ISSN: 2753-7919
British Journal of Healthcare and Medical Research; Vol. 13 No. 05 (2026) (29-41)
https://doi.org/10.14738/bjhr.1305.12176

Omega-3 Fatty Acid Status Biomarkers: Analytical Validity, Clinical Utility, and Public Health Applications: A Narrative Review

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Abstract: Omega-3 polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid and docosahexaenoic acid, are essential to cardiovascular, neurological, metabolic, and immune function. Despite substantial evidence supporting their biological importance, intake remains inadequate in many populations worldwide. Because dietary assessment methods are limited by recall bias, reporting error, and variation in food composition, objective biomarkers are increasingly used to assess physiological omega-3 status. This narrative review examines the scientific basis, analytical validity, clinical relevance, and public health applications of the principal omega-3 biomarkers, with emphasis on the Omega-3 Index, the omega-6:omega-3 ratio, and dried blood spot testing. The Omega-3 Index, based on red blood cell EPA and DHA content, emerges as the most robust marker of long-term omega-3 status and the one most consistently associated with clinically relevant outcomes, particularly in cardiovascular health. The omega-6:omega-3 ratio offers complementary insight into dietary balance and inflammatory context, although its interpretive limitations reduce its value as a stand-alone marker. Dried blood spot testing has broadened access to fatty acid assessment by enabling minimally invasive, scalable sampling suitable for both clinical and population-level use. Together, these biomarkers support a shift toward more objective, individualised, and prevention-orientated approaches to nutrition assessment. Their wider integration into healthcare and public health practice may improve risk stratification, guide intervention, and strengthen population monitoring of omega-3 sufficiency.

Keywords: Omega-3 Index, EPA, DHA, biomarkers, personalised nutrition, omega-6: omega-3 ratio, dried blood spot testing, public health, precision nutrition.

INTRODUCTION

Omega-3 polyunsaturated fatty acids (PUFAs) are essential nutrients with broad physiological functions. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are incorporated into cell membranes throughout the body, where they influence membrane fluidity, receptor function, intracellular signalling, and lipid mediator synthesis (1, 2). DHA is especially enriched in neural and retinal tissues, where it contributes to synaptic activity, neuroprotection, and visual function, whereas EPA plays a prominent role in inflammatory modulation through competition with arachidonic acid and altered eicosanoid production (3, 4).

Despite their importance, omega-3 intake remains inadequate in many populations. Low intake of oily fish and other marine foods, together with high consumption of omega-6-rich vegetable oils, has shifted fatty acid balance in ways that may contribute to chronic low-grade inflammation and cardiometabolic risk (5, 6). These patterns have increased interest in reliable approaches to assessing omega-3 status beyond dietary self-report alone.

Traditional dietary assessment tools, including food frequency questionnaires and dietary recalls, remain useful in nutritional epidemiology, but they are inherently limited by recall bias, social desirability bias, and imprecision in estimating nutrient exposure (7, 8).

Biomarkers offer a more direct indication of physiological exposure and tissue incorporation. Therefore, measures such as plasma fatty acids, whole-blood fatty acids, red blood cell fatty acid composition, the omega-3 index, and the omega-6:omega-3 ratio are increasingly used in research, clinical practice, and public health surveillance (9, 10).

This narrative review examines the principal biomarkers used to assess omega-3 status, with emphasis on their scientific basis, analytical properties, clinical interpretation, and practical relevance in both individual care and population health. Additionally, looking at the progress made over the years in.

METHODOLOGY

This narrative review synthesises evidence from biomarker validation studies, mechanistic research, clinical trials, observational cohorts, and public health analyses relating to the assessment of omega-3 status in clinical practice, nutritional research, and public health. Sources include peer-reviewed journal articles, systematic reviews, meta-analyses, consensus statements, and large epidemiological studies. A literature search was performed using the electronic databases Embase, Google Scholar, PubMed/MEDLINE, Scopus, , and Web of Science to identify relevant publications published between 2002 and 2025. The search combined Medical Subject Headings (MeSH) and free-text keywords, including omega-3 biomarkers, omega-3 status, EPA, DHA, Omega-3 Index, red blood cell fatty acids, plasma phospholipids, whole blood fatty acids, serum fatty acids, adipose tissue fatty acids, dried blood spots, biomarkers, nutrition assessment, clinical biomarkers, and public health. Boolean operators ("AND" and "OR") were used to optimise the search strategy.

Priority was given to biomarkers that satisfy several scientific and practical criteria: biological relevance to medium- or long-term omega-3 exposure, analytical stability and reproducibility, association with clinically meaningful outcomes, feasibility for use in clinical or population settings, and utility within personalised nutrition frameworks. Animal studies, conference abstracts, editorials without primary scientific content, non-English publications, and studies unrelated to omega-3 biomarker assessment were excluded (**Figure 1**).

As a narrative review, the aim is not to provide a formal systematic synthesis but rather an interpretive overview connecting methodological considerations to clinical and public health applications. To ensure that the review was rigorous, we employed only peer-reviewed papers published in English. This review included studies that were qualitative, quantitative and mixed-method study designs.

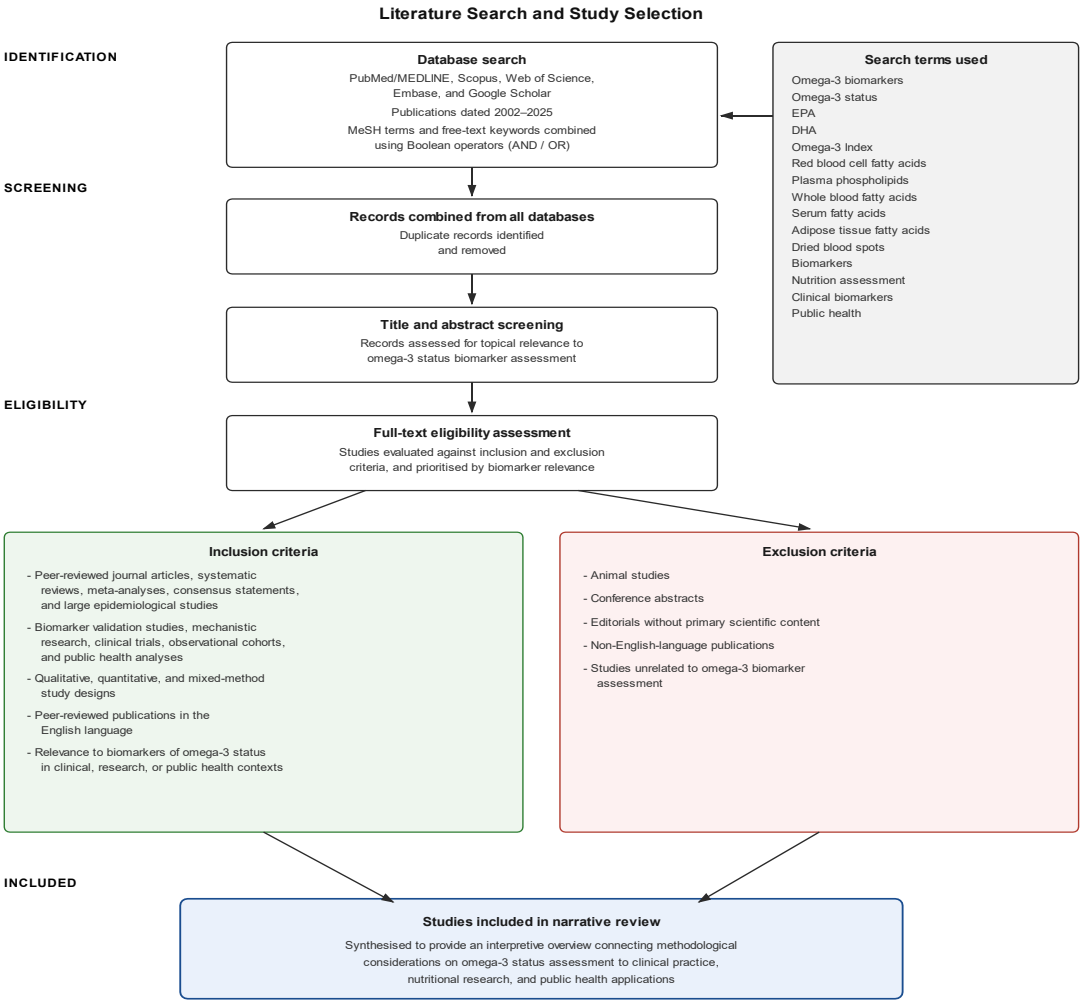

Figure 1: Flowchart for literature search and study selection

This is a comprehensive literature search and study selection for identification, screening and eligibility included in this narrative review.

BIOLOGICAL FOUNDATIONS OF OMEGA-3 STATUS ASSESSMENT

Biomarkers of omega-3 status provide an objective complement to dietary assessment by reflecting physiological exposure and incorporation into circulating or cellular lipid compartments (11). An ideal biomarker should capture habitual intake over a meaningful time frame, demonstrate analytical reliability, show limited short-term variability, correlate with tissue composition or health outcomes, and be feasible to collect and analyse in routine settings (12). The principal biological matrices used to assess omega-3 status are plasma, whole blood, and red blood cells, each of which reflects a different period of intake and metabolic integration.

Plasma Fatty Acids as Indicators of Recent Intake

The composition of fatty acids in plasma reflects relatively recent intake, usually over hours to days. It is strongly influenced by recent meals, fasting status, and short-term metabolic shifts, making it useful in acute feeding studies but less appropriate for the assessment of

long-term omega-3 status (7). Although plasma measures can provide valuable information in controlled settings, their day-to-day variability and weaker correlation with tissue fatty acid composition limit their usefulness for long-term monitoring and risk stratification. Furthermore, the variability may lead to increasing dosing protocols when used for treatment due to its diurnal variation (13).

Whole Blood Fatty Acids as Intermediate-Term Biomarkers

Whole-blood fatty acid measures represent a composite of plasma and cellular lipids and therefore reflect intake over a somewhat longer period, generally on the scale of weeks (14, 15). They are more stable than plasma measures and are particularly relevant to dried blood spot methodologies. Their practicality and compatibility with remote collection have made them increasingly important for large-scale nutritional surveillance and decentralised testing models.

Erythrocyte EPA and DHA as Long-Term Biomarkers

Red blood cell fatty acid composition reflects longer-term intake, corresponding approximately to the lifespan of erythrocytes. Because EPA and DHA incorporated into erythrocyte membranes change relatively slowly, red blood cell measures are less affected by short-term dietary fluctuation and more reflective of habitual omega-3 status (16, 17). Red blood cell EPA and DHA levels also correlate more closely than plasma measures with fatty acid composition in several tissues, supporting their use as longer-term indicators of physiological sufficiency (9).

Omega-3 Index as a Biomarker of Long-Term Status

The Omega-3 Index (O_3I) is defined as the combined EPA and DHA content of red blood cell membranes expressed as a percentage of total identified fatty acids. Since its introduction as a cardiovascular risk marker, it has become the most widely discussed biomarker of long-chain omega-3 status. O_3I is used as a marker for reduced cardiovascular risk when the value is ≥8% (18). Additionally, its appeal lies in its biological relevance, low short-term variability, analytical stability, and relative ease of interpretation. Among currently available biomarkers, it offers one of the strongest combinations of methodological robustness and clinical utility.

Omega-3 Status and Cardiovascular Health

The most substantial clinical literature relating to the Omega-3 Index concerns cardiovascular health. Higher values have been associated with lower risk of sudden cardiac death, reduced arrhythmic susceptibility, improved endothelial function, lower resting heart rate, and reduced triglyceride levels (19). Observational and prospective data have also suggested an inverse relationship between higher red blood cell EPA and DHA levels and cardiovascular mortality (20). Although biomarker associations should not be treated as proof of direct causality, the consistency of these findings has contributed to the clinical prominence of the O_3I as a risk-relevant nutritional biomarker. Alpha-linoleic acid(α-LA) is

involved in proinflammatory processes and signalling pathways its mechanism involves the lowering of the TNF-α gene, COX-2 and iNOS. There are studies in overweight participants supplemented with α-LA that showed improved cardiovascular risk markers (21). It is noteworthy to mention that there have been inconsistent results for the extent to which α-LA affects cognitive decline. However, there are ongoing tries that show that α-LA could boost brain function through DHA endogenous conversion of APOE4 carriers (22). Additionally, α-LA improves insulin sensitivity; this has been shown in an RCT trial in type 2 DM versus placebo administration. However, there are conflicting data outcomes for both observational and interventional studies (23). There is variability in older methods leading to problems in comparability, which includes lack of laboratory standardisations; matrix inconsistency; clinical correlation due to method differentiation; and individual variability (11, 24).

ANALYTICAL ROBUSTNESS AND CLINICAL APPLICATIONS OF THE OMEGA-3 INDEX

The O3I is widely regarded as the preferred biomarker of long-term omega-3 status because it is based on a biologically meaningful compartment, remains relatively stable under standard collection conditions, has been associated with several clinically relevant outcomes, and is sensitive (25). There have been various efforts to improve the way omega-3 status is assayed, which include red blood cells and adipose tissue, and there has also been an expansion into its use in precision nutrition, cardiovascular medicine and global surveillance (Table 1).

Analytical Performance

Analytical validity includes accuracy, reproducibility, precision, and biological stability. The Omega-3 Index performs well across these parameters. Because it is measured in erythrocyte membranes rather than plasma, it is minimally influenced by recent meals or transient metabolic changes. Standardised laboratory approaches have demonstrated good reproducibility, and the biomarker is sufficiently stable for use in routine monitoring when appropriate handling protocols are followed.

Table 1: Key milestones in omega-3 biomarker development (2000–2026)

Period	Major Development	
2000–2009	Validation of erythrocyte EPA+DHA and introduction of the **omega-3 index**	(16)
2010–2015	Comparative validation of plasma, RBC, adipose tissue, and novel biomarkers	(27)
2016–2025	Expansion into precision nutrition, cardiovascular medicine, pregnancy, psychiatry, and inflammation	(25)
2021–2026	Commercial testing, dried blood spots, digital health integration, and global surveillance initiatives	(28)

All-Cause Mortality

Higher blood omega-3 status has also been associated with lower all-cause mortality in pooled prospective analyses (29). These findings reinforce the broader significance of omega-3 biomarkers beyond cardiovascular endpoints alone. However, interpretation should

remain cautious, as mortality outcomes are shaped by multiple biological, behavioural, and socioeconomic factors.

Neurocognitive Outcomes

DHA is a major structural lipid in brain tissue, and omega-3 status has been investigated in relation to memory, cognitive performance, age-related decline, and neurodegenerative disease (30). Observational data generally support an association between higher DHA status and more favourable cognitive outcomes, although intervention evidence remains mixed across populations and endpoints (31, 32). Biomarker-based assessment remains especially valuable in this area because it provides a more reliable estimate of biologically relevant exposure than self-reported intake alone.

Inflammation and Metabolic Health

EPA and DHA influence inflammatory pathways through effects on eicosanoid synthesis, membrane composition, and the generation of specialised pro-resolving mediators (33). Higher omega-3 status has been associated with lower triglyceride concentrations and, in some studies, with more favourable inflammatory and metabolic profiles, including improved insulin sensitivity and reduced hepatic fat accumulation (1, 34). The evidence is not equally strong across all outcomes, but the mechanistic rationale for biomarker-guided assessment remains compelling.

Clinical Decision Thresholds

The Omega-3 Index is commonly interpreted using threshold-based categories, with values below 4% considered less favourable; values between 4% and 8% considered intermediate; and values above 8% often regarded as desirable, particularly in cardiovascular contexts (17). These thresholds have practical value, but they should be interpreted in relation to the population, clinical question, and evidence base for the specific outcome under consideration.

CLINICAL SIGNIFICANCE OF THE OMEGA-6: OMEGA-3 RATIO

The omega-6: omega-3 ratio has long been used as a conceptual and analytical marker of dietary fatty acid balance. Because omega-6 and omega-3 fatty acids share enzymatic pathways involved in elongation, desaturation, and eicosanoid synthesis, the relative abundance of each may influence inflammatory signalling and broader physiological processes (5).

This ratio can provide useful context regarding dietary pattern. A high ratio may reflect low omega-3 intake, excessive omega-6 intake, or both, and may therefore indicate a less favourable fatty acid profile overall (5). For this reason, it remains popular in discussions of Western dietary imbalance and inflammation.

However, the omega-6:omega-3 ratio has substantial interpretive limitations. It does not distinguish among individual fatty acids, including EPA and DHA, which are the omega-

3 components most consistently linked to clinical outcomes. It may be elevated by high omega-6 intake even when omega-3 status is adequate, and a seemingly favourable ratio may coexist with low absolute omega-3 exposure (35). In addition, its association with major clinical endpoints is generally less consistent and less predictive than that of red blood cell EPA and DHA measures.

Accordingly, the omega-6:omega-3 ratio is best viewed as a complementary rather than primary biomarker. It may add interpretive value when used alongside the omega-3 index or absolute fatty acid concentrations, especially in dietary counselling and personalised nutrition.

REMOTE BIOMARKER ASSESSMENT USING DRIED BLOOD SPOTS

Dried blood spot testing has broadened the accessibility of omega-3 biomarker assessment by enabling minimally invasive sample collection and simplified transport (36). In this approach, a small capillary blood sample is collected on filter paper, dried, and later analysed for fatty acid composition. The method requires only a small volume of blood and avoids the logistical burden associated with venous sampling.

One of the main strengths of dried blood spot testing is its practical compatibility with remote collection, home testing, and geographically dispersed population studies. Its analytical value is supported by strong correlations with conventional blood-based fatty acid measures when appropriate protocols are used. These features make it especially attractive for large-scale monitoring, community programmes, and contexts in which clinic-based phlebotomy is difficult or costly.

Dried blood spot testing is also relevant to personalised nutrition because it enables baseline assessment, follow-up monitoring, and user-centred feedback. Objective biomarker results may improve engagement with dietary or supplemental intervention by making change measurable over time.

The method is not without limitations. Accuracy may be affected by collection technique, blood volume, storage conditions, and assay standardisation. In some settings, precision may remain slightly lower than that of directly measured red blood cell assays (15). Even so, its overall balance of convenience, scalability, and acceptable analytical performance has made it an important tool in contemporary omega-3 assessment.

BIOMARKER-GUIDED PRECISION NUTRITION

The growing use of omega-3 biomarkers reflects a broader shift toward more individualised and measurable approaches to nutritional care. Generalised dietary advice often has limited impact because it can be difficult to tailor, difficult to monitor, and difficult for patients to translate into observable progress. Biomarker-guided strategies help address these limitations by providing an objective baseline, informing targeted intervention, and enabling reassessment over time (37, 38).

In the context of omega-3 status, a biomarker-guided model typically includes initial testing, interpretation of results, targeted dietary or supplemental intervention, and follow-up measurement after an appropriate interval. This process creates a feedback loop that

may improve adherence, support behavioural change, and refine intake recommendations according to individual response.

At the same time, biomarker-guided nutrition should be applied with scientific restraint. The presence of a measurable marker does not by itself guarantee clinically meaningful benefit, and commercial enthusiasm should not outpace the evidence base. The most defensible applications are those in which biomarkers are analytically sound, clinically interpretable, and used to support evidence-based intervention rather than promotional claims.

POPULATION HEALTH APPLICATIONS

Omega-3 insufficiency has important public health implications because of its likely contribution to cardiovascular, neurocognitive, inflammatory, and maternal health burdens (6, 39). Population intake of EPA and DHA varies substantially across regions, with higher status generally observed in populations consuming substantial quantities of marine foods and lower status in many Western and inland populations (40).

From a public health perspective, biomarkers offer several advantages over dietary assessment alone. They enable objective identification of at-risk groups, support evaluation of nutrition interventions, and generate population-level data that may inform policy, prevention strategies, and surveillance systems (41). These advantages are particularly relevant in settings where dietary exposure is difficult to estimate reliably or where programme monitoring requires a standardised biological endpoint.

Dried blood spot testing may be especially valuable in this context because it supports large-scale sampling with relatively low logistical burden. Its use in community screening, epidemiological surveys, and preventive health programmes could make population monitoring of omega-3 status more feasible than reliance on venous sampling alone. The roles of other biomarkers used to access biomarkers cannot be overemphasised, and their durations can be considered fit for purpose for how omega-3 status is monitored (Table 2). This chronological arrangement follows the historical development of omega-3 biomarker research, progressing from long-term tissue biomarkers established in the early 2000s to the more recent emphasis on whole blood assays and the standardised Omega-3 Index in 2024.

Table 2: Major biomarkers used to assess omega-3 fatty acid status in clinical practice and public health research (2002–2024)

Biomarker	Biological Sample	Reflects Intake Over	Advantages	Limitations	Major Clinical/Public Health Applications	
Adipose tissue fatty acids	Subcutaneous adipose tissue biopsy	6 months–2 years	Reflects habitual long-term intake; considered reference tissue	Invasive; impractical for routine use	Nutritional epidemiology; validation studies	-42
Cheek (buccal) cell fatty acids	Buccal epithelial cells	Weeks	Non-invasive; suitable for paediatric studies	Limited validation; fewer reference ranges	Pediatric nutrition; community screening	-14
Breast milk DHA/EPA	Human milk	Weeks–months	Direct indicator of maternal omega-3 status and infant exposure	Applicable only during lactation	Maternal-child nutrition; infant development	-43

Dried blood spots (DBS)	Finger-prick whole blood	Weeks–months	Minimal blood volume; transport without cold chain; ideal for large surveys	Standardisation is still evolving.	Global health; population surveillance; remote monitoring	-44
Plasma total fatty acids	Plasma	Hours–days	Highly responsive to recent dietary intake; simple collection	Large day-to-day variability; influenced by fasting status	Dietary intervention studies; nutritional surveillance	-45
Red blood cell (RBC) membrane fatty acids	Erythrocytes	3–4 months	Stable; reflects long-term tissue incorporation; less affected by recent meals	More expensive, specialised laboratory analysis	Risk assessment; precision nutrition; chronic disease monitoring	-46
Plasma phospholipid EPA+DHA	Plasma phospholipids	2–3 weeks	Better indicator of medium-term intake than total plasma; widely validated	Requires lipid fractionation; affected by metabolic status	Cardiovascular disease, pregnancy, cognitive research	-26
Fatty acid ratios (Omega-6/Omega-3 ratio; AA/EPA ratio)	Plasma or RBC	Variable	Reflect inflammatory balance; useful adjunct biomarkers.	Less standardised; influenced by both omega-3 and omega-6 intake	Inflammation research; metabolic disease	-47
Serum fatty acids	Serum	Days	Easily available in clinical laboratories	Reflects recent meals more than tissue stores	Epidemiological studies and supplementation monitoring	-27
Whole blood fatty acids	Whole blood	1–3 months	Simple collection, suitable for field studies, correlates with the RBC Omega-3 Index.	Slightly more variable than isolated RBC measurements	Population surveys; remote testing	-28
Omega-3 Index (EPA+DHA in RBC membranes)	RBC membranes	Approximately 120 days	Gold-standard biomarker; highly reproducible;	Cut-offs primarily validated in cardiovascular disease	Cardiovascular risk prediction; public health surveillance; intervention monitoring	-16

EMERGING DIRECTIONS IN OMEGA-3 BIOMARKER RESEARCH

Several developments are likely to shape the next phase of omega-3 biomarker research and application. One is the integration of fatty acid biomarkers with other biological data, including genomics, metabolomics, proteomics, and microbiome profiling, to better characterise inter-individual variability in metabolism and response (37). Another is the development of more refined predictive models linking baseline biomarker status to likely response to intervention.

There is also growing interest in emerging lipid-related biomarkers, including oxylipins, specialised pro-resolving mediators, and broader lipidomic signatures that may offer more detailed insight into inflammatory regulation and tissue-specific biology (48). Continued advances in remote sampling, assay miniaturisation, and digital interpretation platforms may further expand the accessibility and applicability of testing in both clinical and public health settings. These developments are promising, but they will require continued attention to standardisation, validation, and cautious interpretation. The long-term value of any biomarker depends not only on analytical sophistication but also on whether its use improves clinical decisions, intervention targeting, and health outcomes in real-world settings.

CONCLUSION

Omega-3 biomarkers have become increasingly important tools at the intersection of nutrition science, clinical care, and public health. Among available measures, the omega-3

index stands out as the most analytically robust and clinically interpretable marker of long-term omega-3 status. The omega-6:omega-3 ratio provides broader dietary context, while dried blood spot testing offers a practical route to scalable assessment.

Taken together, these biomarkers support a more objective and individualised approach to nutrition assessment than dietary self-report alone. Their growing use reflects a wider shift toward measurable, prevention-orientated care. As methods continue to improve and the evidence base matures, omega-3 status biomarkers are likely to assume an increasingly important role in both personalised nutrition and population health strategy.

Author Contributions: Conceptualisation (TG, JB), Literature search (TG, JB, WM), Methodology (TG, JB), Project administration (TG, JB, WM), Supervision (TG, JB) Writing – original draft (TG, JB); Writing – review and editing (TG, JB, WM).

Data Availability: No datasets were generated or analysed during the current study.

Clinical Trial Registration: Clinical trial number: not applicable. Registration date: not applicable.

Funding: No Funding

Informed Consent Statement: Not Applicable.

Disclosure: JB has an affiliation to Zinzino.

Ethics approval and consent to participate: Not applicable, as this study is a literature review and did not involve human participants.

Use of AI-assisted tools: AI-assisted tools were used only to support language polishing, formatting, and organisation. The authors critically reviewed, verified, and approved all content, references, interpretations, and conclusions and took full responsibility for the final manuscript.

Abbreviations

AA: Arachidonic Acid

DBS: Dried Blood Spot(s)

DHA: Docosahexaenoic Acid

DNA: deoxyribonucleic acid

EPA: Eicosapentaenoic Acid

FFQ: Food Frequency Questionnaire

LC-PUFAs: Long-Chain Polyunsaturated Fatty Acids

MeSH: Medical Subject Headings

O_3I: Omega-3 Index

OR, Boolean Operator “OR”

PUFAs: polyunsaturated fatty acids

RBC: Red Blood Cell

RNA: ribonucleic acid

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