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Differences in Postprandial Lipid Response to Breast- or Formula-feeding in 8-Week-Old Infants


**ABSTRACT**

**Objective:** Lipids play important roles in infant growth and development. In this exploratory observational single-center study, we investigated postmeal responses of infants to dietary lipids and differences between breast-feeding (BF) and formula-feeding (FF).

**Methods:** Two capillary blood samples were collected from each subject, before and randomly assigned at either 30, 60, 90, 120, 180, or 240 minutes after their respective feeding, followed by measurement of lipid-related plasma parameter concentrations using enzyme-linked immunosorbent assay-based or combined enzymatic and colorimetric methods.

**Results:** The intermeal interval before testing was shorter in the BF group (20.27 ± 7.7 minutes; FF 14.82 ± 3.57 minutes). Composite postmeal concentration profiles were generated from 59 plasma sample pairs with sufficient volume (BF = 30): triglyceride (TG) baselines were not different. A TG difference was indicated for BF over FF subjects at 30 minutes, for FF over BF subjects at 60 minutes when corrected for baseline. TG responses in both groups appeared and seemed to clear much faster than those reported for adults. The TG:apolipoprotein B48 (ApoB48) ratio suggests that chylomicrons in BF subjects may carry a higher fat load (P < 0.05), compensated by a higher chylomicron number in FF subjects.

**Conclusions:** Our results indicate that lipids from either BF or FF may be handled differently in young healthy infants.

**Key Words:** apolipoprotein B48, bottle feeding, dietary fat, nursing, triglycerides

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Dietary lipids, preferably from human milk (HM), are a key energy source to meet the high energy requirements of the fast-growing infant. In addition to their nutritional value, dietary lipids including fat-soluble vitamins play important roles as signaling molecules involved in infant growth and metabolism, gene expression regulation, as well as in the development and modulation of cardiovascular, immune, and nervous system functions (1,2).

The lipid components in HM vary highly over the time of day, lactational stage, maternal nutrition status, and dietary intake (1,3,4). The HM lipid moiety is distinctly different from infant milk formulae (IF) as well with regard to fatty acid composition, the presence of cholesterol, lipid droplet size and architecture (5–7), structural composition of triglycerides (TG) (8,9). There

**What Is Known**

- Breast-feeding is optimal for infant nutrition and associated with short- and long-term health.
- Distinct features of human milk lipids (ie, droplet size and architecture, structural composition of triglycerides, and gradual fat delivery during a breast-feeding session) differ from formulae and may affect digestion.

**What Is New**

- Our study suggests that human milk and formula lipids may be handled differently by neonates.
- The triglyceride response of formula-fed subjects started at 60 minutes, and thus earlier than that of breast-fed subjects.
- Chylomicrons in breast-fed subjects carry a higher fat load, compensated by a higher chylomicron number in formula-fed subjects.
is a gradual release of lipids during a breast-feeding (BF) session (foremilk/hindmilk) in contrast to constant concentrations during formula-feeding (FF) (10). These features may directly affect lipid digestion, absorption, and bioavailability as shown previously in rodents, (preterm) infants, and adults (7,8,11-16). This may potentially have long-term consequences. Many of these physiological and metabolic aspects regarding lipid delivery have not been studied in healthy term infants.

By measuring lipid-related plasma concentrations of TG, total Cholesterol, HDL-Cholesterol (HDL), LDL-Cholesterol (LDL), apolipoproteins A1 (ApoA1), ApoB48, and total ApoB (ie, ApoB48 plus ApoB100) we estimated postmeal responses of 2-month-old healthy term infants to dietary lipids and compared BF with FF.

SUBJECTS AND METHODS

Study Design, Recruitment, and Procedure

An exploratory, observational single-center study with invasive measures (heel prick) was conducted between April 2010 and January 2012 in Groningen, the Netherlands. The study was named EAGLE 1 after a fictional space craft exploring unknown territory.

Participation was voluntary and written informed consent obtained from both parents. To strictly avoid interfering with parents’ feeding choice, FF subjects were only included when the decision to exclusively feed formula was made study independently and before inclusion (Fig. 1). FF subjects received free formula once informed consent was given. For these subjects, inclusion was possible up to the age of 4 weeks. For the exclusively BF subjects, inclusion was possible until the study day at 8 weeks of age. Recruitment was the responsibility of the investigator who made the study known in hospitals and “well-baby clinics.”

Healthy term subjects with normal birth and body weight at testing (>10th percentile of locally applicable growth charts for sex and age) were included. Exclusion criteria were congenital or metabolic diseases or compromised maternal health (hepatitis B, HIV, high blood pressure, hyperlipidemia, diabetes, overweight [body mass index <27] before pregnancy), and nonexclusive/mixed feeding. Subjects that needed other than standard cow’s milk-based formula or that had received medical treatment within 4 days before study day were excluded. At the study day, all subjects received a small cuddle toy. Parents had the right to resign from the study at any time. A sequential identification number and subject code (no initials) were assigned to each subject at study entry, stored at the study center.

Study Day

The study amounted to 1 test day (Fig. 1): before the age of 2 months (55 ± 5 days), parents contacted the study nurse after finishing the first feeding after 06:00 AM and arranged a home visit before the next feeding. Informed consent was confirmed, a final eligibility check performed, and data collected. Before the second feeding of the day, a capillary baseline blood sample (t0, 1 mL) was obtained, collected in heparin-coated tubes (BD Microtainer Contact-Activated Lancet, BD Diagnostics, Franklin Lakes, NJ), and stored on ice. Subjects were then fed in their usual manner either per bottle (FF) or by nursing (BF); feeding start and end were recorded. HM composition was not assessed during the present study. Subjects were randomly assigned to 1 of 6 postprandial blood collection times using an allocation list generated by a DOS-based software RANDOM provided by Nutricia Research, The Netherlands. The

permuted block randomization was stratified for the type of feeding (BF, FF). The second blood sample (t1) was collected either at 0, 60, 90, 120, 180, or 240 minutes after the first sample and immediately stored on ice. All subjects were weighed before and after the meal, the difference being a proxy for volume intake (test weighing); bottle weights were recorded before and after feeding for the FF group for more precise volume intake measures in this group. Blood samples were delivered within 1 hour after collecting the second blood sample to the laboratory, centrifuged, and plasma and pellets stored separately at -80°C until analysis.

Infant Formula

After inclusion in the study, the formula-fed infants received Bambix 1 Zuigelingenmelk (Nutricia B.V., The Netherlands), a generic commercial cow’s milk-based formula for infants’ age range 0 to 6 months compliant to EU directives for infant feeding (Directive 2006/141/EC). The prepared formula contained 66 kcal per 100 mL, and comprised protein 1.3 g, carbohydrates 7.4 g (of which 0.6 g per 100 mL >0.8 g per 100 kcal oligosaccharides), fat 3.4 g (of which 1.5 g saturated fatty acids, 1.3 g mono-unsaturated, and 0.6 g poly-unsaturated fatty acids (of which linoleic acid 447 mg, alpha-linolenic acid 83 mg, 11 mg arachidonic, 6.4 mg docosahexaenoic acid, and traces of cholesterol <0.0003 g). Bambix 1 formula lipids originated from a vegetable oil blend, representative of the most common lipid origins in infant formulae without addition of specific fat components (such as milk fat

FIGURE 1. Study design and procedure at study day. Exclusively breast-fed infants (dark gray) were recruited until shortly before the study day whereas exclusively formula-fed infants (light gray) were included until 4 weeks of age so that they received the same formula for at least 4 weeks before testing. Study day: during a home visit of the study nurse before the second daily feeding, subject’s anthropometric measures and a capillary baseline sample (t0) were taken. After the feeding, subjects were weighed again and randomized to the timing of the second blood sampling at 1 of the 6 sample times (t1) (W = anthropometrics and test weighing; blood drop symbol = heel prick).
globule membrane fractions, structured lipids, etc) that would change the generic character of the formula as reviewed in more detail, recently (17). A full description of all components of the formula is given in Supplemental Digital Content 1, Table, http://links.lww.com/MPG/A741.

**Plasma Parameters**

Primary and secondary parameters were total plasma TG concentrations at t₀ and t₁ and differences between groups at each postmeal time point, respectively. In addition to TG, total cholesterol, HDL, LDL, ApoA1, and ApoB48, total ApoB (ApoB48 plus ApoB100) concentrations were measured. Ratios of HDL:LDL, ApoA1:total ApoB, and TG:ApoB48 were calculated. From the available plasma sample pairs with sufficient volume, composite postmeal concentration profiles were generated combining data from multiple subjects. For this reason, the terms “postprandial” or “kinetics” were avoided in favor of “postmeal” and “lipid handling” as chronological blood collection from the same individual would be implied otherwise. Data are presented as (1) baseline, (2) postmeal concentrations not corrected for baseline (“PM”), or (3) postmeal concentrations corrected to each subjects’ own baseline (delta/Δ).

**Plasma Analyses**

Plasma analyses were performed at UMCG. Briefly, TG, total Cholesterol, HDL, and LDL were measured together applying combined enzymatic and colorimetric assays using a Roche Modular P Chemistry Analyser (Roche, Mannheim, Germany); Apolipoprotein A1 and total ApoB concentrations were assessed with specific antisera in the Siemens BN’II ProSpec system (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). ApoB48 concentrations were measured with the Shibayagi sandwich enzyme-linked immunosorbent assay kit (Shibayagi Co, Ltd, Shibukawa, Gunma, Japan via BioVendor GmbH, Heidelberg, Germany) according to manufacturer’s specifications.

**Approximated Fat Intake/Calculations**

Approximated fat intake per body weight was calculated by relating feeding volume estimated by test weighing (see above) corrected for milk and formula density (HM 1.03 g/mL and IF 1.0 g/mL) (18) to the average body weight at testing (Table 1) assuming that the mature term HM contained 3.5 g/dL fat (19).

**Statistics**

Little to no data were available from literature for sample size calculations. Considering the burden of collecting 2 blood samples from infants, we estimated that the minimal sample size to generate informative results would be 5 subjects/ sample time totaling 30 subjects per feeding group. t test comparison or Wilcoxon Mann-Whitney test were used as indicated. P values <0.05 were significant, between 0.05 and <0.1 were considered worth mentioning because of the small sample size. SAS/STAT version 9.2 software (SAS Institute, Cary, NC) was used.

**RESULTS**

**Study Population**

Seventy six subjects were enrolled, 4 were withdrawn by parents before testing, 2 did not meet study criteria leaving 70 subjects in the intention-to-treat population; 3 subjects were excluded because of nonresolvable errors in blood collection, leaving 67 subjects in the per-protocol population (BF, n = 33) for which subject characteristics are shown (Table 1). In this population, 59 plasma sample pairs with sufficient volume were available for analysis (baseline t₀/postmeal sample time t₁) (BF, n = 30); mean subject age was 55.3 ± 2.8 days. Subject characteristics (gestational age, weight, and length at birth and at test day) as well as sex distribution between feeding regimens were not statistically different between groups. All subjects followed their respective feeding regimen exclusively. More details on the study population are shown in Table 1; no (serious) adverse event occurred throughout the study.

**Food Intake/Feeding Duration/Feeding Number**

Significant differences between groups were observed (P < 0.001) for number of meals in the 24 hours before testing with a higher frequency of feeding moments in the BF group (BF 6.55 ± 0.83, FF 5.53 ± 0.56) (Table 1). Subsequently the feeding interval before the study meal was shorter for this group (BF, 128.91 ± 22.86 minutes; FF, 214.09 ± 30.77 minutes) (Table 1). Duration of the study meal was 5 minutes longer in the BF group (BF, 20.27 ± 7.70 minutes vs FF, 14.82 ± 3.57 minutes).

Feeding volume was, however, not statistically different as indicated by body weight (BF, 174.85 ± 43.49 g; FF, 166.62 ± 25.78 g) and bottle weight differences (FF, 164.07 ± 22.87 g) before and after feeding. Feeding duration did not correlate with weight change (data not shown).

**Approximated Fat Intake**

We found no statistical difference between approximated fat intake per body weight (BF, 1.65 ± 0.42 g/kg body weight; FF, 1.55 ± 0.27 g/kg body weight) when calculated as described above.

**Postprandial Plasma Lipid Concentrations**

**Triglycerides, ApoB48, and TG:ApoB48 Ratio**

TG baselines were not statistically different between groups (Fig. 2A). Postmeal TG concentrations seemed higher in BF than FF subjects at 30 minutes and higher in FF than BF subjects at 60 minutes (“PM; Fig. 3A). Once values were corrected for baseline (Δ), the response of FF over BF subjects at 60 minutes was confirmed, but the TG median of the BF group increased above baseline only at 90 minutes and did not statistically differ from FF subjects at this time. ΔTG concentrations of all FF subjects remained at or above baseline within 2 hours after feeding. In contrast, ΔTG concentrations for several (but not all) BF subjects substantially fell below baseline in the 2 hours after feeding. In both groups, the postmeal response took place between 30 and 180 minutes, after which TG concentrations (Δ) returned to baseline.

Baseline ApoB48 concentrations were significantly higher in FF than BF subjects (Fig. 2B) and postmeal concentrations (“PM) increased between 120 and 180 minutes in FF subjects only, returning to baseline after 4 hours (Supplemental Digital Content 2, Table http://links.lww.com/MPG/A742). In contrast, postmeal concentrations in BF subjects (“PM) did not vary over time resulting in biggest differences between groups at 120 and 180 minutes (P = 0.03 and 0.074, respectively). Once corrected for baseline, postmeal ApoB48 concentrations (Δ) were not statistically different between groups and stayed close to baseline.

The ratio of TG:ApoB48 was higher in BF than FF subjects at baseline and consistently elevated over the whole postmeal period.
Postmeal TG:ApoB48 concentrations were, however, not different between groups once corrected for baseline.

Total Cholesterol and Related Parameters

Baseline total cholesterol and total cholesterol postmeal concentrations were higher in BF than FF subjects at all times (Fig. 2D and Fig. 3C). In BF subjects, total cholesterol concentrations increased in response to the meal, whereas in FF subjects, total cholesterol concentrations remained close to baseline at all times (Fig. 3C).

The other cholesterol-related parameters (HDL, LDL, ApoA1, total ApoB, and ratios of HDL:LDL and ApoA1:total ApoB), also showed some pre- and postmeal differences: baseline concentrations of HDL, its lipoprotein ApoA1, LDL, total ApoB all were higher in BF subjects. Corrected for baseline, total ApoB concentrations showed a postmeal response similar in timing.
but not magnitude as total cholesterol concentrations in BF subjects only (90 minutes $P = 0.027$, 120 minutes $P = 0.050$). A complete overview of all parameter outcomes is shown in detail in Supplemental Digital Content 2, Table http://links.lww.com/MPG/A742 for baseline and postmeal concentrations ($PM$ and delta $\Delta$).

**DISCUSSION**

Many studies have shown beneficial effects of BF on later-life health (20–22). If and how particular components of HM such as lipids or physiological aspects of BF such as the later release of lipids during a BF session may contribute to these effects has not yet been elucidated: few studies investigated postprandial plasma parameters in (hospitalized) term (15) or preterm infants (16,23,24); others assessed fasting concentrations (25–28) or explored specific interventions over time (29–32). Postmeal responses of healthy infants after receiving either HM or IF with focus on lipids have, however, not been studied previously.

In this small-sized study, we investigated postmeal responses of infants to dietary lipids and found indications of differences between breast- and formula-fed infants. BF subjects in our study were fed more frequently than formula-fed infants, as found also in other studies (33–35). Subsequently, this group had shorter intermeal intervals. In addition, BF lasted longer than formula-feeding. The shorter feeding interval and longer feeding duration of BF subjects in our study was not associated with different volume intakes as determined by test weighing. We do not believe that these factors confounded our observations since 80% to 90% of a BF volume is consumed in the first 4 minutes of feeding (36).

The gradual release of fat toward the end of a BF session in contrast to bottle feeding, which provides lipids steadily over time, could be a reason for the differences we observed. The drop of some individual’s $\Delta$TG concentration in the BF group below baseline shortly after the meal could indicate that the late fat release of hindmilk had not been digested fully in all BF infants at the time of baseline blood collection.
FIGURE 3. Postmeal responses. For each of the selected lipid-related parameters, the upper panel shows measured postmeal (∆PM) concentrations; the lower panel data that are corrected for each subject’s own baseline (Delta, Δ) of exclusively breast-fed (dark circles) and exclusively formula fed (open triangles) subjects after their respective feeding. Each symbol represents 1 subject. The median is shown as gray bar. Observations with $P < 0.1$, established by Wilcoxon-Mann-Whitney test, were considered relevant findings due to the small sample size per assessment time.
Despite a potential delay in lipid intake in BF subjects, baselines were not different between groups. Postmeal BF TG concentrations seemed to increase earlier than those in the FF group (12 PM, median BF > FF, 30 minutes) indicating that HM lipids may be more quickly digested, absorbed, and reached the bloodstream faster than those in formula as suggested by others (24). The effect was, however, lost with baseline correction, and the BF ΔTG concentrations stayed at baseline until a later increase above baseline at 90 minutes. This observation suggests an effect of the more lipid-rich hindmilk on plasma TG concentrations.

Our observations seem to contradict with previously published reports that TG from HM disappear earlier from the stomach of preterm infants than those from formulae (24). There was, however, no hindmilk effect in that study because the children received their feeding by nasogastric tube, and therefore with a more constant fat intake.

Recent in vitro studies indicate that formula composition has less impact on digestion and absorption than physical properties (37). Differences in lipid handling in our study could thus be related to physical properties of HM and BF lipids such as milk fat droplet size and architecture, which are larger and more complexly coated in HM (7, 11–13, 38), structural properties of TG such as positioning of specific fatty acids along the glycerol backbone in HM (8) or differing viscoelasticity (39, 40). These factors have shown to affect gastric emptying, gastric lipolysis, intestinal digestion, and absorption of fatty acids, TG, and other lipid-related parameters.

We observed faster changes in postmeal lipid concentrations compared with studies in adults challenged with a fat load (12). Fasted adults that had ingested 0.68 g fat/kg body weight showed a ΔTG net peak height of 1.0 mmol/L after 3 to 4 hours and a return to baseline after 8 hours (12). In contrast, infants in our study showed an earlier and much lower postmeal ΔTG response (0.42 mmol/L between 60 and 90 minutes) and a return to baseline within 180 minutes, despite an estimated 2.3 times higher fat intake relative to body weight (~1.6 g fat/kg body weight).

Differences of postmeal TG concentrations between infants and adults could be due to faster uptake from the gastrointestinal tract or a more efficient clearance of circulating lipids in newborn infants, both of which would be in line with the approximately 6 times higher energy demands in infants than adults and the dependency of the human neonate on fatty acids and ketone bodies as energy source (1). Although a higher clearance efficiency may be reflected by the lower plasma peak heights despite a higher fat load that we observed, this could also be related to differences in fasting state between infants and adults. Because fasting for >4 hours is not ethical in infants, yet common for digestion studies in adults and rodents, other studies are required for a comparison of lipid uptake and plasma clearance between neonates and adults to confirm the indications given by our data.

A single ApoB48 lipoprotein is incorporated into an individual chylomicron and can therefore serve as proxy for chylomicron number (41). By relating TG to ApoB48 concentrations, the TG load carried per chylomicron can be estimated (42, 43). The significant difference in baseline TG:ApoB48 ratio may suggest that chylomicrons in BF subjects carry a higher TG load than FF subjects. This observation is supported by postmeal ApoB48 concentrations in the BF group being consistently higher than in the FF group. Because TG baselines do not indicate a difference in circulating TG concentrations between groups, the lower TG:ApoB48 ratio in the FF group could therefore be compensated for by a higher number of chylomicrons (as indicated by ApoB48) in this group.

Baseline total cholesterol concentrations were higher in the BF than FF group in line with previous studies (25, 30–32, 44–46). Postmeal concentrations were also consistently higher in the BF group and—once corrected for baseline—increased in response to the meal whereas concentrations in the FF group remained unchanged. This observation is most likely explained by the virtual absence of cholesterol in the studies infant formula. The presence of cholesterol in HM has been proposed to exert an inverse yet permanent (long-term programming) effect resulting in low circulating cholesterol concentrations in adulthood (25) thus contributing to the protective effect proposed for BF on later cardiovascular diseases risk (47–49), yet needs further investigation.

Our study has a number of important limitations. First, the number of infants for each measuring time was very small. For ethical reasons and to limit the burden, we collected 2 blood samples from each healthy infant within hours after feeding. This allowed us to obtain consent from both the parents and the ethical committee. Second, this limitation of only collecting 1 baseline and 1 postmeal sample made it impossible to construct true postprandial kinetics or area-under-the-curve graphs of individual infants that are usually generated in food intake or fat challenge studies. Thus, our data could be regarded as a first indication of differences of plasma lipid concentration patterns in infants receiving either BF or FF. Based on our results, future studies on dietary lipid handling in healthy term infants could focus on the first 2 hours after a feeding. In addition, our data could support future power calculations for the parameters used in the present study. Third, because of the ethical reasons, infants cannot be fasted as for rodent or adult fat load experiments, which may have affected the degree of comparability, as discussed in detail above. And last, we studied 1 formula with a vegetable oil-based fat bend, only. More studies are needed to investigate postmeal responses to formulae of different composition including fat blend components and compare responses between infants and adults directly.

In summary, we found that postmeal plasma concentrations of several lipid parameters showed small differences after either BF or FF in healthy infants. Differences were indicated for TG, BF over FF subjects at 30 minutes (12 PM) yet for FF over BF subjects at 60 minutes when corrected for baseline. The differences in TG: ApoB48 ratio together with baseline TG and ApoB48 concentrations suggest that chylomicrons in BF subjects may carry a higher fat load compensated by a higher chylomicron number in FF subjects. Cholesterol and lipoprotein concentrations were higher in BF subjects and seemed to increase after the meal in the BF group only.

**Research Perspectives**

Although knowledge about lipid requirements and physiological impact of lipids is accumulating, more research is needed. Only few studies over the past 4 decades have investigated postprandial responses of (healthy) infants to their respective feeds. Future studies should aim to:

- further increase the understanding of direct physiological effects of HM components and the process of BF on lipid handling and the infant’s metabolism;
- investigate effects and functional mechanisms of milk characteristics such as fat droplet size and architecture, composition of TG, as well as active components such as lactoferrin, lactalbumin, and others; and
- identify impact of dietary lipids on the human epigenome and their functional role as signaling molecules.

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