Contribution of dietary and newly formed arachidonic acid to human milk lipids in women eating a low-fat diet[1–3]

Martha Del Prado, Salvador Villalpando, Ariane Elizondo, Maricela Rodríguez, Hans Demmelmair, and Berthold Koletzko

ABSTRACT

Background: Polyunsaturated fatty acids in milk are derived from direct intestinal absorption, endogenous synthesis, or maternal body stores. Arachidonic acid (AA) intake is frequently low in undernourished women, but milk secretion of this fatty acid is similar to that in well-nourished women.

Objective: The objective was to evaluate the contribution of dietary and endogenously synthesized AA to its total secretion in the milk of women eating a low-fat diet.

Design: Ten lactating women who habitually ate a low-fat diet (17% of energy) received 2.5 mg [^{13}C]linoleic acid (LA)/kg body wt orally 5 mo postpartum. LA and AA concentrations and ^{13}C enrichment were measured in milk samples collected before and after the tracer application. Total lipid, LA, and AA contents were determined in diet composites. Fatty acids were assessed by gas chromatography and ^{13}C enrichment by isotope ratio mass spectrometry.

Results: The cumulative 72-h recovery of [^{13}C]LA in milk was 16.3 ± 6.4% of the dose; only 0.01% of the label was found as [^{13}C]AA. The calculated transfer of dietary LA and AA into milk was 32.8 ± 18.0% and 11.8 ± 6.6%, respectively. AA originating from conversion of dietary LA contributed only 1.1% to the total milk AA secreted.

Conclusions: Little milk AA originates from conversion of LA; 70% of LA and 90% of AA secreted in milk were not derived from direct intestinal absorption. Our results suggest that maternal body stores are the major source of milk LA and AA in these women. *Am J Clin Nutr* 2001;74:242–7.

KEY WORDS Human milk, linoleic acid, arachidonic acid, polyunsaturated fatty acids, lactation, isotope ratio mass spectrometry, Mexico, women

INTRODUCTION

The diet of rural dwellers in Mexico is derived mostly from vegetable sources and contains scarce amounts of animal foods (1). Carbohydrates provide 70% and fat only 17% of energy intakes. An analysis of diet composites from a typical rural community in Mexico showed that oleic acid (18:1n−9) represented 27.2% and linoleic acid (LA; 18:2n−6) 43% of total fat intake, respectively (2).

Dietary LA can be converted into long-chain polyunsaturated fatty acids (LCPUFAs), essential structural components of tissue membranes; thus, LA plays an important role in the rapid development of the human brain in the late fetal and early postnatal periods (3–5). During the first months of life, breast-fed infants obtain preformed LCPUFAs from milk only; therefore, an adequate supply depends on the LCPUFA content in milk.

LCPUFAs secreted in breast milk may originate directly from intestinal absorption of dietary lipids, mobilization of maternal body stores, or endogenous synthesis by maternal tissues other than the breast, with LA or α-linolenic acid (18:3n−3) as substrate (3, 6). The relative contribution of these sources to total milk arachidonic acid (AA; 20:4n−6) secretion has not been appropriately assessed.

Although the dietary intake of AA in undernourished women living in developing countries is generally low, the relative content of this fatty acid in their milk is similar to that of well-nourished women living in developed countries (2, 7–9). Thus, the secretion of n−6 LCPUFAs into milk lipids does not seem to depend solely on maternal dietary intake.

This study was designed to evaluate the contribution of the diet and the endogenous synthesis of AA to its secretion in milk in a population with a low dietary intake of AA. The working hypothesis was that women with a low dietary intake of AA but with an adequate intake of LA can maintain an adequate total secretion of AA in milk by biotransforming LA into AA.

[1] From the Unidad de Investigación Médica en Nutrición, Instituto Mexicano del Seguro Social, México City, and Klinderklinik and Kinderpoliklinik, Dr von Haunersches Kinderspital, Ludwig-Maximilians-University, Munich, Germany.

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[3] Address reprint requests to B Koletzko, Dr von Haunersches Kinderspital, Ludwig-Maximilians-University, Division Metabolic Disorders and Nutrition, Lindwurmstrasse 4, D-80336, München, Germany.

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SUBJECTS AND METHODS

Subjects

Ten healthy lactating women from the local prenatal clinic in San Mateo Capulhuac, México, were recruited at 5–6 mo postpartum from among women who met the inclusion criteria. Subjects had to be aged 18–34 y, be 145–154 cm in height, have a parity <4, have delivered full-term babies weighing >2.5 kg, be free of chronic disease, and not be consuming medication or alcohol regularly. The infants were fully breast-fed for the duration of the study. The characteristics of the village were described elsewhere (10). The typical diet in this community provides 70% of energy as carbohydrate, 17% of energy as fat, and 13% of energy as protein, mostly from vegetable sources (1). The study protocol was reviewed and approved by the Ethics Committee of the Instituto Mexicano del Seguro Social. Written, informed consent was obtained from all subjects after the nature, procedures, and risks related to the study were explained.

Maternal anthropometric measures

Weight and height were measured with an electronic balance (Tanita Inc, Tokyo) and stadiometer (Holtain Ltd, Crymych, United Kingdom), respectively. Skinfold thicknesses were measured at the triceps, biceps, subscapular, and suprailiac sites with a Lange caliper (Cambridge Scientific Instruments, Cambridge, MD). Body circumferences at the waist, hip, arm, and calf were measured with a nonextensible measuring tape. The percentage of body fat was calculated from skinfold thicknesses (11).

Procedures

Subjects were admitted to the local health facility on the evening of study day 0, after 12 h of rest and fasting. Subjects received 2.5 mg uniformly labeled [^{13}C]LA/kg body wt (Martek Bioscience, Columbia, NY) as free acid embedded in a piece of bread served with breakfast (12) on the morning of the first study day. Although dietary fat consists mainly of triacylglycerols, the tracer was given as a nonesterified fatty acid; it was reasonably assumed that intestinal lipolysis of ingested fat would yield free fatty acids (13). Women were encouraged to maintain their habitual daily activity patterns, ie, cooking, house cleaning, and laundering.

Habitual dietary intake and design of the controlled diet

The usual dietary intakes of the subjects were assessed in their homes the week before the study began by a combination of test weighing the food for 2 d and conducting a 24-h recall for 1 d. Macronutrient and energy intakes were calculated from the results of the test weighing and 24-h recall by using values from Mexican food-composition tables (14). On the basis of the food-composition tables, subjects received a controlled diet for the duration of the study that resembled their habitual diet. The diet provided 170–210 kJ/kg body wt, 0.9 g protein/kg body wt, and 17% of energy as fat. Duplicate portions of the diet were collected, homogenized, and frozen at −20°C until the total fat content and relative concentrations of LA and AA were measured.

Milk samples for measurement of [^{13}C]LA and [^{13}C]AA enrichment

Milk samples were obtained by expressing 5 mL milk from the left breast before and after the infants were fed, to include variations in the fat concentration of foremilk and hindmilk. Milk samples were collected 0, 6, 9, 12, 15, 24, 36, 48, and 72 h after tracer administration and were stored at −70°C until analyzed for [^{13}C]LA and [^{13}C]AA enrichment.

Milk volume and composition

The concentration of total milk fat was determined in a pooled sample that was expressed with an electric breast pump (Egnell, Inc, Cary, IL) at 1000, 1400, and 1800 on the third day of the study from the left breast, which the infants were not allowed to suckle during the preceding 2 h. As soon as aliquots had been obtained for analysis, the remainder of the milk was offered to the infants. This sampling scheme represented milk fat secretion over 24 h (15). Milk volume was measured for 48 h by test weighing during the second and third days of the study (24–72 h after tracer intake).

Gas chromatography and isotope ratio mass spectrometry for assessment of fatty acids and ^{13}C enrichment

Total milk fat was extracted with chloroform:methanol (2:1, by vol), and its concentration was measured gravimetrically (16). Fatty acids in this extract were transmethylated with 3 mol methanolic hydrochloric acid/L at 85°C for 1 h and then stored in hexane with 2 g butylhydroxytoluene/L as antioxidant. Individual fatty acids were quantified and isotopes were analyzed in aliquots of this solution by gas chromatography (9).

Individual fatty acids were determined in an HP5890 gas chromatograph (series II; Hewlett-Packard, Waldbronn, Germany) equipped with a flame ionization detector. Fatty acid methyl esters were separated with a BPX70 column (SGE, Weiterstadt, Germany) as described in detail elsewhere (12). Individual fatty acids were identified by comparison with fatty acid methyl ester standards (NuCheck Prep, Elysian, MN) and were expressed as percentages by weight with chain lengths of 12–24 carbons.

The ^{13}C enrichment of LA and AA was assessed by gas chromatography–combustion isotope ratio mass spectrometry (HP5890 interfaced with a Finnigan MAT delta S mass spectrometer, Bremen Germany). Fatty acid methyl esters were separated for ^{13}C analysis by using the same chromatographic conditions described above. After eluting from the gas chromatography column, fatty acid methyl esters were combusted on-line, and the ratio of ^{13}C to ^{12}C of the resulting carbon dioxide was determined. The δ[^{13}C]values were obtained by relating the ratio of ^{13}C to ^{12}C of the sample to the international Pee Dee Belemnite standard (17). The delta over baseline values, used for further calculations, were obtained by subtracting the baseline δ[^{13}C] value from the value obtained in samples after administration of the tracer.

Calculations and statistical analysis

Daily individual intakes of LA and AA were calculated by multiplying the total fat content of diet composites by the percentage by weight of the individual fatty acids measured in the diet. The amount of individual fatty acids secreted in milk daily was calculated as follows:

$$\text{Fatty acid secretion} = (\text{milk volume} \times \text{total milk fat concentration}) \times \text{percentage by weight of the fatty acid} \quad (1)$$

The proportion of [^{13}C] administered with [^{13}C]LA that was recovered in the LA or AA secreted in milk was calculated by multiplying the daily milk secretion of a particular fatty acid by the percentage of ^{13}C contributed by the tracer. The enrichment

TABLE 1
Characteristics, dietary intakes, and breast-milk volume and composition of the lactating women[1]

Characteristic	Value
Age (y)	21.4 ± 3.1
Weight (kg)	50.9 ± 6.1
Height (cm)	149.5 ± 2.6
BMI (kg/m^2)	22.8 ± 2.4
Calculated body fat (%)	28.6 ± 4.2
Parity	2.2 ± 1.0
Intergestational period (mo)	25.8 ± 15.8
Duration of previous lactation (mo)	12.7 ± 5.7
Total energy intake (kJ/d)	9497 ± 1501
Fat intake	
(g/d)	47.0 ± 11.2
(g·kg^{-1}·d^{-1})	0.94 ± 0.15
Milk volume (g/d)	720 ± 126
Milk lipid concentration (g/L)	31.2 ± 10.4
Milk lipid secretion (g/d)	21.95 ± 5.88

[1] $\bar{x}$ ± SD; n = 10.

of [^{13}C]LA, dihomo-γ-linolenic acid (DGLA; 20:3n−6), and AA in milk was expressed as both μmol/h and as a proportion relative to the applied tracer dose. The total amount of LA and AA transferred directly from the diet into milk fat was calculated by multiplying the 24-h intake of LA and AA by the percentage of [^{13}C]LA and [^{13}C]AA recovered in milk. Transfer rates of dietary AA into milk were assumed to be identical to those of LA on the basis of observations by Fidler et al (16).

Results are presented as means ± SDs. All statistical calculations were performed by using SPSS (version 9.0; SPSS Inc, Chicago).

RESULTS

Subject characteristics, dietary intakes, and milk secretion are summarized in **Table 1**. Women were stunted (height: 145–152 cm) but not wasted [lowest body mass index (BMI; in kg/m^2): 19.4]. The percentages of fatty acids in breast milk and in the diet are shown in **Table 2**. In the diet composites, 31% of fatty acids were saturated, 43% were monounsaturated, and 26% were polyunsaturated. Oleic acid (34.3 ± 3.6%) and LA (24.9 ± 5.6%) were the most abundant fatty acids in the diet. In breast milk, 36% of fatty acids were saturated, 34% were monounsaturated, and 30% were polyunsaturated. Medium-chain fatty acids (8–16 carbons) represented 22% of total fatty acids. The dietary intake of saturated fatty acids (10–14 carbons), AA, and docosahexaenoic acid (22:6n−3) were lower than their respective secretions in milk. In contrast, the dietary intake of fatty acids with 16–18 carbons was higher than the amount secreted in milk.

[^{13}C]AA was present in milk 6 h after administration of the tracer, the amount of which increased progressively up to 72 h. The ^{13}C enrichment of milk DGLA reached its maximum 12 h after administration of the tracer and declined thereafter (**Figure 1**). The cumulative recovery of [^{13}C]LA as [^{13}C]LA, [^{13}C]DGLA, and [^{13}C]AA in milk is shown in **Figure 2.** The proportions of the tracer recovered in milk within 72 h were 16% [^{13}C]LA, 0.07% [^{13}C]DGLA, and only 0.01% [^{13}C]AA.

The calculated amounts of dietary LA and AA transferred into milk were 32.8 ± 18.0% and 11.8 ± 6.6%, respectively, of the total amounts secreted in milk (**Table 3**). The endogenous conversion of [^{13}C]LA to [^{13}C]AA in milk was very low, contributing only 1.1% to the total AA secreted in milk.

DISCUSSION

In the present sample of lactating women, who consumed a diet low in total fat but relatively high in LA, ≈67% of the LA and 88% of the AA secreted into the milk were not directly derived from the diet. Only a minor fraction of milk AA stemmed from conversion of dietary LA. The results suggest that an important proportion of AA and LA from the diet do not reach the mammary gland directly after intestinal absorption, but

TABLE 2
Percentages of individual fatty acids in the diet and breast milk of the lactating women and corresponding daily amounts ingested or secreted per day[1]

Fatty acid	Diet	Daily intake	Milk	Daily secretion	Secretion/intake
	%	*g/d*	*%*	*g/d*	
Saturated					
8:0	0.9 ± 1.1	0.51 ± 0.8	0.2 ± 0.05	0.03 ± 0.02	0.2 ± 0.2
10:0	0.4 ± 0.1	0.17 ± 0.05	1.7 ± 0.3	0.37 ± 0.12	2.2 ± 0.6
12:0	0.5 ± 0.1	0.21 ± 0.04	6.9 ± 1.6	1.51 ± 0.53	7.5 ± 3.1
14:0	1.3 ± 0.3	0.6 ± 0.17	6.0 ± 1.6	1.33 ± 0.50	2.4 ± 1.0
16:0	18.6 ± 3.7	8.64 ± 2.27	16.5 ± 1.4	3.61 ± 1.0	0.4 ± 0.2
18:0	8.4 ± 1.2	3.93 ± 0.88	4.8 ± 0.8	1.07 ± 0.42	0.3 ± 0.1
22:0	0.6 ± 0.4	0.29 ± 0.19	0.1 ±0.03	0.02 ± 0.01	0.06 ± 0.05
24:0	0.1 ± 0.03	0.05 ± 0.02	0.05 ±0.02	0.01 ± 0.01	0.2 ± 0.2
Monounsaturated					
16:1	1.4± 0.5	0.66 ± 0.3	2.5 ± 0.5	0.52 ± 0.11	1.0 ± 0.5
trans 18:1	6.6 ± 3.6	3.21 ± 1.97	2.3 ± 1.6	0.57 ± 0.46	0.2 ± 0.3
18:1n−9	34.3 ± 3.6	16.13 ± 4.1	29.3 ± 2.6	6.41 ± 1.67	0.4 ± 0.1
20:1n−9	0.4 ± 0.2	0.18 ± 0.09	0.3 ± 0.01	0.08 ± 0.03	0.5 ± 0.3
Polyunsaturated					
18:2n−6	24.9 ± 5.6	11.64 ± 3.49	27.3 ± 2.6	5.93 ± 1.48	0.5 ± 0.1
20.4n−6	0.2 ± 0.01	0.07 ± 0.04	0.4 ± 0.1	0.09 ± 0.03	1.7 ± 0.9
18:3n−3	0.9 ± 0.5	0.45 ± 0.3	1.01 ± 0.3	0.242 ± 0.1	0.7 ± 0.5
20:5n−3	ND	—	0.5 ± 0.1	0.11 ± 0.04	—
22:6n−3	0.1 ± 0.03	0.04 ± 0.02	0.2 ±0.04	0.04 ± 0.02	1.1 ± 0.7

[1] $\bar{x}$ ± SD; n = 10, not detected.

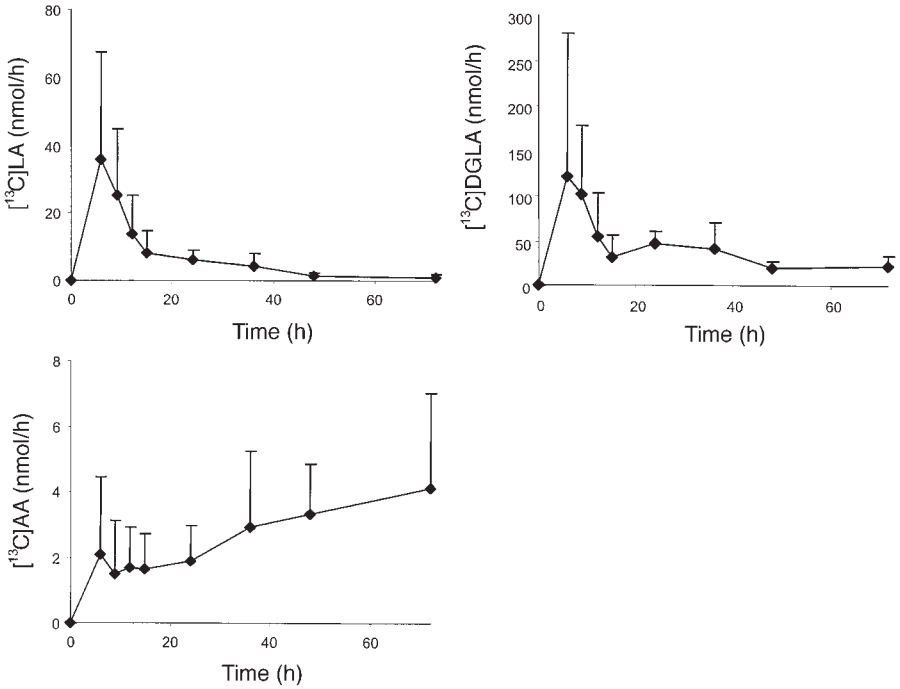

FIGURE 1. Mean (±SD) excretion of [^{13}C]linoleic acid (LA), [^{13}C]dihomo-γ-linolenic acid (DGLA), and [^{13}C]arachidonic acid (AA) in milk after the administration of an oral dose of [^{13}C]LA to 10 lactating mothers.

rather are temporarily stored in a maternal body pool and then released into the circulation. These turning-over maternal body stores seem to be the major sources of milk LA and AA.

Two separate studies in humans found no differences among the transfer rates of orally administered, labeled palmitic acid (16:0), oleic acid, LA, and docosahexaenoic acid into milk lipid (16, 18). Hachey et al (18) and Demmelmair et al (12) found that within 72 h of administration, 10–12% of an oral dose of isotopically labeled LA was transferred into the breast milk of well-nourished American and German women. In the present study we recovered 16% of the [^{13}C]LA dose within 72 h of tracer administration. Thus, it is reasonable to assume that the transfer rate of the LA tracer is applicable to nonlabeled dietary LA. Our calculations of the amount of dietary LA transferred into milk

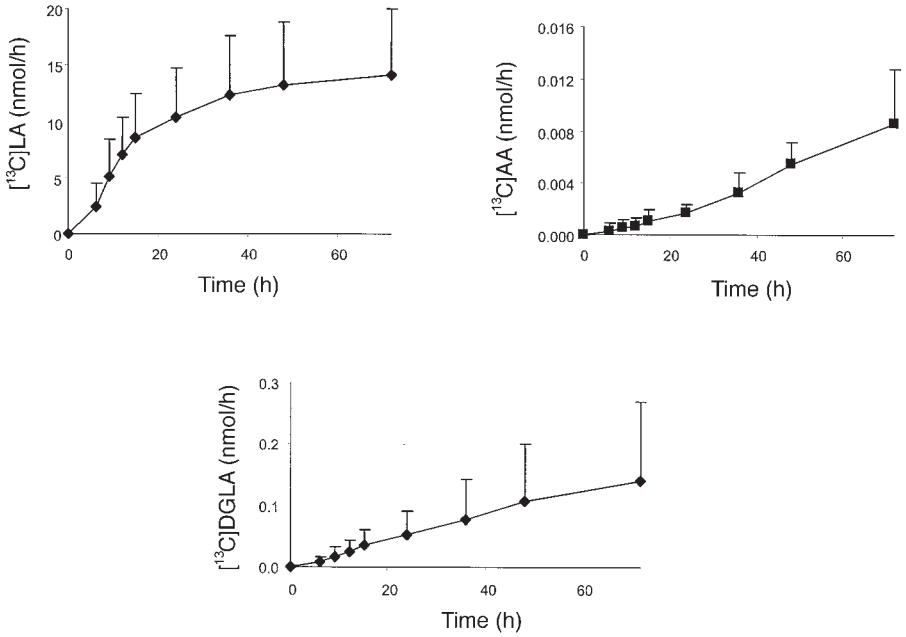

FIGURE 2. Mean (±SD) cumulative recovery of an oral dose of [^{13}C]linoleic acid (LA) in milk as [^{13}C]LA, [^{13}C]arachidonic acid (AA), and [^{13}C]dihomo-γ-linolenic (DGLA) in 10 lactating mothers.

TABLE 3
Estimated contribution of dietary linoleic and arachidonic acids and of endogenously synthesized arachidonic acid to the output of these fatty acids in breast milk[1]

	Linoleic acid	Arachidonic acid
Maternal dietary intake (g/d)	11.5 ± 3.4	0.07 ± 0.04
Secreted in milk (g/d)	6.0 ± 1.7	0.09 ± 0.03
Transferred into milk from diet		
(g/d)	1.8 ± 0.8	0.011 ± 0.007
(% of total secreted in milk)	32.8 ± 18.0	11.8 ± 6.6
Transferred into milk from endogenous synthesis		
(g/d)	—	0.0008 ± 0.0004
(% of total secreted in milk)	—	1.14 ± 0.80

[1] $\bar{x}$ ± SD; n = 10.

(33%) were based on this assumption. This value agrees with the results of a study in well-nourished German women (12). The amount of dietary AA secreted in milk was only 12%, assuming that its transfer rate is similar to that of LA.

However, note that some in vitro studies found a relative resistance of AA esters of chylomicron triacylglycerol against hydrolysis by lipoprotein lipase and a preferential incorporation of dietary AA rather than of LA into phospholipids (19, 20). Although these differences in the metabolism of AA and LA indicate a lower transfer rate of AA than of LA, our calculations overestimated the contribution of dietary AA to its total secretion into milk. Studies performed in nonlactating rats showed a lower oxidation rate of istopically labeled AA than of isotopically labeled LA (21). To our knowledge, no information is available in humans about differences among the oxidation rates of polyunsaturated fatty acids with ≥18 carbons.

Although we expected a high contribution of endogenous synthesis of AA to milk AA in the participating women because of their low dietary intake of AA, the endogenous conversion of dietary LA to AA contributed only 2.2% to the total AA secreted in milk. This value is lower than the value of 3.2% reported previously in well-nourished women (12). The low endogenous transformation of dietary LA to milk AA does not necessarily reflect the hepatic production of AA for the following reasons: *1*) the hepatic nascent AA was diluted in a pool of preexisting AA of unknown size, *2*) AA synthesis from LA derived from body stores as a precursor is hardly detectable with the method we used, and *3*) the potential dilution of the isotopic label in the DGLA pool was not taken into account. Thus, the true amount of dietary LA converted to AA and secreted in milk may be higher than the calculated value of 1%.

Desaturation of LA and α-linolenic acid by Δ^6-desaturase is considered the rate-limiting step in the production of AA and docosahexaenoic acid (22). In the present study, the ^{13}C enrichment of LA and DGLA in milk suggests the efficient conversion of LA to DGLA and hence an active Δ^6-desaturase. Nevertheless, there is no information about the level of saturation of the system.

The daily secretion of milk AA exceeded by far the sum of AA transferred from the diet and newly synthesized AA. We assume that this difference is made up by AA mobilized from maternal pools. We present evidence that part of the [^{13}C]AA synthesized from dietary LA might be temporarily stored in a maternal body pool and subsequently released to appear in milk lipids for as late as 72 h after tracer administration. This time is well beyond the estimated time (6–8 h) for the intestinal absorption of any fatty acid and its incorporation into milk fat.

We conclude that <70% of LA and 90% of AA in milk are not derived directly from intestinal absorption. Additionally, only a minor fraction of milk AA stems from its detectable conversion from dietary LA, which indicates that slowly turning-over maternal body pools are the major sources of milk LA and AA. There is a need for further research in this area, especially to assess the transfer of AA from maternal depots into milk by more direct methods.

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