed as follows: categorical variables with Chi-square or Fisher's exact tests, normally distributed continuous variables with Student's t-tests, and non-normally distributed variables with non-parametric tests (Wilcoxon and Mann–Whitney U). IVF and pregnancy outcomes are reported and analyzed according to the CORE outcome set for infertility research (143).

4.4. Evaluated parameters

The biological samples are analyzed as follows:

- *Peripheral blood*: analyzed for systemic inflammation using the INFLA-score, which integrates C-reactive protein levels, white blood cell count, platelet count, and granulocyte-to-lymphocyte ratio (144). Peripheral blood was collected to analyze FAs composition using the dried blood spot (DBS) technique. Blood drops (20-60 µl) were obtained and spotted onto Whatman 903 protein saver cards (General Electric), previously pretreated with a stabilizing solution. Once dried, the samples were subjected to methylation with HCl/MeOH (Supelco) to convert FAs into their corresponding methyl esters. The resulting derivatives were then injected into a gas chromatography Shimadzu Nexis GC-2030) for the qualitative and quantitative evaluation of the fatty acid profile.
- *Vaginal swabs* and *fecal samples*: assessed for bacterial abundance and diversity by microbiome analysis. Samples undergo bacterial DNA extraction and high-throughput amplicon sequencing targeting the 16S rRNA gene, followed by bioinformatics processing to characterize microbial communities (145).
- *Follicular fluid*: analyzed for sex steroid concentrations using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Hormones measured include aldosterone, androstenedione, cortisol, corticosterone, 11-deoxycorticosterone, 11-deoxycortisol, 21-deoxycortisol, dehydroepiandrosterone (DHEA), DHEA sulfate (DHEA-S), dihydrotestosterone, estradiol, 17-OH progesterone, progesterone, and testosterone (135).

Hematochemical parameters obtained from peripheral blood collection include the INFLA-score, CRP, WBC counts, PLT counts, granulocyte-to-lymphocyte ratio and FAs levels, specifically 16:0, 16:1, 18:0, 18:1 n-9, 18:1 n-7, 18:2 n-6, 18:3 n-3, 20:3 n-9, 20:3 n-6, 20:6 n-6, 20:5 n-3, 22:5 n-3, 24:0, 22:6 n-3, and 24:1, together with their ratios (SFA, PUFA, MUFA, n-3, n-6, SFA/PUFA, MUFA/PUFA, n-6/n-3, n-3/n-6, DHA/AA, LA/ALA, and AA/LA). These biomarkers allow for the assessment of both systemic inflammations, through the INFLA-score, and dietary FAs metabolism, through the quantitative and qualitative FA profile. The integration of these parameters provides a comprehensive evaluation of the inflammatory status and lipidomic balance of participants, supporting the hypothesis that an-inflammatory dietary intervention may contribute to reducing the INFLA-score and, in turn, ameliorating clinically relevant reproductive outcomes, such as pregnancy rate and live birth. Finally, dietary habits over the past six months of the enrolled patients were assessed using a Food Frequency Questionnaire (FFQ). Nutritional compounds were analyzed, including flavonoids, proanthocyanidin, and related phytochemicals. The evaluated variants included isoflavones, anthocyanidins, flavan-3-ols, flavanones, flavones, flavonols, and several proanthocyanidin subtypes (monomers, dimers, trimers, 4-6-mers, 7-10-mers, and polymeric forms). The aim was to investigate whether, and if so, how dietary habits influence systemic inflammation in the women enrolled in the study.

4.5 Results

4.5.1 Fatty acids levels

Peripheral blood samples were collected from 40 women at baseline (T0). FA levels were quantified in absolute terms using gas chromatrography, and the corresponding ratios were subsequently calculated. The analysis included the following FAs: plamitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), oleic acid (18:1 n-9), vaccenic acid (18:1 n-7), linoleic acid (18:2 n-6), α-linolenic acid (18:3 n-3), eicosenoic acid (20:3 n-9), dihomo-γ-linolenic acid (20:3 n-6), arachidonic acid (20:4 n-6), eicosapentaenoic acid (20:5 n-3), docosapentaenoic acid (22:5 n-3), lignoceric acid (24:0), docosahexaenoic acid (22:6 n-3), and nervonic acid (24:1). Additionally, ratios of SFAs, MUFAs, PUFAs, omega-3s and omega-6s, as well as derived indices including SFA/PUFA, MUFA/PUFA, omega-6/omega-3, omega-3/omega-6, DHA/AA, LA/ALA, and AA/LA were calculated to provide a comprehensive assessment of FA composition.

The baseline FAs profile of 40 enrolled participants releveled a balanced yet slightly polyunsaturated dominant composition. On average, the proportion of SFAs accounted for 37.39 ± 2.73% of total FAs, while PUFAs represented 39.37 ± 3.02% and MUFA 23.12 ± 2.45%. the distribution across participants was relatively homogeneous, with median values (SFA = 37.31%, PUFA = 39.50%, MUFA = 22.88%) closely matching the corresponding means, suggesting the absence of major asymmetries in class distribution. This composition indicates that, at the population level, PUFAs slightly predominate over SFAs, a pattern consistent with a dietary intake relatively rich in plant-derived oils and/or polyunsaturated lipid sources (*Graphic 1-2*).

Palmitic acid was the most abundant single component, with a mean relative abundance of 22.94 ± 1.99%, followed by OA at 19.39 ± 2.52%. Among the PUFAs series, LA and AA were predominant, whereas long-chain omega-3 species such as EPA and DHA were present at markedly lower levels, averaging 0.43 ± 0.35% and 2.20 ± 0.81%, respectively.

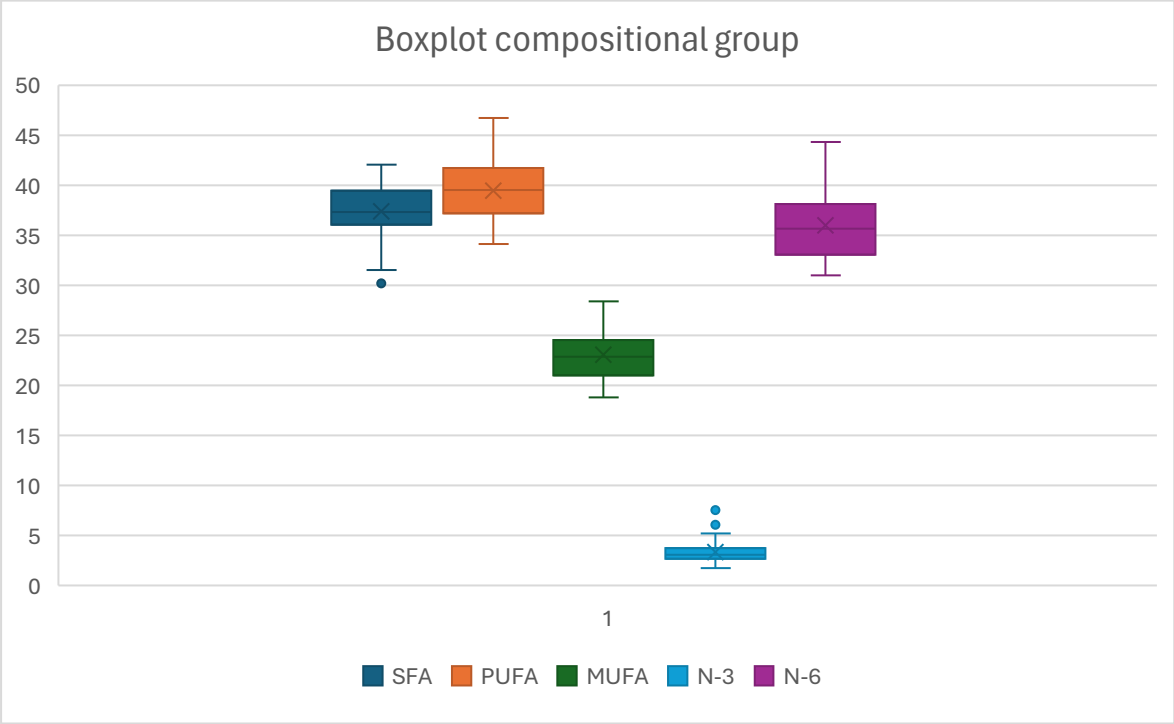
The balance between the omega-6 and omega-3 PUFA families was clearly skewed towards the former. The omega-6/omega-3 ratio averaged 11.9, confirming a predominance of omega-6 FAs. In parallel, total

omega-6 (35.88 ± 3.25) greatly exceeded total omega-3 FAs (3.37 ± 1.21%). This proportion is considerably higher that the ratios typically considered optimal for anti-inflammatory equilibrium, implying a relatively low representation of omega-3 long-chain PUFAs in the lipid profile.

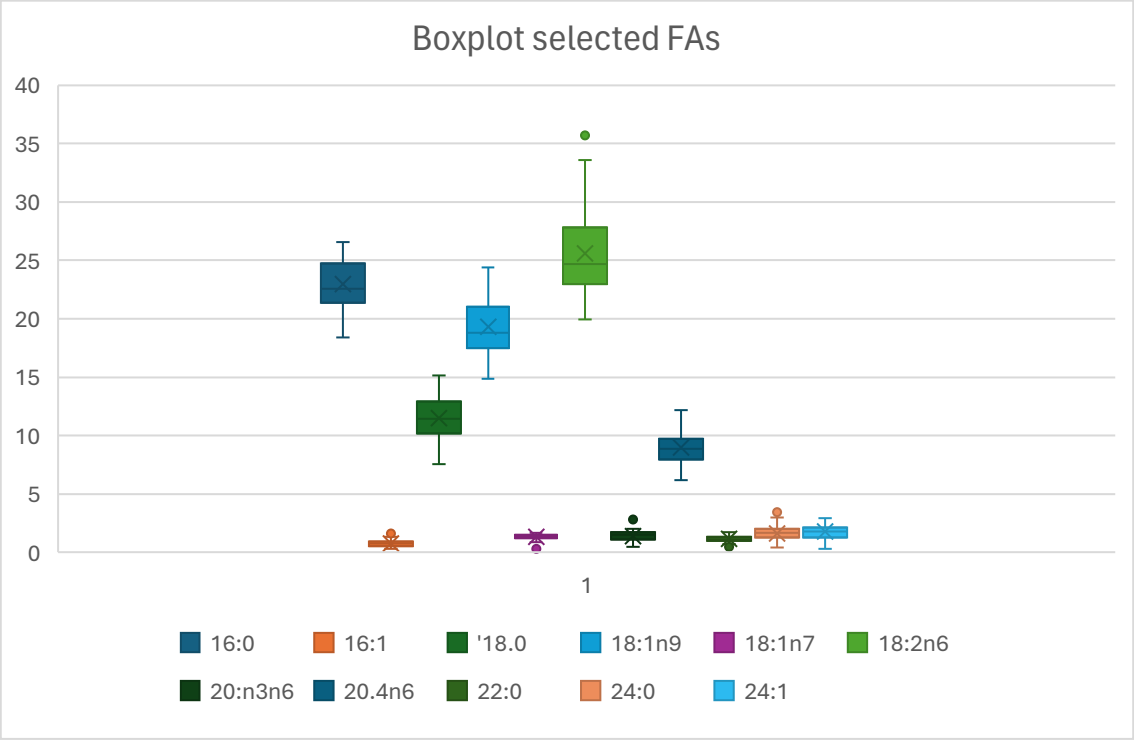
Variability across individuals was moderate for the main lipid classes, but pronounced for minor components. The coefficients of variation for SFA, PUFA, and MUFA were in the range of 6-8%. However, FAs such as EPA and DHA displayed considerably higher inter-individual dispersion. The boxplot representation of these data confirmed the presence of a few subjects with unusually low EPA or high DHA proportions, though no extreme outliers were identified.

Correlation analysis among FAs revealed biologically coherent relationships. Strong positive correlations were observed within each biochemical family (e.g. among omega-6 series), while expected inverse associations were detected between PUFA and SFA fractions due to compositional constraints (*Graphic 3*).

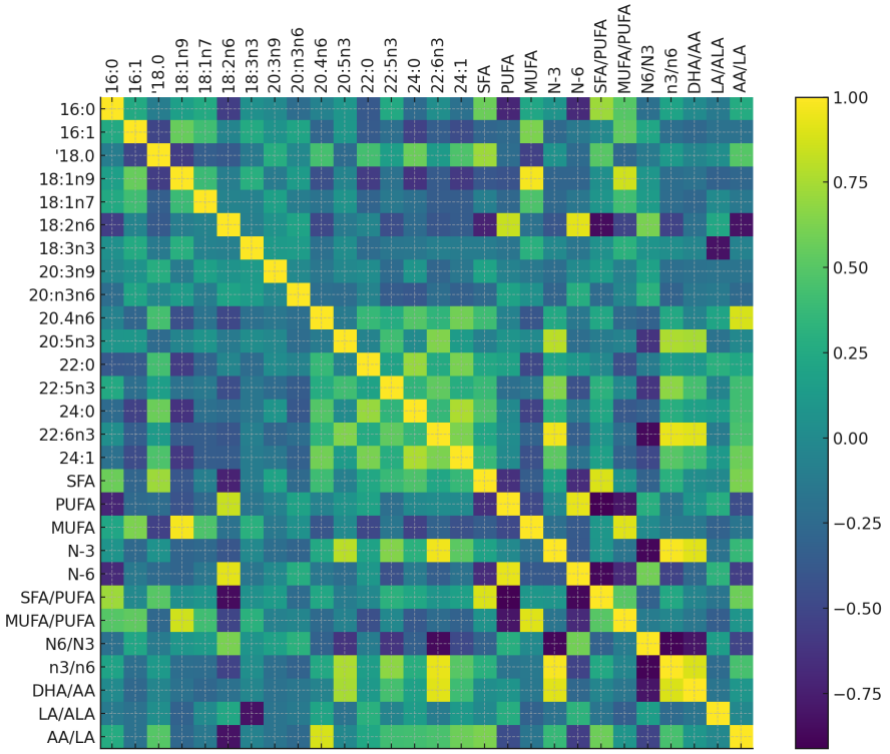
In summary, the baseline FA profile of this cohort is characterized by a moderate predominance of PUFAs, a notably high n-6/n-3 ratio (~12:1), and low EPA levels relative to DHA and total n-6 content. Inter-individual variability was modest for major lipid classes.



Graphic 1. Boxplot refers to compositional group in 40 enrolled women.



Graphic 2. Boxplot refers to selected FAs in 40 enrolled patients.



Graphic 3. A correlation matrix (heatmap) shows expected positive correlations within families and negative relationships driven by compositional constraints.

4.5.2 Inflammatory biomarker (INFLA-score)

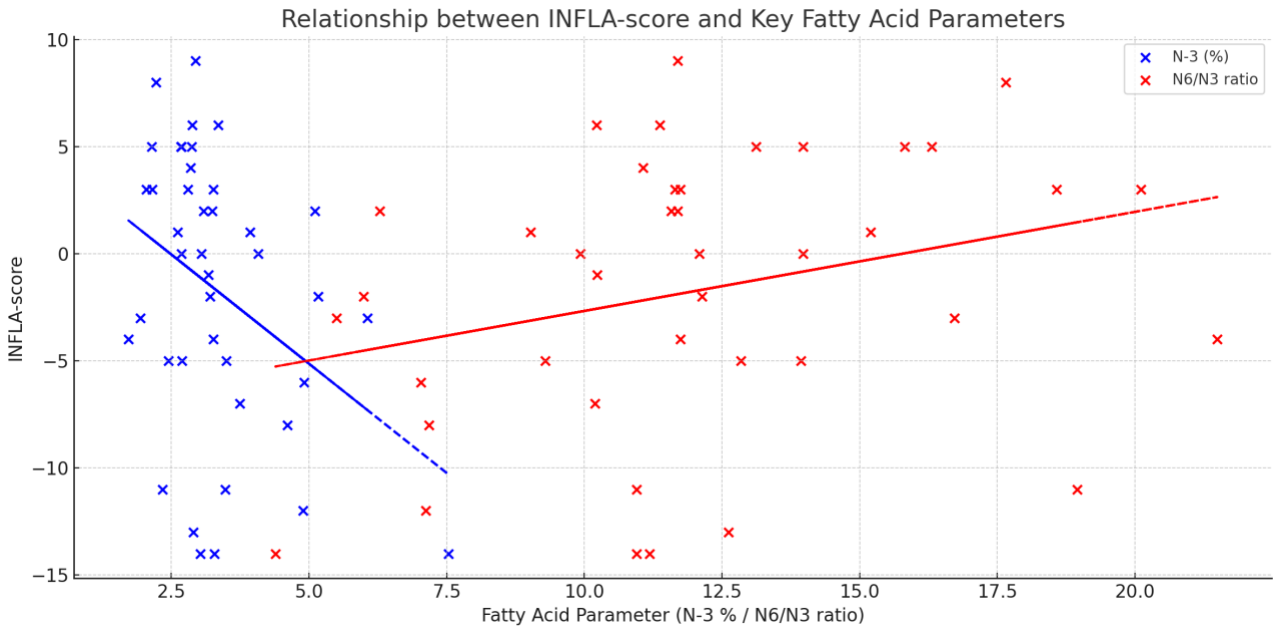
At baseline (T0), the INFLA-score exhibited considerable variability among the 40 participants, ranging from -14 to +9, with a mean of approximately -1.5 and a standard deviation of 6.5. Negative scores indicate a lower inflammatory status, whereas positive values reflect higher inflammation. The distribution was relatively balanced with a slight skew towards a lower degree of inflammation, suggesting heterogeneous inflammatory states within the cohort.

Analysis of the correlation matrix revealed distinct patterns among FAs, their classes, and inflammatory markers. SFAs were positively associated with the SFA/PUFA ratio ($r = 0.695$, $p < 0.01$) and negatively correlated with PUFAs ($r = -0.665$, $p < 0.01$), indicating a reciprocal relationship between saturated and polyunsaturated lipids.

MUFAs, primarily represented by 18:1 – n9, were strongly positively correlated with MUFA/PUFA ratio ($r = 0.911$, $p < 0.01$) and inversely associated with PUFA and omega-6 FAs, reflecting the compensatory balance between mono- and polyunsaturated lipids. Notably, omega-3 and omega-6 FAs exhibited a strong inverse relationship ($r = -0.877$, $p < 0.01$), with corresponding ratios (n6/n3 and n3/n6) reflecting these dynamics. Importantly, the INFLA-score, a composite measure of systemic inflammation, was negatively associated with omega-3 FAs $r = -0.362$, $p < 0.05$) and DHA/AA ratio ($r = -0.388$, $p < 0.05$), suggesting that higher levels of n-3 fatty acids and favorable DHA/AA balance are linked to lower inflammatory status. Individual fatty acids showed expected biochemical relationships; for example, 18:2n6 strongly correlated with total n-6 ($r = 0.842$, $p < 0.01$) and 20:5n3 with total n-3 ($r = 0.784$, $p < 0.01$), supporting the internal consistency of the dataset. These findings highlight the interplay between fatty acid composition and systemic inflammation, with potential implications for dietary modulation of inflammatory risk (*Table 7, Graphic 4)*.

Fatty acid/ Index	*Coefficient r*	*P value*	*Interpretation*
DHA/AA ratio	–0.388	$p < 0.05$	Higher DHA/AA ratio is associated with lower inflammation
Omega-3	–0.362	$p < 0.05$	Omega-3 levels are inversely associated with inflammation
EPA	–0.364	$p < 0.05$	Significant ant-inflammatory effect
SFA	–0.018	n.s.	No clear relationship
Omega-6	+0.046	n.s.	Slight positive trend, not significant
MUFA	–0.045	n.s.	No relevant association
PUFA	–0.112	n.s.	Weak inverse trend
Omega-6/omega-3 ratio	+0.361	$p < 0.05$	Higher ratio (more omega-6 relative to omega-3) associated with increased inflammation

Table 7. Correlation between different fatty acids and inflammation markers: higher omega-3 levels and DHA/AA ratio are linked to lower inflammation, while a higher N6/N3 ratio is associated with increased inflammation.



Graphic 4. Dotplot refers to relationship between INFLA-score and key fatty acid parameters, in particular omega-3 and omega-6/omega-3 ratio. The blue dots show the relationship between INFLA-score and omega-3 (%) with a downward trendline, confirming the negative correlation. The red dots show the relationship between INFLA-score and omega-6/omega-3 ratio with an upward trendline, confirming the positive correlation.

4.5.3 Nutritional compounds

The analysis examines a dataset of nutritional compounds, specifically flavonoids, proanthocyanidins, and related phytochemicals, measured across multiple samples. The analysis of dietary flavonoid and proanthocyanidin intake revealed substantial variability across the study population. Isoflavone content was highly heterogeneous, ranging from approximately 7.33 µg to 39,461.83 µg, indicating pronounced differences in dietary sources among participants. Anthocyanidins exhibited moderate variation (0.87–324.48 mg), with a small subset of individuals showing particularly high intakes. Flavan-3-ols had a mean intake of around 32 mg, with peaks exceeding 96 mg, while flavanones, flavones, and flavonols generally contributed lower absolute amounts, resulting in moderate contributions to total flavonoid intake. Total flavonoids (flatot) ranged from ~4.68 to 619 mg, reflecting the combined contributions of all flavonoid subclasses. Proanthocyanidins displayed high variability, with polymeric forms dominating the total proanthocyanidin pool in most samples. The combined index of total flavonoids and proanthocyanidins (flapro) ranged from ~109 to ~2,655, representing overall polyphenolic intake.

Correlation analysis indicated strong positive associations between flavan-3-ols and proanthocyanidin dimers and trimers ($r \approx 0.65–0.78$), consistent with their role as structural precursors. Isoflavones demonstrated weaker correlations with other flavonoid subclasses ($r \approx 0.2–0.35$), reflecting distinct dietary sources. Notably, total flavonoid intake (flatot) correlated strongly with flapro ($r \approx 0.92$), confirming that the combined index effectively captures overall polyphenolic consumption. Graphical analyses further revealed right-skewed distributions for isoflavones and flavan-3-ols, highlighting a few individuals with exceptionally high intakes, and heatmaps demonstrated strong intra-class correlations among proanthocyanidin subtypes, with moderate correlations among flavonols and flavones.

Overall, these results indicate that flavonoid and proanthocyanidin intake is highly variable in the study population, with polymeric proanthocyanidins and flavan-3-ols representing major contributors to total polyphenol intake, and isoflavones contributing substantially in subset of individuals. The observed patterns and correlations provide a comprehensive overview of polyphenolic consumption, supporting further analyses on the potential relationship between dietary bioactive compounds and systemic inflammation.

4.6 Discussion

The present study might provide a comprehensive overview of the associations between dietary bioactive compounds, FA composition, and systemic inflammation in the enrolled cohort.

The baseline plasma FAs profile of this cohort revealed a relatively balanced composition among major FA classes, with a slight predominance of PUFAs over SFAs. The mean proportion of SFAs (37.39 ± 2.73%) and PUFAs (39.37 ± 3.02%) suggests a diet relatively rich in plant-derived oils and polyunsaturated lipid sources, while the mono-unsaturated fraction (MUFAs, 23.12 ± 2.45%) aligns with general dietary recommendations. The close alignment between mean and median values indicates a homogeneous distribution of FAs within the population, suggesting a stable and representative baseline lipid profile.

Among individual FAs, palmitic acid (16:0) and OA (18:1 n-9) were the most abundant, consistent with previous reports on human plasma lipids. Within the PUFA series, LA and AA were predominant, whereas long-chain omega-3 species such as EPA and DHA were relatively low, averaging 0.43 ± 0.35% and 2.20 ± 0.81%, respectively. The omega-6/omega-3 ratio (~12:1) indicates a clear imbalance favoring omega-6 FAs, a pattern commonly observed in Western diets and associated with potential pro-inflammatory effects.

Correlation analyses among FA classes confirmed biologically coherent relationships. SFAs were inversely correlated with PUFAs, while MUFAs positively correlated with the MUFA/PUFA ratio and negatively with omega-6 FAs. Importantly, omega-3 FAs were inversely associated with the INFLA-score ($r = -0.362$, $p < 0.05$), and the DHA/AA ratio also correlated negatively with inflammation ($r = -0.388$, $p < 0.05$). These findings suggest that higher plasma levels of omega-3 FAs and a favorable DHA/AA balance are linked to lower systemic inflammatory status, supporting the potential role of dietary omega-3s in modulating inflammatory risk (146,147).

Inter-individual variability was modest for major FA classes but more pronounced for minor components, particularly EPA and DHA, highlighting differences in metabolism or dietary intake. Strong correlations between specific FAs and their respective families (e.g., 18:2-n6 with total n-6, 20:5-n3 with total n-3) support the internal consistency and reliability of the dataset.

Analysis of dietary flavonoid and proanthocyanidin intake revealed high variability across participants. Isoflavones showed extreme heterogeneity (7.33–39,461.83 µg), whereas flavan-3-ols and polymeric proanthocyanidins were the main contributors to total polyphenol intake. Strong intra-class correlations, such as between flavan-3-ols and proanthocyanidin dimers/trimers, reflect their co-occurrence in plant foods and suggest potential synergistic effects on inflammation. The combined polyphenol index (flapro) was strongly correlated with total flavonoid intake, confirming its utility as a comprehensive measure of dietary polyphenols.

Taken together, these findings highlight a complex interplay between FA composition, polyphenol intake, and systemic inflammation. The high omega-6/omega-3 ratio and low EPA levels observed in individuals with higher INFLA-scores suggest potential targets for dietary interventions, while variability in polyphenol consumption points to opportunities for modulating inflammation through flavonoid-rich foods. Future longitudinal studies are warranted to clarify causal relationships and to explore the combined impact of FAs and polyphenols on inflammatory and metabolic health.

4.7 Limitations and future perspectives

Despite its strengths, this study presents several limitations that should be acknowledged. a) Participants could not be blinded due to the inherent nature of dietary intervention, potentially introducing performance and reporting biases. b) The context-specific design of the anti-inflammatory dietary intervention, developed according to local food availability and culinary habits in Italy, may limit the generalizability of the findings to other populations or geographic settings. c) Adherence to dietary recommendations, although carefully monitored, relied in part on self-reported data, which may be subject to inaccuracies. Moreover, while the study assessed both objective outcomes (e.g., INFLA-score, FAs profile, microbiome composition, hormonal and embryological parameters) and subjective outcomes (e.g., symptom severity, sexual function, quality of life), the complex interplay between these domains may be influenced by unmeasured confounders, such as lifestyle habits or psychosocial factors.

A major limitation in this specific context was the inability to compare baseline (T0) and post-intervention (T1, 1.5 months) data for participants in the intervention group. Such comparisons could have provided valuable insights into potential correlations between FA profiles and INFLA-score values, allowing a more direct evaluation of the dietary intervention's efficacy in modulating inflammation and increasing levels of anti-inflammatory PUFAs, particularly ALA, EPA, and DHA.

Nevertheless, this trial offers a unique opportunity to explore the impact of an anti-inflammatory diet on reproductive outcomes in women with endometriosis in a rigorous, randomized controlled framework. Future studies should aim to replicate these findings in more diverse populations, incorporating multi-omics approaches to elucidate the mechanistic links between diet, inflammation, microbiome composition, and reproductive health. Long-term follow-up is also warranted to determine whether dietary modifications can sustainability improve fertility outcomes and overall health, potentially informing personalized nutrition strategies as adjunctive therapies in endometriosis management.

4.8 Conclusion

In summary, this study provides evidence that the baseline FA composition of peripheral blood is closely related to systemic inflammatory status, as reflected by the INFLA-score. The cohort exhibited a moderately PUFA-dominant lipid profile, but a markedly elevated omega-6/omega-3 ratio, indicating an imbalance toward pro-inflammatory FAs. Correlation analysis revealed that higher omega-3 PUFA levels were consistently associated with lower inflammation, whereas omega-6 PUFAs and SFAs showed positive associations with inflammatory status. These findings underscore the biological relevance of the omega-6/omega-3 balance as a key determinant of inflammatory tone.

Taken together, the data suggest that optimizing the omega-6/omega-3 ratio through dietary strategies or targeted supplementation may represent an effective approach to mitigating low-grade systemic

inflammation. The moderate inter-individual variability observed in long-chain omega-3 highlights the need for personalized nutritional or metabolic interventions.

Future longitudinal and interventional studies should aim to confirm these associations causally and to clarify the mechanisms through which specific FA species modulate inflammatory pathways. Ultimately, maintaining a balanced FA profile may play an essential role in promoting metabolic health and preventing inflammation-related chronic diseases.

CHAPTER 5

Salivary fatty acids as emerging biomarkers

5.0 Background and rationale

Traditionally, the assessment of FA profiles has relied on invasive blood sampling, which may not be feasible for all populations or settings. Recent advancements have highlighted the potential of saliva as a non-invasive alternative for FA analysis. A comprehensive review by Abodi et al. (148) emphasized the emerging role of salivary FAs as biomarkers for health and disease. The authors discussed various analytical techniques for FA profiling and explored the relationship between dietary intake and salivary FA composition. They also highlighted the potential of salivary FAs in reflecting systemic health conditions, thereby underscoring their diagnostic value.

Human saliva contains a complex lipid composition, including mono-, di-, and TG, free fatty acids (FFAs), phospholipids, was esters, cholesterol esters, and squalene. Neutral lipids dominate the salivary lipid profile, representing 96-99% of total lipids, whereas polar lipids such as phospholipids are present in minor amounts. Concentrations of total FAs in resting saliva average around 9 µg/mL, with FFAs accounting for approximately 3.5 µg/mL. Major FFAs identified in saliva include palmitic, OA, LA, and stearic acids, while short-chain fatty acids (SCFAs) are present biologically significant in spite of their limited concentrations.

5.1 Analytical methods for salivary FA profiling

Accurate profiling of salivary FAs necessitates sensitive and reproducible analytical methods due to their low concentration and complex matrix. The analysis involves several critical steps, including collection, storage, extraction, derivatization, and chromatographic or spectrometric analysis. Saliva can be collected either unstimulated, through drooling, aspiration, or pipette collection, or stimulated using chewing gum, parafilm, or Salivette®. Collection protocols vary widely in terms of fasting duration, time of day, and avoidance of behaviors such as oral hygiene, smoking, and eating, all of which can influence FA composition. Storage at -80°C is typically required to prevent degradation, and antioxidants such as BHT or acidifying agents may be added to improve stability, especially for SCFAs. Lipids are commonly extracted using Folch or Moilanen & Nikkari methods and derivatized to fatty acid methyl esters (FAMEs) for gas chromatography (GC) or GC-MS analysis. Advanced techniques, including LC-MS/MS, NMR, and HPLC-TOF-MS, are increasingly employed for comprehensive FA profiling.

Salivary FA composition is influenced by multiple biological and environmental factors. Lingual lipase, present in approximately half of healthy adults and abundant in infants, affects basal FFAs levels and fat taste perception. Smoking has been associated with decreased unsaturated FA levels and enhanced inflammatory markers in saliva. Dietary intake strongly impacts salivary FAs, with correlations observed between specific FAs and nutrient consumption; for example, ALA is higher in vegetarians, while linoleic and arachidonic acids correlate with vegetable oil and red meat intake, respectively. Age and reproductive stage also influence salivary FAs: hormonal fluctuations alter FA composition across the menstrual cycle, while aging affects salivary gland function and FA levels.

5.2 Correlation with systemic FA levels and inflammatory markerd

Emerging evidence suggests that specific salivary Fas correlate significantly with systemic FA concentrations, particularly essential PUFA and long-chain saturated Fas. These correlations provide a biological rationale for using saliva as a surrogate matrix for monitoring systemic lipid metabolism. Moreover, certain salivary Fas have been associated with markers of inflammation, including CRP, IL-6, and TNF- α, indicating their potential utility in non-invasive monitoring of inflammatory states (149).

Alterations in salivary FAs have been reported in multiple pathological states. In Sjögren's syndrome, palmitic acid promotes IL-6 expression, contributing to local inflammation. In cystic fibrosis, total salivary FFAs and unsaturated/saturated ratios are altered, in correlation with disease severity. Obesity and hepatic steatosis are associated with elevated palmitic acid and SCFAs, whereas periodontal disease and dental caries exhibit dysregulated FFAs and SCFAs, reflecting bacterial activity and inflammation. In diabetes mellitus, inflammatory FAs such as LA and AA are elevated in patients with gingivitis or periodontitis. Additionally, acute heart failure is associated with increased docosapentaenoic acid (DPA) levels, illustrating the potential of salivary FAs as prognostic markers.

A study by Huang et al. (150) utilized mass-spectrometry-based metabolomic profiling to identify alterations in salivary redox status and fatty acid metabolism in response to iinflammation and oxidative stress in periodontal disease. The findings highlighted the importance of redox status in periodontitis and provided a rationale for preventing periodontal disease by dietary interventions aiming to restore redox balance.

5.3 Preliminary findings and implications for non-invasive diagnostics

Salivary FAs represent a promising non-invasive biomarker for assessing nutritional status, metabolic health, and disease activity. Evidence suggests correlations between salivary FA composition, dietary intake and systemic inflammation. However, methodological heterogeneity, including differences in collection, storage, extraction, and derivatization, limits comparability across studies. Furthermore, few investigations have explored the relationship between salivary and plasma Fas, and SCFAs require careful handling due to their volatility.

Preliminary clinical data indicate distinct salivary FA signatures in individuals with systemic inflammatory conditions compared to healthy controls. For instance, elevated levels of specific SFA and MUFAs may refelct heightened inflammatory anti-inflammatory status. These findings highlight the potential of salivary FA profiling as a rapid, non-invasive diagnostic tool, capable of complementing conventional blood-based assays.

Future research should focus on longitudinal studies, larger cohorts, and integration with other salivary biomarkers to establish standardized reference ranges and validate predictive models for clinical application. Additionally, the development of point-of-care diagnostic devices utilizing salivary FA profiles could strongly improve the monitoring of chronic diseases and facilitate early detection, leading to improved patient outcomes.

CHAPTER 6

Nutraceutical approaches and translation applications of PUFAs and ALA

The clinical significance of PUFAs, particularly ALA, has stimulated the development of nutraceutical strategies aimed at supporting cardiovascular, metabolic, neurocognitive, and reproductive health. Nutraceutical interventions allow for targeted supplementation with careful attention to dosage, bioavailability, safety, and potential interactions with other bioactive compounds. Current research highlights both established and emerging applications of PUFA supplementation, emphasizing ALA-based interventions, their safety profiles, and translational potential across pediatrics, gynecology, and immunology.

6.0 Supplementation with PUFAs and ALA: formulations, dosages, and safety

PUFAs are available in multiple formulations, including triglycerides, ethyle esters, phospholipids, and free FAs, with bioavailability influenced by the chemical form, concomitant dietary fat, and digestive physiology (151). ALA is commonly sourced from plant oils, but encapsulated supplements and enriched foods are increasingly used for controlled intake (152).

For ALA, EFSA recommends 0.5% of total energy intake (≈2 g/day in adults) to maintain cardiovascular health (EFSA, 2011). For long-chain n-3 PUFAs, intake of 250-500 mg/day of EPA+DHA are advised for general adult populations (EFSA, 2012). Clinical trials in cardiometabolic and inflammatory diseases often use higher doses (up to 4 g/day EPA+DHA), whereas safe upper intake levels have been established up to 5 g/day (153).

PUFA supplementation is generally well-tolerated, with mild gastrointestinal discomfort and, at higher doses, increased bleeding tendency due to antithrombotic effects being the most commonly reported side effects (154). ALA is considered safe even at high intakes, though attention should be paid to overall energy balance and n-6/n-3 ratio. Importantly, supplementation does not appear to adversely affect glycemic control or immune competence (155).

6.1 Interactions with other nutraceutical molecules, vitamins, and pharmacological agents

6.1.1 Probiotics and prebiotics

PUFAs interact synergistically with probiotics and prebiotics, modulating gut microbiota composition and enhancing metabolic outcomes. Animal and human studies indicate that n-3 PUFAs, particularly EPA and DHA, enrich short-chain fatty acid (SCFA)-producing taxa such as *Bifidobacterium* and *Roseburia*, while reducing pro-inflammatory Gram-negative bacteria (156). Conversely, prebiotic fibers including inulin and fructo-oligosaccharides promote microbial growth that enhances PUFA bioavailability and anti-inflammatory activity, contributing to improved lipid metabolism, glycemic control, and immune homeostasis (157).

Clinical studies in metabolic syndrome and non-alcoholic fatty liver disease demonstrate additive benefits of combining PUFAs with probiotics or prebiotics, including reductions in TG, LDL-C, and systemic inflammation, although study heterogeneity limits definitive conclusions (158). These effects

appear to be mediated by the dual action of PUFAs in inflammatory signaling and microbiota-derived SCFAs on gut barrier integrity and host metabolism. Moreover, the background dietary n-6/n-3 ratio seems to modulate this interaction, with more balanced intake favoring microbial diversity and anti-inflammatory taxa (159).

Overall, the available evidence supports a bidirectional synergy: while n-3 PUFAs shape a gut microbiome favorable to SCFA production and metabolic resilience, probiotics and prebiotics improve the handling and efficacy of PUFAs, leading to potentially additive effects in cardiometabolic prevention and immune modulation. Nevertheless, heterogeneity in probiotic strains, prebiotic types, PUFA formulations, and study designs still limit firm conclusions, highlighting the need for large, well-controlled RCTs (160).

6.1.2 Hypocholesterolemic agents

PUFAs, particularly ALA, may act additively with hypocholesterolemic nutraceuticals such as plant sterols/stanols, viscous soluble fibers and red yeast rice, thereby contributing to the reduction of LDL-C and cardiovascular risk. Plant sterols and stanols reduce intestinal cholesterol absorption by competing with dietary and biliary cholesterol at the NPC1L1 transporter. Their effect is dose-dependent: intakes of 1.5/3.0 g/day lower LDL-C by approximately 7-12% with maximal reductions of around 12% at 3 g/day. The hypocholesterolemic effect is similar between sterols and stanols and is unaffected by timing of intake during the day (161,162).

Viscous soluble fibers bind bile acids in the intestine, increasing bile acid excretion and stimulating hepatic cholesterol catabolism. Oat β-glucans at > 3 g/day reduce LDL-C by ~0.25 mmol/L (~10 mg/dL), with similar evidence for barley β-glucans at equivalent doses. Psyllium supplementation has been consistently associated with further LDL-C reductions in meta-analyses of RCTs (163,164).

For ALA, experimental and clinical evidence supports modest but favorable effects on LDL-C, and other cardiometabolic risk markers, in addition to anti-inflammatory and endothelial benefits. Comparative dietary studies have shown that ALA-rich diets can lower LDL-C more effectively than LA-rich diets. A recent meta-analysis of RCTs in overweight and obese adults reported improvements in cardiovascular risk factors with ALA supplementation or ALA-enriched foods (53).

RCTs have evaluated multi-target formulations combining omega-3 FAs with plant sterols (165). In adults with mild hypercholesterolemia or hypertriglyceridemia, a low-fat spread enriched with plant sterols and omega-3 significantly reduced both LDL-C and triglycerides compared with controls, indicating additive effects between cholesterol absorption inhibition and PUFA mediated lipid metabolism modulation. In individuals with impaired glucose regulation, the same combination improved lipid profile, systemic inflammation, and insulin sensitivity (166).

Red yeast rice contains monacolins, particularly monacolin K which inhibit HMG-CoA reductase and reduce LDL-C. Several studies (167,168) confirm clinically meaningful LDL-C reductions. However, the safety profile of this substance is under regulatory scrutiny in the EU, as EFSA has concluded that exposure to monacolin K may induce adverse muscular and hepatic events, including myopathy and, in rare case, rhabdomyolysis. Consequently, the European Commission has restricted health claims and limited the use, prohibiting products providing > 3 mg/portion and previously capping daily intake at 10 mg (EFSA Journal. 2018;16(6):5368).

Overall, integrated nutraceutical strategies combining PUFAs with plant sterols or stanols and soluble fibers are biologically plausible, clinically supported, and safe options for LDL-C lowering and cardiovascular prevention.

6.1.3 Vitamins

6.1.3a Vitamin E

Vitamin E is the primary lipid-soluble antioxidant responsible for protecting PUFAs from peroxidation within cellular membranes and lipoproteins. Increased intake of n-3 FAs raises the requirement for vitamin E, as the higher degree of unsaturation makes membranes more susceptible to oxidative damage (169). Experimental and clinical studies have shown that combined supplementation with vitamin E and n-3 PUFAs more effectively reduces oxidative stress biomarkers and inflammatory mediators (CRP, IL-6) compared with either nutrient alone (170). This synergy suggests that vitamin E co-supplementation may be particularly important in high-dose PUFA regimens or in conditions characterized by increased oxidative stress, such as diabetes, obesity, and cardiovascular disease.

6.1.3b Vitamin D

Vitamin D and long-chain n-3 PUFAs exert convergent immunomodulatory effects, influencing T-cell differentiation, cytokine secretion, and endothelial function. Evidence indicates that their co-administration provides additive benefits in pregnancy, supporting placental function and reducing the risk of hypertensive complications, as well as in autoimmune diseases, where they reduce inflammation and improve immune tolerance (171). Moreover, combined supplementation has been associated with improvements in vascular reactivity and insulin sensitivity, supporting their role in integrated cardiometabolic prevention strategies.

6.1.3c B group vitamins

Vitamins B6, B12, and folates regulate homocysteine metabolism, a key cardiovascular risk factor. Elevated homocysteine is associated with endothelial dysfunction, oxidative stress, and increased thrombogenicity. When combined with n-3 PUFAs, these vitamins exhibit synergistic effects in lowering plasma homocysteine, thereby enhancing cardiovascular protection beyond lipid-lowering mechanisms alone (172). Such synergy has been particularly emphasized in populations with suboptimal B-vitamin status, suggesting the importance of assessing baseline micronutrient adequacy when prescribing PUFA supplementation.

6.1.4 Minerals

Selenium, as an essential cofactor of glutathione peroxidase (GPx), plays a central role in neutralizing lipid peroxides generated during PUFA oxidation. Adequate selenium intake enhances antioxidant defense, thereby preserving PUFA integrity in cellular membraned and circulating lipoproteins. Deficiency may exacerbate oxidative stress in individuals with high PUFA intake, attenuating the clinical efficacy of supplementation (173).

Zinc and magnesium are involved in the enzymatic activity of desaturase and elongase, which catalyze the conversion of dietary ALA into long-chain derivatives EPA and DHA. Deficiencies in these minerals may impair PUFA metabolism, reducing tissue levels of bioactive n-3 FAs. Magnesium also modulates vascular tone and inflammation, potentially enhancing the endothelial effects of n-3 PUFAs. Collectively, these mineral PUFA interactions highlight the necessity of ensuring adequate trace element status in clinical and nutritional interventions (174).

6.1.5 Anticoagulants

High dose n-3 PUFA supplementation exerts anti-thrombotic effects, including reduced platelet aggregation and altered eicosanoid balance. When combined with anticoagulant therapies (e.g., warfarin, direct oral anticoagulants), these effects may potentiate bleeding risk in susceptible patients. Although large-scale meta-analyses suggest the risk is modest, clinical monitoring is warranted in patients receiving both n-3 and anticoagulant drugs (175).

6.1.6 Fibrates

Both fibrates and n-3 FAs act via PPARα activation, leading to reductions in plasma triglycerides (176). While their combination may provide enhanced triglyceride lowering, overlapping mechanisms could also increase the risk of hepatotoxicity or myopathy. Thus, while biologically plausible, this strategy requires careful evaluation in clinical practice.

6.1.7 Polyphenols and other bioactives

Emerging evidence supports synergistic effects between PUFA supplementation and polyphenolic compounds, such as resveratrol, catechins, and curcumin. These bioactives enhance antioxidant capacity, improve mitochondrial function, and reduce systemic inflammation. When combined with omega-3 FAs, they may potentiate protective effects in metabolic syndrome, cardiovascular prevention, and aging related disorders (177). Similarly, Q10, a mitochondrial cofactor and antioxidant, has been investigated in combination with PUFAs for its potential to improve bioenergetics and endothelial function. These nutrient synergies represent promising future directions for nutraceutical formulations.

6.2 Emerging clinical application

6.2.1 Pediatrics

6.2.1a Neurodevelopment and vision

Among pediatric populations, the strongest evidence base concerns DHA, given its structural role in retinal and neuronal membranes. RCTs and meta-analyses indicate that DHA-supplemented formula improves visual acuity in term infants up to ~12 months, with diminishing returns at higher doses (178). Regulatory assessments likewise recognize DHA's contribution to normal brain development in infants and children. ALA enriched diets do contribute to long-chain n-3 pools and can support neurodevelopment, particularly when the dietary LA/ALA ratio is not excessive (179 Narrative and

systematic reviews suggest potential neurodevelopmental benefits of early-life n-3 exposure, while also highlighting heterogeneity across trials and outcomes beyond visual acuity (180).

6.2.1b Allergy and immune maturation

Omega-3 exposure during pregnancy and/or early infancy may shape immune development and allergic risk. A Cochrane review concluded that maternal n-3 supplementation improves certain perinatal outcomes (e.g. lowers risk of early preterm birth) and may influence atopic trajectories, though allergy endpoints show mixed effects across trials (181). More recent systematic reviews focused on atopy report signals for reduced allergic sensitization/eczema with prenatal or early-life n-3 intake, but results remain inconsistent and context-dependent (timing, background diet, baseline status (182). In infants and toddlers, some RCTs observed fewer common infections and respiratory symptoms, suggesting immunomodulatory benefits in addition to growth and neurodevelopment outcomes (183).

6.2.1c Behavioral and learning disorders

Children with ADHD often exhibit lower blood DHA/EPA than typically developing peers, and meta-analyses report small, clinically modest improvements in overall ADHD symptoms and certain cognitive and behavioral domains with n-3 supplementation. Findings are most consistent for formulations providing n-3, and combination with micronutrients may enhance effects in subsets of children with nutritional insufficiencies (184). More recent reviews caution that core ADHD symptoms may not meaningfully improve in all settings, underscoring the need for longer trials, stratification by baseline n-3 index, and attention to co-nutrients status (185).

6.2.1d Respiratory and atopic conditions

Early RCTs and subsequent work in children suggest that n-3 intake can modulate airway inflammation. Isolated trials report improvements in asthma control and lung function, but evidence is heterogeneous and not uniformly positive, especially for primary prevention of asthma in school-aged children (186).

In pediatrics, n-3 PUFAs show the most robust support for visual development and select neurodevelopmental endpoints, with modest behavioral benefits in ADHD and context-dependent effects on allergic and infectious outcomes. Effects vary by timing (prenatal vs infancy vs childhood), formulation/dose, and baseline status, reinforcing the rationale for individualized strategies and careful outcome selection in future trials (187).

6.2.2 Gynecology and reproductive medicine

PUFA intake in women of reproductive age exerts pleiotropic effects on fertility, pregnancy outcomes, and lactation.

6.2.2a Fertility and ART outcomes

Observational studies suggest that higher dietary n-3 intake is associated with improved oocyte quality, embryo morphology, and endometrial receptivity, plausibly through modulation of prostaglandin synthesis and anti-inflammatory lipid mediators (188). Clinical evidence from women undergoing ART indicates that PUFA supplementation may improve implantation and pregnancy rates, although heterogeneity in formulations and dosages warrants cautions interpretation (189).

6.2.2b Pregnancy outcomes

Long-chain n-3 PUFAs promote placental blood flow and endothelial function via nitric oxide bioavailability and a shift from pro-inflammatory to anti-inflammatory eicosanoids. Meta-analyses have confirmed a reduction in preterm birth and early preterm birth with maternal-3 supplementation (190). Furthermore, higher intake of n-3 FAs during pregnancy has been linked to a lower risk of preeclampsia and gestational hypertension (191).

6.2.2c Perinatal mental health

Low maternal omega-3 status is associated with an increased risk of postpartum depression. RCTs testing DHA and EPA supplementation show modest benefits on mood outcomes, though variability is seen depending on baseline omega-3 index, EPA/DHA ratio, and timing of supplementation (192).

6.2.2d Endometriosis and gynecologic conditions

Dietary modulation of the n-6/n-3 ratio may attenuate inflammation and pain in endometriosis by reducing AA derived prostaglandins and leukotrienes (193). Preliminary interventional studies suggest that higher EPA/DHA intake may reduce dysmenorrhea severity, highlighting a potential role for PUFA based adjunctive therapy.

6.2.3 Immunology and autoimmune disorders

PUFAs are increasingly recognized for their immunomodulatory and anti-inflammatory properties. Omega-3 PUFAs influence both innate and adaptive immunity: they reduce T-cell activation, decrease production of pro-inflammatory cytokines (IL-6, TNF- α), and enhance the generation of specialized pro-resolving mediators such as resolvins, protectins, and maresins, which actively promote resolution of inflammation (194). ALA also contributes to these effects, though conversion efficiency is limited.

Multiple RCTs and meta-analyses have demonstrated that omega-3 supplementation reduces joint pain, morning stiffness, and NSAID use in rheumatoid arthritis (RA) (195,196). Also, this supplementation is useful due to its link with improved endothelial function, decreased disease activity indices, and reduces oxidative stress in systemic lupus erythematosus (SLE) patients (197).

Omega-3 PUFAs have shown potential in reducing mucosal inflammation, although clinical trial results are heterogeneous; effects appear stronger in ulcerative colitis than Crohn's disease (198).

Preliminary studies suggest that PUFA supplementation may reduce relapse rates and modulate inflammatory biomarkers, though confirmatory large RCTs are lacking (199).

At least, PUFA-enriched medical foods and nutraceuticals are under investigation as adjunctive therapies in autoimmune conditions, aiming to complement pharmacological treatments with anti-inflammatory and pro-resolving lipid mediators (200).

Emerging evidence underscores the multifaceted roles of PUFAs, particularly ALA and its long-chain derivatives, across pediatrics, gynecology, and immunology. These nutrients support neurodevelopment, modulate allergic and immune responses, improve fertility and pregnancy outcomes, and complement pharmacological management of chronic inflammatory and autoimmune disorders. While promising, the heterogeneity of studies, doses, and baseline nutritional status necessitates further well-controlled and stratified clinical trials. Collectively, PUFAs represent versatile bioactive nutrients at the intersection of preventive and personalized medicine.

GENERAL CONCLUSIONS

7.0 Summary of main findings

The present doctoral research aimed to explore the physiological and pathological significance of Fas, with a particular emphasis on ALA and LA, two essential PUFAs that play a crucial role in maintaining metabolic homeostasis and regulating inflammation.

Given that humans cannot endogenously synthesize ALA and LA, their dietary intake is indispensable for ensuring adequate production of their long-chain derivatives, including EPA, DHA, and AA.

This work demonstrated that the balance between omega-3 and omega-6 Fas represents a key reegulatory node linking diet, inflammation, and disease progression. Specifically, data obtained from the clinical component of this research, involving women with endometriosis undergoing an anti-inflammatory diertary intervention, indicated that dietary modulation can effectively influence fatty acid composition, leading to a relative increase in omega-3 PUFAs (notably ALA, EPA, and DHA) and a concomitant reduction in the omega-6/omega-3 ratio. These biochemical modifications were paralleled by lower INFLA-scores and improvements in subjective symptom reports, suggesting a systemic anti-inflammatory effect associated with the dietary protocol.

Although the limited study duration precluded definitive causal inference, the consistency of biochemical and clinical trends supports the hypothesis that targeted nutritional strategies can modify inflamamtory tone and metabolic regulation even in complex disorders such as endometriosis. These findings alòign with previous evidence showing that ALA-rich diets can attenuate systemic inflammation, improve endothelial function, and promote a more favorable lipidomic profile (201,202).

At a physiological level, ALA and LA exert complementary roles in maintaining membrane fluidity, supporting mitochondrial bioenergetics, and modulating eicosanoid synthesis. While LA serve as the precursor for AA and pro-inflammatory lipid mediators (e.g., PGE, LTB), ALA gives rise to EPA and DHA, which are precursors of anti-inflammatory and pro-resolving mediators such as resolvins and

protectins. Maintaining an appropriate dietary omega-6/omega-3 ratio (typically between 2:1 and 4:1) appears essential to prevent the dominance of pro-inflammatory cascades and to sustain physiological resolution processes.

Taken together, the results of this doctoral project highlight that the qualitative and quantitative balance of ALA and LA in the human diet may act as a biomarker of metabolic and inflammatory health, as well as a potential determinant of therapeutic responsiveness to dietary or pharmacological interventions.

7.1 Clinical and research implications

From a clinical standpoint, these findings underscore the potential of nutritional modulation of FA composition as a complementary and non-invasive strategy to manage inflammation-driven conditions. In particular, for chronic diseases such as endometriosis, metabolic syndrome, or cardiovascular disease, dietary approaches aimed at enhancing ALA intake and optimizing the omega-6/omega-3 ratio could reduce systemic inflammation, improve lipid metabolism, and possibly influence hormonal and reproductive function (203) (*Table 8)*.

In the studied cohort of women with endometriosis, the anti-inflammatory dietary protocol, designed according to local Italian food availability and culinary traditions, demonstrated that evem short-term dietary changes can significantly alter FA profiles and inflammation markers. This suggests that dietary counseling and education could be a poweerful adjunct in integrative reproductive medicine. Moreover, the inclusion of objective biocheemical indicators, such as the FA profile and INFLA-score, allows for the quantification of dietary adherence and metabolic response, bridging the gap between self-reported data and physiological outcomes.

From a research perspective, FA profiling could serve as a dynamic biomarker of metabolic flexibility, providing valuable insight into the biological effects of dietary interventions. Integrating lipidomic data with other molecular and clinical parameters, inclusing hormonal status, immune cell activation, and microbiome composition, could enable a more holistic understanding of diet-disease interactions.

Furthermore, inter-individual variability in PUFA metabolism, influenced by genetic polymorphisms (e.g., FADS1/FADS2), hormonal milieu, and lifestyle factors, represents a key area for future exploration. Clarifying these determinants will be fundamental to the development of personalized nutrition strategies, tailored to the metabolic phenotype and inflammatory status of each individual.

The evidence presented herein is consistent with the growing recognition that ALA supplementation or ALA-rich foods, such as flaxseed oil and walnuts, can reduce prof-inflammatory cytokines, and modulate lipid metabolism (204).

Nevertheless, the relative inefficiency of ALA conversion to EPA and DHA in humans highlights the need to better characterize optimal intake levels and synergistic dietary factors that enhance its bioavailability and metabolic conversion.

7.2 Future perspectives: the role of gut microbiota and integrated nutrition

A promising frontier emerging from this work concerns the bidirectional relationship between dietary FAs and the gut microbiota, which plays a central role in shaping host immunity and metabolic function. Recent studies have shown that dietary ALA can alter gut microbial composition, increasing the

abundance of beneficial taxa such as *Bifidobacterium* and *Lactobacillus,* and enhancing the production of short-chain FAs, notably butyrate and propionate. These microbial metabolites exhibit potent anti-inflammatory and barrier-protective effects, suggesting that the microbiota may mediate part of the systemic benefits attributed to PUFAs.

Conversely, excessive intake of omega-6 FAs such as LA has been linked to gut dysbiosis, reduced microbial diversity, and enhanced intestinal permeability, which may perpetuate low-grade inflammation and metabolic dysregulation. Understanding this diet-microbiota-host axis is crucial for designing next-generation nutritional interventions aimed at restoring immune balance and metabolic integrity (*Figure 6*).

Future research should employ multi-omics approaches, integrating lipidomic, metabolomics, metagenomics, and transcriptomics, to unravel the mechanistic links between dietary FAs, microbial metabolism, and host inflammation (204). Such integrative studies could identify novel metabolic signatures or microbial markers predictive of responsiveness to dietary modulation, opening the way to precision nutrition, an emerging paradigm where dietary strategies are tailored according to individual microbiota profiles and metabolic status.

In addition, a broader perspective on intregrated nutrition is warranted. Combining ALA and LA balanced dietary patterns with lifestyle interventions, including physical activity, sleep hygiene, and stress reduction, could synergistically enhance anti-inflammatory responses and improve long-term health outcomes. In the context of women's reproductive health, this approach may support hormonal regulation, fertility, and overall well-being.

Ultimately, this doctoral research contributes to a growing body of evidence advocating for the integration of nutritional biochemistry, systems biology, and clinical medicine. It reinforces the concept that dietary FAs are not mere energy sources but bioactive signaling molecules capable of modulating inflammation, immunity, and cellular metabolism. The balance between ALA and LA thus emerges not only as a metabolic marker but also as a therapeutic target with implications extending from molecular physiology to clinical practice.

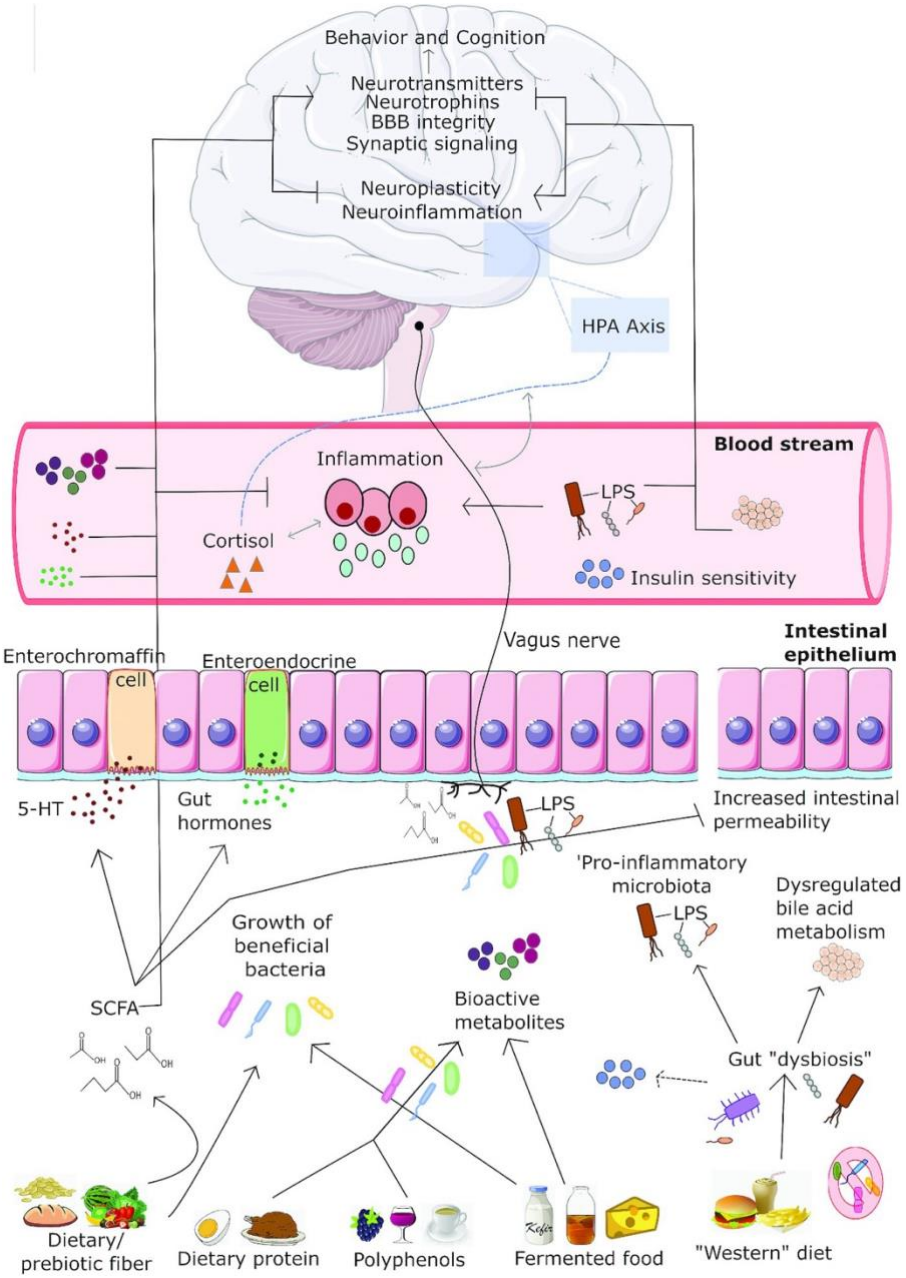


Figure 6. Gut–brain communication can be modulated by diet through multiple pathways, including microbial metabolites, hormonal, immune, metabolic, and neuronal mechanisms. A healthy diet (rich in fiber, polyphenols, and fermented foods) supports beneficial microbes that produce bioactive metabolites, neurotransmitters (e.g., serotonin), and gut hormones, which influence brain function and behavior. Conversely, unhealthy dietary patterns (e.g., Western diets) can cause dysbiosis, leading to increased intestinal permeability, inflammation, and altered metabolism, ultimately contributing to neuroinflammation and impaired brain processes.

Clinical area	***Biological mechanism/Role***	***Dosage/ Formulation***	***Safety/ Interactions***	***Key references***
Neurodevelopment and vision	Supports bran and retinal development Modulates neuroinflammation	ALA contribute to n-3 pool	Safe Efficacy depends on LA/ALA ratio and baseline nutrition	Huffman et al, 2011 Qawasmi et al, 2013 Hu et al, 2024
Immunity and allergy	Modulates immune maturation Reduces atopic risk Influences infection susceptibility	Maternal/infant n-3 supplementation	Safe Effects vary by timing, background diet	Middleton et al, 2018 Bärebring et al, 2022 Lapillone et al, 2014
Behavioral and ADHD	Modest improvements in cognitive/behavioral symptoms Supports n-3 balance	N-3 + micronutrients in children with nutritional insufficiency	Safe Response may depend on baseline n-3 status	Chang et al, 2018 Liu et al, 2023
Gynecology/ Fertility	Enhances oocyte quality, embryo morphology, endometrial receptivity	PUFA-rich diet or supplementation (ALA/EPA/DHA)	Safe	Chiu et al, 2018 Abodi et al, 2022
Pregnancy and perinatal outcomes	Improves placental blood flow Anti-inflammatory property Lowers pre-term birth risk	Maternal long-chain PUFAs supplementation ALA supports n-3 reserves	Safe Monitor energy balance and n-6/n-3 ratio	Makrides et al, 2019 Oken et al, 2008
Immunology and autommune disorders	Reduces T-cell activation Increases pro-resolving mediators	N-3 PUFA Adjunctive nutraceuticals	Safe Effects vary by disease	Calder et al, 2013 Serhan et al, 2014 Goldberg et al, 2007 Arriens et al, 2015

Table 8. Summary of the clinical applications of PUFAs, including ALA, across different health domains. The table outlines the clinical areas of application, proposed biological roles and mechanisms of action, typical dosages or formulations, safety considerations and potential interactions, as well as key supporting references from the scientific literature.

REFERENCES

1. Calder, P.C. Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology? *Br J Clin Pharmacol*. 2013;75(3):645-662. doi: 10.1111/j.1365-2125.2012.04374.x
2. Dakal, T.C., Xiao, F., Bhusal, C.K., Sabapathy, P.C., Segal, R., Chen, J., Bai, X. Lipids dysregulation in diseases: core concepts, targets, and treatment strategies. *Lipids Health Dis*. 2025;24:61. doi: 10.1186/s12944-024-02425-1
3. Givens, D.I. Saturated fats, dairy foods and cardiovascular health: No longer a curious paradox? *Nutr Bull*. 2022;47(4):407-422. doi: 10.1111/nbu.12585
4. Gutierrez-Guerra, A., Cambron-Mora, D., Rodriguez-Echevarria, R., Hernandez-Bello, J., Campos-Perez, W., Canales-Aguirre, A.A., Perez-Robles, M., Martinez-Lopez, E. Dietary n-6:n-3 PUFA ratio modulates inflammation-related gene expression and influences improvements in biochemical parameters in a murine model of diet-induced obesity. *Nutrients*. 2025;17(21):1996. doi: 10.3390/nu17121996
5. Saini, R.K., Keum, Y.S. Omega-3 and omega-6 polyunsaturated fatty acids: dietary sources, metabolism, and significance: a review. *Life Sci*. 2018:203:255-267. doi: 10.1016/j.lfs.2018.04.049
6. DiNicolantonio, J.J., O'Keefe, J.H. Omega-6 vegetable oils as a driver of coronary heart disease: the oxidized linoleic acid hypothesis. *Open Heart*. 2018;5(2):e000898. doi: 10.1136/openhrt-2018-000898
7. Cao, X., Guo, H., Dai, Y., Jiang, G., Liu, W., Li, X., Zhang, D., Huang, Y., Wang, X., Hua, H., Wang, J., Chen, K., Chi, C., Liu, H. Excessive linoleic acid induces muscle oxidative stress through 5-lipoxygenase-dependend peroxidation. *Redox Biol*. 2024:71:103096. doi: 10.1016/j.redox.2024.103096
8. Cavina, M., Battino, M., Gaddi, A.V., Savo, M.T., Visioli, F. Supplementation with alpha-linolenic acid and inflammation: a feasibility trial. *Int J Food Sci Nutr*. 2021;72(3):386-390. doi: 10.1080/09637486.2020.1802581
9. Decsi, T., Kennedy, K. Sex-specific differences in essential fatty acid metabolism. *Am J Clin Nutr*. 2011;94(6 Suppl):1914S:1919S. doi: 10.3945/ajcn.110.000893
10. Panth, N., Gavarkovs, A., Tamez, M., Mattei, J. The influence of diet on fertility and the implications for public health nutrition in the United States. *Front Public Health*. 2018;6:211. doi 10.3389/fpubh.2018.00211
11. Chalon, S. The role of fatty acids in the treatment of ADHD. *Neuropharmacology*, 2009;57(7-8):636-9. doi: 10.1016/j.neuropharm.2009.08.012
12. Singh, M. Essential fatty acids, DHA, and human brain. *Indian J Pediatr*. 2005;72(3):239-42
13. Mallick, R., Basak, S., Duttaroy, A.K. Fatty acids as evolving roles of their proteins in neurological, cardiovascular disorders and cancers. *Prog Lipid Res*. 2021:83:101116. doi: 10.1016/j.plipres.2021.101116
14. Burdge, G.C., Calder, P.C. Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reprod Nutr Dev*. 2005;45(5):581-97. doi: 10.1051/rnd:2005047
15. Calder, P.C. Omega-3 fatty acids and inflammatory processes: from molecules to man. *Biochem Soc Trans*. 2017;45(5):1105-1115. doi: 10.1042/BST20160474
16. Brenna, J.T., Salem, N., Sinclair, A.J., Cunnane, S.C. Alpha-linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans. *Prostaglandinds Leukot Essent Fatty Acids*. 2009;80(2-3):85-91. doi: 10.1016/j.plefa.2009.01.004
17. Vedtofte, M.S., Jakobsen, M.U., Lauritzen, L., O'Reilly, E.J., Virtamo, J., Knekt, P., Colditz, G., Hallmans, G., Buring, J., Steffen, L.M., Robien, K., Rimm, E.B., Heitmann, B.L. Association

between the intake of alpha-linolenic acid and the risk of CHD. *Br J Nutr*. 2014;112(5):735-43. doi: 10.1017/S000711451400138X
18. Ramsden, C.E., Zamora, D., Leelarthaepin, B., Majchrzak-Hong, S.F., Faurot, K.R., Suchindran, C.M., Ringel, A., Davis, J.M., Hibbeln, J.R. Use of dietary linoleic acid for secondary prevention of coronary heart disease and death: evaluation of recovered data from the Sydney Diet Heart Study and updated meta-analysis. *BMJ*. 2013:346:e8707. doi: 10.1136/bmj.e8707
19. Stillwll, W., Wassall, S.R. Docosahexaenoic acid: membrane properties of a unique fatty acid. *Chem Phys Lipids*. 2003;126(1):1-27. doi: 10.1016/s0009-3084(03)00101-4
20. Calder, P.C. Functional roles of fatty acids and their effects on human health. *JPEN J Parenter Enteral Nutr*. 2015;39(1):18S-32S. doi: 10.1177/0148607115595980
21. Dyall, S.C., Balas, L., Bazan, N.G., Brenna, J.T., Chiang, N., da Cista Souza, F., Dalli, J., Durand, T., Galano, J.M., Lein, P.J., Serhan, C.N., Taha, A.Y. Polyunsaturated fatty acids and fatty acid-derived lipid mediators: recent advances in the understanding of their biosynthesis, structures, and function. *Progress in Lipid research*. 2022;86:101165
22. Serhan, C.N., Levy, B.D. Resolvins in inflammation: emergence of the pro-resolving superfamily of mediators. *J Clin Invest*. 2018;128(7):2657-2669. doi: 10.1172/JCI97943
23. Jump, D.B. The biochemistry of n-3 polyunsaturated fatty acids. *J Biol Chem*. 2002;277(11):8755-8. doi: 10.1074/jbc.R100062200
24. Brenna, J.T. Long-chain polyunsaturated fatty acids and the preterm infant: a case study in developmentally sensitive nutrient needs in the United States. *Am J Clin Nutr*. 2016;103(2):606S-15S. doi: 10.3945/ajcn.114.103994
25. Lattka, E., Koletzko, B., Zeilinger, S., Hibbeln, J.R., Klopp, N., Ring, S.M., Steer, C.D. Umbilical cord PYFA are determined by maternal and child fatty acid desaturase (FADS) genetic variants in the Avon longitudinal Study of Parents and Children (ALSPAC). *Br J Nutr*. 2013;109(7):1196-210. doi: 10.1017/S0007114512003108
26. Guilloud, H., Zadravec, D., Martin, P.G.P., Jacobsson, A. The key roles of elongases and desaturases in mammalian fatty acid metabolism: insights from transgenic mice. *Prog Lipid Res*. 2010;49(2):186-99. doi: 10.1016/j.plipres.2009.12.002
27. Gonzalez-Soto, M., Mutch, D.M. Diet regulation of long-chain PUFA synthesis: role of macronutrients, micronutrients, and polyphenols on Δ-5/Δ-6 Desaturases and Elongases 2/5. *Adv Nutr*. 2021;12(3):980-994. doi: 10.1093/advances/nmaa142
28. Djuricic, I., Calder, P.C. Beneficial outcomes of omega-6 and omega-4 polyunsaturated fatty acids on human health: an update for 2021. *Nutrients*. 2021;13(7),2421. doi: 10.3390/nu13072421
29. Alijani, S., Hahn, A., Harris, W., Schuchardt, J.P. Bioavailability of EPA and DHA in humans. A comprehensive review. *Prog Lipid Res*. 2025:97:101318. doi: 10.1016/j.plipres.2024.101318
30. Lane, K.E., Wilson, M., Hellon, T.G., Davies, I.G. Bioavailability and conversion of plant- based sources of omega-3 fatty acids: a scoping review to update supplementation options for vegetarians and vegans. *Crit Rev Food Sci Nutr*. 2022;62(18):4982-4997. doi: 10.1080/10408398.2021.1880364
31. Yuan, Q., Xie, F., Huang, W., Hu, M., Yan, Q.M., Chen, Z., Zheng, Y., Liu, L. The review of alpha-linolenic acid: sources, metabolism, and pharmacology. *Phytotherapy Research*. 2021;36, 164-188. doi: 10.1002/ptr.7295
32. Barcelo-Coblijn, G., Murphy, E.J. Alpha-linolenic acid and its conversion to longer chain n-3 fatty acids: benefits for human health and a role in maintaining tissue n-3 fatty acid levels. *Prog Lipid Res*. 2009,48(6):355-74. doi: 10.1016/j.plipres.2009.07.002
33. Reynolds, A., Mann, J., Cummings, J., Winter, N., Mete, E., Morenga, L. Carbohydrate quality and human health: A series of systematic reviews and meta-analyses. *Lancet*. 2019;3939,434-445. doi: 10.1016/S0140-6736(18)31809-9

34. Del Bo, C., Bernardi, S., Marino, M., Porrini, M., Tucci, M., Guglielmetti, S., Cherubini, A., Carrieri, B., Kirkup, B.M., Kroon, P.A. Systematic review on polyphenol intake and health outcomes is there sufficient evidence to define a health-promoting polyphenol-rich dietary pattern? *Nutrients*. 2019. 11(6):1355. doi: 10.3390/nu11061355
35. Ros, E., Hu, F.B. Consumption of plant seeds and cardiovascular health: Epidemiological and clinical trial evidence. *Circulation*, 2013. 128, 553-565. doi:10.1161
36. United States Department of Agriculture. USDA National Nutrient Database for Standard Reference; USDA: Washington, DC, USA, 2015; Institute of Medicine. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients); National Academy Press: Washington, DC, USA, 2005
37. Simopoulos, A.P. The importance of the omega-6/omega-3 fatty acid ration in cardiovascular disease and other chronic diseases. *Exp Biol Med*. 2008, 233,674-688. doi: 10.3181/0711-MR-311
38. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific Opinion on the Tolerable Upper Intake Level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). EFSA J. 2012, 10, 2815
39. Baker, E.J., Miles, E.A., Burdge, G.C., Yaqoob, P., Calder, P.C. Metabolism and functional effects of plant-derived omega-3 fatty acids in humans. *Prog Lipid Res*. 2016:64:30-56. doi: 10.1016/j.plipres.2016.07.002
40. Burdge, G.C., Calder, P.C. α-Linolenic acid metabolism in adult humans: the effects of gender and age on conversion to longer-chain polyunsaturated fatty acids. *European journal of lipid science and technology*. 2005;107:6:pp.426.439
41. Glaser, C., Lattka, E., Rzehak, P., Steer, C., Koletzko, B. Genetic variation in polyunsaturated fatty acid metabolism and its potential relevance for human development and health. *Matern Child Nutr*. 2011;7 Suppl 2:27-40. doi: 10.1111/j.1740-8709.2011.00319.x
42. Khan, S.U., Lone, A.N., Khan, M.S., Virani, S.S., Blumentahl, R.S., Nasir, K., Miller, M., Michos, E.D., Ballantyne, C.M., Boden, W.E., Bhatt, D.L. Effect of omega-3 fatty acids on cardiovascular outcomes: A systematic review and meta-analysis. *E Clinical Medicine*. 2021:38:100997. doi: 10.1016/j.eclinm.2021.100997
43. Dyall, S.C. Long-chain omega-3 fatty acids and the brain: a review of the independent and shared effects of EPA, DPA and DHA. *Front Aging Neurosci*. 2015;7:52. doi: 10.3389/fnagi.2015.00052
44. Simopoulos, A.P. Omega-3 fatty acids in health and disease and in growth and development. *Am J Clin Nutr*. 1991;54(3):438-63. doi: 10.1093/ajcn/54.3.438
45. Gillingham, L.G., Harris-Janz, S., Jones, P.J.H. Dietary monounsaturated fatty acids are protective against metabolic syndrome and cardiovascular disease risk factors. *Lipids*. 2011;46(3):209-28. doi: 10.1007/s11745-010-3524-y
46. Dighriri, I.M., Alsubaie, A.M., Hakami, F.M., Hamithi, D.M., Alshekh, M.M., Khobrani, F.A., Dalak, F.E., Hakami, A.A., Alsueaadi, E.H., Alsaawi, L.S., Alhsammari, S.F., Alqahtani, A.S., Alawi, I.A., Aljuaid, A.A., Tawhari, M.Q. Effects of omega-3 polyunsaturated fatty acids on brain functions: a systematic review. *Cureus*. 2022;14(10):e30091. doi: 10.7759/cureus.30091
47. Gonçalves, N.B., Bannits, R.F., Silva, B.R., Becari, D.D., Poloni, C., Gomes, P.M., Foss, M.C., Freitas, M.C. α-Linolenic acid prevents hepatic steatosis and improves glucose tolerance in mice fed a high-fat diet. *Clinics*. 2018;73:e150. doi: 10.6061/clinics/2018/e150
48. Yin, S., Xu, H., Xia, J., Lu, Y., Xu, D., Sun, J., Wang, Y., Liao, W., Sun, G. Effect of alpha-linolenic acid supplementation on cardiovascular disease risk profile in individuals with obesity or overweight: a systematic review and meta-analysis of randomized controlled trials. *Adv Nutr*. 2023;14(6):1644-1655. doi: 10.1016/j.advnut.2023.09.010

49. Jaca, A., Durão, S., Harbron, J. Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease. *S Afr Med J.* 2020;110(12):1158-1159. doi: 10.7196/SAMJ.2020.v110i12.14730
50. Kromhout, D., Geleijnse, J.M., de Goede, J., Griep, L.M.O., Mulder, B.J.M., de Boer, M.J., Deckers, J.W., Boersma, E., Zock, P.L., Giltay, E.J. N-3 fatty acids, ventricular arrythmia-related events, and fatal myocardial infarction in postmyocardial infarction patients with diabetes. *Diabetes Care*. 2011;34(12):2515-20. doi: 10.2337/dc11-0896
51. Simopoulos, A.P. Omega-3 fatty acids in inflammation and autoimmune diseases. *J Am Coll Nutr.* 2002;21(6):495-505. doi: 10.1080/07315724.2002.10719248
52. Liput, K.P., Lepczyński, A., Ogłuszka, M., Nawrocka, A., Poławska, E., Grzesiak, A., Ślaska, B., Pareek, C.S., Czarnik, U., Pierzchała, M. Effects of dietary n-3 and n-6 polyunsaturated fatty acids in inflammation and cancerogenesis. *Int J Molt Sci*. 2021;22(13):6965. doi: 10.3390/ijms22136965
53. Pan, A., Chen, M., Chowdhury, R., Wu, J.H.Y., Sun, Q., Campos, H., Mozaffarian, D., Hu, F.B. Alpha-linolenic acid and risk of cardiovascular disease: a systematic review and meta-analysis. *Am J Clin Nutr.* 2012;96(6):1262-73. doi: 10.3945/ajcn.112.044040
54. Bertoni, C., Mazzocchi, A., Leone, L., Agostoni, C., Filocamo, G. Cardiovascular risk and inflammation in a population with autoimmune diseases: a narrative review. *Fron Immunol.* 2024:15:1380372. doi: 10.3389/fimmu.2024.1380372
55. Han, H., Xue, T., Li, J., Guo, Y., Li, X., Wang, L., Pei, L., Zheng, M. Plant sterol ester of α-linolenic acid improved non-alcoholic fatty liver disease by attenuating endoplasmic reticulum stress-triggered apoptosis via activation of the AMPK. *J Nutr Biochem*. 2022:107:109072. doi: 10.1016/j.jnutbio.2022.109072
56. Bertoni, C., Pini, C., Mazzocchi, A., Agostoni, C., Brambilla, P. The role of alpha-linolenic acid and other polyunsaturated fatty acids in mental health: a narrative review. *Int J Mol Sci.* 2024;25(22):12479. doi: 10.3390/ijms252212479
57. Lee, A.Y., Lee, M.H., Lee, S., Cho, E.J. Alpha-linolenic acid regulates amyloid precursor protein processing by mitogen-activated protein kinase pathway and neuronal apoptosis in amyloid beta-induced SH-SY5Y neuronal cells. *Applied Biological Chemistry*. 2018;61;61-71
58. Naghshi, S., Aune, D., Beyenne, J., Mobarak, S., Asadi, M., Sadeghi, O. Dietary intake and biomarkers of alpha linolenic acid and risk of all cause, cardiovascular, and cancer mortality: systematic review and dose-response meta-analysis of cohort studies. *BMJ*. 2021:375:n2213. doi: 10.1136/bmj.n2213
59. Murff, H.J., Shu, X.O., Li, H., Kallianpur, A., Yang, G., Cai, H., Wen, W., Gao, Y.T., Zheng, W. A prospective study of dietary polyunsaturated fatty acids and colonrectal cancer risk in Chinese women. *Cancer Epidemiol Biomarkers Prev.* 2009;18(8):2283.2291. doi: 10.1158/1055-9965.EPI-08-1196
60. Wang, M., Ma, L.J., Yang, Y., Xiao, Z., Wan, J.B. N-3 polyunsaturated fatty acids for the management of alcoholic liver disease: a critical review. *Crit Rev Food Sci Nutr*. 2019;59(sup1):S116-S129. doi: 10.1080/10408398.2018.1544542
61. Mititelu, M., Lupuliasa, D., Neacșu, S.M., Olteanu, G., Busnatu, S.S., Mihai, A., Popovici, V., Maru, N., Boroghina, S.C., Mihai, S., Ioniță-Mîndrican, C.B., Scafa-Udriște, A. Polyunsaturated fatty acids and human health: a key to modern nutritional balance in association with polyphenolic compounds from food sources. *Foods*. 2024;14(1):46. doi: 10.3390/foods14010046
62. Mischoulon, D., Freeman, M.P. Omega-3 fatty acids in psychiatry. *Psychiatr Clin North Am.* 2013;36(1):15-23. doi: 10.1016/k.psc.2012.12.002
63. Hallahan, B., Garland, M.R. Essential fatty acids and mental health. *Br J psychiatry.* 2005:186:275-7. doi:10.1192/bjp.186.4.275

64. Gustafsson, H.C.,Dunn, G.A., Mitchell, A.J., Holton, K.F., Loftis, J.M., Nigg, J.T., Sullivan, E.L. The association between heightened ADHD symptoms and cytokine and fatty acid concentrations during pregnancy. *Front. Psychiatry.* 2022, *13*, 855265
65. Pinar-Marti, A., Gignac, F., Fernandez-Barres, S., Romaguera, D., Sala-Vila, A., Lazaro, I., Ranzani, O.T., Persavento, C., Delgado, A., Carol, A. Effect of walnut consumption on neuropsychological development in healthy adolescents: A multi- school randomised controlled trial. *eClinicalMedicine*. 2023, 59, 101954
66. Pinar-Martí, A., Fernández-Barrés, S., Gignac, F., Persavento, C., Delgado, A., Romaguera, D., Lázaro, I., Ros, E., López-Vicente, M., Salas-Salvadó, J. Red blood cell omega-3 fatty acids and attention scores in healthy adolescents. *Eur. Child. Adolesc. Psychiatry.* 2023, *32*, 2187–2195
67. Zielinska, M.A., Hamulka, J., Grabowicz-Chadrzynska, I., Brys, J. Wesolowska, A. Association between Breastmilk LC PUFA, Carotenoids and Psychomotor Development of Exclusively Breastfed Infants. *Int. J. Environ. Res. Public Health*. 2019, 16, 1144
68. Asch, R.H., Schurdak, J.D., McNamara, R.K. Perinatal dietary omega-3 fatty acid deficiency reduces maternal nurturing behavior in rats: dissociation from elevated pro-inflammatory signaling. *Nutr Neurosci.* 2021;24(9):735-746. doi:10.1080/1028415X,2019.1674507
69. Nomura, M., Tanaka, K., Banno, Y., Hara, R., Asami, M., Otsuka, T., Tomata, Y. Polyunsaturated fatty acids and risk of anorexia nervosa: A Mendelian randomization study. *J. Affect. Disord*. 2023, *330*, 245–248
70. Caspar-Bauguil, S., Montastier, E., Galinon, F., Frisch-Benarous, D., Salvayre, R., Ritz, P. Anorexia nervosa patients display a deficit in membrane long chain polyunsaturated fatty acids. *Clin. Nutr.* 2012, *31*, 386–390
71. Chaves, R.D.C.S., Aguiar, O.B., Moreno, A.B., Brunoni, A.R., Molina, M.D.C.B., Viana, M.C., Bensoñor, I., Griep, R.H., da Fonseca, M.J.M. Consumption of Omega-3 and Maintenance and Incidence of Depressive Episodes: The ELSA-Brasil Study. *Nutrients* 2022, 14, 3227
72. Deacon, G., Kettle, C., Hayes, D., Dennis, C., Tucci, J. Omega 3 polyunsaturated fatty acids and the treatment of depression. *Crit. Rev. Food Sci. Nutr.* 2017, 57, 212–223
73. Evans, S.J., Prossin, A.R., Harrington, G.J., Kamali, M., Ellingrod, V.L., Burant, C.F., McInnis, M.G. Fats and factors: Lipid profiles associate with personality factors and suicidal history in bipolar subjects. *PLoS ONE*. 2012, 7, e29297
74. Fatemi, F., Siassi, F., Qorbani, M., Sotoudeh, G. Higher dietary fat quality is associated with lower anxiety score in women: A cross-sectional study. *Ann. Gen. Psychiatry*. 2020, 19, 14
75. Gracious, B.L., Chirieac, M.C., Costescu, S., Finucane, T.L., Youngstrom, E.A., Hibbeln, J.R. Randomized, placebo-controlled trial of flax oil in pediatric bipolar disorder. *Bipolar Disord*. 2010, 12, 142–154
76. Osuna, E., Herter-Aeberli, I., Probst, S., Emery, S.;,Albermann, M., Baumgartner, N., Strumberger, M., Ricci, C., Schmeck, K., Walitza, S. Omega-3 study team. Associations of n-3 polyunsaturated fatty acid status and intake with paediatric major depressive disorder in Swiss adolescents: A case-control study. *J. Affect. Disord*. 2023, *339*, 355–365
77. Zeng, L., Lv, H., Wang, X., Xue, R., Zhou, C., Liu, X., Yu, H. Causal effects of fatty acids on depression: Mendelian randomization study. *Front. Nutr*. 2022, *9*, 1010476
78. Harauma, A., Yoshihara, H., Hoshi, Y., Hamazaki, K., Moriguchi, T. Effects of varied omega-3 fatty acid supplementation on postpartum mental health and the association between prenatal erythrocyte omega-3 fatty acid levels and postpartum mental health. *Nutrients* 2023, *15*, 4388
79. Cherubini, A, Andres-Lacueva, C., Martin, A., Lauretani, F., Iorio, A.D., Bartali, B.; Corsi, A., Bandinelli, S., Mattson, M.P., Ferrucci, L. Low plasma N-3 fatty acids and dementia

in older persons: The InCHIANTI study. *J. Gerontol. A Biol. Sci. Med. Sci.* 2007, *62*, 1120–1126
80. Crupi, R., Marino, A., Cuzzocrea, S. N-3 fatty acids: Role in neurogenesis and neuroplasticity. *Curr. Med. Chem.* 2013, *20*, 2953–2963
81. Current, W., Godos, J., Alanazi, A.M., Lanza, G., Ferri, R., Caraci, F., Grosso, G., Galvano, F., Castellano, S. Dietary Fats and Cognitive Status in Italian Middle-Old Adults. *Nutrients* 2023, *15*, 1429
82. Dhillon, V.S., Thomas, P., Lee, S.L., Deo, P., Fenech, M. Red blood cell fatty acid profiles are significantly altered in south australian mild cognitive impairment and alzheimer's disease cases compared to matched controls. *Int. J. Mol. Sci.* 2023, mboxemph24, 14164
83. Nishi, S.K., Sala-Vila, A., Julvez, J., Sabaté, J., Ros, E. Impact of nut consumption on cognition across the lifespan. *Nutrients* 2023, *15*, 1000
84. Ogawa, T., Sawane, K., Ookoshi, K., Kawashima, R. Supplementation with flaxseed oil rich in alpha-linolenic acid improves verbal fluency in healthy older adults. *Nutrients* 2023, *15*, 1499.
85. Yamagishi, K., Ikeda, A., Chei, C.L., Noda, H., Umesawa, M., Cui, R., Muraki, I., Ohira, T., Imano, H., Sankai, T. IRCS Investigators. Serum α-linolenic and other ω-3 fatty acids, and risk of disabling dementia: Community-based nested case-control study. *Clin. Nutr.* 2017, *36*, 793–797
86. Li, X., Feng, X., Sun, X., Hou, N., Han, F., Liu, Y. Global, regional, and national burden of Alzheimer's disease and other dementias, 1990–2019. *Front. Aging Neurosci.* 2022, *14*, 937486
87. Amminger, G.P., Mechelli, A., Rice, S., Kim, S.W., Klier, C.M., McNamara, R.K., Berk, M., McGorry, P.D., Schäfer, M.R. Predictors of treatment response in young people at ultra-high risk for psychosis who received long-chain omega-3 fatty acids. *Transl. Psychiatry* 2015, *5*, e495
88. Jones, H.J., Borges, M.C., Carnegie, R., Mongan, D., Rogers, P.J., Lewis, S.J., Thompson, A.D., Zammit, S. Associations between plasma fatty acid concentrations and schizophrenia: A two-sample Mendelian randomisation study. *Lancet Psychiatry* 2021, *8*, 1062–1070
89. Rice, S.M., Schäfer, M.R., Klier, C., Mossaheb, N., Vijayakumar, N., Amminger, G.P. Erythrocyte polyunsaturated fatty acid levels in young people at ultra-high risk for psychotic disorder and healthy adolescent controls. *Psychiatry Res.* 2015, *228*, 174 176
90. Rog, J., Blazewicz, A., Juchnowicz, D., Ludwiczuk, A., Stelmach, E., Koziol, M., Karakula, M., Nizinski, P., Karakula-Juchnowicz, H. The role of GPR120 receptor in essential fatty acid metabolism in schizophrenia. *Biomedicines*. 2020;8(8):243. doi:10.3390/biomedicines8080243
91. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). Opinion on the substantiation of health claims related to alpha linolenic acid and maintenance of normal blood cholesterol concentrations and maintenance of normal blood pressure pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA J. 2009, 7, 1252
92. Greupner, T., Kutzner, L., Pagenkopf, S., Kohrs, H., Hanh, A., Schebb, N.H., Schuchardt, J.P. Effects of a low and a high dietary LA/ALA ration on long-chain PUFA concentrations in red blood cells. *Food Funct.* 2018, 19, 4742–4754

93. Bertoni, C., Abodi, M., D'Oria, V., Milani, G.P., Agostoni, C., Mazzocchi, A. Alpha-linolenic acid and cardiovascular events: a narrative review. *Int J Mol Sci*. 2023;24(18):14319. doi: 10.3390/ijms241814319
94. Li, G., Wang, X., Yang, H., Zhang, P., Wu, F., Li, Y., Zhour, Y., Zhang, X., Ma, H., Zhang, W. Linolenic acid but not linolenic acid protects against hypertension: Critical role of SIRT3 and autophagic flux. *Cell Death Dis*. 2020, 11, 83
95. Skilton, M.R., Pahkala, K., Viikari, J.S., Ronnemaa, T., Simell, O., Jula, A., Niinikoski, H., Celermajer, D.S., Raitakari, O.T. The association of dietary alpha-linolenic acid with blood pressure and subclinical atherosclerosis in people born small for gestational age: The Special Turku Coronary Risk Factor Intervention Project study. *J. Pediatr*. 2015, 166, 1252–1257
96. Rodriguez-Leyva, D., Weighell, W., Edel, A.L., LaVallee, E., Dibrov, E., Pinneker, R., Maddaford, T.G., Ramjiawan, B., Aliani, M., Guzman, R. Potent antihypertensive action of dietary flaxseed in hypertensive patients. *Hypertension*. 2013, 62, 1081–1089
97. Daien, C., Czernichow, S., Letarouilly, J.G., Nguyen, Y., Sanchez, P., Sigaux, J., Beauvais, C., Desouches, S., Le Puillandre, R., Rigalleau, V. Dietary recommendations of the French Society for Rheumatology for patients with chronic inflammatory rheumatic diseases. *Jt. Bone Spine*. 2022, 89, 105319
98. Bernasconi, A.A., Wiest, M.M., Lavie, C.J., Milani, R.V., Laukkanen, J.A. Effect of omega-3 dosage on cardiovascular outcomes: An updated meta-analysis and meta-regression of interventional trials. *Mayo Clin. Proc*. 2021, 96, 304–313
99. Harris, W.S., Bulchandani, D. Why do Omega-3 fatty acids lower serum triglycerides? *Curr. Opin. Lipidol*. 2006, 17, 387–393
100. Kyu-Bong, K., Yoon, A.N., Kim, H.S., Hayes, A.W., Lee, B.M. Alpha-linolenic acid: Nutraceutical, pharmacological and toxicological evaluation. Food Chem. Toxicol. 2014, 70, 163–178
101. Stark, A.H., Crawford, M.A., Reifen, R. Update on alpha-linolenic acid. *Nutr. Rev*. 2008, 66, 326–332
102. Liput, K.P., Lepczynski, A., Ogluszka, M., Nawrocka, A., Polawska, E., Grzesiak, A., Slaska, B., Pareek, C.S., Czarnik, U., Pierzchala, M. Effects of dietary n-3 and n-6 polyunsaturated fatty acids in inflammation and cancerogenesis. *Int. J. Mol. Sci*. 2021, 22, 6965
103. D'Angelo, S., Motti, M.L., Meccariello, R. Omega-3 and Omega-3 polyunsaturated fatty acids, obesity and cancer. *Nutrients*. 2020,12, 2751
104. Azzolino, D., Bertoni, C., De Cosmi, V., Spolidoro, G.C.I., Agostoni, C., Lucchi, T., Mazzocchi, A. Omega-3 polyunsaturated fatty acids and physical performance across the lifespan: a narrative review. *Front Nutr*. 2024:11:1414132. doi: 10.3389/fnut.2024.1414132
105. Zhao, G., Etherton, T.D., Martin, K.R., West, S.G., Gillies, P.J., Kris-Etherton, P.M. Dietary alpha-368 linolenic acid reduces inflammatory and lipid cardiovascular risk factors in 369 hypercholesterolemic men and women. *J Nutr*. 2004);134(11):2991-7
106. Middleton, P., Gomersall, J.C., Gould, J.F., Sphepherd, E., Olsen, S.F., Makrides, M. Omega-3 fatty acid addition during pregnancy. *Cochrane database Syst Rev*. 2018;11(11):CD003402. doi: 10.1002/14651858.CD003402.pub3
107. Olsen, S.F., Østerdal, M.L., Salvig, J.D., Mortensen, L.M., Rytter, D., Secher, N.J., Henriksen, T.B. Fish oil intake compared with olive oil intake in late pregnancy and

asthma in the offspring: 16 y of registry-based follow-up from a randomized controlled trial. *Am J Clin Nutr*. 2008;88(1):167-75. doi: 10.1093/ajcn/88.1.167
108. Makrides, M., Gibson, R.A., PcPhee, A.J., Yelland, L., Quinlivan, J., Ryan, P. Effect of DHA supplementation during pregnancy on maternal depression and neurodevelopment of young children: a randomized controlled trial. *JAMA*. 2010;304(15):1675-83. doi: 10.1001/jama.2010.1507
109. Markhus, M.W., Skotheim, S., Graff, I.E., Frøyland, L., Braarud, H.C., Stormark, K.M., Malde, M.K. Low omega-3 index in pregnancy is a possible biological risk factor for postpartum depression. *PLoS One*. 2013; 8(7):e67617. Doi 10.1371/journal.pone.0067617
110. Gould, J.F., Smithers, L.G., Makrides, M. The effect of maternal omega-3 (n-3) LC-PUFA supplementation during pregnancy on early childhood cognitive and visual development: a systematic review and meta-analysis of randomized controlled trials. *Am J Clin Nutr*. 2013;97(3):531-44. doi: 10.3945/ajcn.112.045781
111. Shulkin, M., Pimpin, L., Bellinger, D., Kranz, S., Fawzi, W., Duggan, C., Mozaffarian, D. N-3 fatty acid supplementation in mothers, preterm infants, and term infants and childhood psychomotor and visual development: a systematic review and meta-analysis. *J Nutr*. 2018;148(3):409-418. doi 10.1093/jn/nxx031
112. Koletzko, B., Lien, E., Agostoni, C., Campoy, C., Cetin, I. The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations. *J Perinat Med*. 2008;36(1):5-14. doi: 10.1515/JPM.2008.001
113. Chen, M., Zheng, Z., Shi, J., Shao, J. Insight on polyunsaturated fatty acids in endometrial receptivity. *Biomolecules*. 2021;12(1):36. doi: 10.3390/biom12010036
114. De Faveri, C., Fermino, P.M.P., Piovezan, A.P., Volpato, L.K. The inflammatory role of pro-resolving mediators in endometriosis: an integrative review. *Int J Mol Sci*. 2021;22(9):4370. doi: 10.3390/ijms22094370
115. De Cosmi, V., Cipriani, S., Syren, M.L., Turolo, S., Parazzini, F., Reschini, M., Abodi, M., Vegetti, W., Chiaffarino, F., Bertoni, C., Somigliana, E., Agostoni, C. Peripheral fatty acids and outcome of assisted reproduction. *Nutrition*. 2025:130:112616. doi: 10.1016/j.nut.2024.112616
116. Hammiche, F., Vujkovic, M., Wijburg, W., de Vries, J.H.M., Macklon, N.S., Laven, J.S.E., Steegers-Theunissen, P.M. Increased preconception omega-3 polyunsaturated fatty acid intake improves embryo morphology. *Fertil Steril*. 2011;95(5):1820-3. doi: 10.1016/j.fertnstert.2010.11.021
117. Vujkovic, M., de Vries, J.H., Lindermans, J., Macklon, N.S., van der Spek, P.J., Steegers, E.A.P., Steegers-Theunissen, P.M. The preconception Mediterranean dietary pattern in couples undergoing in vitro fertilization/intracytoplasmic sperm injection treatment increases the chance of pregnancy. *Fertil Steril*. 2010;94(6):2096-101. doi: 10.1016/j.fertnstert.2009.12.079
118. Polak, G., Koziol-Montewka, M., Gogacz, M., Blaszkowska, I., Kotarski, J. Total antioxidant status of peritoneal fluid in infertile women. *Eur J Obstet Gynecol Reprod Biol*. 2001;94(2):261-3. doi: 10.1016/s0301-2115(00)00352-3
119. Stanhiser, J., Jukic, A.M.Z., McConnaughey, D.R., Steiner, A.Z. Omega-3 fatty acid supplementation and fecundability. *Hum Reprod*. 2022;27(5):1037-1046. doi: 10.1093/humrep/deac027

120. Horne, A.W., Missmer, S.A. Pathophysiology, diagnosis, and management of endometriosis. *BMJ*. 2022;379:e070750. doi: 10.1136/bmj-2022-070750
121. Viganò, P., Casalechi, M., Dolmans, M.M. European Union underinvestment in endometriosis research. *J. Endometr Uter Dis*. 2024; 5: 100058. doi.org/10.1016/j.jeud.2023.100058
122. Al-Lami, R.A., Taha, S.H., Jalloul, R.J. Women with endometriosis in the United States: National Survey of Family Growth, 2011–2019. *J. Endometr Uter Dis*. 2024;8: 100081. doi.org/10.1016/j.jeud.2024.100081
123. As-Sanie, S., Black, R., Giudice, L.C. Assessing research gaps and unmet needs in endometriosis. *Am J Obstet Gynecol*. 2019; 221:86-94. doi: 10.1016/j.ajog.2019.02.033
124. Somigliana, E., Li Piani, L., Paffoni, A. Endometriosis and IVF treatment outcomes: unpacking the process. *Reprod Biol Endocrinol*. 2023;21:107. doi: 10.1186/s12958-023-01157-8
125. Casalechi, M., Di Stefano, G., Fornelli, G. Impact of endometriosis on the ovarian follicles. *Best Pract Res Clin Obstet Gynaecol*. 2024;92:102430. doi: 10.1016/j.bpobgyn.2023.102430
126. Ruotolo, A., Vannuccini, S., Capezzuoli, T. Diet characteristics in patients with endometriosis. *J Endomet Uter Dis*. 2025; 9; 100094. doi.org/10.1016/j.jeud.2024.100094
127. Türkoğlu, I., Sacinti, K.G., Panattoni, A. Eating for Optimization: Unraveling the Dietary Patterns and Nutritional Strategies in Endometriosis Management. *Nutr Rev*. 2025;83:869-79. doi: 10.1093/nutrit/nuae120
128. Dougan, M.M., Fest, S., Cushing-Haugen, K. A prospective study of dietary patterns and the incidence of endometriosis diagnosis. *Am J Obstet Gynecol*. 2024;231:443.e1-443.e10. doi: 10.1016/j.ajog.2024.04.030
129. Alviggi, C., Andersen, C.Y., Buehler, K. A new more detailed stratification of low responders to ovarian stimulation: from a poor ovarian response to a low prognosis concept. *Fertil Steril*. 2016; 105:1452–3. doi: 10.1016/j.fertnstert.2016.02.005
130. Pounis, G., Bonaccio, M., Di Castelnuovo, A. Polyphenol intake is associated with low-grade inflammation, using a novel data analysis from the Moli-sani study. *Thromb Haemost*. 2016;115:344-52. doi: 10.1160/TH15-06-0487
131. Shi, H., Schweren, L.J.S., Ter Horst, R. Low-grade inflammation as mediator between diet and behavioral disinhibition: A UK Biobank study. *Brain Behav Immun*. 2022;106:100-10. doi: 10.1016/j.bbi.2022.07.165
132. Jones, G., Kennedy, S., Barnard, A. Development of an endometriosis quality-of-life instrument: The Endometriosis Health Profile-30. *Obstet Gynecol*. 2001;98:258-64. doi: 10.1016/s0029-7844(01)01433-8
133. Breivik, H., Borchgrevink, P.C., Allen, S.M. Assessment of pain. *Br J Anaesth*. 2008;101:17–24. doi: 10.1093/bja/aen103
134. Rosen, C., Brown, J., Heiman, S. The Female Sexual Function Index (FSFI): A multidimensional self-Report instrument for the assessment of female sexual function. *J Sex Marital Ther*. 2000; 26: 191–208. doi: 10.1080/009262300278597
135. Galati, G., Somigliana, E., Ciaffaglione, M. Follicular steroidogenesis in random start protocols for oocyte cryopreservation. *J Assist Reprod Genet*. 2023;40(9):2149-2156. doi: 10.1007/s10815-023-02883-z

136. Invernici, D., Reschini, M., Benaglia, L. The impact of endometriosis on IVF efficacy: qualitative and quantitative assessment of ovarian response and embryo development. *Reprod Biomed Online*. 2022;45:275-281. doi: 10.1016/j.rbmo.2022.04.010
137. Allaire, L.C., As-Sanie, S., Stratton, P. World Endometriosis Research Foundation Endometriosis Phenome and Biobanking Harmonization Project: V. Physical examination standards in endometriosis research. *Fertil Steril*. 2024;122:304-15. doi: 10.1016/j.fertnstert.2024.03.007
138. Decarli, A., Franceschi, S., Ferraroni, M. Validation of a food-frequency questionnaire to assess dietary intakes in cancer studies in Italy. Results for specific nutrients. *Ann Epidemiol*. 1996;6:110-8. doi: 10.1016/1047-2797(95)00129-8
139. Visser, E., de Jong, K., van Zutphen, T. Dietary Inflammatory Index and Clinical Outcome Measures in Adults With Moderate-to-Severe Asthma. *J Allergy Clin Immunol Prac*. 2023;11:3680-9.e7. doi: 10.1016/j.jaip.2023.08.032
140. Shivappa, N., Steck, S.E., Hurley, T.G. A population-based dietary inflammatory index predicts levels of C-reactive protein in the Seasonal Variation of Blood Cholesterol Study (SEASONS). *Public Health Nutr*. 2014;17:1825-33. doi: 10.1017/S1368980013002565
141. Kyle, U.G., Bosaeus, I., De Lorenzo, A.D. Composition of the ESPEN Working Group. Bioelectrical impedance analysis--part I: review of principles and methods. C*lin Nutr*. 2004; 23:1226-43. doi: 10.1016/j.clnu.2004.06.004; 45
142. Hoffman, T.C., Glasziou, P.P., Boutron, I. Better reporting of interventions: template for intervention description and replication (Tidier) checklist and guide. *BMJ*. 2014;348:g1687. doi: 10.1136/bmj.g1687
143. Duffy, J.M.N., Bhattacharya, S., Bhattacharya, S. Standardizing definitions and reporting guidelines for the infertility core outcome set: an international consensus development study. *Fertil Steril*. 2021; 115: 201–212. doi.org/10.1016/j. fertnstert.2020.11.013
144. Peng, X., Li, X., He, J. Ultra-Processed Food Consumption and the Risk of Psoriasis: A Large Prospective Cohort Study. *Nutrients*. 2025;17:1473. doi: 10.3390/nu17091473
145. Wilmes, L., Caputi, V., Bastiaanssen, T.F.S. Sex specific gut-microbiota signatures of resilient and comorbid gut-brain phenotypes induced by early life stress. *Neurobiol Stress*. 2024;33:100686. doi: 10.1016/j.ynstr.2024.100686
146. Galli, C., Calder, P.C. Effects of fat and fatty acid intake on inflammatory and immune responses: a critical review. *Ann Nutr Metab*. 2009;55(1-3):123-39. doi: 10.1159/000228999
147. Allaire, J., Couture, P., Leclerc, M., Charest, A., Marin, J., Lepine, M.C., Talbot, D., Tchernof, A., Lamarche, B. A randomized, crossover, head-to-head comparison of eicosapentaenoic acid and docosahexaenoic acid supplementation to reduce inflammation markers in men and women: the comparing EPA to DHA (ComparED) Study. *Am J Clin Nutr*. 2016;104(2):280-7. doi: 10.3945/ajcn.116.131896
148. Abodi, M., Mazzocchi, A., Risè, P., Marangoni, F., Agostoni, C., Milani, G.P. Salivary fatty acids in humans: a comprehensive literature review. Clin Chem Lab Med. 2024;63(1):14-26. doi: 10.1515/cclm-2024-0177)

149. Wu, Y., Xing, L., Lu, L., Liu, S., Zhao, D., Lin, L., Wang, S., Li, C., Pan, Y. Alterations in the salivary microbiome and metabolism in patients with carotid atherosclerosis from rural Northeast China. J aM HEART assoc. 2024;13(15):e034014. doi: 10.1161/JAHA.123.034014
150. Huang, Y., Zhu, M., Li, Z., Sa, R., Chu, Q., Zhang, Q., Zhang H., Tang, W., Zhang, M., Yin, H. Mass spectrometry-based metabolomic profiling identifies alterations in salivary redox status and fatty acid metabolism in response to inflammation and oxidative stress in periodontal disease. Free Radic Biol Med. 2014:70:223-32. doi:10.1016/j.freeradbiomed.2014.02.024
151. Ghasemifard, S., Turchini, G.M., Sinclair, A.J. Omega-3 long chain fatty acid "bioavailability": a review of evidence and methodological considerations. *Prog Lipid Res*. 2014:56:92:108. doi 10.1016/j.plipres.2014.09.001
152. Nguemeni, C., Delplanque, D., Rovere, C., Simon-Rousseau, S., Ganind, C., Agnani, G., Nahon, J.L., Heurteaux, C., Blondeau, N. Dietary supplementation of alpha-linolenic acid in an enriched rapeseed oil diet protects from stroke. *Pharmacol Res*. 2010;61(3):226-33. doi: 10.1016/j.phrs.2009.12.007
153. Van Dael, P. Role of n-3 long-chain polyunsaturated fatty acids in human nutrition and health: review of recent studies and recommendations. *Nutr Res Pract*. 2021;15(2):137-159. doi: 10.4162/nrp.2021.15.2.137
154. Chang, J.P.C., Tseng, P.T., Zeng, B.S., Chang, C.H., Su, H., Chou, P.H., Su, K.P. Safety of supplementation of omega-3 polyunsaturated fatty acids: a systematic review and meta-analysis of randomized controlled trials. *Adv Nutr*. 2023;14(6):1326-1336. doi: 10.1016/j.advnut.2023.08.003
155. Jovanovski, E., Li, D., Ho, H.V.T., Djedovic, V., de Castro Ruiz Marques, A., Shishtar, E., Meja, S.B., Sievenpiper, J.L., de Souza, R.J., Duvnjak, L., Vuksan, V. The effect of alpha-linolenic acid on glycemic control in individuals with type 2 diabetes: a systematic review and meta-analysis of randomized controlled clinical trials. *Medicine(Baltimore)*. 2017;96(21):e6531. doi: 10.1097/MD.0000000000006531
156. Marrone, M.C., Coccurello, R. Dietary fatty acids and microbiota-brain communication in neuropsychiatric diseases. *Biomolecules*. 2019;10(1):12. doi: 10.3390/biom10010012
157. Vitetta, L., Gorgani, N.N.; Vitetta, G., Henson, J.D. Prebiotics progress shifts in the intestinal microbiome that benefits patients with type 2 diabetes mellitus. *Biomolecules*. 2023;13(9):1307. doi: 10.3390/biom13091307
158. Kobyliak, N., Abenavoli, L., Falalyeyeva, T., Mykhalchyshyn, G., Boccuto, L., Kononenko, L., Jyriienko, D., Komisarenko, I., Dynnyk, O. beneficial effects of probiotic combination with omega-3 fatty acids in NAFLD: a randomized clinical study. *Minerva Med*. 2018;109(6):418-428, doi: 10.23736/S0026-4806.18.05845-7
159. Mirzaei, R., Dehkhodaie, E., Bouzari, B., Rahimi, M., Gholestani, A., Hosseini-Fard, S.R., Keyvani, H., Teimoori, A., Karampoor, S. Dual role of microbiota derived short-chain fatty acids on host and pathogen. *Biomed Pharmacother*. 2022:145:113352. doi: 10.1016/j.biopha.2021.112352
160. Fu, Y., Wang, Y., Gao, H., Li, D., jiang, R., Ge, L., Tong, C., Xu, K. Associations among dietary omega-3 polyunsaturated fatty acids, the gut microbiota, and intestinal immunity. *Mediators Inflamm*. 2021;2021:8879227. doi 10.1155/2021/8879227

161. Ras, T.R., Geleijnse, J.M., Trautwein, E.A. LDL-cholesterol lowering effect of plant sterols and stanols across different dose ranges: a meta-analysis of randomized controlled studies. *Br J Nutr*. 2014;112(2):214-9. doi: 10.1017/S0007114514000750
162. Demonty, I., Ras, R.T., van der Knaap, H.C.M., Duchateau, G.S.M.J.E., Meijer, L., Zock, P.L., Geleijnse, J.M., Trautweing, E.A. Continuous dose-response relationship of the LDL-cholesterol lowering effect of phytosterol intake. *J Nutr*. 2009;139(2):271-84. doi: 10.3945/jn.108.095125
163. Whitehead, A., Beck, E.J.; Tosh, S., Wolever, T.M.S. Cholesterol-lowering effects of oat β-glucan: a meta-analysis of randomized controlled trials. *Am J Xlin Nutr*. 2014;100(6):1413-21. doi: 10.3945/ajcn.114.086108
164. EFSA panel on dietetic products, nutrition and allergies. Scientific opinion on the substatation of health claims related to beta-glucans from oats and barley and maintenance of normal blood cholesterol concentrations. EFSA Journal 2011;9(6):2207
165. Wang, J.F., Zhang, H.M., Li, Y., Xia, S., Wei, Y., Yang, L., Wang, D., Ye, J.J., Li, H., Yuan, J., Pan, R. A combination of omega-3 and plant sterols regulate glucose and lipid metabolism in individuals with impaired glucose regulation: a randomized and controlled trial. *Lipids Health Dis*. 2019;18:106. doi: : 10.1186/s12944-019-1048-x
166. Blom, W.A.M., Koppenol, W.P., Hiemstra, H., Stojakovic, T., Scharnagl, H., Trautwein, E.A. A low-fat spread with added plant sterols and fish omega-3 fatty acids lowers serum triglyceride and LDL-cholesterol concentrations in individuals with modest hypercholesterolaemia and hypertriglyceridaemia. *Eur J Nutr*. 2019;58(4):1615-1624. doi: 10.1007/s00394-018-1706-1
167. Li, Y., Jiang, L., Jia, Z., Xin, W., Yang, S., Yang, Q., Wang, L. A meta-analysis of red yeast rice: an effective and relatively safe alternative approach for dyslipidemia. *PLoS One*. 2014;9(6):e98611. doi: 10.1371/journal.pone.0098611
168. Gerards, M., Terlou, R.J., Yu, H., Koks, C.H.W., Gerdes, V.E.A. Traditional Chinese lipid-lowering agent red yeast rice results in significant LDL reduction but safety is uncertain - a systematic review and meta-analysis. *Atherosclerosis*. 2015;240(2):415-23. doi: 10.1016/j.atherosclerosis.2015.04.004
169. Raederstorff, D., Wyss, A., Calder, P.C., Weber, P., Eggersdorfer, M. Vitamin E function and requirements in relation to PUFA. *Br J Nutr*. 2015;114(8):1113-1122. doi: 10.1017/S000711451500272X
170. Valk, E.E., Hornstra, G. Relationship between vitamin E requirement and polyunsaturated fatty acid intake in man: a review. *Int J Vitam Nutr Res*. 2000;70(2):31.42. doi: 10.1024/0300-9831.70.2.31
171. Mousa, A., Naderpoor, N., de Courten, M.P., Teede, H., Kellow, N., Walker, K., Scragg, R., de Courten, B. Vitamin D supplementation has no effect on insulin sensitivity or secretion in vitamin D-deficient, overweight or obese adults: a randomized placebo-controlled trial. *Am J Clin Nutr*. 2017;105(6):1372-1381. doi: 10.3945/ajcn.117.152736
172. Huang, T., Li, K., Asimi, S., Chen, Q., Li, D. Effect of vitamin B-12 and n-3 polyunsaturated fatty acids on plasma homocysteine, ferritin, C-reaction protein, and other cardiovascular risk factors: a randomized controlled trial. *Asia Pac J Clin Nutr*. 2015;24(3):403-11. doi: 10.6133/apjcn.2015.24.3.19
173. Lee, J.G., Jang, J.Y., Baik, S.M. Selenium as an antioxidant: roles and clinical applications in critically ill and trauma patients: a narrative review. *Antioxidants* (Basel). 2025;14(3):294. doi: 10.3390/antiox14030294

174. Takic, M., Zekovi, M., Terzic, B., Stojsavljevic, A., Mijuskovic, M., Radjen, S., Ristic-Medic, D. Zinc deficience, plasma fatty acid profile and desaturase activities in hemodialysis patients: is supplementation necessary? *Front Nutr*. 2021;8:700450. doi: 10.3389/fnut.2021.700450
175. Siscovick, D.S., Barringer, T.A., Fretts, A.M., Wu, J.H.Y., Lichtenstein, A.H., Costello, R.B., Kris-Etherton, P.M., Jacobson, T.A., Engler, M.B., Alger, H.M., Appeal, L.J., Mozaffarian, D. Omega-3 polyunsaturated fatty acid (fish oil) supplementation and the prevention of clinical cardiovascular disease: a science advisory from the American heart association. *Circulation*. 2017;135(15):e867-e884. doi: 10.1161/CIR.0000000000000482
176. Burri, L., Thoresan, G.H., Berge, R.K. The role of PPARα activation in liver and muscle. *PPAR Res*. 2010;2010:542359. doi: 10.1155/2010/542359
177. Winiarska-Mieczan, A., Kwiecien, M., Rogowska, K.J., Donaldson, J., Tomaszewska, E., Baranowska-Wojcik, E.B.. Anti-inflammatory, antioxidant, and neuroprotective effects on polyphenols – Polyphenols as an element of diet therapy in depressive disorders. *Int J Mol Sci*. 2023;24(3):2258. doi: 10.3390/ijms24032258
178. Qawasmi, A., Bloch, M.H. Meta-analysis of LCPUFA supplementation of infant formula and visual activity. *Pediatrics*. 2013;131(1):e262-e272. doi10.1542/peds.2012-0517
179. Huffman, S.L., Harika, R.K., Eilander, A., Osendarp, S.J.M. Essential fats: how do they affect growth and development of infants and young children in developing countries? A literature review. *Matern Child Nutr*. 2011;7(Supple 3):44-65. doi: 10.1111/j.1740-8709.2011.00356.x
180. Hu, R., Xu, J., Hau, Y., Li, Y., Li, J. Could early life DHA supplementation benefit neurodevelopment? A systematic review and meta-analysis. *Front Neurol*. 2024;15:1295788. doi: 10.3389/fneur.2024.1295788
181. Middleton P, Gomersall JC, Gould JF, Shepherd E, Olsen SF, Makrides M. Omega-3 fatty acid addition during pregnancy. Cochrane Database Syst Rev. 2018;11(11):CD003402. doi: 10.1002/14651858.CD003402.pub3
182. Bärebring, L., Nwaru, B.I., Lamberg-Allardt, C., Thorisdottir, B., Ramel, A., Söderlund, F., Arnesen, E.K., Dierkes, J., Akesson, A. Supplementation with long chain n-3 fatty acids during pregnancy, lactation, or infancy in relation to risk of asthma and atopic disease during childhood: a systematic review and meta-analysis of randomized controlled clinical trials. *Food Nutr Res*. 2022;66:10:29219. doi: 10.29219/fnr.v66.8842
183. Lapillone, A., Pastor, N., Zhuang, W., Scalabrin, D.M.F. Infants fed formula with added long chain polyunsaturated fatty acids have reduced incidence of respiratory illnesses and diarrhea during the first year of life. *BMC Pediatr*. 2014:14:168. doi10.1186/1471-2431-14-168
184. Chang, J.P.C., Su, K.P., Mondelli, V., Pariante, C.M.. Omega-3 polyunsaturated fatty acids in youths with attention deficit hyperactiviry disorder: a systematic review and meta-analysis of clinical trials and biological studies. *Neuropsychoparmacology*. 2018;43(3):534-545. doi: 10.1038/npp.2017.160
185. Liu, T.H., Wu, J.Y., Huang, P.Y., Lai, C.C., Chang, J.P.C., Lin, C.H., Su, K.P. Omega-3 polyunsaturated fatty acids for core symptoms of attention-deficit/hyperactivity disorder: a meta-analysis of randomized controlled trials. *J Clin Psychiatry*. 2023;84(5):22r14772. doi: 10.4088/JCP.22r14772

186. Muley, P., Shah, M., Muley, A. Omega-3 fatty acids supplementation in children to prevent asthma: is it worthy? A systematic review and meta-analysis. *J Allergy* (Cairo). 2015;2015:312025. doi: 10.1155/2015/312052
187. Qawasmi, A., Landeros-Weiswnberg, A., Bloch, M.H. Meta-analysis of LCPUFA supplementation of infant formula and visual acuity. *Pediatrics*. 2013;131(1):e262-e272. doi: 10.1542/peds.2012-0517
188. Chiu, Y.H., Karmon, A.E., Gaskins, A.J., Arvizu, M., Williams, P.L., Souter, I., Rueda, B.R., Hauser, R., Chavarro, J.E. Serum omega-3 fatty acids and treatment outcomes among women undergoing assisted reproduction. *Hum Reprod*. 2018;33(1):156-165. doi: 10.1093/humrep/dex335
189. Abodi M, De Cosmi V, Parazzini F, Agostoni C. Omega-3 fatty acids dietary intake for oocyte quality in women undergoing assisted reproductive techniques: A systematic review. Eur J Obstet Gynecol Reprod Biol. 2022:275-97-105. doi: 10.1016/j.ejogrb.2022.06.019
190. Makrides, M., Best, K., Yelland, L., PcPhee, A., Zhou, S., Quilivan, J., Dodd, J., Atkinson, E., Safa, H., van Dam, J., Khot, N., Dekker, G., Skubisz, M., Anderson, A., Kean, B., Bowman, A., McCallum, C., Cashman, K., Gibson, R. A randomized trial of prenatal n-3 fatty acid supplementation and preterm delivery. *N Engl J Med*. 2019;381(11):1035-1045. doi: 10.1056/NEJMoa1816832
191. Oken, E., Radesky, J.S., Wright, R.O., Bellinger, D.C., Amarasiriwardena, C.J., Kleinman, K.P., Hu, H., Gillman, M.W. Maternal fish intake during pregnancy, blood mercury levels, and child cognition at age 3 years in a US cohort. *Am J Epidemiol*. 2008;167(10):1171-81. doi: doi: 10.1093/aje/kwn034
192. Mozurkewich, E., Chilimigras, J., Klemens, C., Keeton, K., Allbaugh, L., Hamilton, S., Berman, D., Vazquez, D., Marcus, S., Djuric, Z., Vahratian, A. The mothers, omega-3 and mental health study. *BMC Pregnancy Childbirth*. 2011;11:46. doi: 10.1186/1471-2393-11-46
193. Missmer, S.A., Chavarro, J.E., Malspeis, S., Bertone-Johnson, E.R., Hornstein, M.D., Spiegelman, D., Barbieri, R.L., Willett, W.C., Hankinson, S.E. A prospective study of dietary fat consumption and endometriosis risk. *Hum Reprod*. 2010;25(6):1528-35. doi: 10.1093/humrep/deq044
194. Calder, P.C. N-3 fatty acids, inflammation and immunity: new mechanisms to explain old actions. *Proc Nutr Soc*. 013;72(3):326-36. doi: 10.1017/S0029665113001031
195. Goldberg, R.J., Katz, J. A meta-analysis of the analgesic effects of omega-3 polyunsaturated fatty acid supplementation for inflammatory joint pain. *Pain*. 2007;129(1-2):210-23. doi:) 10.1016/j.pain.2007.01.020
196. Lee, Y.H., Bae, S.C., Song, G.G. Omega-3 polyunsaturated fatty acids and the treatment of rheumatoid arthritis: a meta analysis. *Arc Med Res*. 2012;43(5):356-62. doi: 10.1016/j.arcmed.2012.06.011
197. Arriens, C., Hynan, L.S., Lerman, R.H., Karp, D.R., Mohan, C. Placebo-controlled randomized clinical trial of fish oil's impact on fatigue, quality of life, and disease activity in systemic lupus erythematosus. *Nutr J*. 2015:14:82. doi: 10.1186/s12937-015-0068-2
198. Jia, X., Hu, C., Wu, X., Qi, H., Lin, L., Xu, M., Xu, Y. Evaluating the effects of omega-3 polyunsaturated fatty acids on inflammatory bowel disease via circulating

metabolites: a mediation mendelian randomization study. *Metabolites*. 2023;13(10):1042. doi: 10.3390/metabo13101041
199. Torkildsen, O., Wergeland, S., Bakke, S., Beiske, A.G., Bjerve, K.S., Hovdal, H., Midgard, R., Lilleas, F., Pedersen, T. ω-3 fatty acid treatment in multiple sclerosis (OFAMS Study): a randomized, double-blind, placebo-controlled trial. *Arch Neurol*. 2012;69(8):1044-51. doi 10.1001/archneurol.2012.283
200. Filocamo, G., Consolaro, A., Schiappapietra, B., Dalprù, S., Lattanzi, B., Magni-Manzoni, S., Ruperto, N., Pistorio, A., Pederzoli, S., Civino, A., Guseinova, D., Masala, E., Viola, S., Martini, A., Ravelli, A. A new approach to clinical care of juvenile idiopathic arthritis: the Juvenile Arthritis Multidimensional Assessment Report. *J Rheumatol*. 2011;38(5):938-53. doi: 10.3899/jrheum.100930
201. Li, Y., Yu, Z., Liu, Y., Wang, T., Liu, Y., Bai, Z., Ren, Y., Ma, H., Bao, T., Lu, H., Wang, R., Yang, L., Yan, N., Yan, R., Jia, S., Zhang, X., Wang, H. Dietary α-linolenic acid-rich flaxseed oil ameliorates high-fat diet-induced atherosclerosis via gut microbiota-inflammation-artery axis in apoe−/− mice. *Front Cardiovasc Med*. 2022;9:830781. doi: 10.3389/fcvm.2022.830781
202. Gao, X., Chang, S., Liu, S., Peng, L., Xie, J., Dong, W., Tian, Y., Sheng, J. Correlations between α-linolenic acid-improved multitissue homeostasis and gut microbiota in mice fed a high-fat diet. *mSystems*. 2020;5(6):e00391-20. doi: 10.1128/mSystems.00391-20
203. Zhou, Q., Zhang, Z., Wang, P., Zhang, B., Chen, C., Zhang, C., Su, Y. EPA+DHA, but not ALA, improved lipids and inflammation status in hypercholesterolemic adults: a randomized, double-blind, placebo-controlled trial. *Mol Nutr Food Res*. 2019;63(10):e1801157. doi: 10.1002/mnfr.201801157
204. Yang, J., Liu, S., Zhao, Q., Li, X., Jiang, K. Gut-microbtioa-related metabolite alpha-linolenic acid mitigates intestinal inflammation induced by oral infection with Toxoplasma gondii. *Microbiome*. 2023;11(1):273. doi: 10.1186/s40168-023-01681-0

APPENDIX

Abbreviations and acronymous

1. FAs: Fatty acids
2. SFAs: Saturated fatty acids
3. MUFAs: Monounsatured fatty acids
4. PUFAs: Polyunsaturated fatty acids
5. ALA: Alpha-linolenic acid
6. LA: Linoleic acid
7. EFAs: Essential fatty acids
8. DHA: Docosahexaenoic acid
9. ART: Assisted reproductive technologies
10. EPA: Eicosapentaenoic acid
11. AA: Arachidonic acid
12. OA: Oleic acid
13. COX: Cyclooxygenases
14. LOX: Lipoxygenases
15. SPMS: Specialized pro-resolving mediators
16. RXRs: retinoid X receptors
17. TAGs: triacylglycerols
18. EFSA: European food safety authority
19. TC: Total cholesterol
20. PPARs: Peroxisome proliferator-actovated receptors
21. SREBP-1c: Sterol reegulatory element-binding protein-1c
22. RCTs: Randomized controlled trials
23. CVDs: Cardiovascular diseases
24. CHD: Coronary heart diseases
25. AMPK: AMP-activated protein kinase
26. NAFLD: Non-alcoholic fatty liver disease
27. BD: Bipolar disorder
28. AN: Anorexia nervosa
29. SCZ: Schizophrenia
30. Tg: Triglycerides
31. SBP: Systolic blood pressure
32. DBP: Diastolic blood pressure
33. NO: Nitric oxide
34. MI: Myocardial infarction
35. IHD: Ischemic heart disease
36. ROS: Reactive oxygen species
37. IGF-1: Insulin-like growth factor 1
38. GPCRs: G-protein coupled receptors

39. CRP: C-reactive protein
40. MAR: Medically assisted reproduction
41. DII: Dietary inflammatory index
42. DANTE: Dietary intervention in ameliorating fertility parameters in subjects with endometriosis
43. PROMs: Patient-reported outcomes
44. MII: Mature oocyte
45. EHP: Endometriosis health profile
46. NRS: Numeric rating scale
47. FSFI: Female sexual function index
48. REDCap: research electronic data capture
49. WES: World endometriosis societ
50. WERF: World endometriosis research foundation
51. GI: Gastrointestinal
52. FFQ: food frequency questionnaire
53. ESPEN: European society of parental and enteral nutrition
54. TiDier: Template for intervention description and replication
55. PP: Per-protocol
56. ITT: Intention-to-treat
57. RRs: Relative risks
58. Cis: Confidence intervals
59. DBS: Dried blood spot
60. DHEA: Dehydroepiandrosterone
61. DHEA-S: DHEA sulfate
62. SCFAs: Short-chain fatty acids
63. FAMEs: Fatty acid methyl esters
64. GC: gas chromatography
65. DPA: Docosapentaenoic acid
66. GPx: Gluthathione peroxidase
67. RA: Rheumatoid arthritis
68. SLE: Systemic lupus erythematosus