Docosahexaenoic acid transfer into human milk after dietary supplementation: a randomized clinical trial

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Abstract **Docosahexaenoic acid (DHA) is important for infant development. The DHA transfer from maternal diet into human milk has not been investigated in detail. We studied the effects of DHA supplementation on the fatty acid composition of human milk and the secretion of dietary ^{13}C-labeled fatty acids, including DHA, into human milk. Ten lactating women were randomized to consume, from 4 to 6 weeks postpartum, an oil rich in DHA (DHASCO™, 200 mg of DHA/day) (n = 5) or a placebo oil (n = 5). Dietary intakes were followed by 7-day protocols. On study day 14 a single dose of [U-^{13}C]DHASCO™ was given orally, milk samples were collected over 48 h, and milk production was recorded. Milk fatty acid composition was determined by gas-liquid chromatography and isotopic enrichment was determined by gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS). Milk DHA content did not differ between the supplemented and placebo group at study entry (0.29 vs. 0.28 wt%, median). After 2 weeks of supplementation the milk DHA content was almost 2-fold higher in the supplemented versus placebo group (0.37 vs. 0.21 wt%, *P* = 0.003). Cumulative recovery of [^{13}C]palmitic, [^{13}C]oleic, and [^{13}C]docosahexaenoic acids in human milk at 48 h was similar between supplemented and placebo groups (palmitic acid 7.40 vs. 8.14%, oleic acid 9.14 vs. 9.97%, and docosahexaenoic acid 9.09 vs. 8.03% of dose, respectively). Notable lower recovery was observed for [^{13}C]myristic acid in both the supplemented and placebo groups, 0.62 versus 0.77% of dose. Dietary DHA supplementation increases the DHA content in human milk. DHA transfer from the diet into human milk is comparable to palmitic and oleic acid transfer.**—Fidler, N., T. Sauerwald, A. Pohl, H. Demmelmair, and B. Koletzko. **Docosahexaenoic acid transfer into human milk after dietary supplementation: a randomized clinical trial. *J. Lipid Res.* 2000.** 41: **1376–1383.**

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Docosahexaenoic acid (DHA, C22:6n−3), a long-chain polyunsaturated fatty acid (LCPUFA), is an essential structural component in tissue membranes of the human body and is highly concentrated in brain and retina (1, 2). Inadequate early intake may affect later visual function and neurodevelopment (3, 4). Erythrocyte DHA levels are positively correlated with visual functions in term infants (5). The dietary intake of DHA and other n−3 and n−6 LCPUFA has been associated with several biochemical and functional effects. During pregnancy LCPUFA, especially DHA and arachidonic acid (C20:4n−6), are transported through the placenta to the developing fetus (6–8). It has been suggested that elevated n−3 LCPUFA consumption during pregnancy prolongs the duration of pregnancy, possibly because of inhibition of prostaglandin synthesis (9, 10). Maternal fish oil supplementation during pregnancy also promotes higher concentrations of DHA in the blood of newborn infants (11, 12).

DHA can be provided by dietary sources, body stores, and endogenous synthesis from precursor n−3 fatty acids (13). The amount of DHA in human milk is influenced by maternal diet (14). Reported DHA levels in mature human milk range from 0.05 wt% in vegetarian women (15, 16) to 1.40 wt% in Inuit women (17). Even higher levels up to 2.78 wt% were observed in human milk of Chinese women living on the island of Zhangzi (18). Several studies have examined the effects of different DHA intake on human milk composition, reporting a dose-related increase in the DHA content of human milk (5, 19, 20).

Previous studies (13, 21–23) have demonstrated the feasibility of gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) to trace ^{13}C-labeled fatty acids in vivo. The safety of the use of ^{13}C-stable isotopes has been reaffirmed in several studies (24, 25).

The purpose of the present study was to assess in a placebo-controlled trial the effects of maternal dietary supplementation with DHA on human milk fatty acid composition. Further, the transfer of orally supplied ^{13}C-labeled DHA,

Abbreviations: APE, atom percent excess; DHA, docosahexaenoic acid, C22:6n−3; DHASCO™, single-cell triglyceride oil rich in DHA; DOB, delta over baseline, that is, increase in the δ^{13}C value above the baseline; EPA, eicosapentaenoic acid, C20:5n−3; GLC, gas-liquid chromatography; GC-C-IRMS, gas chromatography-combustion-isotope ratio mass spectrometry; LCPUFA, long-chain polyunsaturated fatty acid; U-^{13}C, uniformly ^{13}C labeled.

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oleic, palmitic and myristic acid as a function of time into human milk was monitored in vivo.

MATERIALS AND METHODS

Subjects

Healthy breastfeeding women with healthy single, full-term newborns were recruited shortly after delivery at the Department of Gynecology and Obstetrics (University of Munich, Munich, Germany). The study protocol was approved by the Ethics Committee of the Medical Faculty of the University of Munich. A written informed consent was obtained from all participating women after careful explanation of the study. The infants were exclusively breast fed during the duration of the study. Regular contact was maintained with all women during the study period and visits and assistance were carried out on days of milk sampling. Ten subjects were estimated to be sufficient to detect a 40% difference for the primary outcome measure of DHA content in human milk after supplementation, with 80% power and a probability of 0.05. The estimation was based on results of a previous study by Makrides, Neumann, and Gibson (5), who obtained about 60% higher levels of DHA in a supplemented versus a placebo group using the same source and dose of DHA supplementation. The randomization scheme followed the sequence that was obtained from the coded numbers contained in a closed envelope.

Dietary supplementation

At 4 weeks postpartum (study day 0), mothers were randomly and blindly assigned to receive 2 capsules per day, containing either the dietary supplement rich in DHA (n = 5, DHASCO™, 100 mg of DHA per capsule; Martek Biosciences, Columbia, MD) or a placebo oil (n = 5, corn oil/soy oil mixture = 1/1) for the following 14 days. DHASCO™ is a triglyceride oil derived from a unicellular algae, mainly containing DHA (C22:6n–3), myristic (C14:0), palmitic (C16:0), and oleic acids (C18:1n–9). One capsule was consumed in the morning and one in the evening. The fatty acid composition of the DHASCO™ supplement and the tracer obtained by analysis in our laboratory is presented in **Table 1**. Compositional data were nearly identical to those provided by the manufacturer. With the intake of 2 capsules of DHASCO™ per day, the following amounts of fatty acids were consumed with the supplement: 200 mg of C22:6n–3; 100 mg of C18:1n–9; 75 mg each of C14:0 and C16:0; 15 mg of C12:0; 13 mg of C18:2n–6; 5 mg each of C10:0, C16:1n–7, C18:0, and C24:1n–9.

TABLE 1. Fatty acid composition (wt%) of dietary supplement (DHASCO™) and tracer ([^{13}C]DHASCO™): results of own analyses

Fatty Acid	DHASCO™ Supplement	[^{13}C]DHASCO™[a]
C8:0	0.1	0.0–0.3
C10:0	0.6	1.1–1.8
C12:0	4.1	7.8–9.3
C14:0	11.8	18.1–19.6
C16:0	7.6	9.0–13.2
C16:1n–7	1.7	0.9–1.0
C18:0	0.9	0.0–0.3
C18:1n–9	29.5	5.1–7.4
C20:0	0.1	0.1
C20:1n–9	0.3	0.0–0.3
C18:2n–6	1.3	—
C18:3n–3	—	—
C20:3n–3	—	—
C20:5n–3	—	—
C22:5n–3	0.5	0.2–0.4
C22:6n–3	40.1	49.3–55.4

[a] Range of values obtained after measurement of three batches of tracer used.

Dietary assessment

Only women consuming omnivorous diets were recruited for the study. Diets were assessed during the 14 days of supplementation by 7-day weighed dietary protocols. During the study period women continued their normal diet but were asked not to eat fish and sea food, because of their high n–3 LCPUFA content. Further, they were asked not to eat corn and corn-based products the day before tracer application because of the elevated natural ^{13}C abundance of these foods. Each participant was instructed to carefully record the exact amount of all foods and drinks consumed (quantity in grams, milliliters, or household measures) and the corresponding brands. Caloric intakes and food compositional data were calculated with nutrition database software (Prodi 4.4, Karlsruhe, Germany), which is based on a German nutrient survey with about 12,000 food items.

Tracer ingestion

After 14 days of supplementation (at 6 weeks postpartum) the women from both groups received an oral bolus dose of uniformly ^{13}C-labeled DHASCO™ (2 mg/kg body weight; 57.1–65.7% ^{13}C labeling; Martek Biosciences) with a standardized breakfast.

Human milk sampling

Samples were obtained at 4 weeks postpartum (before starting the supplementation) in the morning and again at 6 weeks postpartum just before and 6, 12, 24, 36 and 48 h after ingestion of ^{13}C-labeled DHASCO™. Seven milk samples were obtained from each of the 10 participating subjects. Samples were collected with an electrical breast pump (Medap, Bad Homburg, Germany) as aliquots of fore and hind milk from the same breast at each time point. Equal volumes of fore and hind milk were gently mixed immediately after collection, while still warm, and then frozen and stored at −80°C until further analysis. Infants were weighed before and after each feeding on study days 14 and 15 in order to determine the total volume of milk consumed per day.

Analysis of human milk samples

Frozen milk samples were thawed at room temperature, tempered in a water bath to 38°C, mixed, and an aliquot of 1 ml was pipetted into an 8-ml reaction tube (26). Extraction of lipids, preparation of fatty acid methyl esters, and gas chromatography were performed according to a procedure described previously (27). Briefly, fat was extracted with the addition of potassium oxalate into 1 ml of ethanol, 1 ml of tertiary butylmethyl ether, and 1 ml of petrol ether (28). Fat content was determined gravimetrically (29).

The lipid extract was dissolved in 1 ml of 3 M methanolic hydrochloric acid, 1 ml of methanol, and 0.5 ml of hexane and heated at 90°C for 1 h. After the addition of 2 ml of water the fatty acid methyl esters were extracted into 3 ml of hexane containing 0.2% 2,6-ditertiary butyl *p*-kresol. The fatty acid composition of the methyl esters was analyzed by gas-liquid chromatography (GLC), using an HP 5890 Series II gas chromatograph (Hewlett Packard, Waldbronn, Germany) equipped with an HP 7376 autosampler, cool on-column injection, and a flame ionization detector. The separation was performed on a BPX-70 capillary column (length, 60 m; 0.32-mm i.d.). The ^{13}C enrichment of individual fatty acid methyl esters in milk was measured by GC-C-IRMS (Finnigan MAT; Delta S, Bremen, Germany) equipped with an HP 5890 gas chromatograph (30). All samples were chromatographed in duplicate.

Expression of results

The average volume of milk secreted per day on study days 14 and 15 was calculated from the amount of milk fed to the baby in these 2 days plus the amount of milk taken for sampling.

From the mean fat content (grams of fat per 100 ml of milk), mean weight percentage (wt%) of single fatty acids (grams of fatty acid per 100 g), and the amount of milk synthesized on study days 14 and 15, the amounts (g/day) of single fatty acids secreted into milk on study days 14 and 15 were calculated.

From the $^{13}C/^{12}C$ ratio of the samples the $\delta^{13}C$ relative to the international standard PDB (Pee Dee Belemnite) and the ^{13}C atom percentage (atom%) were calculated. The atom percentage excess (APE) was obtained by subtracting the basal ^{13}C abundance from the ^{13}C abundance in the samples after the tracer application (31). The ^{13}C contents were also calculated as the difference between the $\delta^{13}C$ values before and after tracer ingestion (delta over baseline: DOB as ‰) (30). The ^{13}C fatty acid secretion into human milk was further expressed as percentage of dose of ingested fatty acid and as percentage of cumulative recovery of ingested fatty acid.

Statistics

Because of the relatively small number of subjects per group, the differences between the supplemented and the placebo group were assessed by a nonparametric test (Mann-Whitney test-exact, Monte Carlo). The significance level (one-sided significance for DHA and two-sided significance for the other fatty acids) was set at $P < 0.05$. Differences in the transfer of fatty acids from diet into human milk were examined by the Kruskal-Wallis test. Associations between dietary intake of DHA and its human milk content were assessed by the Pearson correlation coefficient. All analyses were performed with the statistical software SPSS, version 8.0 (SPSS, Chicago, IL). The results are presented as medians and interquartile ranges (IQR), while the subject data are given as means ± SD.

RESULTS

Subject data

Anthropometric data of the 10 women enrolled in the study, daily milk production, and milk fat content are presented in **Table 2**. All these parameters, as well as dietary intakes (**Table 3**), were not different between the supplemented and the placebo group.

Milk fatty acid composition: quantitative analysis

We determined 38 different fatty acids in the human milk samples, starting with C8:0. **Table 4** presents the n−6 and n−3 fatty acid composition of human milk before and after supplementation with DHASCO™ or placebo.

TABLE 2. Characteristics of lactating women, daily milk production, and milk fat content (mean ± SD)

Parameter	DHASCO™ Supplement (n = 5)	Placebo (n = 5)
Age (yr)	29.2 ± 4.7	32.0 ± 4.2
Height (cm)	166 ± 5.0	173 ± 4.0
Weight $_{day\ 0}$ (kg)	68.5 ± 4.6	74.3 ± 2.6
Weight $_{day\ 14}$ (kg)	67.3 ± 3.9	73.4 ± 2.4
Body mass index $_{day14}$ (kg/m^2)	22.9 ± 1.9	21.4 ± 1.4
Milk secretion[a] (mL/day)	677 ± 134	666 ± 112
Milk fat content[a] (g/100mL)	3.7 ± 0.6	4.1 ± 0.6

[a] Average for the study days 14 and 15.

TABLE 3. Dietary intakes of selected substrates of the participating breastfeeding women determined by 7-day weighed dietary records during the period of supplementation without the supplement [median (IQR)]

	DHASCO™ Supplement (n = 5)	Placebo (n = 5)
	MJ/day	
Energy intake	10.1 (8.0–10.3)	8.7 (8.4–9.0)
	g/day	
Protein	72.0 (64.5–92.2)	76.9 (72.4–81.8)
Carbohydrate	273.0 (228.9–309.2)	235.5 (198.8–239.9)
Fat	90.2 (76.9–99.7)	95.4 (88.4–100.7)
C14:0	4.8 (4.2–5.3)	6.0 (5.3–6.4)
C16:0	18.6 (16.0–19.9)	20.2 (19.7–21.3)
C18:0	7.9 (7.0–9.3)	9.4 (7.81–9.8)
C16:1n−7	1.8 (1.6–2.1)	1.9 (1.9–2.3)
C18:1n−9	27.8 (23.9–30.2)	29.2 (27.7–30.5)
C18:2n−6	10.7 (9.2–13.5)	7.2 (6.8–8.6)
C18:3n−3	1.3 (1.2–1.7)	1.2 (1.2–1.3)
C20:4n−6	0.15 (0.10–0.18)	0.15 (0.10–0.21)
C20:5n−3	0.01 (0.01–0.02)	0.01 (0.00–0.01)
C22:5n−3	0.03 (0.02–0.05)	0.02 (0.01–0.07)
C22:6n−3[a]	0.05 (0.04–0.12)[a]	0.09 (0.04–0.11)
Total saturated[b]	39.1 (33.1–42.3)	44.1 (43.1–47.3)
Total monounsaturated[c]	31.4 (27.4–34.1)	33.7 (31.7–34.5)
Total polyunsaturated[d]	13.5 (11.1–15.0)	9.4 (8.2–11.2)

[a] Additional 0.2 g DHA/day ingested with the DHASCO™ capsules.
[b] Σ C14:0, C15:0, C16:0, C18:0, C20:0, and C22:0.
[c] Σ C14:1n−5, C16:1n−7, C18:1n−9, C20:1n−9, C22:1n−9, and C24:1n−9.
[d] Σ C18:2n−6, C18:3n−3, C20:3n−6, C20:4n−6, C20:5n−3, C22:5n−3, and C22:6n−3.

DHA content before and after the DHASCO™ supplementation. At study entry (day 0) there was no difference in the fatty acid composition (wt%) between the supplemented and the placebo group. After 2 weeks of supplementation with 200 mg of DHA per day, the milk contained a significantly higher percentage of DHA (0.37 wt%) than milk from the placebo group (0.21 wt%; $P = 0.003$). Women in the supplemented group consumed on average 3.6-fold more DHA per day versus women in the placebo group (0.25 vs. 0.07 g). This resulted after 2 weeks of supplementation in an almost 1.8-fold higher DHA content. There was no significant difference in the content of any other fatty acid between the milk from the two groups at any time point after the DHASCO™ supplementation.

Between 4 and 6 weeks after delivery the DHA content in the supplemented group increased in four of five women, whereas it decreased in four of five women in the placebo group. A comparison within the group (related samples) of the supplemented women showed a nonsignificant increase, from 0.29 to 0.37 wt% DHA, and a decrease in the placebo group, from 0.28 to 0.21 wt% DHA, which was close to significance ($P = 0.06$) (Table 4).

DHA content before and after tracer ingestion. DHA levels in the supplemented group remained higher than in the placebo group at 6, 12, 24, 36, and 48 h after the oral tracer ingestion (**Fig. 1**). In the supplemented group the

TABLE 4. Polyunsaturated fatty acid composition of human milk before and after DHASCO™ supplementation [% wt/wt of 38 fatty acids, median (IQR)]

	Supplement		Placebo	
Fatty acid	Study Day 0	Study Day 14	Study Day 0	Study Day 14
n−6				
C18:2	10.37 (9.49–11.90)	11.62 (10.09–13.13)	8.69 (7.94–11.70)	10.16 (8.31–11.66)
C18:3	0.14 (0.13–0.15)	0.14 (0.14–0.15)	0.18 (0.15–0.19)	0.15 (0.14–0.16)
C20:3	0.36 (0.35–0.37)	0.37 (0.31–0.39)	0.33 (0.31–0.57)	0.33 (0.28–0.42)
C20:4	0.54 (0.45–0.58)	0.41 (0.39–0.49)	0.47 (0.45–0.57)	0.43 (0.40–0.51)
C22:4	0.12 (0.11–0.12)	0.09 (0.09–0.11)	0.10 (0.09–0.12)	0.08 (0.08–0.10)
Σ n-6 LCPUFA	1.38 (1.32–1.42)	1.15 (1.14–1.40)	1.15 (1.09–1.62)	1.06 (1.03–1.19)
n−3				
C18:3	0.80 (0.69–0.88)	0.77 (0.66–1.37)	0.57 (0.52–0.65)	0.70 (0.58–1.08)
C20:5	0.06 (0.05–0.09)	0.05 (0.05–0.07)	0.07 (0.06–0.08)	0.05 (0.05–0.06)
C22:5	0.21 (0.16–0.23)	0.15 (0.14–0.22)	0.17 (0.16–0.18)	0.15 (0.14–0.16)
C22:6	0.29 (0.27–0.42)	0.37 (0.34–0.40)[a]	0.28 (0.25–0.28)	0.21 (0.19–0.23)[a]
Σ n-3 LCPUFA	0.62 (0.57–0.79)	0.64 (0.61–0.74)[b]	0.52 (0.52–0.60)	0.46 (0.44–0.49)[b]

[a] Study day 14: supplemented versus placebo group, $P = 0.003$.
[b] Study day 14: supplemented versus placebo group, $P = 0.007$.

DHA content (wt%) in milk was maximal 6 h after tracer application and declined afterwards. At 24 h and onward the DHA content remained below the value at 0 h in the supplemented women. In the placebo group the DHA content in human milk peaked at 12 h after tracer application and remained above the basal value also at 24, 36, and 48 h.

Other fatty acids. Other fatty acids in human milk from the supplemented and the placebo group did not differ significantly at any time point.

Absolute amounts of fatty acids secreted per day. Women from both groups secreted comparable amounts of fatty acids per day. The only significant difference was the higher amount of DHA secreted in the supplemented [0.09 (0.08–0.10) g/day, median (IQR)] versus the placebo group [0.06 (0.06–0.07) g/day; $P = 0.017$]. The three fatty acids secreted in largest amounts were oleic, palmitic, and linoleic acid, 8.2 (7.8–9.0), 5.9 (5.4–6.2), and 2.7 (2.2–3.3) g/day, respectively. These fatty acids were also those most abundant in the diet (Table 3). The total amount of fatty acids secreted into milk was about 25 g/day.

Relationship between dietary DHA intake and its content in human milk. There was a linear relationship between the dietary DHA intake (g/day) and the milk DHA content (wt%) ($r = 0.84$, $P = 0.002$; day 14; **Fig. 2**). Similarly, there was a linear relationship between daily dietary DHA intake and the amount secreted into human milk (g/day) ($r = 0.71$, $P = 0.02$; day 14). When the amounts of ingested DHA were corrected for *a*) body weight (g DHA per day/kg body weight) or for *b*) body mass index (g DHA per day/BMI) the following correlation coefficients and significance levels for a relationship with the milk DHA content (wt%) were obtained: *a*) $r = 0.82$, $P = 0.003$ and *b*) $r = 0.75$, $P = 0.012$.

Transfer of fatty acids from diet into human milk: isotopic analysis

Isotopic enrichment in milk fatty acids. The [^{13}]DHASCO™ tracer contained five major and seven minor (≤1.8 wt%)

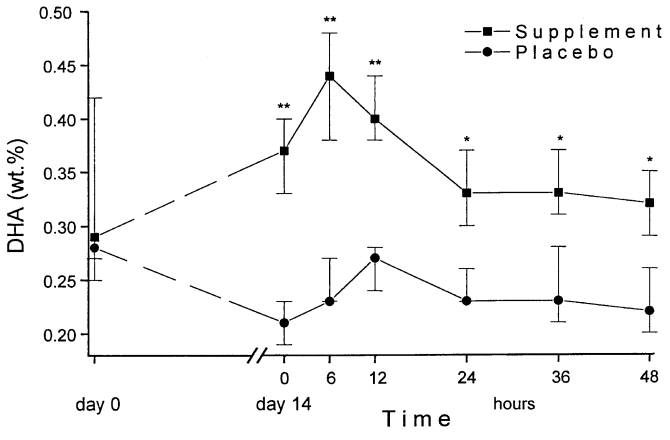

Fig. 1. DHA content in human milk before and after 14 days of DHASCO™ supplementation (200 mg of DHA/day) or placebo, as well as before and at various time points after [^{13}C]DHASCO™ ingestion (2 mg/kg body weight) on day 14 of the study [median (IQR); * $P < 0.05$; ** $P < 0.01$].

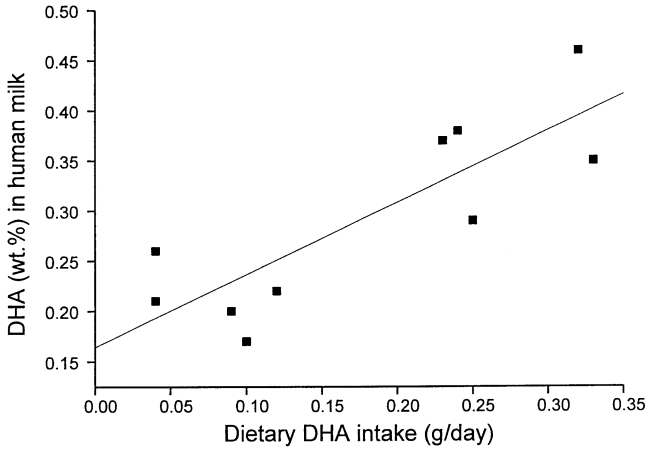

Fig. 2. Relationship between DHA intake (diet + supplement/placebo) and DHA content in human milk ($r = 0.84$, $P = 0.002$; study day 14) in 10 lactating women.

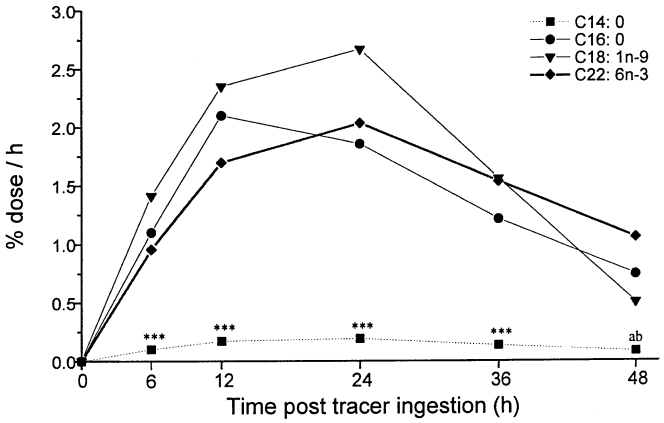

Fig. 3. Percent dose of ingested ^{13}C-labeled fatty acids ([^{13}C]DHASCO™ at 2 mg/kg body weight) secreted into human milk as a function of time after ingestion of the tracer (medians, combined data from supplemented and placebo group, n = 10; *** $P < 0.001$, C14:0 vs. all other fatty acids, ^{ab}C14:0 vs. C18:1n–9 at 48 h, NS).

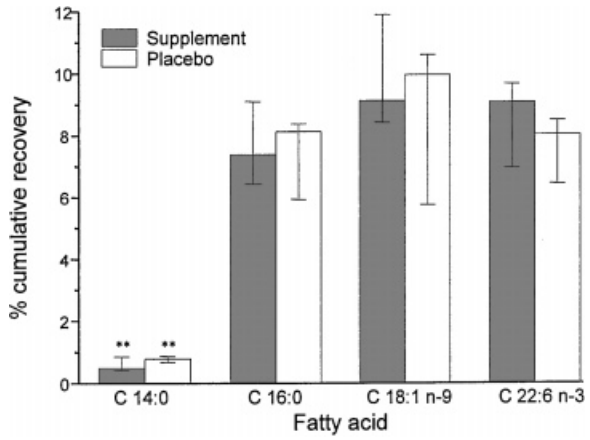

Fig. 4. Cumulative recovery of fatty acids in human milk in the supplemented and placebo group at 48 h after tracer ([^{13}C]DHASCO™ at 2 mg/kg body weight) ingestion [median (IQR); ** $P < 0.01$, C14:0 vs. all other fatty acids].

fatty acids labeled with ^{13}C (Table 1). We measured the enrichment patterns of seven fatty acids as a function of time after the tracer ingestion. The enrichment patterns of six fatty acids, myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1n–7), oleic (C18.1n–9), docosapentaenoic (C22:5n–3), and DHA (C22:6n–6), showed peak ^{13}C enrichment at 12 h after tracer ingestion. Peak DOB for these fatty acids ranged from 5.7 to 4798‰, whereas the enrichment of stearic acid (C18:0) was close to the detection limit. The peak DOB for stearic acid was in the range from 0.4 to 2.7‰. In addition to the fatty acids contained in the tracer we measured the ^{13}C enrichment for pentadecanoic (C15:0), linoleic (C18:2n–6), α-linolenic (C18:3n–3), eicosatrienoic (C20:3n–6), and arachidonic (C20:4n–6) acid and did not detect any rise in the ^{13}C content of these fatty acids in milk. For the statistical analysis we considered only those fatty acids that were present in the tracer with >5%, that is, myristic, palmitic, oleic, and DHA.

To correct for the amounts of tracer fatty acids ingested we calculated the enrichments of single fatty acids secreted into human milk as a percentage of the ingested dose (**Fig. 3**) as well as the cumulative recovery after 48 h of these fatty acids in human milk (**Fig. 4**). These two measures allowed for a direct comparison of the secretion pattern of single fatty acids into human milk. There was no difference in the secretion pattern of labeled fatty acids between the placebo and the supplemented group within the 48 h of observation.

Percent dose recovery. There was no difference in the transfer of fatty acids between the two groups. Figure 3 shows the percentage of the dose of the four ingested fatty acids that were secreted into the milk of all 10 woman over 2 days after [^{13}C]DHASCO™ ingestion. The biggest proportion of the ingested tracer dose was secreted into milk at 12 h for palmitic acid (2.10%) and at 24 h for myristic (0.19%), oleic (2.67%), and DHA (2.04%). Palmitic, oleic, and docosahexaenoic acid had a similar secretion pattern into human milk, whereas myristic acid was secreted in an appreciable smaller proportion ($P < 0.001$).

Cumulative recovery. Figure 4 presents the cumulative recovery of the four labeled fatty acids secreted into human milk at 48 h after tracer ingestion in the supplemented and the placebo group. Tracer recovery was defined as the percentage of the ingested tracer fatty acid that was secreted into milk. No significant difference was found for the secretion patterns of a specific fatty acid between the supplemented and the placebo group. Similar cumulative recoveries in human milk were found for palmitic [7.77 (6.38–8.82)%], oleic [9.56 (8.02–10.90)%], and docosahexaenoic acid [8.04 (7.01–9.56)%] for the whole group of 10 women [median (IQR)]. Eminently lower recovery in milk was found for myristic acid [0.75 (0.49–0.82)%, $P < 0.0001$].

DISCUSSION

This study shows that dietary DHA intake has a specific effect on breast milk DHA content. The in vivo secretion pattern of labeled fatty acids into human milk showed that the transfer of DHA from the diet into human milk is not preferential compared to palmitic and oleic acids.

At study entry there were no differences between subjects from the two groups. Dietary intakes, milk fatty acid composition, and milk fat secretion of the women were within the range reported in other studies (15, 30, 32, 33). After the 14-day supplementation period DHA tended to increase in the supplemented group by 28%, whereas it tended to decrease by 25% in the placebo group. We speculate that the observed increase of DHA in the supplemented group did not reach significance in our study because of the small sample size. Further, our data are in agreement with previous supplementation studies with dietary changes over longer periods of time, which resulted in similar and significant changes in human milk DHA levels (5, 19, 20). As in other studies (5, 20), dietary treatments had no effect on milk fat content and fatty acids other than DHA.

Human milk DHA may originate directly from the diet, body stores, and biosynthesis from precursor fatty acids such as α-linolenic acid (13, 34). In this study we estimated the percentage of DHA derived directly from the diet. Women from the supplemented group secreted an average of 656 ml of milk with a fat content of 3.7 g/100 ml, which is in line with the results obtained in other European studies (15, 30, 32, 33). The excess DHA content of 0.16 wt% observed after DHA supplementation indicates that about 20% of the supplemented 0.2 g of DHA/day was secreted into milk. This result is in agreement with the results of Makrides, Neumann, and Gibson (5), who supplemented lactating women from day 5 to 12 weeks postpartum with a placebo, 0.2, 0.4, 0.9, or 1.3 g of DHA/day, also using the DHASCO™ oil. They reported that over the entire supplementation range some 20% of the supplemented DHA was secreted into human milk. We obtained the same results, although we used a shorter duration of the supplementation period (14 vs. 79 days in the study by Makrides Neumann, and Gibson). These findings support the conclusion that stable levels of human milk fatty acids are achieved already within 1 week of supplementation (20).

In agreement with Makrides, Neumann, and Gibson (5) we found that DHASCO supplementation had no appreciable effect on the content of fatty acids other than DHA in human milk (Table 4). Furthermore, we found in agreement with their study (5) a linear relationship between maternal DHA intake and DHA content in human milk. In addition to the previous studies here we also monitored the women's dietary DHA intake (Table 3), which strengthens the results of this relationship. In studies supplementing fish oil, which in contrast to our supplement contains not only DHA but also eicopentaenoic acid (EPA) and docosapentaenoic acid, increased contents of DHA, EPA, and docosapentaenoic acid were observed in human milk (19, 20). Although DHA can be retroconverted to docosapentaenoic acid and EPA, the degree of retroconversion in humans has been reported to be low. Brossard et al. (35) measured plasma lipid classes in three healthy volunteers, and found that only about 1.4% of the ingested [^{13}C]DHA was retroconverted to docosapentaenoic acid and EPA within 3 days. The results of our study support the hypothesis of Makrides, Neumann, and Gibson (5), that increased levels of EPA and docosapentaenoic acid in human milk after fish oil supplementation (19, 20) are the result of the direct intake of these fatty acids with the diet rather than the result of a retroconversion from DHA.

We observed a pronounced increase in human milk DHA content (wt%), with a peak between 6 and 12 h after dietary DHA intake (Fig. 1, day 14), which is in agreement with the findings of others (20, 36). The maximal ^{13}C enrichment (maximal DOB) of myristic, palmitic, oleic, and DHA occurred 12 h after tracer ingestion in human milk, which also is in good agreement with the data on linoleic acid transfer from Demmelmair et al. (30). Moreover, Hachey et al. (36) found maximal enrichments of labeled fatty acids in human milk between 8 and 10 h after the oral administration of ^{2}H-labeled triglycerides of palmitic, oleic, and linoleic acid to three breastfeeding women. In that study milk samples were collected more frequently than in our study. The simultaneous comparison of secretion patterns of different fatty acids in the same subject and at the same time points after ingestion carried out in our study and in that of Hachey et al. (36) diminishes uncertainties in interpretation of data achieved from separate subjects.

From estimated dietary intakes, dose of tracer ingestion, fatty acid composition of the milk, and isotopic enrichments in milk we calculated the proportions of fatty acids in human milk that were transferred directly from the diet during the last 48 h. This calculation is reasonable only for those fatty acids that fulfill the following two conditions: *1*) The dietary intake of the fatty acid should be reliably estimated from the dietary protocols. We consider the estimation of the major dietary fatty acids to be more precise than that of the minor dietary fatty acids (<0.1 wt% of total fatty acids) (Table 3); and *2*) the fatty acid should be close to a steady state situation on the study day 14, that is, its intake and the secretion into human milk over the observational period should be stable. On study day 14 women from both groups received 2 mg of tracer/kg body weight in addition to their regular diet. Tracer ingested on study day 14 represented only a minor additional consumption of the major fatty acids. Expressed as a percentage of total dietary intake, the contribution of the tracer was 0.5, 0.07, and 0.03% for myristic, palmitic, and oleic acid, respectively. Thus, all these three fatty acids were ingested mainly through the diet.

We calculated that approximately 24% of palmitic acid and 25% of oleic acid in the human milk originated from the diet during the past 48 h. Only about 2% of milk myristic acid would originate from the myristic acid ingested within 48 h, based on our estimation. The remaining part of fatty acids secreted into human milk (76% palmitic, 75% oleic, and 98% myristic acid) originated from other sources than the actual diet, that is, body stores, presumably mostly adipose tissue and de novo synthesis (especially myristic acid) or interconversion (elongation, desaturation) from other fatty acids.

The kinetics and the cumulative recovery of the ^{13}C-labeled fatty acids secreted into human milk over a 48-h period were similar for palmitic, oleic, and docosahexaenoic acids, but differed for myristic acid. Hachey et al. (36) reported cumulative recoveries of palmitic and oleic acid in breast milk over a 72-h period of 8.4 and 9.9%, respectively, which is similar to the 7.8% for palmitic acid and 9.6% for oleic acid we found over the 48-h study period. Because our measurements were close to baseline at 48 h, prolonged measurements in our study would probably also have resulted in similar values compared with those of Hachey et al. (72 h) (36).

Crawford, Hassam, and Stevens (6) postulated a selective materno-fetal transfer of LCPUFA through the placenta. There has also been a controversial discussion for several years concerning whether transfer of DHA from diet into human milk would also follow a selective mecha-

nism. Hachey et al. (36) found no difference in the secretion pattern of linoleic, oleic, and palmitic acids in breastfeeding women. Makrides, Neumann, and Gibson (5), on the basis of their DHA supplementation study in breastfeeding women, proposed that there is no selective transfer of DHA from the diet into human milk. In our study we measured for the first time the transfer of DHA from the diet into human milk simultaneously with the transfer of other long-chain and medium-chain fatty acids in vivo. Our results (Figs. 3 and 4) clearly prove that there is no selective transfer of DHA from the diet into human milk, compared with palmitic and oleic acids. Our results also suggest that the transfer of DHA is comparable to the transfer of linoleic acid, which was previously reported (30). The substantially lower transfer of the medium-chain myristic acid from the diet into human milk could possibly be due to differences in metabolic pathways, possibly faster absorption (37) and a higher rate of oxidation (38, 39). Further, it is known from studies in humans that the mammary gland has the capacity for de novo synthesis of myristic acid (40, 41). Another possibility for the metabolic disposal of myristic acid is the elongation to palmitic acid, which cannot be quantitated in our study because of the GC-C-IRMS methodology used. Also, palmitic acid may be elongated to stearic acid and further desturated to oleic acid. This interconversion might influence to some extent the values for recovery of myristic, palmitic, and oleic acids in human milk, but are assumed to be quantitatively minor. Rhee et al. (42) measured the desaturation and interconversion of palmitic and stearic acids in adults on omnivorous diets. On the basis of measurements of various lipoproteins over 7 days they reported values for the elongation of palmitic to stearic acid of about 6% and desaturation of stearic to oleic acid of about 14%. Similarly, Emken et al. (43) reported only low rates for elongation of palmitic to stearic and conversion of stearic to oleic acid.

Our estimation that diet-derived palmitic and oleic acids accounted for 24 and 25% of the fat in milk within 2 days is in agreement with the study by Hachey et al. (36), where diet-derived (within 3 days) palmitic, oleic, and linoleic acid each accounted for 29% of the amount contained in milk. Similarly, Demmelmair et al. (30) reported that about 30% of linoleic acid in human milk originated directly from the diet. The remaining part of fatty acids in human milk is derived from body stores, presumably mainly adipose tissue, and a minor part is derived from de novo synthesis. Martin et al. (32) reported that 60% of the variance in the linoleate content in human colostrum triglycerides was accounted for by the variance in the linoleate content of white adipose tissue triglycerides. In contrast to the high proportion of diet-derived long-chain fatty acids (=16 carbon atoms) in human milk, the low portion of diet-derived myristic acid in human milk for the first time reported in our study (about 2% within 2 days) is in accordance with the hypothesis that medium-chain, saturated fatty acids (C8–C14) are mainly synthesized de novo in the mammary gland (40, 41).

We conclude that DHA supplementation has a specific effect on breast milk DHA and that there is no preferential DHA secretion into human milk. Whereas the secretion of several long-chain fatty acids is comparable, medium-chain fatty acids such as myristic acid are transferred in substantially smaller amounts.

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