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High *Sn*-2 Docosahexaenoic Acid Lipids for Brain Benefits, and Their Enzymatic Syntheses: A Review

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ABSTRACT

The normal development and maintenance of central neural functions are highly correlated with the amount of docosahexaenoic acid (DHA; ω-3 fatty acid) accumulated in the brain. DHA incorporated at the *sn*-2 position of lipids is well absorbed by intestinal mucosa and utilized efficiently *in vivo*. However, modern consumers have a reduced direct intake of DHA and increased intake of saturated fats or ω-6 fatty acid oils, resulting in behavioral and neurophysiological deficits. To provide an understanding of the integrated beneficial effects of DHA on the human brain, this review introduces the positional difference (*sn*-2 and *sn*-1,3 positions) of DHA on a glycerol skeleton in natural fats and oils, and further discusses the possible functional mechanism regarding DHA supplementation and the gut–brain axis. The multiple bidirectional routes in this axis offer a novel insight into the interaction between DHA supplementation, the gut microbiota, and brain health. To achieve high *sn*-2 DHA in diets, it is suggested that *sn*-2 DHA lipids be enzymatically produced in more efficient and economical ways by improving the specific activities of lipases and optimizing the purification procedures. These types of diets will benefit individuals with strong needs for *sn*-2 ω-3 lipids such as infants, children, and pregnant and lactating women.

1. Introduction

Docosahexaenoic acid (DHA), a 22:6 ω-3 fatty acid (FA), is abundant in the cell membranes of the human brain, and contributes to the normal development of neural and retinal tissues throughout the human life due to its unique structure and multiple double bonds [1,2]. DHA deficiency in the developing brains of fetuses, newborns, and children is generally linked to neuropathology (e.g., cognitive disorders and anxiety) and disorders related to visual function [3,4]. DHA also plays an important role in maintaining cognitive function and emotional performance during adulthood [5].

DHA is traditionally obtained by consuming α-linolenic acid (α-LNA; 18:3 ω-3)-rich diets and marine foods such as fish and algae. However, the conversion efficiency of α-LNA to DHA in individuals usually cannot meet daily requirements, especially for pregnant women and patients with liver or maple syrup urine diseases [3,6,7]. On the other hand, because the agricultural revolution and food industry have caused a shift in modern diets from marine or α-LNA-rich oils (flaxseed oil, etc.) to ω-6 FA-rich oils (soybean oil, palm olein, and corn oil, etc.) and saturated fats, there is a decreased intake of ω-3 FAs and further decreased concentrations of DHA in human milk [8,9]. Therefore, it has been suggested that preformed DHA from fish oils, algal oils, or high-DHA structured lipids (SLs) be added into foods [10]. Studies have shown that mothers who consumed preformed DHA diets accumulated many times more DHA in their milk, in comparison with the milk of vegans [11]. DHA in vegan milk is primarily synthesized from the ω-3 FAs present in vegetable oils.

In general, DHA is esterified to different positions (*sn*-1, 2, or 3) in a triacylglycerol (TAG) molecule depending on various food sources. After oral intake, TAGs are hydrolyzed by *sn*-1,3-specific pancreatic lipase, forming *sn*-2 monoacylglycerols (MAGs) and free fatty acids (FFAs) [12]. The *sn*-2 MAGs are then well absorbed through the intestinal mucosa and are preferentially used for the re-synthesis of TAGs or phospholipids (PLs; important components

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of the brain cell membrane) [13,14]. In contrast, no specific absorption is observed for FFAs hydrolyzed from the *sn*-1 and *sn*-3 positions [15]. Therefore, TAGs with DHA located at the *sn*-2 position are more favorable in terms of absorption and utilization compared with those that have a random DHA distribution [16]. Similarly, *sn*-2 DHA MAGs showed significantly higher absorption efficiency than other derivatives such as DHA-diacylglycerol (DAG) and DHA-ethyl ester [17,18]. However, most of the current DHA recommendations and supplementations do not pay attention to its positional distribution, and are only focused on the total amount of its daily intake.

Given that the positional arrangement of DHA in TAG and PL structures influences its pharmacological and nutritional benefits for human brain development and maintenance, it is worth providing a background on DHA distribution in common fats and oils, and on the brain benefits provided by high *sn*-2 DHA lipid diets. The technological procedures of enzymatic syntheses to produce *sn*-2 DHA-rich SLs and their typical analysis methods are also discussed in this review.

2. *Sn*-2 DHA in natural and synthesized lipids

DHA is generally provided by marine fish oils and single-cell oils [19]. There are four main types of DHA lipids from natural sources: *sn*-2 DHA TAGs, DAGs, MAGs in fish and algal oils, and *sn*-2 DHA PLs in krill oils and egg yolk (Fig. 1).

The position distribution of DHA on a glycerol skeleton in common fats and oil are summarized in Table 1 [20–31]. Single-cell algal oils (e.g., *Schizochytrium* sp. oil and *Crypthecodinium cohnii* oil) contain the highest total DHA levels, ranging from 44.89% to 48.20%, followed by various fish oils such as tuna oil, sardine oil, anchovy oil, and salmon oil (9.76%–26.85%). In contrast, the relative percentages of *sn*-2 DHA were higher in fish oils than in algal oils. Approximately 44.79%–72.99% of the total DHA in fish oil TAGs were esterified at the *sn*-2 position, while the numerical values were 31.66%–42.09% in algal oil TAGs. This difference might result from the absorption characteristics of *sn*-2 DHA lipids mentioned above. That is, the DHA synthesized in algal oil is eaten by fish through the food chain; *sn*-2 DHA MAGs or DAGs are then produced through digestion and absorption, and are further used to resynthesize TAGs, which increases the *sn*-2 DHA percentages in fish oils to some extent [15].

In particular, the lipids in egg yolk and krill oils are primarily present as PLs (Fig. 1), which are quite different from the lipids in fish and algal oils. Different lipid classes might influence DHA absorption and its concentration in the brain. Diets containing krill oil have been found to increase the DHA levels in rat brain as PLs, and PLs were found to be the major components of both the krill oil and brain cell membranes [32].

DHA also makes up a small proportion (0.36%–0.70%) of total FAs found in human milk fat (HMF) TAGs, and more than half (52.63%–65.15%) is incorporated at the *sn*-2 position (Table 1). However, the percentages decreased from colostrum to mature milks (0.56%–0.70%→0.36%–0.44%), while the relative percentages of *sn*-2 DHA increased from 52.63%–55.71% to 61.39%–65.15%. In addition, DHA levels were found to be progressively lower in nursing mothers who had given birth to twins or had given birth in rapid succession [33,34]. Clinical studies showed that feeding with α-LNA but without DHA over the first six months of life cannot sustain normal DHA concentrations in infant brains [35]. The low conversion rates of α-LNA to DHA in newborn and breast-fed infants were also confirmed in this case. It is further concluded from Table 1 that most of the current infant formula fats (IFFs) contain a lower total amount of DHA and *sn*-2 DHA (the relative percentages were 27.56%–48.17%) in comparison with HMFs. In 11 evaluated IFFs in Spain, only one IFF contained DHA at the *sn*-2 position [29]. However, 70–80 mg of DHA per day from breast milk is suggested to meet the increasing demand of the rapid growth of a baby's nervous system [34]. It is therefore suggested that DHA supplementation—especially of *sn*-2 DHA lipids—in

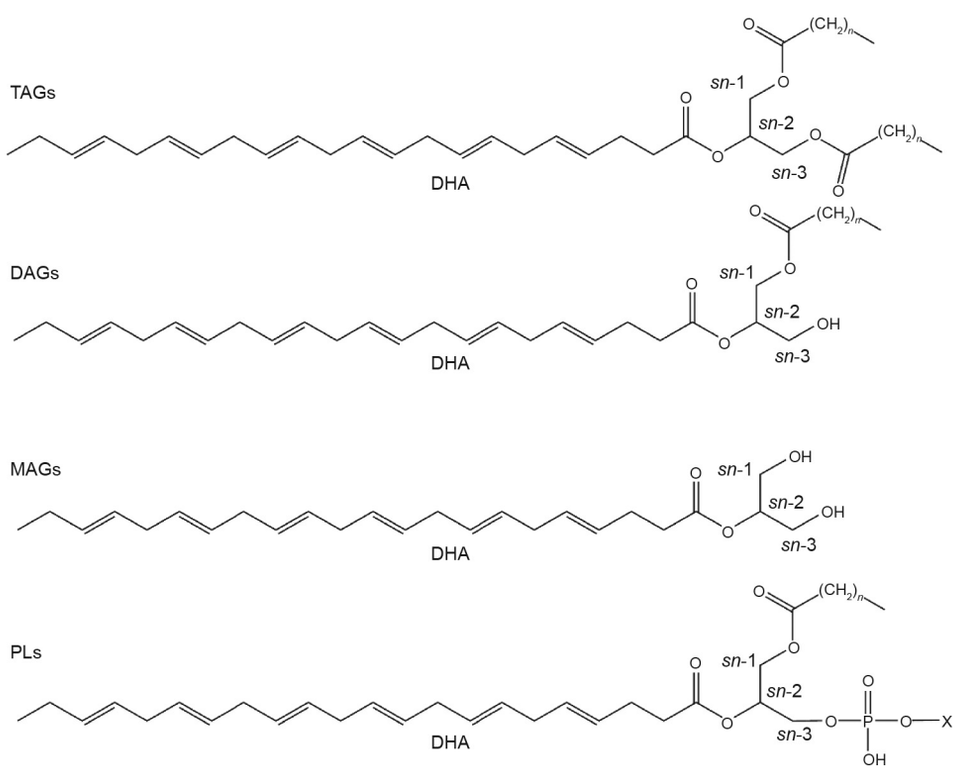

Fig. 1. Primary molecular structures of *sn*-2 DHA lipids. X: ethanolamine, choline, serine, inositol, etc.

Table 1
Position distribution of DHA on a glycerol skeleton in foods and infant formulas.

Sources	Total DHA (%)	*Sn*-2 DHA (%)	Relative percentage of *sn*-2 DHA[a] (%)
Salmon oil [20]	9.99	12.62	50.61
Anchovy oil [20,21]	9.76–10.04	11.59–20.88	49.28–71.31
Tuna oil [20,22]	21.94–26.85	25.88–36.08	44.79–49.00
Sardine oil [23]	10.30–13.90	21.10–29.40	60.67–72.99
Schizochytrium sp. oil [24]	48.20	60.86	42.09
Crypthecodinium cohnii oil [25]	44.89	42.64	31.66
Egg yolk PL [26][b]	2.74	2.89	—
Shrimp (*P. borealis*) oil [27][b]	8.3	7.1	—
HMF in Wuxi (China) [28][c]			
Colostrum	0.70	1.17	55.71
Transitional	0.61	1.07	58.47
Mature	0.44	0.86	65.15
HMF in Spain [29]			
Colostrum	0.56	0.93	52.63
Transitional	0.50	0.81	56.80
Mature	0.36	0.64	61.39
IFF in China [30]	—	0.09–0.21	27.56–33.13
IFF in Spain [29]	ND–0.20	ND–0.28	ND–48.17
IFF in America [31]	0.39	0.49	41.88

HMF: human milk fat; IFF: infant formula fat; ND: not detectable.
[a] Relative percentage of DHA at *sn*-2 position was calculated as [*sn*-2 DHA percentage/(DHA percentage in TAG × 3)] × 100% [30], or reported by the literature.
[b] The data was shown as mol%.
[c] HMF collected after birth at Days 1–5 was colostrum, at Days 6–15 was transitional, and at more than 15 days was mature.

maternal diets may protect infants from deficits in neurodevelopment [4].

3. Positive effects of *sn*-2 DHA on brains

3.1. DHA accumulation in brains by utilizing sn-2 DHA lipids

Lipids account for approximately 60% of the dry weight of brain tissue [34]. Although DHA is a critical component in maintaining proper brain and nervous functions, its location on a glycerol skeleton exhibits significantly different efficiencies in terms of absorption and utilization. It is much easier for DHA to be absorbed by the intestinal mucosa when it is incorporated at the *sn*-2 position than when it is randomly distributed at the *sn*-1,2,3 positions [16]. Further studies have revealed that DHA levels in brain PLs, such as phosphatidylserine and phosphatidylcholine (PC) of newborn rats fed *sn*-2 DHA diets, were significantly improved compared with those in rats that were fed milk diets (Table 2) [36]. Also, *sn*-2 lysophosphatidylcholine DHA was preferentially utilized in the rat brains in comparison with unesterified DHA (Table 2) [37]. In addition, large-scale trials have concluded that DHA supplementation through the consumption of large doses of marine oils is safe during pregnancy [38].

Table 2
Brain benefits of *sn*-2 DHA lipids.

Treatments	Findings	Reference
Newborn rats were fed diets containing 7.0% fat (3.70% DHA and 6.18% *sn*-2 DHA for structured oil group; 3.98% DHA and 3.57% *sn*-2 DHA for randomized oil group; and 0.66% DHA in rat milk for reference group)	DHA levels of brain phosphatidylserine and PC were significantly increased compared with the reference after three weeks, but no differences were observed in phosphatidylethanolamines and phosphatidylinositols	[36]
A solution containing *sn*-2 lysophosphatidylcholine DHA or unesterified DHA was injected into the tail veins of 20-day-old male rats for 30 s, respectively. Their tissue lipids were analyzed from 2 to 60 min after the injection	The developing (young) brain preferentially utilized *sn*-2 lysophosphatidylcholine DHA rather than unesterified DHA	[37]

3.2. DHA supplementation improves brain functions through the gut–brain axis

Emotional disorders, which are one of the results of brain function deficits, have been found to be specifically associated with gut microbiota alterations [39]. There has been recent interest in the possible correlation between brain problems (e.g., brain injury, declined cognition, schizophrenia, stroke, anxiety, stress, and depression) and intestinal microflora. The human intestines contain more than 1000 microbiota species with 100 trillion living microorganisms [40]. Bacterial colonization of different species could alter brain functions, and in turn, the central nervous system is speculated to indirectly influence the gut microbial composition. These integrative and bidirectional signaling pathways, which mainly involve the routes of the vagus nerve and spinal pathway, are defined as the gut–brain axis or the brain–gut–microbiota axis (Fig. 2) [41,42].

Previous evidence suggests that gut microbes play an important role in developing therapies for complex brain function disorders. In general, dietary interventions with DHA may have beneficial effects on behavioral and neurophysiological disorders due to alteration of the microbial composition in the intestines [43,44] as seen in Table 3 [45–49].

As shown in Table 3, DHA supplementation for early-life stressed, socially isolated, or aging mice restored and normalized their gut microbiota composition, by increasing the abundance of beneficial species such as *Lactobacillus*, *Bifidobacterium*, and *Bacteroides*, concomitantly decreasing the abundance of *Proteobacteria* (e.g., *Undibacterium*) and *Cyanobacteria*, among others, and subsequently alleviating the mice's brain-related disorders. In addition, García-Ródenas et al. [49] has suggested that psychological stress could be reduced by consuming DHA-containing diets through the normalization of gut permeability without the restoration of the intestinal microbiota. This difference indicates that the gut-brain axis includes various bidirectional routes, some of which have not yet been fully elucidated. More studies are required to explain the potential mechanism of the intestinal microbiome on DHA diet-induced effects on the brain. Also, further studies on

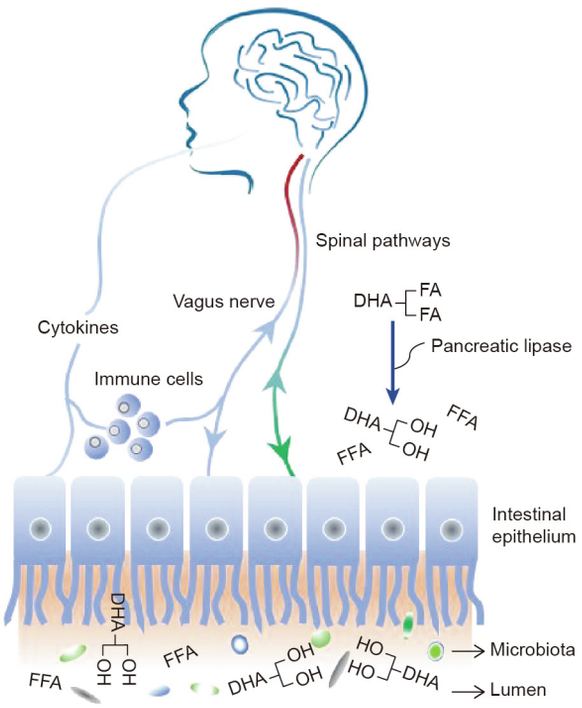

Fig. 2. The gut–brain axis: potential multiple bidirectional routes between the brain and the intestinal microflora [41,42].

the impacts of diets with a DHA positional difference (e.g., high *sn*-2 DHA lipid diets and randomly distributed DHA lipid diets) on the gut–brain axis are necessary.

4. Enzymatic synthesis of high *sn*-2 DHA fats and oils

Many infants and pregnant and nursing women consume foods containing only DHA precursors or limited DHA levels [11]. The decreased dietary DHA consumption that results from following a Western diet is responsible for this problem [50]. The production of modified fats and oils with abundant *sn*-2 DHA using low-pollution and highly efficient techniques such as enzymatic syntheses from saturated fats and the DHA-rich oils listed in Table 1 is encouraged. These processes mainly include the enzymatic reactions of acidolysis, interesterification, ethanolysis, and their combination.

4.1. Acidolysis reactions

Most of the developed methods to produce high *sn*-2 DHA SLs focus on the acidolysis of single-cell oils (e.g., DHA single-cell oil (DHASCO) from alga *Crypthecodinium cohnii*) and FAs (e.g., caprylic acid (C)) in a one-step reaction using *sn*-1,3 specific lipases or lipases with high activity on DHA.

As shown in the acidolysis reactions in Table 4 [25,30,31,51–61], optimal reactions are generally carried out with substrate mole ratios of 1:3–1:18 (oils to FFAs) at mild temperatures of 30–55 °C with 4%–15% enzymes for dozens of hours [51–54]. The *sn*-2 DHA levels vary significantly based on the enzyme species [62]. In some cases, the lipases, such as *Pseudomonas* sp. KWI-56 lipase, showed non-regiospecificity but were active toward DHA and docosapentaenoic acid (DPA), and may also cleave the DHA at the *sn*-2 position, resulting in acyl migration to some extent [52]. This side reaction might easily occur in the presence of caprylic acid and different lipases [63]. It is suggested that possible alternative or better lipases be developed in order to minimize acyl migration. In addition, recovery of the target SLs from these reaction products is usually complicated. Usually, for a small-scale reaction, FFAs are removed by neutralization with alkaline solution, followed by the extraction of TAGs with hexane; the solvent is then further evaporated to obtain the final SLs.

The other typical method to prepare *sn*-2 DHA SLs is to hydrolyze single-cell oils or marine fish oils to prepare DHA, followed by esterification with TAGs (Table 4). In this context, DHA is first released from the marine oils by saponification using potassium hydroxide and acidification using hydrochloric acid in the presence of antioxidants (e.g., butylated hydroxytoluene). Acidolysis of the prepared DHA and other oils is then conducted at substrate mole ratios of 1:5–1:18 (oils to DHA) and with an enzyme load of 10%, and the reaction is kept at 60–65 °C for around 24 h [25,31,55]. For large-scale and industrial reactions, the extra FFAs are commonly removed through short-path distillation.

4.2. Interesterification reactions

Interesterification between DHA-rich oils/ethyl ester and FA ethyl ester is another method to provide targeted SLs (Table 4).

Table 3
DHA absorbed through the intestinal mucosa improves brain functions through the gut–brain axis.

Treatments	Findings	Reference
Early-life stressed female rats were fed with DHA and EPA supplementation of 0.4 or 1.0 g·kg^{-1} daily for 17 weeks, and their fecal pellets were collected for microbiota analysis	High-dose DHA and EPA supplementation restored and normalized the gut microbiota composition of stressed rats. Levels of *Butyrivibrio* and several members of *Actinobacteria* were elevated, with a concomitant reduction of some *Proteobacteria*	[45]
Newborn male mice were fed with DHA and EPA diets for 13 weeks. Their social, depressive, and cognitive behaviors were tested, and fecal microbiota compositions were analyzed	The supplementation improved the neurodevelopment of the mice, with increases in beneficial *Bifidobacterium* and *Lactobacillus* in their gut. In contrast, DHA- and EPA-deficient mice showed social and emotional problems with an increased *Firmicutes*: *Bacteroidetes* ratio	[46]
Socially isolated male and female mice were supplemented with 0.1% or 1.0% by weight DHA. Their fecal pellets were collected for microbiota analysis at 0, 1, and 7 day(s) following the introduction of DHA supplementation	DHA intervention produced beneficial effects on anxiety in male mice, which were correlated with changes in gut microbiota relative abundances, e.g., an increase in *Allobaculum* abundance, which could decrease anxiety- and anhedonia-like behaviors	[47]
Aging mice received tuna oil and/or algal oil for 12 weeks. Their brain biochemical indices and fecal samples were evaluated	DHA-rich diets alleviated age-related decline in cognition by enriching the abundance of *Bacteroides*, *Tannerella*, *Coprobacter*, *Lactobacillus*, and *Prevotella*, and by decreasing the abundance of *Falsiporphyromonas* and *Cyanobacteria*	[48]
Early-life stressed male rat pups were fed with 100 g diets containing 2 g DHA or ARA, along with other components	The adapted diets reverted the negative imprinting of neonatal stress by normalizing intestinal permeability, and further restore the relevant growth rate	[49]

EPA: eicosapentaenoic acid; ARA: arachidonic acid.

Table 4
Enzymatic syntheses of high *sn*-2 DHA SLs.

Substrates	Enzymes	Technical procedure	Products	Reference
Acidolysis reactions				
DHASCO, and C	*Pseudomonas* sp.	SL was produced by esterification of the substrates and purified using hexane	*Sn*-2 DHA level was increased from 25.9% in unmodified oil to 39.9% in SL	[51]
Single-cell oil and C	*Pseudomonas* sp. KWI-56 lipase	Acidolysis was carried out using the substrates with more than 60 mol% lipase	SL contained 36% C-DHA/DPA-C and C–C-DHA/DPA, and the former accounted for 77%–78%	[52]
Tuna oil and C	*Rhizopus delemar*	SL was produced by acidolysis of tuna oil with C and FFA was neutralized with potassium hydroxide-hydroalcoholic solution	SL contained 16.2 mol% DHA, and its *sn*-2 position was occupied by 24.9 mol% DHA	[53]
Fish oil and capric acid	*Rhizomucor miehei*	Acidolysis reactions were carried out in hexane or solvent-free systems, respectively	DHA level obtained from the solvent-free system (28.3 mol%) was higher than that from the hexane system (23.5 mol%)	[54]
DHASCO, palm olein, etc.	Novozym 435	Preparation of DHA by hydrolyzing DHASCO, urea complexation, and solvent crystallization; then it was esterified with palm olein to produce SL	SL contained 17.2% DHA while 22.71% of it was incorporated at the *sn*-2 position[a]	[25]
DHASCO, tripalmitin, etc.	Lipozyme TL IM	DHA was prepared by saponification and acidification of DHASCO; then it was esterified with tripalmitin to produce SL	SL containing 4.80% *sn*-2 DHA was used in infant formula	[31]
DHASCO, olive oil, and tripalmitin	Lipozyme TL IM	FFAs were prepared by saponification of DHASCO and olive oil; SL was then produced by esterification of the mixed FFAs and tripalmitin	SL containing 1.79 mol%–2.57 mol% *sn*-2 DHA was used in infant formula	[55]
Interesterification reactions				
Ethyl DHA, ethyl caprylate, and tricaprylolylglycerol	*Alcaligenes* sp. and Novozym 435	SL was prepared by interesterification of ethyl DHA and tricaprylolylglycerol, followed by a regioselective ester reaction with ethyl caprylate	SL contained 76.4% C-DHA-C/C–C-DHA, and 82.7% of it was *sn*-C-DHA-C	[56]
Menhaden oil and ethyl caprate	Lipozyme 435	SL was produced by interesterification of the substrates using the Taguchi method	SL contained 9.83 mol%–10.57 mol% DHA, and its *sn*-2 position was occupied by 19.53 mol%–20.79 mol% DHA	[57]
DHASCO	Lipozyme TL IM and Novozym 435	Interesterification of DHASCO was done using mixed enzymes (weight ratio = 1:1) to increase the *sn*-2 DHA percentage	*Sn*-2 DHA in SL oil was improved from 34.3 mol% to 49.7 mol%	[58]
From *sn*-2 DHA MAG to *sn*-2 DHA lipids				
Bonito oil and ethyl caprylate	Novozym 435 and Lipozyme IM	*Sn*-2 MAG was prepared by the ethanolysis of bonito oil using Novozym 435; the MAG was then mixed with ethyl caprylate to produce SL using Lipozyme IM	Proportion of SL with DHA at the *sn*-2 position to that at the *sn*-1 (3) positions was more than 50:1	[59]
Cod liver oil, tuna oil, and C	Novozym 435 and *Rhizopus oryzae*	Novozym 435 was suitable for producing *sn*-2 MAG from fish oils, and *Rhizopus oryzae* was selected to catalyze the esterification reaction of the MAG and C to produce SL	Purified SL contained 37.9% DHA at the *sn*-2 position	[60]
Cod liver oil and C	Novozym 435 and *Rhizopus oryzae*	Alcoholysis of cod liver oil with Novozym 435 was used to prepare *sn*-2 MAGs, followed by incorporating the C at the *sn*-1,3 positions of the MAGs to produce SL	Purified SL contained 38.0% DHA at its *sn*-2 position	[61]

DPA: docosapentaenoic acid.
[a] Relative percentage of DHA at the *sn*-2 position was calculated as [*sn*-2 DHA percentage/(DHA percentage in TAG × 3)] × 100% [30].

The reactions require strict enzyme selection due to their positional specificities and the steric hindrance of DHA [52]. For example, in a two-step reaction, unspecific DHA-rich oil was first prepared from a nonselective reaction of DHA-ethyl ester and tricaprylolylglycerol using *Alcaligenes* sp. lipase (50 °C, 90 h), followed by a *sn*-1,3 regioselective interesterification of the unspecific DHA-rich oil and ethyl caprylate using Novozym 435 to produce *sn*-1,3-dicapryloyl-2-docosahexaenoylglycerol (40 °C, 40 h) [56]. Both reactions were carried out in a nitrogen atmosphere to avoid oxidation, and extra esters and tricaprylolylglycerol were removed by molecular distillation.

4.3. From sn-2 DHA MAG to sn-2 DHA lipids

Another typical strategy to obtain *sn*-2 DHA-rich lipids is to prepare *sn*-2 DHA MAG from marine oils, followed by the incorporation of needed FAs at the *sn*-1,3 positions of the MAG (Fig. 3 and Table 4).

To achieve this technical route, preparation of *sn*-2 DHA MAG from oils is a key step due to the oxidation problems of DHA, acyl migration during enzymatic catalysis, and the cost [64]. Conventional methods were carried out in an ethanol system with enzymes such as Novozym 435, which showed *sn*-1,3 regiospecificity in the presence of ethanol [59,60]. Recent research has reported a highly efficient approach to produce MAG enriched with ω-3 polyunsaturated fatty acids (PUFAs) at the *sn*-2 position using *Candida antarctica* lipase A in a more economical way [65]. In similar cases, *Candida antarctica* lipase A effectively concentrated the *sn*-2 DHA of anchovy oil from 20.88% in oil to 65.69% at *sn*-2 MAGs via catalytic reaction at low temperature (35 °C) for 12 h; the *sn*-2 DHA value in microalgae oil was increased from 3.24% to 22.20% in the same way [66]. This research demonstrated that *Candida antarctica* lipase A exhibits non-regiospecific and non-ω-3 PUFA preference in an ethanol system, and can thus selectively cleave non-target FAs and further keep the ω-3 PUFAs such as DHA on the glycerol backbone to form DHA-rich MAGs [21,65,66].

Fig. 3. Typical technique to produce *sn*-2 DHA SLs for various uses.

Table 5
Sn-2 PUFA compositions of fish oils determined by the pancreatic lipase method and the Novozym 435 method [71].

Methods	Cod liver oil		Tuna oil	
	EPA (%)	DHA (%)	EPA (%)	DHA (%)
Pancreatic lipase	10.8	23.4	7.5	27.1
Novozym 435	9.0	30.1	6.8	35.9

For purification, DHA-containing byproducts such as FFAs and their ethyl esters can be removed by short-path or molecular distillation for further re-utilization [67]. The advantage of this technique is its flexibility in manufacturing different fats and oils such as shortenings, margarines, spreads, IFFs, and bakery and confectionary fats using the *sn*-2 DHA MAG.

5. Analytical methods for *sn*-2 DHA

Regiospecific analysis of FAs in TAG molecules is generally conducted on a gas chromatograph equipped with a flame ionization detector. In brief, TAGs are first hydrolyzed by *sn*-1,3-specific lipases to form MAGs, followed by the isolation of *sn*-2 MAG using thin-layer chromatography and its conversion to FA methyl esters for further analysis [68]. Pancreatic lipase is a widely used lipase, which has been well confirmed through the determination of the *sn*-2 FA composition of many fats and oils. However, it should be noted that pancreatic lipase exhibits limited ability to hydrolyze all FAs, particularly PUFAs from marine oils [57]. Its ability for selective hydrolysis depends on the FA species and the location of the double bonds [69]. In contrast, *Candida antarctica* lipase B (Novozym 435 or Lipozyme 435) is suggested to be a better hydrolytic enzyme for this purpose [70,71]. Although Lipozyme 435 is a non-regioselective lipase in many cases, it behaves as *sn*-1,3-specific in the presence of excess ethanol [70]. Table 5 [71] shows the PUFA compositions of fish oils as detected by the Novozym 435 method and the pancreatic lipase method. Novozym 435 can release PUFAs from fish oils at different rates based on the degree of chain length and unsaturation. For example, eicosapentaenoic acid (EPA) levels detected using the pancreatic lipase method (7.5%–10.8%) were higher than those determined using the Novozym 435 method (6.8%–9.0%), while the contents of DHA exhibited the opposite trends [71]. That is, Novozym 435 shows exclusive selectivity for DHA compared with pancreatic lipase.

In general, the Novozym 435 method needs strict hydrolysis conditions, such as ethanol-to-oil ratio, reaction time, and temperature, to completely release the *sn*-1,3 FAs from TAGs; otherwise, the hydrolysis reaction might result in lower results compared with ^{13}C nuclear magnetic resonance (NMR) or predicted values. In a cod liver oil test, the result for *sn*-2 DHA by the Novozym 435 method was 69.4%, which was lower than that measured by ^{13}C NMR (72.5%); however, for analysis of tuna oil, the *sn*-2 DHA results were similar, at 53.1% for the Novozym 435 method and 52.0% for ^{13}C NMR [72].

6. Conclusion

Marine fish and algal oils are typical DHA sources with about half of their FA incorporated at the *sn*-2 position. Their unique structure makes it easier for DHA to be absorbed by the intestinal mucosa and to be used for the re-synthesis of TAGs or PLs *in vivo*, in comparison with molecules that have DHA located at the *sn*-1,3 positions. *sn*-2 DHA lipids, therefore, play important roles in the development of brain functions and in the mitigation of brain deficits such as anxiety, stress, declined cognition, schizophrenia, and stroke. A focus on the gut–brain axis is the most effective strategy to understand the beneficial effects of DHA supplementation on brain functions. It is suggested that brain problems could be alleviated by restoring and normalizing the gut microbial composition through DHA intervention. However, the multiple bidirectional routes of the gut–brain axis are not yet fully understood or explained. Further research is required on the impacts of dietary *sn*-2 DHA lipid supplementation on the gut microbiota and brain functions.

DHA accumulates in the human brain at a rapid rate from gestation to age two. However, although the amount of DHA in HMFs decreases to a low level 15 days after birth, the relative percentages of *sn*-2 DHA show increased trends, indicating the importance of *sn*-2 DHA in the brain development of infants and children. Therefore, it is suggested that preformed *sn*-2 DHA SLs containing *sn*-2 DHA be included in maternal diets; this could be done by preparing *sn*-2 DHA MAG from DHA-rich oils, and then incorporating selected FAs at the *sn*-1,3 positions of the MAG. For further study, it is suggested that novel lipases with high activity at the *sn*-1,3 positions or with a non-ω-3 PUFA preference be developed, together with mild reaction conditions and purification procedures to make the synthesis techniques and products more efficient and economical.

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Compliance with ethics guidelines

Jun Jin, Qingzhe Jin, Xingguo Wang, and Casimir C. Akoh declare that they have no conflict of interest or financial conflicts to disclose.

Nomenclature

α-LNA	α-linolenic acid
ARA	arachidonic acid
DAG	diacylglycerol
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
EPA	eicosapentaenoic acid
FA	fatty acid
FFA	free fatty acid
PL	phospholipid
HMF	human milk fat
IFF	infant formula fat
MAG	monoacylglycerol
NMR	nuclear magnetic resonance
PC	phosphatidylcholine
PUFA	polyunsaturated fatty acid
SL	structured lipid
TAG	triacylglycerol

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富含 *sn*-2 DHA 脂质对大脑的益处及其酶法合成综述

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摘要

大脑中二十二碳六烯酸（DHA, ω-3脂肪酸）的含量与中枢神经系统的正常发育和功能维持高度相关。甘油酯*sn*-2位上的DHA可以被肠黏膜更好地吸收，从而实现机体对DHA的高效利用。然而，如今人们在饮食中摄入较多的饱和脂肪或富含ω-6脂肪酸的油脂，而摄入较少的DHA，从而导致了部分个体在行为和神经生理学方面的缺陷。为了全面了解DHA对大脑的有益功能，本文系统介绍了天然油脂甘油骨架上DHA的位置分布（*sn*-2和*sn*-1,3位）特征，并讨论了DHA补充和通过肠-脑轴传递信息的潜在功能机制。肠-脑轴包含的多条双向信息通道为DHA、肠道菌群和大脑健康的相互作用提供了新的研究思路。为了在日常饮食中摄入更多的*sn*-2 DHA，我们建议通过更为高效和经济的酯交换制造技术生产富含*sn*-2 DHA脂质，其中需要解决的关键技术包括强化酶的特异性和优化纯化工艺。这类饮食可满足对*sn*-2 ω-3脂质有强烈需求的人群，特别是婴儿、儿童、孕妇和哺乳期妇女。

1. 引言

二十二碳六烯酸（DHA）是一种22:6 ω-3脂肪酸（FA），它拥有独特的分子结构和多个双键，主要存在于大脑细胞膜中，对神经和视网膜组织的发育至关重要[1,2]。胎儿、新生儿和儿童在大脑发育期若缺乏DHA，可能会造成神经生理学方面的疾病（如认知障碍、焦虑症等）和视觉功能的降低[3,4]。对于成年人，DHA在维持认知功能和情绪表现方面也起着重要的作用[5]。

一般，DHA主要来源于富含α-亚麻酸（α-LNA；18:3 ω-3）的饮食和鱼类、藻类等海洋食品。然而，人体内从α-LNA转化为DHA的效率很低，并不能满足日常所需，尤其是对于孕妇、肝病或枫糖尿病患者[3,6,7]。另一方面，随着农业改革和食品工业的发展，人们日常摄入的脂肪已经从海洋油脂或α-LNA类油脂（如亚麻籽油）转变为ω-6类油脂（如大豆油、棕榈液油和玉米油）与饱和脂肪，从而使ω-3脂肪酸的摄入降低，进一步导致了母乳中DHA的含量的减少[8,9]。因此，现代食品加入了更多的鱼油、藻油和富含DHA的结构脂质（structured lipids, SL），以便人们可以直接摄取DHA[10]。研究显示，摄入外源DHA的孕妇，其母乳中的DHA含量会比素食主义者母乳的DHA含量高好几

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倍[11]。素食主义者母乳中的DHA主要来源于植物油脂中ω-3 FA在体内的转化。

根据油脂来源不同，DHA可分布在甘油三酯（TAG）分子上不同的位点（*sn*-1,2或3位）。人体摄入DHA后，*sn*-1,3位特异性胰脂酶会水解TAG，从而生成*sn*-2单甘酯（MAG）和游离脂肪酸（FFA）[12]。*sn*-2 MAG可在小肠黏膜上被很好地吸收，进而被用于重新合成TAG或磷脂（PL，脑细胞膜的重要组成部分）[13,14]。相比较而言，从*sn*-1和*sn*-3位水解下来的FFA则没有被针对性地吸收[15]。因此，DHA分布在*sn*-2位的TAG比DHA随机分布的TAG更有利于人体的吸收和利用[16]。类似地，*sn*-2 DHA MAG相比于DHA甘油二酯（DAG）和DHA乙酯也更容易被机体吸收[17,18]。然而，目前关于DHA膳食或保健品的指南基本局限于对DHA总量的推荐，很少涉及不同DHA位置分布的相关信息。

基于药理和营养角度，TAG和PL分子中DHA的位置分布会影响大脑的发育和功能维持，因此阐明常见油脂中DHA的分布以及富含*sn*-2 DHA脂质饮食的特点很有必要。本文同时介绍了富含*sn*-2 DHA SL的酶法合成技术以及相关的检测分析方法。

2. 天然油脂和合成脂质中的 *sn*-2 DHA

DHA主要来源于海洋鱼油和单细胞油脂[19]。*sn*-2 DHA脂质主要以TAG、DAG、MAG的形式存在于鱼油和藻油中，也有以PL的形式存在于虾油和蛋黄脂质中（图1）。

常见油脂中DHA的含量见表1。单细胞藻油（如*Schizochytrium* sp.油和*Crypthecodinium cohnii*油）的DHA含量最高，为44.89%~48.20%, 其次为金枪鱼油、沙丁鱼油、凤尾鱼油和鲑鱼油等（9.76%~26.85%）。然而，在这些鱼油中，*sn*-2 DHA的含量要高于藻油中的*sn*-2 DHA的含量。在鱼油的TAG中，44.79%~72.99%的DHA分布在*sn*-2位上，而在藻油TAG中，31.66%~42.09%的DHA分布在*sn*-2位上。这可能与如上所述的*sn*-2 DHA脂质消化特性有关。鱼类以藻类为食，通过消化吸收藻油中的DHA脂质生成富含*sn*-2 DHA的MAG或DAG，进而重新合成TAG，从而提高了*sn*-2 DHA的占比[15]。

蛋黄和虾油中的脂质一般以PL形式存在（图1），这与常见的鱼油和藻油中的脂质不同。不同的脂质存在形式会影响大脑对DHA的吸收。含有虾油的饮食可以

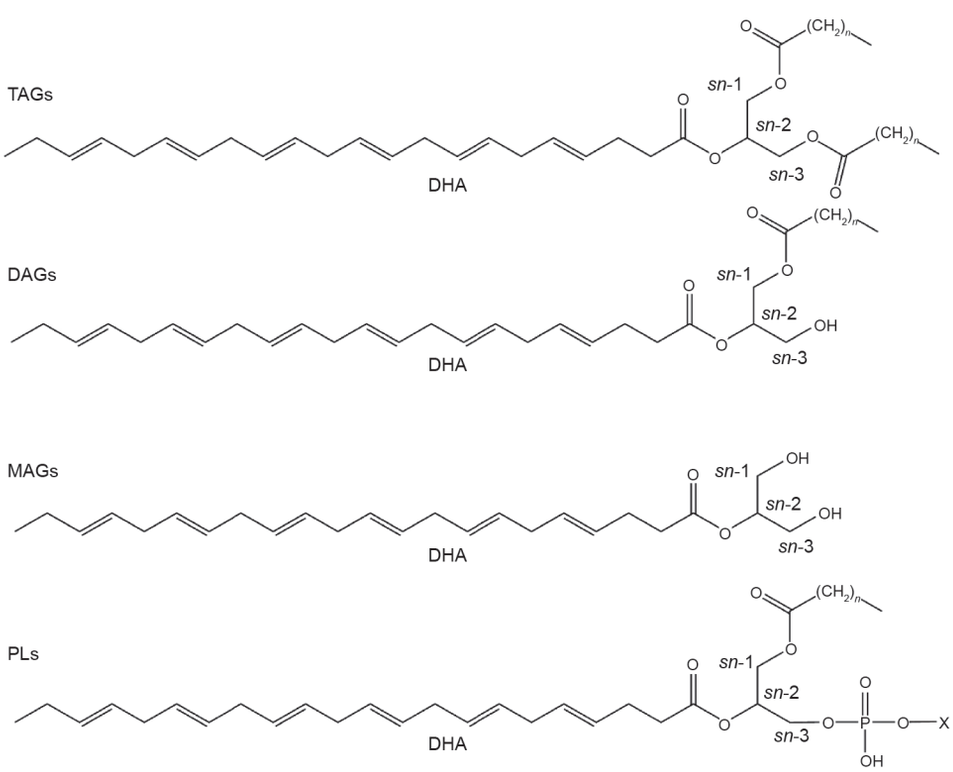

图1. *sn*-2 DHA脂质的主要分子结构。X：乙醇胺、胆碱、丝氨酸、肌醇等。

表1 食物和配方奶粉脂质中甘油骨架上DHA的位置分布

Sources	Total DHA (%)	*sn*-2 DHA (%)	Relative percentage of *sn*-2 DHA (%)[a]
Salmon oil [20]	9.99	12.62	50.61
Anchovy oil [20,21]	9.76–10.04	11.59–20.88	49.28–71.31
Tuna oil [20,22]	21.94–26.85	25.88–36.08	44.79–49.00
Sardine oil [23]	10.30–13.90	21.10–29.40	60.67–72.99
Schizochytrium sp. oil [24]	48.20	60.86	42.09
Crypthecodinium cohnii oil [25]	44.89	42.64	31.66
Egg yolk phospholipid [26][b]	2.74	2.89	—
Shrimp (*P. borealis*) oil [27][b]	8.3	7.1	—
HMF in Wuxi (China) [28][c]			
Colostrum	0.70	1.17	55.71
Transitional	0.61	1.07	58.47
Mature	0.44	0.86	65.15
HMF in Spain [29]			
Colostrum	0.56	0.93	52.63
Transitional	0.50	0.81	56.80
Mature	0.36	0.64	61.39
IFF in China [30]	—	0.09–0.21	27.56–33.13
IFF in Spain [29]	ND–0.20	ND–0.28	ND–48.17
IFF in America [31]	0.39	0.49	41.88

HMF: human milk fat; IFF: infant formula fat; ND: not detectable.

[a] Relative percentage of DHA at *sn*-2 position was calculated as [*sn*-2 DHA percentage / (DHA percentage in TAG × 3)] × 100 [30], or reported by the literature.

[b] The data was shown as mol%.

[c] HMF collected after birth at Days 1–5 was colostrum, at Days 6–15 was transitional, and at more than 15 days was mature.

增加大鼠大脑中DHA PL的含量，而PL正是大脑细胞膜的主要组成部分[32]。

在人乳脂（HMF）中也含有少量DHA，约占总FA的0.36%~0.70%，其中52.63%~65.15%的DHA分布在*sn*-2位上（表1）。然而，DHA的含量从初乳至成熟乳逐渐减少（0.56%~0.70%→0.36%~0.44%），但在*sn*-2位上的相对含量却从52.63%~55.71%增至61.39%~65.15%。此外，在分娩双胞胎或连续分娩的母亲的母乳中，DHA的含量逐渐降低[33,34]。临床研究显示，补充了α-LNA但没有补充DHA的婴儿在出生后的前6个月无法维持其大脑中正常的DHA浓度[35]。在新生儿体内，α-LNA转换为DHA的效率同样很低。相比于HMF，许多市售婴幼儿配方奶粉脂肪（IFF）中的DHA和*sn*-2 DHA（相对含量为27.56%~48.17%）水平均较低（表1）。在11款西班牙产的IFF中，只有一款IFF在*sn*-2位上含有DHA。一般而言，为维持神经系统的正常发育，婴儿需从母乳中日均摄取70~80 mg的DHA [34]。因此，为保护婴儿免受神经系统发育的缺陷，产妇的饮食中通常需要补充外源DHA，尤其是*sn*-2 DHA脂质[4]。

3. *sn*-2 DHA 对大脑的有益功能

3.1. 吸收 *sn*-2 DHA 脂质增加大脑中 DHA 的积累

脂质约占脑组织干基重量的60% [34]。尽管DHA是维持大脑和神经功能正常的关键物质，但其被机体吸收和利用的程度显著受到其在甘油骨架上的位置分布的影响。相比于*sn*-1,2,3位上随机分布的DHA，分布在*sn*-2位上的DHA更容易被肠黏膜所吸收[16]。食用富含*sn*-2 DHA饮食的新生大鼠，其大脑PL [如磷脂酰丝氨酸和磷脂酰胆碱（PC）]中的DHA含量显著高于对照组食用牛奶饮食大鼠的大脑PL中的DHA水平（表2）[36]。类似的研究发现，相比于未酯化的DHA，大鼠大脑可以优先利用*sn*-2溶血磷脂酰胆碱DHA（表2）[37]。此外，已有大规模试验表明，在孕期通过食用较多海洋油脂补充DHA是安全的[38]。

3.2. DHA 通过肠 - 脑轴增强脑功能

情绪障碍是大脑功能缺陷的症状之一，目前认为该症状与肠道菌群的改变关系密切[39]。近年来，人们对

表2 大脑对*sn*-2 DHA脂质的吸收

Treatments	Findings	Reference
Newborn rats were fed diets containing 7.0% fat (3.70% DHA and 6.18% *sn*-2 DHA for structured oil group; 3.98% DHA and 3.57% *sn*-2 DHA for randomized oil group; and 0.66% DHA in rat milk for reference group)	DHA levels of brain phosphatidylserine and PC were significantly increased compared with the reference after 3 weeks, but no differences were observed in phosphatidylethanolamines and phosphatidylinositols	[36]
A solution containing *sn*-2 lysophosphatidylcholine DHA or unesterified DHA was injected into the tail veins of 20-day-old male rats for 30 s, respectively. Their tissue lipids were analyzed from 2 to 60 min after the injection	The developing (young) brain preferentially utilized *sn*-2 lysophosphatidylcholine DHA rather than unesterified DHA	[37]

大脑问题（如脑损伤、认知能力下降、精神分裂症、中风、焦虑症、压力和抑郁症）和肠道菌群之间的相关性颇感兴趣。人体肠道内生活着1000多种微生物菌群，共计约100万亿个微生物[40]。不同的菌群会改变大脑的功能，反之中枢神经系统也可能间接影响肠道微生物的组成。这一系列综合性的双向信号传导途径被定义为肠-脑轴或脑-肠-菌群轴，主要涉及迷走神经和脑脊髓传导通路（图2）[41,42]。

前期研究认为，肠道微生物在开发复杂脑功能障碍的疗法中起着重要作用。一般而言，含有DHA的饮食干预会通过改变肠道微生物的组成而对行为和神经生理障碍产生有益的影响[43,44]，见表3。

从表3可知，在大鼠试验中，补充DHA可重新构建大鼠的肠道菌群并使之正常化，主要表现为有益微生物（如乳酸杆菌、双歧杆菌和拟杆菌）丰度的增加，同时变形杆菌（微小未裂杆菌）和蓝细菌等的丰度减少，从而有助于缓解早期应激、社交孤立或衰老等大脑功能的相关障碍。此外，García-Ródenas等[49]认为，摄取富含DHA的饮食，可以使肠道的通透性正常化，进而减轻心理压力，但该路径未表明需要重新构建肠道菌群。这种差异表明，肠-脑轴机制包含多种双向信息通道，其中一些通道尚未被完全探明，因此需要更多的研究来解释DHA饮食作用于肠道微生物并影响大脑功能的潜在机制。另外，DHA位置分布差异化的饮食（如富含*sn*-2 DHA脂质饮食和DHA随机分布脂质饮食）对肠-脑轴的影响也有待进一步研究。

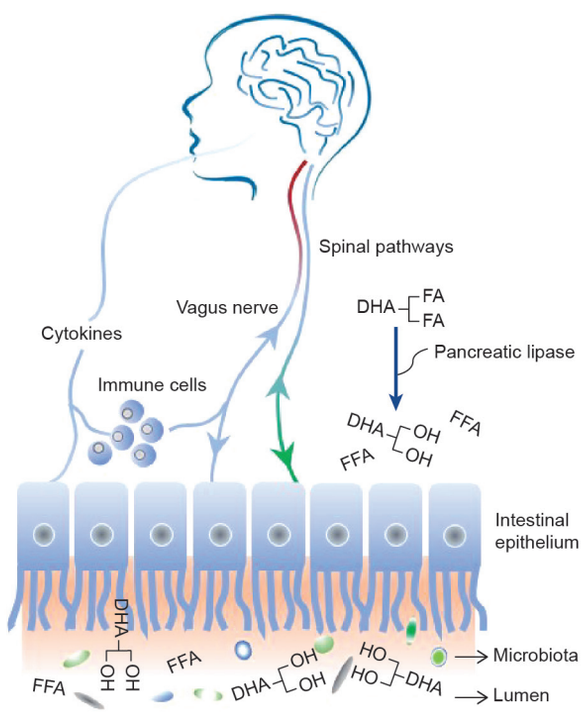

图2. 肠-脑轴：大脑和肠道菌群之间可能存在的多条双向信息通路[41,42]。

4. 酶法合成富含 *sn*-2 DHA 的油脂

许多婴儿、孕妇和哺乳期妇女仅食用DHA前体物质或含有有限DHA的食物[11]。目前DHA摄入量减少主要与西式饮食的流行密切相关[50]。因此，开发低污染和高效的油脂改性技术势在必行，如以饱和脂肪与表1中富含DHA的油脂为原料，通过酶法合成富含*sn*-2 DHA的改性油脂。这些技术主要包括酸解、酯交换、醇解及其组合反应。

4.1. 酸解反应

许多制备*sn*-2 DHA SL的方法是将单细胞油脂[如来自*Crypthecodinium cohnii*（DHASCO）的DHA单细胞藻油]和FA（如辛酸）混合，进行一步酸解反应。其中，所使用的酶主要是*sn*-1,3位特异性脂肪酶或对DHA具有高活性的酶。

根据表4所列举的酸解反应，最优条件的料液比为1:3~1:18（油:FFA）、反应温度为30~55 ℃、载酶量为4%~15%、时间一般为几十个小时[51–54]。*sn*-2 DHA的

表3 肠黏膜吸收的DHA通过肠-脑轴提高大脑功能

Treatments	Findings	Reference
Early-life stressed female rats were fed with DHA and EPA supplementation of 0.4 or 1.0 g·kg^{-1} daily for 17 weeks, and their fecal pellets were collected for microbiota analysis	High-dose DHA and EPA supplementation restored and normalized the gut microbiota composition of stressed rats. Levels of *Butyrivibrio* and several members of *Actinobacteria* were elevated, with a concomitant reduction of some *Proteobacteria*	[45]
Newborn male mice were fed with DHA and EPA diets for 13 weeks. Their social, depressive, and cognitive behaviors were tested, and fecal microbiota compositions were analyzed	The supplementation improved the neurodevelopment of the mice, with increases in beneficial *Bifidobacterium* and *Lactobacillus* in their gut. In contrast, DHA- and EPA-deficient mice showed social and emotional problems with an increased *Firmicutes*: *Bacteroidetes* ratio	[46]
Socially isolated male and female mice were supplemented with 0.1% or 1.0% by weight DHA. Their fecal pellets were collected for microbiota analysis at 0, 1, and 7 day(s) following the introduction of DHA supplementation	DHA intervention produced beneficial effects on anxiety in male mice, which were correlated with changes in gut microbiota relative abundances, e.g., an increase in *Allobaculum* abundance, which could decrease anxiety- and anhedonia-like behaviors	[47]
Aging mice received tuna oil and/or algal oil for 12 weeks. Their brain biochemical indices and fecal samples were evaluated	DHA-rich diets alleviated age-related decline in cognition by enriching the abundance of *Bacteroides*, *Tannerella*, *Coprobacter*, *Lactobacillus*, and *Prevotella*, and by decreasing the abundance of *Falsiporphyromonas* and *Cyanobacteria*	[48]
Early-life stressed male rat pups were fed with 2 g·100 g^{-1} diets containing DHA or ARA, along with other components	The adapted diets reverted the negative imprinting of neonatal stress by normalizing intestinal permeability, and further restore the relevant growth rate	[49]

EPA: eicosapentaenoic acid; ARA: arachidonic acid.

含量受脂肪酶种类的显著影响[62]。在一些研究中，脂肪酶，如*Pseudomonas* sp. KWI-56对甘油的三个结合位点无特异性，但对DHA和二十二碳五烯酸却具有较高活性，因此该脂肪酶可以解离*sn*-2位上的DHA，从而在一定程度上导致酰基转移[52]。这种副反应在辛酸和不同脂肪酶存在时尤其容易发生[63]。开发更好的脂肪酶是尽可能减少酰基转移发生最好的方法。此外，从反应产物中提纯目标SL一直以来都较为复杂。对于小规模反应，反应产物中的FFA可以通过碱液中和被去除，TAG则可通过正己烷萃取获得，然后通过进一步蒸发溶剂就可以获得最终产物SL。

另外一条典型的制备*sn*-2 DHA SL的技术路径是，首先水解单细胞油脂或海洋鱼油，以获得DHA，然后再将DHA酯化成TAG（表4）。在这条技术路径中，首先在添加有抗氧化剂（如丁基化羟基甲苯）的体系中，通过氢氧化钾皂化和盐酸酸化海洋油脂获得DHA，随后DHA与其他油脂酯化成目标SL，具体反应条件是料液比为1:5~1:18（油:DHA）、载酶量为10%、反应温度为60~65 ℃、反应时间为24 h [25,31,55]。对于大规模试验或工业生产，多余的FFA可通过短程蒸馏法除去。

4.2. 酯交换反应

将富含DHA的油脂或DHA乙酯与FA乙酯进行酯交换是制备SL的另一条技术路径（表4）。由于这类反应涉及反应位置的特异性和DHA的空间位阻，因此对酶的种类的筛选比较严格[52]。例如，在两步法反应中，将DHA乙酯和三辛酸甘油三酯以*Alcaligenes* sp.脂肪酶作为催化剂进行非选择性反应（50 ℃，90 h）来制取DHA随机分布的油脂，随后利用Novozym 435脂肪酶作为催化剂进行*sn*-1,3位特异性酯交换（40 ℃，40 h），制得*sn*-1,3-二辛酸-2-DHA甘油三酯[56]。该反应在充氮体系中进行，多余的乙酯和三辛酸甘油三酯则通过分子蒸馏法去除。

4.3. 从 *sn*-2 DHA MAG 到 *sn*-2 DHA 甘油三酯

另一条典型的制备富含*sn*-2 DHA油脂的技术路径是，首先从海洋油脂中制取*sn*-2 DHA MAG，随后在MAG的*sn*-1,3位上接上FA（图3和表4）。

由于DHA易氧化以及酶法催化过程中的酰基转移和成本等问题，从油脂中制备*sn*-2 DHA MAG是该技术路径的关键环节[64]。传统方法是在乙醇体系中采用Novozym 435脂肪酶进行反应，这种酶在乙醇中显示出*sn*-1,3位特异性[59,60]。最近有研究报道了一种高效的制备富含*sn*-2 ω-3多不饱和脂肪酸（PUFA）MAG的方法，这种方法以*Candida antarctica*脂肪酶A作为催化剂，是一种较为经济的方法[65]。在一项同以*Candida ant-*

表4 富含*sn*-2 DHA SL的酶法合成

Substrates	Enzymes	Technical procedure	Products	Ref.
Acidolysis reactions				
DHASCO, and caprylic acid	*Pseudomonas* sp.	SL was produced by esterification of the substrates and purified using hexane	*Sn*-2 DHA level was increased from 25.9% in unmodified oil to 39.9% in SL	[51]
Single-cell oil and caprylic acid	*Pseudomonas* sp. KWI-56 lipase	Acidolysis was carried out using the substrates with more than 60 mol% lipase	SL contained 36% C-DHA/DPA-C and C-C-DHA/DPA, and the former accounted for 77%–78%	[52]
Tuna oil and caprylic acid	*Rhizopus delemar*	SL was produced by acidolysis of tuna oil with caprylic acid and FFA was neutralized with potassium hydroxide-hydroalcoholic solution	SL contained 16.2 mol% DHA, and its *sn*-2 position was occupied by 24.9 mol% DHA	[53]
Fish oil and capric acid	*Rhizomucor miehei*	Acidolysis reactions were carried out in hexane or solvent-free systems, respectively	DHA level obtained from the solvent-free system (28.3 mol%) was higher than that from the hexane system (23.5 mol%)	[54]
DHASCO, palm olein, etc.	Novozym 435	Preparation of DHA by hydrolyzing DHASCO, urea complexation, and solvent crystallization; then it was esterified with palm olein to produce SL	SL contained 17.2% DHA while 22.71% of it was incorporated at the *sn*-2 position[a]	[25]
DHASCO, tripalmitin, etc.	Lipozyme TL IM	DHA was prepared by saponification and acidification of DHASCO; then it was esterified with tripalmitin to produce SL	SL containing 4.80% *sn*-2 DHA was used in infant formula	[31]
DHASCO, olive oil, and tripalmitin	Lipozyme TL IM	FFAs were prepared by saponification of DHASCO and olive oil; SL was then produced by esterification of the mixed FFAs and tripalmitin	SL containing 1.79 mol%–2.57 mol% *sn*-2 DHA was used in infant formula	[55]
Interesterification reactions				
Ethyl DHA, ethyl caprylate, and tricapryloylglycerol	*Alcaligenes* sp., Novozym 435	SL was prepared by interesterification of ethyl DHA and tricapryloylglycerol, followed by a regioselective ester reaction with ethyl caprylate	SL contained 76.4% C-DHA-C/C-C-DHA, and 82.7% of it was *sn*-C-DHA-C	[56]
Menhaden oil and ethyl caprate	Lipozyme 435	SL was produced by interesterification of the substrates using the Taguchi method	SL contained 9.83 mol%–10.57 mol% DHA, and its *sn*-2 position was occupied by 19.53 mol%–20.79 mol% DHA	[57]
DHASCO	Lipozyme TL IM, and Novozym 435	Interesterification of DHASCO was done using mixed enzymes (weight ratio=1:1) to increase the *sn*-2 DHA percentage	*Sn*-2 DHA in SL oil was improved from 34.3 mol% to 49.7 mol%	[58]
From *sn*-2 DHA MAG to *sn*-2 DHA lipids				
Bonito oil and ethyl caprylate	Novozym 435, and Lipozyme IM	*Sn*-2 MAG was prepared by the ethanolysis of bonito oil using Novozym 435; the MAG was then mixed with ethyl caprylate to produce SL using Lipozyme IM	Proportion of SL with DHA at the *sn*-2 position to that at the *sn*-1 (3) positions was more than 50:1	[59]
Cod liver oil, tuna oil, and caprylic acid	Novozym 435, and *Rhizopus oryzae*	Novozym 435 was suitable for producing *sn*-2 MAG from fish oils, and *Rhizopus oryzae* was selected to catalyze the esterification reaction of the MAG and caprylic acid to produce SL	Purified SL contained 37.9% DHA at the *sn*-2 position	[60]
Cod liver oil and caprylic acid	Novozym 435, and *Rhizopus oryzae*	Alcoholysis of cod liver oil with Novozym 435 was used to prepare *sn*-2 MAGs, followed by incorporating the caprylic acid at the *sn*-1,3 positions of the MAGs to produce SL	Purified SL contained 38.0% DHA at its *sn*-2 position	[61]

DPA: docosapentaenoic acid; C: caprylic acid.

[a] Relative percentage of DHA at the *sn*-2 position was calculated as [*sn*-2 DHA percentage / (DHA percentage in TAG × 3)] × 100 [30].

*arctica*脂肪酶A为催化剂的研究中，*sn*-2 DHA含量为20.88%的鲽鱼油在低温（35 ℃）下反应12 h时可被转换为*sn*-2 DHA含量为65.69%的MAG；与之类似，*sn*-2 DHA含量为3.24%的藻油在低温（35 ℃）下反应12 h时可被转换为*sn*-2 DHA含量为22.20%的MAG [66]。这项研究表明，*Candida antarctica*脂肪酶A在乙醇体系中表现出无位置特异性且对ω-3 PUFA无偏好，因此该脂肪酶可较多地裂解非目标FA，从而将ω-3 PUFA（如DHA等）保留在甘油骨架上形成富含DHA的MAG [21,65,66]。

在纯化时，含有DHA的副产物，如FFA和乙酯等，可通过短程蒸馏法和分子蒸馏法回收，以备后续的重复利用[67]。这条技术路径的特点是具备较高的灵活性，并利用*sn*-2 DHA MAG作为中间体制造不同类型的油脂，如起酥油、人造黄油、涂抹脂、IFF、烘焙油脂和糖果油脂等。

5. *sn*-2 DHA 的分析技术

TAG分子中FA的立体异构性分析主要是通过安装有火焰离子检测器的气相色谱技术开展。该方法首先利用*sn*-1,3位特异性脂肪酶将TAG水解为MAG，然后通过薄层层析法将*sn*-2 MAG分离出来，并将其甲酯化后进行检测器检测[68]。其中，常用的*sn*-1,3位特异性脂肪酶是胰脂酶。然而，有研究认为胰脂酶并不能充分水解TAG分子上所有的FA，尤其是海洋鱼油中的PUFA[57]。胰脂酶水解的程度取决于FA的种类和双键的位置[69]。相比较而言，*Candida antarctica*脂肪酶B（Novozym 435或Lipozyme 435）则可以较好地水解PUFA[70,71]。尽管在很多情况下Lipozyme 435是一种非特异性脂肪酶，但在过量的乙醇体系中，它会表现出*sn*-1,3位特异性[70]。有研究分别利用Novozym 435和胰脂酶两种方法测定了鱼油中PUFA的组成，见表5。Novozym 435水解鱼油中PUFA的效率根据碳链长度与饱和度的不同而不同。例如，用胰脂酶法测得的二十碳五烯酸（EPA）含量（7.5%~10.8%）高于用Novozym 435法测得的EPA含量（6.8%~9.0%），而DHA的检测结果正好相反[71]。这说明Novozym 435相比于胰脂酶可以更好地水解DHA。

总体上，Novozym 435法需要严格的水解条件才能完全释放TAG的*sn*-1,3位上的FA，如醇油比、反应时间和温度等，否则，会因水解反应不充分而导致检测值低于C-13核磁共振（^{13}C NMR）的检测值或预测值。在一项鱼肝油试验中，由Novozym 435法得到的*sn*-2 DHA含量为69.4%，该值低于由^{13}C NMR法测得的*sn*-2 DHA含量（72.5%）；而当研究对象为金枪鱼油时，两者的*sn*-2 DHA含量的检测结果接近，其中Novozym 435法测得的值为53.1%，^{13}C NMR法测得的值为52.0% [72]。

6. 结论

海洋鱼油和藻油是典型的DHA来源油脂，其中约一半的DHA FA结合在*sn*-2位上。相比于分布在*sn*-1,3位上的DHA油脂，*sn*-2 DHA脂质这种独特的结构可促使DHA更易被肠黏膜所吸收，并被用于体内TAG或PL的重新合成。因此，*sn*-2 DHA脂质在大脑功能发育和缓解焦虑、压力、认知能力下降、精神分裂症和中风等脑部疾病方面起到积极的作用。研究肠-脑轴是了解DHA饮食对大脑功能有益影响的最有效策略。该机制认为，通过DHA饮食的干预可以重新构建或正常化肠道菌群，从而解决与大脑功能相关的问题。然而，肠-脑轴包含的诸多双向信息通道尚未被完全研究清楚。今后我们还需要进一步研究*sn*-2 DHA脂质补充对肠道微生物和大

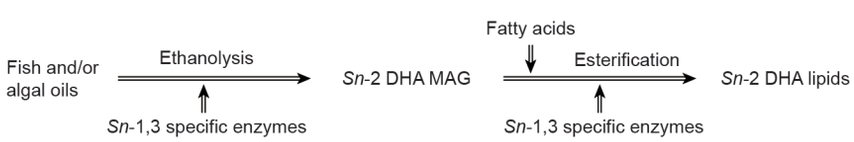

图3. 典型的*sn*-2 DHA SL制备路径。

表5 鱼油中*sn*-2 PUFA组成的测定（胰脂酶法和Novozym 435法）[71]

Methods	Cod liver oil		Tuna oil	
	EPA (%)	DHA (%)	EPA (%)	DHA (%)
Pancreatic lipase	10.8	23.4	7.5	27.1
Novozym 435	9.0	30.1	6.8	35.9

脑功能的影响。

人类在两岁以前，大脑中的DHA含量积累迅速。虽然HMF中DHA的含量在婴儿出生15天后已降至较低水平，但*sn*-2 DHA含量却呈增长趋势，这也说明*sn*-2 DHA在婴幼儿和儿童大脑发育中的重要性。因此，我们建议在日常饮食中摄入富含*sn*-2 DHA的SL。这类SL的制备可首先通过将富含DHA的油脂水解为*sn*-2 DHA MAG，再在*sn*-1,3位上结合所需的FA。在未来的研究中，我们应开发高*sn*-1,3位活性或对ω-3 PUFA没有偏好的脂肪酶，从而在温和的反应条件和纯化技术下提高合成的效率和经济性。

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Compliance with ethics guidelines

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Nomenclature

α-LNA: α-linolenic acid
ARA: arachidonic acid
DAG: diacylglycerol
DHA: docosahexaenoic acid
DPA: docosapentaenoic acid
EPA: eicosapentaenoic acid
FA: fatty acid
FFA: free fatty acid
HMF: human milk fat
IFF: infant formula fat
MAG: monoacylglycerol
NMR: nuclear magnetic resonance
PC: phosphatidylcholine
PUFA: polyunsaturated fatty acid
SL: structured lipid
TAG: triacylglycero

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