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Contents of linoleic acid, α-linolenic acid and their ratio in human milk, influence factors, and effects on infant health

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ABSTRACT

Both linoleic acid (18:2 *n*-6, LA) and α-linolenic acid (18:3 *n*-3, ALA) are essential fatty acids for infants. The contents of LA and ALA, and their ratio exhibited significant changes in human milk over the past 4 decades, which were not well summarized. Here, we summarized these values in 9 898 human breast milk samples of 6 664 mothers from 50 countries in 81 studies. A literature search was conducted using PubMed, Embase, and Web of Science between January 1980 and October 2023. The 95% confidence interval of LA/ALA ratio across lactation and gestation ranged from 14.24 to 31.26. The LA content was higher in China and Turkey (> 20%) whereas the ALA content was below 1% in Africa. The LA/ALA ratio in countries along the Mediterranean coast exceeded 20 or even 30. LA and ALA contents increased significantly ($P < 0.01$) while the ratio remained stable over the last 40 years. Multivariate meta-regression results showed that regions significantly ($P < 0.01$) determined the LA, ALA, and LA/ALA ratio. Especially, maternal diet could definitely explain the variation while the effects of gestational age, lactation period was not significant. Clinical trials demonstrated that decreasing the LA/ALA ratio increased docosahexaenoic acid (22:6 *n*-3, DHA) status, reduced arachidonic acid (20:4 *n*-6, AA) contents, exerted no effect on the visual function of infants, and reached no consensus on growth. The current review aims to provide an overview on the LA and ALA contents and their ratio in human breast milk to raise concern in infant formula.

1. Introduction

Human milk is the optimal diet for infants during the first 6 months and provides the appropriate ratio of nutritional components for infants with immature digestive abilities[1]. Lipids constitute a large fraction of human milk macronutrients and provide approximately 50% of the total energy for infants[2]. Linoleic acid (18:2 *n*-6, LA) and α-linolenic acid (18:3 *n*-3, ALA) are essential fatty acids in human milk, accounting for 5%–30% and 0.3%–2% of the total fatty acid, respectively[3].

LA and ALA compete with the same enzyme system (Fig. 1), and the ratio of LA/ALA determines the balance between arachidonic acid (20:4 *n*-6, AA) and docosahexaenoic acid (22:6 *n*-3, DHA)[4]. Moreover, LA and ALA are precursors of eicosanoids which are important signal molecules in the immune system and in adipose tissue development[3]. Lower LA/ALA ratio, improved the weight and length of term infants at 12 month, as well as retinal function of preterm infants[5]. Some authoritative organizations advocated that the LA/ALA ratio of 5–15 in infant formula had the best effect on infant development since the 1990s[6]. However, many questioned this opinion due to

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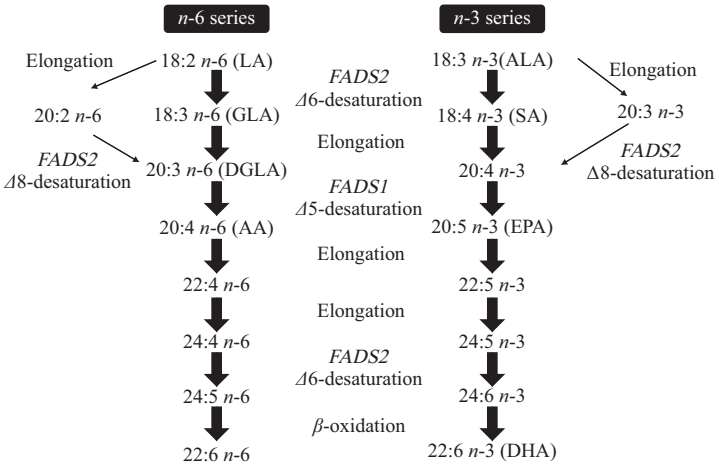

Fig. 1 Schematic representation of LA and ALA metabolism in human. GLA, γ-linolenic acid; DGLA, dihomo-γ-linolenic acid; SA, stearidonic acid; EPA, eicosapentaenoic acid.

the limited sample size and lack of tracking reports[7-8]. Meanwhile, the latest European Commission directive (2016/127/EC) increased the minimum LA content from 300 to 500 mg/100 kcal but lowered the maximum ALA content from 240 to 100 mg/100 kcal in infant formula compared to 2006/141/EC, which may influence the LA/ALA ratio[9-10].

Human milk remains as the initial guidance on infant formula composition. Along with the advance of capillary gas chromatography columns, more evidence of human milk fatty acid composition is presented. Over the past four decades, LA and ALA contents varied from 7.9% to 29.3% and 0.22% to 3.06%, respectively, which may contribute to a significant change in the LA/ALA ratio in human milk in different populations[11]. The LA/ALA ratio changed from 6.3–61.0 (1980–1995) to 8.5–21.7 (after 1995)[12]. However, the recent LA/ALA ratio was not well summarized. Moreover, the intertwined influence factors, such as diet, lactation stage, and maternal health conditions, could modify the ratio in human milk[13]. For example, the LA/ALA ratio in human milk was significantly lower in the rapeseed oil group than olive oil group and significantly lower in mature milk[14]. It seemed that obese mothers contained a higher LA/ALA ratio in their milk[15].

Therefore, it's necessary to investigate the contents of LA and ALA and their ratio in human milk in the last 40 years. Moreover, papers about their influence factors were reviewed and clinical trials about the effect of different LA/ALA ratios on infants were summarized. The current review aimed to provide an overview of the LA/ALA ratio in human milk to raise concerns about this ratio in infant formula.

2. Methods

2.1 Inclusion and exclusion criteria

A literature search was conducted using PubMed, Embase, and Web of Science between January 1980 and October 2023. The PICO was as follows: Population (P) is lactating mothers; there was no intervention (I); there was no comparison (C) in the present review; outcome (O), fatty acid composition. The following subject headings and terms were used: ("human milk" or "breast milk" or "mother milk" or "women milk" or "maternal milk" or "breast feeding") and ("fatty acid") and ("composition" or "constitution" or "structure" or "profile") (Table S1). A time limit was applied due to the significant changes in human milk composition in the last 40 years. All the mothers in the studies were healthy and consumed free diets. Data from mothers with specific diets or fatty acid dietary supplements were excluded. Mothers with health conditions (e.g., postpartum hypertension, diabetes), smoking, and alcoholism were excluded. Pooled milk, donated milk, and milk with no specific information about lactation or gestational stage were also excluded. Literature that did not involve the use of capillary gas chromatography was eliminated. The relative content has small variability and statistical advantages[16]; thus, literatures that did not express fatty acid composition in relative content were excluded, as well as literature focusing only on limited fatty acid types not including LA and ALA.

2.2 Data extraction

Two investigators extracted the data independently using a data collection table, including the first author's name, year of sample collection, country, sample size, gestational age, lactation days, LA, ALA, and LA/ALA ratio. When the year of sample collection was not available, the publication year was used. A lactation period of no more than 6 months was applied. If the author failed to classify the lactation stages, the stages were defined as colostrum (1–7 days), transitional (8–15 days), or mature milk (> 15 days). Term milk was defined as the gestational stage of 37–42 weeks and preterm milk as < 37 weeks. When the literature only reported the numbers of mothers not including human milk samples, we assumed one mother donated one milk sample during each lactation stage[11]. Because partial literature failed to publish the ratio, we obtained some of the unpublished data from friendly investigators[17-18]. Some investigators also shared the ratio friendly, however, the data was excluded due to no specific gestational age and lack of standard deviation (SD) value[19-23].

2.3 Statistical analyses

When the data in literature were reported as median (P25–P75), median (IQR), median (range), and mean (95% confidence interval (CI)), we standardized to mean ± SD for further analysis

using an online tool (https://www.math.hkbu.edu.hk/~tongt/papers/median2mean.html) according to previous studies[24-25]. If the data were expressed as mean ± SEM, the standardization was calculated using the following equation based on the previous method[26]:

$$SD = SEM \times \sqrt{n} \quad (1)$$

Where n represents the numbers of human milk samples.

The estimated pooled mean and 95% CI of LA, ALA, and their ratio in term milk and preterm milk across the lactation stages were calculated using the STATA software version 16.0 (STATA, College Station, TX). The inverse-variance weighting model was used. Heterogeneity was evaluated using the I^2 and τ^2 parameters. Due to the high heterogeneity ($I^2 \geq 50\%$), the random effects model was applied to calculate the pooled mean values and 95% CI.

3. Results

A study flow diagram of the included and excluded studies is presented in Fig. 2. A total of 81 articles met the requirements (Table S2). Human milk in the different lactation stages (colostrum, transitional milk, and mature milk) of 6 664 mothers (5 675 mothers with term infants and 989 mothers with preterm infants) from 50 countries were evaluated, and the contents of LA and ALA and their ratio were recorded. A total of 9 898 human milk samples (8 206 term milk and 1 692 preterm milk) were collected. The variations in LA and ALA contents, and their ratio across regions, year of sample collection, gestation, and lactation were explored.

3.1 Determinants of LA, ALA, and the ratio

The estimated pooled mean of LA, ALA, and the ratio of LA/ALA were 15.31%, 1.00%, and 18.70, respectively. Figs. S1-3, S4-6, and S7-9 displayed the forest plots of LA, ALA, and the ratio across different lactation. To explain the high heterogeneity ($I^2 > 80\%$) of studies, the associations of LA, ALA, and the ratio with regions, year of sample collection, gestation, and lactation were investigated using a multivariate meta-regression model (Table 1). The region showed significant influences on LA and ALA contents in human milk. Positive associations were

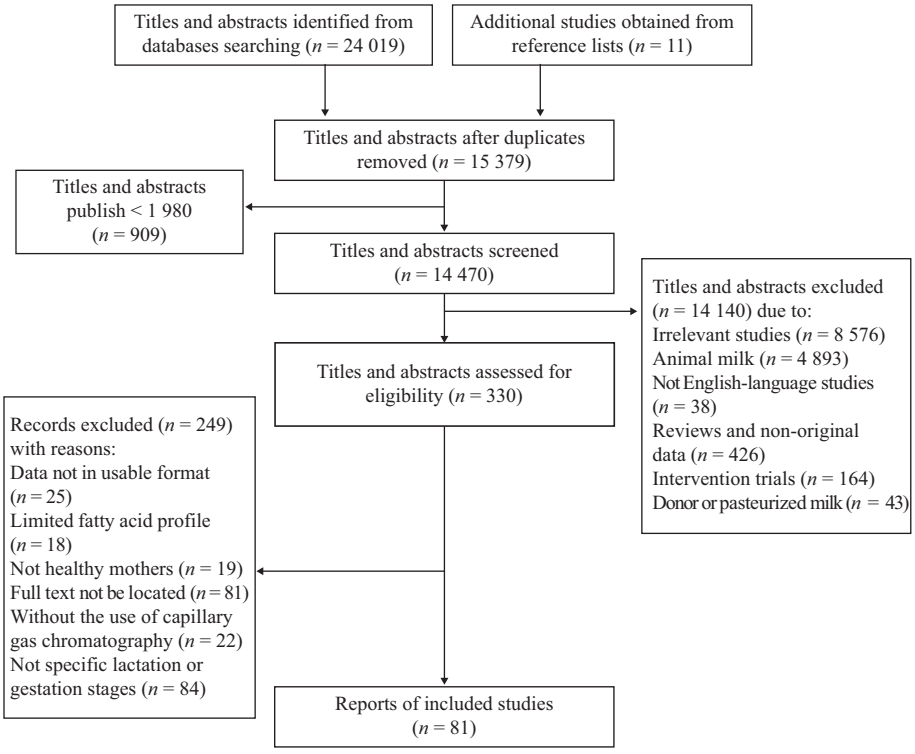

Fig. 2 Study flow diagram of included and excluded studies.

Table 1
Estimated pooled mean (95% CI) of LA, ALA and their ratio in human milk and meta-regression analysis.

Fatty acids	Pooled mean (95% CI)	P value	Heterogeneity		Coefficient, P value			
			I^2 (%)	P value	Region	Year	Gestation	Lactation
LA	15.31% (14.57%, 16.06%)	< 0.001	84.4	< 0.001	–0.12, < 0.001	0.27, < 0.001	1.13, 0.144	–0.07, 0.894
ALA	1.00% (0.93%, 1.07%)	< 0.001	96.0	< 0.001	–0.11, < 0.001	0.01, 0.006	–0.02, 0.825	0.07, 0.118
LA/ALA	18.70 (17.19, 20.21)	< 0.001	85.9	< 0.001	0.23, 0.028	–0.05, 0.754	–1.27, 0.672	–0.74, 0.635

observed between LA and ALA contents and the year of sample collection. However, the effects of gestation and lactation on LA and ALA contents were insignificant ($P > 0.05$). LA/ALA ratio was significantly ($P = 0.028$) associated with regions. No significant influences were observed between the LA/ALA ratio and the year of sample collection, gestation, and lactation. Noteworthy, the regression coefficient of the year of sample collection towards LA was higher than ALA, which may provide some clues about the change in the LA/ALA ratio of 81 studies over the 40 years.

3.2 Variations of LA, ALA, and the ratio across regions

The mean value of LA, ALA, and LA/ALA ratios around the world really differed. As shown in Table 2, the LA contents in human milk ranged from 7.90% to 22.01%. the LA contents in Northern Europe, Southeast Asia, and Australia were below 10%. However, human milk from most countries in America contained 15%–20% LA in total lipids. Especially, the LA contents in China and Turkey were more than 20%.

Table 2
The estimated pooled mean of LA, ALA, and the ratio of LA to ALA in human milk across countries.

Countries	LA			ALA			LA/ALA ratio		
	k	*n*	Mean (95% CI)	*k*	*n*	Mean (95% CI)	*k*	*n*	Mean (95% CI)
Argentina	1	21	16.61% (16.16%–17.06%)	1	21	0.47% (0.45%–0.49%)	1	48	12.85 (12.68–13.02)
Australia	3	518	9.66% (8.74%–10.58%)	3	518	0.80% (0.69%–0.91%)			
Bolivia	1	35	10.23% (8.73%–11.73%)	1	35	1.90% (1.62%–2.18%)			
Brazil	5	230	18.92% (17.68%–20.16%)	5	230	1.06% (0.85%–1.28%)	1	80	15.30 (14.50–16.10)
Cambodia	1	67	9.05% (8.57%–9.53%)	1	67	0.44% (0.41%–0.47%)			
Canada	2	241	12.23% (10.74%–13.72%)	2	241	1.32% (0.92%–1.73%)	1	48	9.89 (9.76–10.02)
Chile	1	50	17.75% (17.59%–17.91%)	1	50	1.14% (1.12%–1.26%)	1	50	17.85 (17.59–18.11)
China	22	3 425	22.01% (20.95%–23.08%)	22	3 425	1.42% (1.34%–1.54%)	13	2 164	17.07 (15.34–18.80)
Croatia	1	83	17.28% (16.90%–17.66%)	1	83	1.41% (1.39%–1.43%)	1	83	12.24 (12.10–12.38)
Cuba	1	52	19.37% (18.17%–20.57%)	1	52	0.92% (0.86%–0.99%)	1	52	22.73 (20.23–25.23)
Dominica	1	7	13.52% (10.82%–16.22%)	1	7	1.06% (0.84%–1.28%)			
Egypt	1	45	16.90% (12.80%–21.00%)	1	45	0.74% (0.30%–1.18%)	1	45	24.50 (22.65–26.36)
Finland	2	113	9.34% (8.68%–10.00%)	2	113	0.99% (0.81%–1.16%)			
France	2	58	11.34% (10.38%–12.30%)	2	58	0.61% (0.45%–0.76%)	2	58	19.80 (16.13–23.48)
Germany	2	229	9.62% (7.59%–11.66%)	2	229	0.75% (0.69%–0.81%)			
Greece	1	127	14.98% (14.29%–15.68%)	1	127	0.12% (0.05%–0.18%)	1	127	51.80 (42.58–61.03)
India	2	255	13.87% (11.52%–16.21%)	2	255	0.99% (0.21%–1.76%)			
Iran	1	52	13.80% (12.70%–14.90%)	1	52	1.10% (0.99%–1.21%)			
Iraq	1	64	10.80% (10.55%–11.04%)	1	64	0.61% (0.57%–0.66%)			
Israel	2	70	17.88% (15.83%–19.93%)	2	70	1.67% (1.32%–2.01%)			
Italy	3	470	11.76% (10.53%–12.98%)	3	470	0.54% (0.42%–0.66%)	1	204	37.03 (32.17–41.90)
Japan	3	124	13.79% (11.67%–15.91%)	3	124	1.42% (1.02%–1.81%)	1	51	9.94 (9.87–10.01)
Kuwait	1	19	17.44% (15.44%–19.44%)	1	19	0.37% (0.32%–0.42%)	1	19	46.83 (45.83–47.83)
Malaysia	2	161	9.46% (8.93%–9.98%)	2	161	0.34% (0.29%–0.40%)			
Mauritius	1	26	25.23% (22.19%–28.28%)	1	26	2.27% (1.56%–2.97%)	1	26	11.80 (10.02–13.59)
Mexico	1	46	16.05% (15.90%–16.20%)	1	46	1.05% (1.03%–1.07%)	1	46	16.91 (16.66–17.16)
Netherlands	2	309	12.92% (10.32%–15.51%)	2	309	0.93% (0.77%–1.09%)			
Nigeria	1	20	15.57% (15.50%–15.65%)	1	20	0.93% (0.92%–0.94%)			
North Sudan	1	60	13.65% (12.20%–15.11%)	1	60	0.17% (0.15%–0.20%)			
Pakistan	1	97	9.06% (8.54%–9.58%)	1	97	0.43% (0.37%–0.49%)			
Panama	1	22	18.56% (16.63%–20.48%)	1	22	1.52% (1.26%–1.77%)			
Philippines	1	54	7.90% (7.84%–7.96%)	1	54	0.43% (0.42%–0.44%)	1	54	19.51 (19.37–19.65)
Poland	2	92	12.41% (9.88%–14.94%)	2	92	1.42% (1.29%–1.56%)	1	32	8.10 (7.27–8.93)
Portugal	1	155	16.17% (15.56%–16.78%)	1	155	0.66% (0.62%–0.71%)	1	155	25.45 (23.70–27.20)
Singapore	1	50	15.15% (13.75%–16.55%)	1	50	1.12% (0.92%–1.32%)			
South Korea	3	546	15.18% (14.37%–15.98%)	3	546	1.54% (1.25%–1.82%)			
South Sudan	1	63	14.42% (13.53%–15.30%)	1	63	0.26% (0.22%–0.31%)			
Spain	6	412	15.19% (14.38%–16.00%)	6	412	0.58% (0.51%–0.65%)	2	249	33.43 (28.89–37.97)
Sweden	5	803	9.85% (9.36%–10.35%)	5	803	1.13% (0.94%–1.32%)	1	57	8.37 (7.52–9.22)
Tanzania	1	133	13.90% (12.89%–14.91%)	1	133	0.52% (0.48%–0.56%)			
Turkey	1	140	21.09% (18.54%–23.63%)	1	140	1.14% (0.80%–1.49%)			
UK	1	44	10.45% (10.33%–10.57%)	1	44	1.22% (1.20%–1.24%)	1	44	8.95 (8.84–9.06)
USA	4	218	15.86% (14.15%–17.58%)	4	218	1.14% (0.95%–1.33%)	1	49	15.44 (15.25–15.63)
Vietnam	1	92	17.09% (15.19%–18.99%)	1	92	1.17% (0.95%–1.39%)			

Note: *k*, number of studies; *n*, number of human milk samples.

The ALA contents varied from 0.12% to 1.90%. Specifically, the ALA contents were over 1% in most countries in America, except for Argentina. Human milk from African mothers contained less than 1% of ALA contents. In the East Asian region, including China, South Korea, and Japan, ALA showed relatively high contents (> 1%) in human milk.

As to the LA/ALA ratio, among the mothers from countries along the Mediterranean coast, such as Spain, Italy, Greece, Portugal, and Egypt, the LA/ALA ratio in their milk exceeded 20 or even 30. The milk of mothers from the Inner Mongolia province of China had the highest ALA content[27]. According to the unpublished data, the LA/ALA ratio in Inner Mongolia of China was 4.25–7.31[19]. The present studies reported the LA/ALA ratio in 22 countries whereas human milk from 36.36% of countries met the current standard of LA/ALA ratio (5–15).

3.3 Variations of LA, ALA, and the ratio across year of sample collection, lactation, and gestation

Meta-regression analysis on the association between LA, ALA, and LA/ALA ratio and year of sample collection was shown in Fig. 3. LA contents in human milk have increased significantly ($P < 0.001$) in the last 40 years. Meanwhile, ALA contents also showed a growing trend from the 1980s ($P = 0.002$). However, only 26 of the studies provide the LA/ALA ratio in human milk. The present data showed no obvious change ($P = 0.065$) over the 40 years.

The estimated pooled mean and 95% CI of LA, ALA, and their ratio across gestation and lactation are shown in Table 3. No significant differences were observed in the LA and ALA contents between term and preterm milk as well as among the different lactation stages. The mean LA content was about 15% in total lipid whereas the ALA content was 1%. The 95% CI of the estimated pooled mean of the LA/ALA ratio ranged from 14.24 to 31.26.

4. Discussion

The present study investigated LA, ALA, and the LA/ALA ratio in human milk worldwide and explored the influences of regions, year of sample collection, gestation, and lactation on human milk composition. LA, ALA, and the ratio showed a wide range in human milk and meta-regression showed that regions and year of sample collection significantly determined the contents in human milk.

A systematic review in 2005 indicated that LA and ALA contents showed insignificant differences between preterm and term milk[28]. Using 4 374 term milk and 1 017 preterm milk samples, Floris et al.[29] found that LA and ALA remained stable over time. In agreement with these previous studies, the present study also found insignificant changes in LA and ALA across lactation and gestation. Sun et al.[30] evaluated 21 studies about Chinese human milk fatty acid composition and found that LA and ALA contents varied in north and south of China. The meta-regression analysis revealed that LA and ALA contents increased throughout the year[31]. This paper conducted a comprehensive review of LA, ALA contents in human milk and the association between these values and region, year, gestation, and lactation. Especially, this paper focused on the ratio of LA/ALA worldwide which was not well summarized in previous studies.

Regions differed in dietary patterns, genes, sociodemographics, and other factors, contributing to the variation of human milk fatty acids[13]. The three main sources of fatty acid in human milk are diet, mammary gland synthesis, and tissue metabolism[32]. As essential fatty acids in human milk, LA and ALA cannot be synthesized in the human body but are rather obtained only through diet. Therefore, maternal diet becomes a principal factor influencing the LA/ALA ratio. The contents of LA and ALA and their ratio in milk respond to their quantities in the maternal diet.

Food frequency questionnaire (FFQ) and dietary intervention were common tools to estimate the relationship between food intake

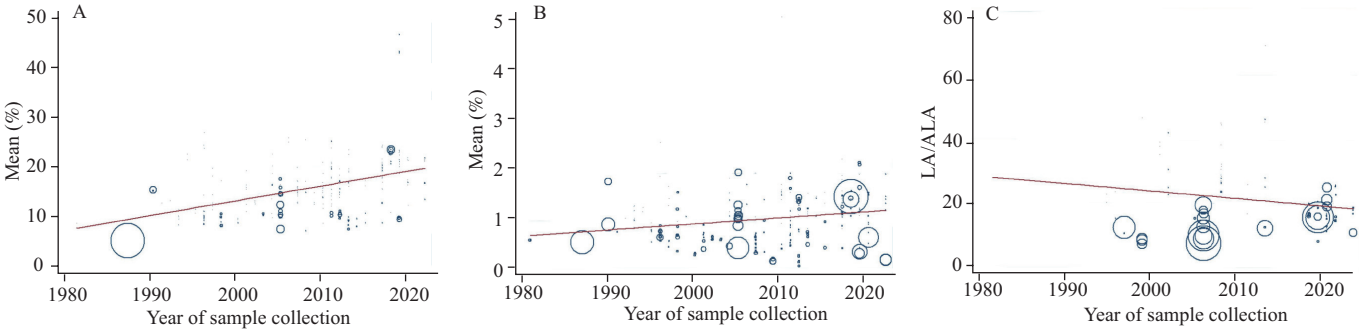

Fig. 3 Meta-regression plots of the association between (A) LA, (B) ALA , and (C) LA/ALA ratio and year of sample collection.

Table 3
Estimated pooled mean and 95% CI of LA, ALA, and their ratio in term and preterm milk across lactational stages.

Fatty acids	Gestation	Colostrum			Transitional milk			Mature milk		
		k	*n*	Mean (95% CI)	*k*	*n*	Mean (95% CI)	*k*	*n*	Mean (95% CI)
LA	Term	37	1 597	16.61% (14.26%–18.97%)	24	1 005	16.64% (13.66%–19.62%)	67	5 604	16.00% (14.81%–17.19%)
	Preterm	14	526	15.24% (13.54%–16.94%)	9	484	14.74% (12.81%–16.67%)	13	682	14.75% (13.35%–16.15%)
ALA	Term	37	1 597	0.94% (0.77%–1.11%)	24	1 005	1.02% (0.76%–1.28%)	67	5 604	1.12% (1.01%–1.22%)
	Preterm	14	526	0.90% (0.73%–1.06%)	9	484	1.00% (0.80%–1.20%)	13	682	1.13% (1.00%–1.26%)
LA/ALA	Term	16	793	21.81 (19.10–24.52)	13	680	21.10 (19.46–22.75)	26	2 148	18.79 (17.45–20.13)
	Preterm	2	50	24.65 (18.03–31.26)	2	50	20.98 (15.05–26.91)	2	50	18.85 (14.24–23.45)

and human milk composition. Using a semiquantitative FFQ, it was found that LA and ALA in human milk were positively correlated with their corresponding dietary contents during pregnancy[33]. Interestingly, LA content in human milk was negatively associated with the consumption of fruits[34]. Linseed oil, coconut oil, mammalian milk, and fermented dairy products were reported to be positively correlated with the ALA in human milk[35]. On the other hand, seafood intake was negatively correlated with LA/ALA ratio[21]. Interestingly, the milk of mothers following vegetarian diets had higher ALA content than those following vegetarian and omnivore diets[36]. The DHA supplementation could increase the human milk ALA content of Mexican mothers, which could be due to reduced ALA to DHA conversion[37].

Especially, some vegetable oils, abundant in LA and ALA, become a major dietary factor affecting human milk LA and ALA contents. For example, LA content was significantly higher in human milk from mothers consuming high oleic acid and LA oil[14]. It was reported that lower human milk LA/ALA ratios in the Inner Mongolia province of China were related to the consumption of linseed oil[19]. The abundant amounts of human milk LA and ALA in India could be explained by the consumption of mustard oil[38]. The Brazilian mothers who consumed soybean oil had high LA content in their milk[39]. The ALA content in human milk increased after Chia oil supplementation[40]. Flaxseed oil (20 g/day) could enhance ALA content in milk from 1.0% to 7.7%[41].

Regional differences affect dietary habits which may change human milk composition. A higher LA/ALA ratio (> 20) in Spain could be explained by the Mediterranean diet rich in LA[22]. Latin countries, such as Mexico and Chile, were characterized by a high-maize diet rich in LA, which led to higher LA content in milk[42]. The African mothers followed a high-carbohydrate/low-fat diet and often consumed cotton oil and peanut oil, which could explain the high LA content in their milk[43-44]. Generally, abundant evidences proved the significant influence of diet on the contents of LA and ALA and their ratio in human milk.

The LA and ALA contents in human milk changed along with the change in dietary fatty acid composition. For example, the total polyunsaturated fatty acid (PUFA) intake in America increased from 2.5% to 7.5% of the total energy in the period of 1940–1985, whereas the LA content in the mature milk of American mothers increased from 6% to 15% in the period of 1944–1990[12]. The LA/ALA ratio in the milk of mothers from Spain increased from 19.1 in 1985 to 27.5 in 1998[45-46], indicating the change in the dietary pattern from a classical Mediterranean diet rich in monounsaturated fatty acid (MUFA) to a more Western diet with a higher intake of LA-rich seed oil[47]. In the period of 1993–2001, ALA consumption was two to three times lower than the recommended value, and then at the beginning of the 21st century, France suggested increasing the dietary ALA content; thus, the LA/ALA ratio decreased from 24 (1993–1998) to 13.5 (2007) and then to 11.75 (2014)[48-49].

An incomplete gestational age can modify the maturity of the mammary gland, thereby altering the composition of human milk[50]. Moreover, preterm infants are characterized by lower birth weight and faster growing speed which means that they have higher nutrition quality than term infants. However, this study found that gestation showed no significant influences on LA, ALA, and the ratio. Floris et al.[11] explored 55 studies worldwide and also found that the LA and ALA contents in term human milk were comparable to those in preterm human milk. Other fatty acids, such as DHA, were found higher in preterm human milk in a previous systematic review[28]. Meanwhile, LA, ALA, and the ratio showed no significant changes over the prolong of lactation period. Two previous systematic reviews found that LA and ALA contents remained stable over time[11,31].

There is a high heterogeneity between studies, however, only regions, year, gestation, and lactation were taken into consideration. Some confounding factors, such as maternal nutritional status during the stages of pre-pregnancy, pregnancy, and lactation, could also determine the fatty acid compositions in human milk. Spanish mothers with obesity (body mass index (BMI) > 30, n = 17) during pre-pregnancy showed lower ALA content and higher LA/ALA ratio in their milk[15]. Contrarily, the pre-pregnancy BMI of Greek mothers was positively correlated with the ALA content in milk[51]. However, some found no statistically significant differences in the LA and ALA contents between different pre-pregnancy weight groups[44]. A positive correlation was observed between BMI and LA/ALA ratio throughout the pregnancy stage[52]. Storck Lindholm et al.[53] found that Swedish mothers with obesity (BMI > 30, n = 41) had lower ALA content in their human milk than those with normal body weight (BMI < 25, n = 41).

Moreover, higher LA content and LA/ALA ratio were observed in human milk in obese mothers (BMI > 30, n = 9) from Argentina compared with those with normal weight (BMI < 25, n = 21) during lactation[54]. Generally, the available literature is not entirely consistent which could be attributed to several factors, such as the weight group classified according to the BMI criterion, timing of BMI measurement, sample size, analysis method, and dietary and genetic differences among the population.

In Italy, the LA content was significantly higher in the milk of younger mothers than that of older ones[55]. The LA/ALA ratio was significantly lower in the milk of mothers with a very low socioeconomic status[45]. Smoking could significantly reduce the LA/ALA ratio in human milk[56]. During the feed progress, ALA content was higher in mid-milk and hind-milk than in fore-milk, whereas the LA content remained constant within the feed[57].

5. Effects of the LA/ALA ratio on infant health

The present study investigated the influence factors of LA, ALA, and the LA/ALA ratio, including maternal diet, lactation, gestation, and other factors, such as maternal weight, age, and smoking. The variation of LA, ALA, and the ratio determined the health effect on infants (Fig. 4). For example, insufficient ALA led to lower levels of DHA in the brain and retina which influenced visual function in infants[58]. LA deficiency increases the permeability of the skin to water, resulting in water metabolism disorders and skin dryness[59]. High LA may affect neurodevelopment and increase the risk of obesity, atopic eczema, and allergic responses[3].

Previous research studied the effect of LA and ALA on the growth and development of infants in 2005 and found that ALA supplementation improved the DHA status of infants[5]. Based on these findings, we summarized the effect of different LA/ALA ratios on infants and adults (Table 4). Regarding the experiment design, five of the six studies were randomized controlled trials, the remaining one was a multicenter prospective cohort study, and six trials were on infants. Regarding the study location, one was conducted in Korea, one in the

USA, one in Germany, two in Australia, and one in France. These clinical trials estimated the fatty acid status, growth, visual function, and neurobehavioral development of infants.

(i) Fatty acid status. The dietary LA/ALA ratio of infants restricts the synthesis ability of DHA and AA. When the ratio was decreased from 55 to 2.2, the total *n*-3 fatty acids increased, the *n*-6/*n*-3 fatty acid ratios decreased, and the ALA concentration showed no change in the plasma[60]. When the ratio was decreased from 44 to 4.8, the plasma phospholipid DHA content increased, but the AA content decreased at 21, 60, and 120 days of age[7]. When the ratio was decreased from 22 to 6, the ALA supplementation exerted a minimal effect on *n*-6 fatty acid but did not significantly affect the AA concentrations in preterm infants after 2 and 15 days of enteral feeding and at the age of 37 week[61]. When the ratio was decreased from 19 to 4, the concentrations of DHA, *n*-3 C_{20}, and C_{22} fatty acids in the erythrocytes increased, but the AA concentration decreased[65]. When the ratio was decreased from 10 to 5, the DHA concentration in the plasma and erythrocyte phospholipids of infants increased,

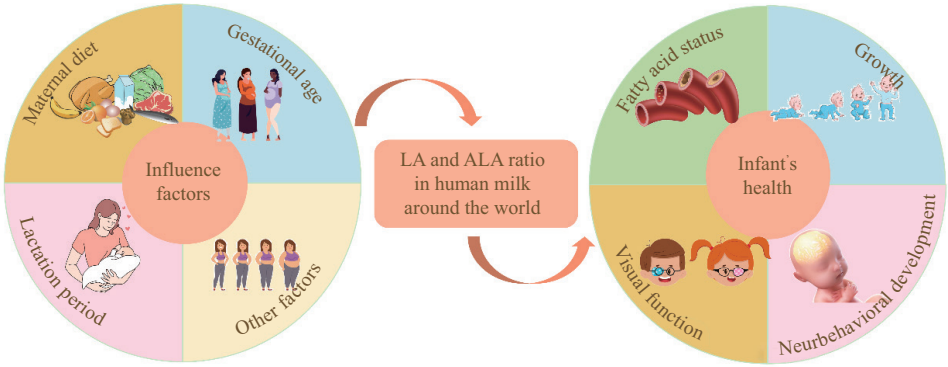

Fig. 4 The overview of influence factors of LA/ALA ratio and the health effect of the ratio on infants.

Table 4
Summary of the main characteristics and results for the effect of different ratios of LA to ALA.

Clinical trials	Ratio of LA/ALA	Participants	Design	Main outcomes
Randomized, controlled double blinded, intervention trial in Germany (2009)[60]	55, 2.2	Infants (2 months), *n* = 102	From 4 to 10 months, the control group received commercial meals (LA/ALA = 55); the intervention group received the same meals adding rapeseed oil (LA/ALA = 2.2)	The total *n*-3 fatty acids and of *n*-3 long-chain (LC)-PUFA were higher and the ratios of *n*-6/*n*-3 fatty acids were lower in the plasma of the intervention group ALA content showed no change in plasma
Randomized, double-blined study in Australia (1994–1995)[8]	10, 5	Term infants, *n* = 73	Infants received formulae with different LA/ALA ratios from near birth to 34 weeks of age	In plasma phospholipid, DHA increased from (1.9 ± 0.3)% to (2.4 ± 0.3)% while AA content remained constant when lowering ratio In erythrocyte phospholipid, DHA increased from (3.0 ± 0.3)% to (3.5 ± 0.3)% while AA decreased from (14.8 ± 0.7)% to (14.1 ± 0.7)% when lowering ratio Lowering ratio resulted in no effect on visual evoked potential acuity or growth rate
Randomized trial in France (1997)[61]	6, 22	Preterm infants, *n* = 88	Infant who was not breast-fed were assigned to a formula with a high or low LA/ ALA ratio. Infants fed with human milk were as a reference group	When formula was supplemented with a LA/ALA ratio of 6, biological values and DHA levels were near to infants fed human milk. The anthropometric parameters were not affected Formula with a LA/ALA ratio of 22 is inadequate to maintain LC-PUFA status
Randomized and masked fashion in America (1997)[7]	44, 18.2, 9.7, 4.8	Term infants (4 months), *n* = 80	Infants received one of four formulas as his or her sole source of nutrition from birth to 120 days of age	The lowest LA/ALA ratio increased plasma phospholipid DHA content and gained less body weight There was not associated with improving visual function
Randomized study in Australia (1992)[62]	19, 4, 3	Term infants, *n* = 51	Infants were randomly allocated to one of three formulas with different LA/ALA ratio (group A: 19:1, group B: 4:1, group C: 3:1) for 10 weeks.	DHA in erythrocytes was highest in group B and C ((4.78 ± 0.45)% and (4.48 ± 0.49)%) relative to group A ((3.47 ± 0.46)%) Decreasing the LA/ALA ratio increased *n*-3 C_{20} and C_{22} fatty acid incorporation Infants fed formulas with LA/ALA ratio 19, 4, 3 did not reach fatty acid levels found in breast-fed infants
Multi-center prospective cohort study in Korea (2006–2011)[63]	11.12 ± 6.9	Infants (6 months), *n* = 960	Dietary intake of pregnant women was assessed by a one-day 24-h recall method. Food consumption of infants was estimated based on the volume of breast milk and weaning foods. Infant neurodevelopment was assessed by the Korean Bayley scales	Maternal intake of LA/ALA was 11.12 ± 6.9. There was negative association between maternal intake of LA/ALA ratio and neurobehavioral outcomes of infant at 6 months of age

whereas the AA concentration showed no significant change in the plasma but marginally decreased in the erythrocyte phospholipids[8].

(ii) Growth. The rate of weight gain, length, and head circumference were not significantly different between the different ratio groups, although breast-fed infants had lower weight and length gains between 16 and 34 weeks of age[8]. This finding was consistent with the previous study, which indicated that the anthropometric parameters (crown-heel length, head circumference, and body weight) were not affected when the ratio was changed from 6 to 24[61]. Contrarily, Jensen et al.[7] found that infants with lower dietary LA/ALA ratio had significantly ($P < 0.05$) lower mean body weight at 120 days of age, however, length, fronto-occipital circumference, or skinfold thickness among the groups at any age showed no statistically significant differences.

(iii) Visual function. The alteration in dietary LA/ALA ratio exhibited no influence on the visually evoked potential acuity of infants at 16 and 34 weeks among formula or breastfed groups[8], as well as the visually evoked response latency and amplitude at 120 and 240 days of age[7].

(iiii) Neurobehavioral development. Kim et al.[63] used the Korean Bayley Scales of Infant Development second edition (BSID-II), including the mental developmental index (MDI) and the psychomotor developmental index (PDI), to evaluate the cognitive and motor development of infants at 6 months of age. A negative correlation between maternal intake of LA and ALA and neurobehavioral outcomes of infants was observed (including the MDI and the PDI).

We concluded that decreasing the LA/ALA ratio increased DHA and EPA status, reduced the AA contents, and exerted no effect on the visual function of infants. There is no consensus on the effect of different ratios on infant growth, which still needs further research. Mothers are encouraged to achieve a low dietary LA/ALA ratio for better neurobehavioral development of their infants. There is a very limited dataset from which to limit an optimal range of the LA/ALA ratio. Further studies involving more participants with different gestation age, lactation age, and ethnicity are warranted.

6. Conclusions

This paper has several strengths. First, this meta-analysis involved a large sample size from 50 countries, which provided an overview of LA, ALA, and their ratios worldwide. Second, the influences of region, year, gestation, and lactation were explored, which did not reach consensus before. Third, this study combined LA, ALA, and their ratios in human milk with influence factors and clinical trials to figure out the optimal LA/ALA ratio for infants. However, several limitations of this study should be paid attention. First, although a large quantity of samples was analyzed, only a portion of publications reported the LA/ALA ratio in human milk. Second, concerning the high heterogeneity, some confounding factors, such as maternal weight, age, and storage condition, were not collected in the meta-regression model.

In conclusion, this study reviewed the contents of LA and ALA and their ratio in human milk from 50 countries. The influences of region, year, gestation, and lactation were explored using a meta-regression model. Clinical trials demonstrated that the variations of LA, ALA, and the LA/ALA ratio determined the fatty acid status, growth, visual function, and neurobehavioral development in infants. The present review aimed to raise concerns about this ratio in infant formula.

Human milk is considered as the golden standard for infant formula, however, the "standard" human milk samples remain open. The data of LA/ALA ratio in human milk are lack which should be a focus in future studies. A challenge for verification of optimal LA/ALA ratio is the absence of randomized controlled trials to provide precious data. The trials can be set by three perspectives: LA intakes, ALA intakes, and the ratio of LA/ALA.

Conflict of interest

The authors declare that they have no known competing financial interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/FSHW.2024.9250309.

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