Lipoprotein-cholesterol responses in healthy infants fed defined diets from ages 1 to 12 months: comparison of diets predominant in oleic acid versus linoleic acid, with parallel observations in infants fed a human milk-based diet[1]

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Abstract A prospective study in healthy infants predefining both diet fatty acid and cholesterol, from birth to age 1 year, compared response of cholesterol fractions in three groups: random assignment to *1)* monounsaturated-(Hi-Mono) (n = 20), or *2)* polyunsaturated-(Hi-Poly) (n = 22) fatty acid-enriched diets, or *3)* non-randomized selection to breast feeding (Human Milk) (n = 25). In each group, designated weaning foods and supplements maintained fatty acid and cholesterol intake similar to that of each group's defined formulas, with long-term compliance confirmed by plasma phospholipid fatty acid concentrations. By 12 months, total cholesterol was significantly lower in the Hi-Poly group compared to either of the other groups ($P < 0.05$). Low density lipoprotein (LDL)- and high density lipoprotein (HDL)-cholesterol concentrations were significantly lower by 12 months in the Hi-Poly group, compared to the Hi-Mono groups. However, at the earlier 4-month interval, total cholesterol and LDL-cholesterol in both Hi-Mono and Hi-Poly groups were not different from each other, although each was significantly lower than the parallel Human Milk-group ($P < 0.05$). The Hi-Mono group increased gradually in total and LDL-cholesterol such that, after 12 months' feedings, all lipid fractions of this Hi-Mono group were no different from those of the Human Milk group. In independent group comparisons, there were no significant differences in HDL-cholesterol concentrations after 4 and 9 months on these diets. Independent of diet, HDL-cholesterol showed a falling trend as an overall time-effect across all groups ($P < 0.001$). These data suggest that prolonged feeding of a diet enriched in polyunsaturated acids in early infancy has a significant cholesterol-lowering effect compared to monounsaturates. These differences in total, LDL-, and HDL-cholesterol plasma concentrations between polyunsaturates and monounsaturates were not significantly evident until feedings had continued for a year.—**Mize, C. E., R. Uauy, R. Kramer, M. Benser, S. Allen, and S. M. Grundy.** Lipoprotein-cholesterol responses in healthy infants fed defined diets from ages 1 to 12 months: comparison of diets predominant in oleic acid versus linoleic acid, with parallel observations in infants fed a human milk-based diet. *J. Lipid Res.* 1995. **36:** 1178–1187.

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Although breast feeding is widely recommended, the majority of infants in the United States start out on liquid-formula diets (1). Most of the fats used in infant formulas are vegetable oils (2), and these frequently are rich in polyunsaturated fatty acids. The reasons for using polyunsaturated oils are related in part to availability, physical characteristics, and costs. But, in addition, there has been a widely held belief that polyunsaturated oils have health benefits (3). Feeding vegetable oil-based formulas over intervals ranging from 1 to 5 months after birth, compared to breast milk, has been associated with lower cholesterol levels in prior studies (4–12). On the other hand, except for infants receiving formulas, there are few populations in the world that subsist on diets high in polyunsaturated fatty acids (13, 14), and the differences specifically in the content of omega-6 (n–6) unsaturated fatty acids are usually not widely varying in different populations on traditional diets (15, 16). Thus, there is

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; LP, lipoprotein; P/S, polyunsaturated:saturated; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid.

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some uncertainty as to whether these polyunsaturates in large amounts are entirely safe, or at least optimal.

In normal adults, responses of serum cholesterol to dietary unsaturated fatty acids have been extensively studied, yet the effects of confounding genetic, environmental, and dietary variables on the LP-cholesterol subfractions have not provided a clear understanding of the role and mechanisms of the different types of fatty acids, particularly as they pertain to human metabolism.

Moreover, considerable research in laboratory animals, and to a limited extent in humans, suggests that dietary fatty acids produce various responses in different systems in the body. Thus, saturated, monounsaturated, and polyunsaturated fatty acids may have different independent effects on membrane structure (17), cellular function (18), gene expression (19), and intermediary metabolism (20–22). Although the various effects of the different types of fatty acids have not been thoroughly defined, particularly as they pertain to human metabolism, the question of what constitutes the best mix of dietary fatty acids in infants and children must still be asked. The optimal mix may not be the same at all ages, or under all conditions.

The prime study objective was to compare the effects of two different types of predefined unsaturated fatty acids in vegetable oil-based formulas, monounsaturated and polyunsaturated, on the concentrations of lipoprotein lipids in plasma in two groups of infants. A third group that used human breast milk-based feedings (closely approximating the monounsaturated fatty acid content of one of the predefined formulas) was chosen as a comparison basis for the feeding variable of physiologic breast milk cholesterol content. The null hypothesis was that there would be no differences among the three infant groups in their blood total and lipoprotein major subclass cholesterol responses at the end of 4, 9, or 12 months of infant diets predefined for oleic acid, linoleic acid, and cholesterol.

METHODS

Subjects

The study protocol and use of experimental infant formula were reviewed and approved by the Human Subjects Institutional Review Boards of the University of Texas Southwestern Medical Center and of the Presbyterian Medical Center, and all families gave informed consent for the study. The subjects were delivered at Presbyterian Hospital in Dallas over an 8-month period and seen for pediatric follow up at a local private practice directed by one of the investigators (RK). All subjects were normal full term newborn infants appropriate for gestational age with gestational age greater than 37 weeks and less than 41 weeks. All were free of major neonatal morbidity and with no evidence of genetic, metabolic, or other chronic disease.

Willing mothers were recruited by the physicians and research nurse (SA) on two initial levels of potential compliance: *a)* commitment to a single infant feeding option (bottle or breast feeding) from birth, and *b)* commitment to a minimum time of breast feeding for $\geq$ 4 months. Of approximately 70 healthy births per month in the physician private practice, 8 to 9 infants per month were recruited under these restrictions. A further incentive was the provision of all of the food for their infant throughout the study. After parents were informed of the nature of the study, infants from families with a self-reported history of early heart attack, or known high cholesterol levels, were excluded at the time of obtaining parental written consent. Neonates with malformations or evidence of congenital problems that could affect postnatal growth were also excluded. Group assignments were made while the infants were still in the nursery within 4 days after birth.

Parents who volunteered for sampling of blood lipids in the course of the study provided a window of inquiry for parental lipoprotein concentrations; for those from whom fasting samples were obtained, no individual group assignment bias could be recognized and overall parental results (mg/dL) were: total cholesterol 180 $\pm$ 36 (n = 82), triglycerides 99 $\pm$ 56 (82), VLDL-cholesterol 14 $\pm$ 12 (80), LDL-cholesterol 114 $\pm$ 31 (80), HDL-cholesterol 52 $\pm$ 12 (80).

Study design

This was a double blind, partially randomized prospective study. Babies whose mothers were not agreeable to breast feeding were assigned randomly to the high monounsaturated (Hi-Mono) and high polyunsaturated (Hi-Poly) groups. Random assignments were generated using computerized random number tables. These were provided in opaque envelopes, sequentially numbered, to the research dietitian so that the appropriate formulas could be given to the mothers. Mothers who agreed to breast feed their babies were recruited for the human milk (Human Milk) group. With the obvious exception of the Human Milk group, physicians, nurses, and families were blinded to the diet assignment. The double-blind was maintained until the 12-month assessment was complete.

One group received a formula (Wyeth SMA®) high in monounsaturated fatty acid (Hi-Mono group). A second group received a formula high in polyunsaturated fatty acids (Hi-Poly group); this formula (Wyeth Experimental SMA) was manufactured by Wyeth Laboratories specifically for this study. A third group remained on mothers' milk (Human Milk group).

Experimental regimens

The detailed fatty acid composition of the predefined diets over the year of the study are shown in **Table 1.** Additional details of precise month-by-month analyses are

TABLE 1. Calculated mean diet intake at successive time intervals (months) of the study

	High Monounsaturated Group				High Polyunsaturated Group				Human Milk Group			
Diet	0-4	4-9	9-12	0-12	0-4	4-9	9-12	0-12	0-4	4-9	9-12	0-12
kcal/kg/day	120	115	111	115	120	110	109	113	118	110	115	114
Protein (%)	9	10	12	10	9	10	12	10	7	9	11	9
Carbohydrate (%)	43	50	47	47	43	50	46	46	41	49	47	46
Fat (%)	48	40	41	43	48	40	41	43	52	43	42	46
Fatty acids (%)												
Saturated	43	43	43	43	45	44	43	44	44	44	43	44
18:1	40	41	42	41	18	18	18	18	42	42	43	42
18:2	14	13	13	13	37	37	37	37	12	12	11	11
18:3	<1	<1	<1	<1	<1	<1	<1	<1	1	1	1	1
Others	3	3	2	3	<1	<1	<1	<1	1	1	2	1
Cholesterol (mg/day)	26	40	62	43	26	35	60	40	106	127	139	124

Mean age of weaning was 6.2 ± 0.5 months (SEM). Data rounded to whole integers are means for the time intervals.

available on request. The Hi-Mono and Hi-Poly formulas contained 48% of total calories as fat, 9% as protein, and the remainder as carbohydrate. The mean percent of energy as polyunsaturated fatty acids for the high-monounsaturated, high-polyunsaturated, and human milk groups was 7.1%, 16.3%, and 6.0%, respectively, with polyunsaturated:saturated (P/S) ratios of 0.38, 0.85, and 0.30, respectively, and PUFA/MUFA ratios of 0.40, 2.08, and 0.31, respectively. In the two groups initially on liquid formula, infants received a mean intake of 43 and 40 mg cholesterol/day, respectively, over the course of the year's study.

All infants received 109 to 120 kCal/kg per day and 2.4 to 2.9 g protein/kg per day. Total intakes were increased as a function of increasing age. The diets provided vitamins and minerals to meet or exceed the NAS-NRC RDAs according to progressing age.

For the two groups initially on liquid formula, the composition of the diets was progressively adjusted to age 12 months to maintain a relative fatty acid composition similar to that of the initial formula. This was achieved by appropriate selection of solid foods combined with use of high-monounsaturated or high-polyunsaturated safflower oil supplements (California Fats & Oils, Richmond, CA). The percent of calories delivered as fat decreased in all groups after solids were introduced from approximately 48–52% to 40–43% (Table 1). Infants in the Human Milk Group were weaned at a mean age of 6.2 months (range 4–8.5 months); after weaning, they received a mixed diet resembling human milk both in general nutrient and fatty acid composition, as well as cholesterol content (100–140 mg/day). This was achieved by making use of egg yolk and baby foods (Gerber®) plus the same Hi-Mono formula employed in the Hi-Mono cohort.

Liquid formulas were prepared by Wyeth Laboratories Nutritional Division, Philadelphia, PA, whereas juices, cereals, vegetables, and meats were provided by Gerber Products Company, Nutrition Division, Fremont, MI. Both the food and the formula taken by the children were provided at no cost to the families throughout the study.

Foods were delivered to participants' homes on a monthly basis. For example, rice cereal was not delivered until month 4, rice and oatmeal cereal together with selected strained vegetables and apple juice were added at month 5, and fruit was delivered at month 6 along with teething biscuits. By month 7, the first fat-containing foods were offered, with different meats offered to the different groups depending on their specific fatty acid profile. Each participating family was provided with informational booklets, a monthly sample menu showing a meal-by-meal suggested intake pattern, and the specific quantities of both formula and food for each group (available on request from authors). The family was also provided with detailed information on the addition of the required oil and/or egg yolk required to maintain the desired fatty acid range of the diet for their respective group.

Parents had 24-h telephone access to study nurses, a dietitian, and the investigator team. Additionally, motivation letters and quarterly newsletters were sent to each family. Compliance was enhanced by providing immediate access to the private office of four participating pediatricians, where the infants were seen regularly on well-baby returns. The families were instructed, by the private practice pediatricians, to contact the study team to resolve questions about any diet modifications, unless the private practice pediatricians determined medical need for formula or diet change, or removal from the study.

Each family was prepared for the study dietitian to call 3–4 times a month (minimum once monthly), providing a detailed diet history at each call for recording the estimated quantities and timing of each new food. With some variation, the families' diet-recalls followed the suggested intake-patterns, from which the nutrient calculations were made. The exact nutritional composition of the for-

mula was provided by the manufacturer. The composition of the food products used was obtained from the Gerber Company and standard reference compilations (23, 24). Averaged figures were used for the nutritional composition of breast milk (25, 26). From these several data sources, individualized manual calculations were made of the month-by-month intake of the major nutrients and lipid components.

Individual infants' plasma data may be affected by factors other than genetic pattern, and the first blood samplings (t_0 data) were designed for age 4 months to follow a stabilizing relationship of parents to the study and to the logistics of providing all food to each infant. Among all this study's "free-living normal infants" drawn from the private pediatric practice, there were 2 of 25 in the Human Milk group, 1 of 20 in the Hi-Mono group, and 0 of 22 in the Hi-Poly group, with total cholesterol levels >180 mg/dL at each time interval; their data were included to avoid non-random bias.

Outcomes

Outcome measures were total cholesterol, lipoprotein-cholesterol subfractions, and phospholipids in plasma. The subjects were evaluated at 4 months (baseline), 9 months, and 1 year. Plasma lipid and lipoprotein measurements were made from venous blood samples obtained by the research nurse at those visits. Blood samples from subjects were obtained invariably following intervals after their normally scheduled, regular feeding times, viz. 2–4 h postprandially to age 3–4 months (usually continuing at similar intervals in breast-fed infants), and 3–6 h in the older infants. There is no standard infant 'fasting' or 'fasting interval,' especially in long-term infant studies. Thus, these intervals were not a uniform long-interval fast usually used in older patient studies, but were practice-based possible in a normal free-living population. Weight, length, and head circumference were obtained at birth and at each follow-up visit; they were made independently by each of two trained team members. Length was measured with a stadiometer, head circumference with paper tape measure, and weight was obtained using a digital scale. Length and head circumference were measured again when differences were larger than 0.5 cm for the two observers; weights had to be within 100 g. The two final measurements were averaged for purposes of statistical analysis.

Laboratory analyses

For analysis of plasma lipids and lipoproteins, venous blood samples were obtained at 4, 9, and 12 months of age. Plasma was separated by centrifugation (3000 g × 10 min) prior to lipid extraction using methanol–chloroform (27). Plasma total cholesterol and triglycerides were measured by enzymatic methods (28, 29). Cholesterol in high density lipoproteins (HDL) was measured enzymatically after precipitation of apolipoprotein B-containing lipoproteins with heparin–manganese (30). Very low density lipoprotein (VLDL) cholesterol was estimated according to the equation of Myers, Phillips, and Havel (31). Low density lipoprotein (LDL) cholesterol was estimated as the difference between total cholesterol and that in the VLDL + HDL fractions.

For determination of the plasma phospholipid fatty acid pattern, phospholipids were isolated by thin-layer chromatography (TLC) (32). Triacylglycerols, nonesterified fatty acids, cholesteryl esters, and total phospholipids from aliquots of total plasma lipids were separated by TLC using hexane–diethyl ether–acetic acid 80:20:1 (v:v:v). Lipids were identified by spraying with 1 mM 6-*p*-toluidine-2-naphthalene sulfonic acid (Eastman Kodak) in 50 mm Tris-HCl (pH 7.4) and visualized with short-wave ultraviolet light. Lipids were protected from oxidation at all steps (extraction, methylation, thin-layer chromatography) by inclusion of antioxidant (0.02% butylated hydroxytoluene) in solvents and flushing with nitrogen prior to all mixing steps and storage at −20°C. After scraping from the plate, fatty acids were directly methylated under N_2 with 14% BF_3 in methanol heated at 100°C for 10 min (33). Fatty acid methyl esters in hexane were fractionated and quantified using capillary column gas chromatography and flame ionization detection in a Hewlett-Packard 5890 gas chromatograph equipped with 0.25 mm inner diameter, 30-m capillary column containing SP-2330 stationary phase (Supelco, Bellefonte, PA). Peak identification was confirmed by comparison to individual purified standards and standard mixtures from Nu-Chek Prep (Elysian, MN) or Supelco. The relative concentration of individual fatty acids was expressed as percent of total fatty acid (weight percent) equal to or greater than 14 carbons.

Estimate of sample size

The sample size was selected based on detecting a difference of 14% in the mean value of an outcome measure with a standard deviation of 12% for a statistical test at the 0.05 level of significance and a power of 0.90 (34). In order to have the required 16 subjects at the completion of the study, it was decided to select 25 for the Human Milk group and randomize at least 20 to each formula group. Given the sample sizes achieved at 12 months a priori, the statistical power for the same comparisons based on the above criteria is in excess of 0.97.

The study subjects comprised 67 healthy infants, of whom 62 completed the study. The initial and final number of subjects were: Human Milk, 25 and 23; Hi-Mono, 20 and 19; and Hi-Poly, 22 and 20, respectively.

The individual reasons for five study drop-outs were *1)* parental refusal for further blood samplings following traumatic blood draw at 9 months; *2)* parental incompatibility with private practice personnel between 5–8

TABLE 2. Demographics and anthropometrics of study infant groups

Variable	High Monounsaturated	High Polyunsaturated	Human Milk
Sex distribution M/F	10/10	11/11	9/16
Weight (kg)[a]			
Birth	3.44 ± 0.48 (20)	3.60 ± 0.39 (22)	3.59 ± 0.45 (25)
Age 12 months	9.68 ± 0.95 (19)	10.11 ± 1.30 (20)	9.66 ± 0.81 (23)
Length (cm)[a]			
Birth	50.9 ± 2.6 (20)	50.8 ± 1.5 (22)	51.6 ± 2.3 (25)
Age 12 months	75.7 ± 2.5 (19)	76.0 ± 2.8 (20)	75.9 ± 3.1 (23)
Head circumference (cm)[a]			
Birth	34.5 ± 1.1 (20)	34.8 ± 1.3 (22)	35.2 ± 1.6 (25)
Age 12 months	45.9 ± 1.2 (19)	46.4 ± 1.2 (20)	47.1 ± 1.4 (23)

The number of infants entered in each group is shown in parentheses.
[a]Values given as mean ± SD.

months; *3)* family left private practice for economic reasons at 5 months; *4)* private practice physician removal due to infant refusal of juices, solids, and liquids at 6–7 months; and *5)* noncompliance with study diet protocol after 4 months. Thus 4-month data were available for the five infants who dropped out, with 4- and 9-month measures available in the first drop-out cited; no 12-month measures were available in these five infants.

Lipid fraction data were not available on all of the 62 who completed the study (Tables 3–5), due to technical/measurement difficulties at different intervals in different infants. Specifically in the case of LDL- and HDL-cholesterol measurements requiring further sample separation, sufficient blood volume could not be obtained in some of these small infants.

Data analysis

The data were analyzed using SAS (35). The sex distribution in the three groups was compared using Chi-square contingency table analysis. Weight and length at birth were compared using one-way analysis of variance. Anthropometric measures were compared for groups over the four time periods using repeated measures analysis of variance (36) with Bonferroni *t* tests for pairwise comparisons (37). Other outcome measures were compared using repeated measures analysis of variance for the three groups at three time periods followed by Bonferroni *t* tests for comparisons of time periods or of groups. Confidence limits were calculated differences between the means of the Hi-Mono and Hi-Poly groups at 4, 9, and 12 months for total cholesterol, LDL- and HDL-cholesterol, and triglycerides. Standard 95% confidence limits for the differences between two independent means were calculated (38).

The $P < 0.05$ level of significance was selected. The results are expressed as mean ± standard deviation unless noted otherwise.

RESULTS

Growth in weight, length, and head circumference occurred at the expected normal progression and were equivalent for each group (**Table 2**). There were no significant differences among any of the groups.

Fatty acid composition of plasma phospholipids and triglycerides in the three groups at 12 months is presented in **Table 3.** These data were analyzed to reflect infant feeding interval responses, as a measure of consistency of long-term dietary compliance in these groups. Compared

TABLE 3. Fatty acid composition of plasma phospholipids and triglycerides at age 12 months of study groups

	A. Phospholipids			B. Triglycerides		
Fatty Acid	Hi-Mono (17)	Hi-Poly (15)	Human Milk (20)	Hi-Mono (17)	Hi-Poly (15)	Human Milk (16)
	relative weight %					
16:0	24.2 ± 1.5	26.1 ± 1.8	25.0 ± 1.8	19.7 ± 2.3	20.6 ± 3.1	22.1 ± 2.3
18:0	16.0 ± 1.4	15.8 ± 0.8	15.4 ± 1.3	4.3 ± 1.0	3.2 ± 0.8	4.2 ± 1.2
18:1	15.5 ± 1.5	8.7 ± 1.0	14.4 ± 2.4	50.7 ± 3.3	29.5 ± 3.8	46.3 ± 3.0
18:2 (ω-6)	26.1 ± 1.3	30.2 ± 3.9	24.6 ± 3.4	18.6 ± 1.7	39.1 ± 6.9	19.8 ± 1.6

Hi-Mono, high monounsaturated group; Hi-Poly, high polyunsaturated group. Number of subjects in parentheses.

TABLE 4. Plasma concentrations as a function of diet and time: total cholesterol and LDL-cholesterol

	A. Total Cholesterol			B. LDL-Cholesterol		
Age	Hi-Mono	Hi-Poly	Human Milk	Hi-Mono	Hi-Poly	Human Milk
months			*mg/dL*			
4	136 ± 30 (19)[a]	135 ± 19 (21)[b]	164 ± 33 (23)[a,b]	72 ± 22 (16)[f,h]	77 ± 14 (19)[g]	100 ± 34 (23)[f,g]
9	147 ± 18 (20)	139 ± 20 (19)[c]	158 ± 24 (22)[c]	88 ± 20 (20)	84 ± 25 (18)	98 ± 24 (22)
12	156 ± 31 (17)[e]	128 ± 20 (16)[d,e]	156 ± 30 (23)[d]	98 ± 24 (16)[h,j]	79 ± 16 (16)[j]	96 ± 28 (23)

Experimental formula: Hi-Mono, high monounsaturated; Hi-Poly, high polyunsaturated. Data are mean ± standard deviation and number of subjects (n). Groups with the same superscripts are significantly different ($P < 0.05$ by repeated measures analysis and Bonferroni t tests).

to the Human Milk group, phospholipid and triglyceride fatty acids of the Hi-Mono group were slightly enriched with oleic acid (18:1), whereas the fatty acids in the Hi-Poly group were enriched with linoleic acid (18:2) with relatively less oleic acid. Similar effects across groups in fatty acid composition of these and other plasma and erythrocyte lipid fractions were also evident at 4, 9, and 12 months (data not shown).

Total cholesterol plasma values are shown for the three groups in **Table 4A.** The Hi-Poly group consistently had significantly lower concentrations throughout the study year compared to the Human Milk group; by 12 months total cholesterol in the Hi-Poly group was also significantly lower than in the Hi-Mono group, concomitant with a gradual increase in total cholesterol in the Hi-Mono group over the year. This increase in the Hi-Mono group was not statistically significant. Thus, while the Hi-Poly group had low cholesterol levels at 4 months, similar to the Hi-Mono group, the Hi-Poly group did not show a change in total cholesterol levels by 1 year. There were no statistically significant temporal changes throughout the first year within each of the three groups.

Table 4B gives values for plasma LDL-cholesterol. Similar to total cholesterol responses, each of the Hi-Mono and Hi-Poly groups had concentrations no different from each other at 4 and 9 months, but by 12 months, LDL-cholesterol was significantly lower in the Hi-Poly group. Each was significantly lower than the Human Milk group at 4 months. LDL-cholesterol in the Hi-Poly group and in the Human Milk group remained essentially unchanged throughout the year. The Hi-Mono group LDL-cholesterol's gradual rise over the course of the year (statistically significant from 4 to 12 months) seemed to parallel the gradual rise in total cholesterol. These data at 12 months show a trend in differences across groups similar to that of total cholesterol, with Hi-Mono and Hi-Poly groups being significantly different at 12 months.

Levels of plasma HDL-cholesterol are presented in **Table 5A.** At 12 months there was a significant difference between Hi-Mono and Hi-Poly groups. After feeding for 12 months, the Hi-Poly group evidenced a lower trend, although not statistically significant, compared to the Human Milk group. The levels were not significantly different within each of the three cohorts through the year, but there was an overall time effect ($P < 0.001$) across all groups for a decline in HDL-cholesterol concentrations regardless of group, i.e., all infants as a whole population showed a fall in HDL-cholesterol concentrations throughout the first year.

Results for plasma triglycerides are shown in Table 5B. There was a significant difference at 4 months between the Hi-Mono and Hi-Poly groups, but there was only a tendency for the Hi-Poly group to have a lower concentration than either of the other groups at 12 months.

For all four major plasma-outcome measures, confidence intervals are shown in **Table 6** for the difference between the means of the Hi-Mono and Hi-Poly groups at each of the time periods.

TABLE 5. Plasma concentrations as a function of diet and time: HDL cholesterol and triglycerides

	A. HDL Cholesterol			B. Triglyceride		
Age	Hi-Mono	Hi-Poly	Human Milk	Hi-Mono	Hi-Poly	Human Milk
months				*mg/dL*		
4	50 ± 12 (16)	46 ± 10 (19)	50 ± 13 (19)	169 ± 67 (19)[b]	97 ± 60 (21)[b]	126 ± 93 (23)
9	43 ± 10 (20)	39 ± 10 (18)	45 ± 8 (22)	102 ± 50 (20)	114 ± 79 (19)	107 ± 58 (22)
12	44 ± 10 (16)[a]	35 ± 8 (16)[a]	42 ± 11 (23)	164 ± 94 (17)	105 ± 64 (16)	151 ± 108 (23)

Experimental formula: Hi-Mono, high monounsaturated; Hi-Poly, high polyunsaturated. Data are mean ± standard deviation and number of subjects (n). Groups with the same superscripts are significantly different ($P < 0.05$ by repeated measures analysis and Bonferroni t tests).

TABLE 6. 95% Confidence limits for the difference between the means of the Hi-Mono and Hi-Poly groups

Variable	4 Months	9 Months	12 Months
Total cholesterol	−17.0,19.0	−5.7,21.7	8.2,47.8
LDL-cholesterol	−22.3,12.3	−11.4,19.4	2.3,35.7
HDL-cholesterol	−4.1,12.1	−2.1,10.1	1.1,16.9
Triglycerides	23.5,120.5	−52.9,28.9	−7.2,125.2

Values given as lower limit, upper limit.

At the end of the first year, the infants returned to ad libitum diets. A small subset of each group was volunteered by their parents at age 24 months for laboratory analysis (Human Milk n = 10, Hi-Mono n = 5, Hi-Poly n = 10). These numbers were too few to include in any statistical analysis, but in these observational data there were no differences across all cholesterol subclasses among all the infants sampled at this time (data not shown).

DISCUSSION

The essential question being asked in this study was whether different intakes of dietary major unsaturated fatty acids used in infant feeding have differing effects on plasma lipoprotein-lipid concentrations. This study does not draw comparison to a non-intervention course of natural feeding in normal healthy infants, because the purpose was to create more tightly maintained dietary intake regimens. A test of the null hypothesis showed that there were no differences at 4 and 9 months for major outcome measures, but there were differences for 12-month outcome measures of plasma total, LDL-, and HDL-cholesterol concentrations following a full year of feeding the defined diets. The three diets could also have other effects that were different, but the focus of the present study was on plasma lipids and lipoproteins. The number of patients decreased from 67 to 62 as the study progressed, but the power analysis of final sample size supports the conclusion that sufficient samples were obtained for statistical comparisons at all time points of the study.

For many years polyunsaturated fatty acids were thought to differ from monounsaturated fatty acids in their actions on plasma lipids. While both types of unsaturated fatty acids are known to lower plasma cholesterol levels when substituted for saturated fatty acids, the early studies of Keys, Anderson, and Grande (39) and Hegsted et al. (40) strongly suggested that polyunsaturated fatty acids produce lower levels of plasma total cholesterol than do monounsaturated fatty acids. Although it was originally assumed that the difference in total cholesterol levels must reflect differences in LDL-cholesterol levels, several more recent investigations indicate that polyunsaturated fatty acids can lower both HDL-cholesterol (41, 42) and VLDL-triglyceride levels (43). Subsequent studies with monounsaturated fatty acids have not revealed a reduction in VLDL and HDL-cholesterol levels, compared to saturated acids (44, 45). Whether polyunsaturated fatty acids lower LDL-cholesterol levels relative to monounsaturates has recently come into question. Over the past years, several studies suggest that the two types of unsaturated fatty acids have similar effects on LDL-cholesterol levels (46–49), although others have reported that polyunsaturates lower LDL levels relative to monounsaturates (44, 50–52). A recent review of all available data on this question seems to indicate that polyunsaturated fatty acids have a slightly greater LDL-lowering effect relative to monounsaturates (53).

One of the features of the present study was that it was carried out for a full year, and it was possible that there would be differences in the longitudinal effects of the three diets. The design did not encompass a completely ad libitum diet group. Cholesterol responses of such ad libitum feeding in infancy can be surmised from a few reports (54, 55). The types of feedings of this study design (breast milk or a formula followed by normal progression to solid foods) generally resembled ad libitum progression in free-living populations, with the study's difference, and major strength, being the tight within-group compliance of defined diets, both pre- and post-weaning. The general diet composition of two of the groups, Human Milk and Hi-Mono, generally accord with random ad libitum feedings of parents who opt for either breast milk for several months or for a commercially available formula (which the Hi-Mono formula was) up to 4 months; however, the diets were defined more tightly thereafter.

Growth in length, weight, and head circumference throughout the first year was similar for the three diets. Thus, as expected, the overall diets inclusive of the two different oils provided an appropriate number of calories and allowed for normal growth and development. Further, there was no clinical evidence that the overall health of the infants was different on the three diets. Thus, no evidence was obtained that any of the diets had adverse health effects relative to the others.

Up to 4 months, Hi-Mono and Hi-Poly formulas appear to have essentially identical effects on plasma lipids and lipoproteins. At 4 months, the plasma total cholesterol levels on the two diets containing unsaturated oils were significantly lower than in infants on human milk. A similar trend was noted for LDL-cholesterol levels. Similar data have been reported in another short-term study (7). As the Hi-Mono diet closely resembled human milk in fatty acid composition, the higher total cholesterol levels with human milk most likely were due to a higher cholesterol content of the latter. Thus, infants up to this age of 4 months appear to be unusually sensitive to the effects of dietary cholesterol on plasma total cholesterol levels. It should be noted that the cholesterol load for the breast-fed infants at this age was 20–23 mg/kg

per d, and that this decreases with time on a body weight basis. For HDL-cholesterol levels up to 4 months, no differences were observed among the three diets.

Over the next 5 months infants consumed solid food diets and formulas or breast milk such that the overall fatty acid pattern did not change compared to the first 4 months. At the 9-month time point, there was not a statistically significant difference between the Hi-Mono and Hi-Poly groups for total cholesterol or LDL-cholesterol. Differences in total cholesterol levels between the Human Milk group and Hi-Mono declined by 9 months, although the Hi-Poly group maintained a significantly lower concentration relative to the Human Milk cohort. By 9 months, there were no differences for LDL-cholesterol across groups. Triglyceride and HDL-cholesterol levels were again not different among the three groups. Overall, there was a tendency for differences among the three diets for some parameters, but the differences narrowed somewhat compared to 4 months.

The most striking differences at 12 months were significantly lower plasma levels in the Hi-Poly group, compared to the Hi-Mono group, in total cholesterol, LDL-cholesterol, and HDL-cholesterol. There were no statistically significant differences in VLDL-cholesterol throughout the study (data not shown). At 12 months, any differences in total cholesterol levels between the Human Milk and Hi-Mono group had disappeared altogether. This occurred in spite of a higher cholesterol intake in the Human Milk group. Thus, it appears that the effect of dietary cholesterol to raise cholesterol levels is muted by 12 months. Of interest, over the course of the first year, HDL-cholesterol levels showed an overall across-group time-effect decline.

The results obtained at 12 months suggest that polyunsaturated fatty acids have a cholesterol-lowering effect in infants through the first year of life compared to monounsaturates. This effect may be important in infants, because the magnitude of the difference in cholesterol levels between the two types of unsaturated fatty acids may well be blunted with aging, as differences in LDL concentrations may be relatively more pronounced in younger than older adults. The data of the present study lend support to this concept. The current data suggest that the cholesterol-lowering action of polyunsaturated fatty acids extends to both LDL and HDL lipoprotein fractions. If further studies confirm a difference in lipoprotein fractions between the two types of unsaturates after such a lengthy 12-month feeding regimen, considerable accumulation of polyunsaturated fatty acids in the body may be necessary before its differential effect on LDL- and HDL-cholesterol concentrations becomes apparent.

The comparison with human milk, similar in composition to the high-monounsaturated formula but containing cholesterol, can provide useful parallel observations. Although it cannot be said a priori that the cholesterol and fatty acid contents of human milk are optimal, there is a broad acceptance of the concept that breast feeding is preferable to formula feeding (56). The argument can be made that, in cases where breast feeding is rejected or not practical, the formula replacement should be similar in composition and/or have similar metabolic effects as human milk.

Studies investigating dietary cholesterol intake in early infancy have usually been based on breast or cow milk comparisons with formulas, without precise definition of fatty acid intake or management of overall diet post-weaning (4–6, 9–11, 57, 58). Precise statements cannot be made about the separate effect of dietary cholesterol in this study, as the Human Milk and Hi-Mono groups did not ingest diets precisely differing only in cholesterol content. Nonetheless, the Human Milk group may reflect an observational parallel group for monounsaturated fatty acid comparison, due to the close similarity in overall fatty acid composition of this study's specially manufactured monounsaturated formula to that of human breast milk.

There are also several trends observed across all the interval data within groups. Within-group comparisons as a function of age address a separate biologic issue in each cohort, viz. biologic early maturation over the months of the first year of life. Such data are relatively difficult to accrue in free-living normal infants consuming defined diets for such a long period. Total cholesterol levels were unchanged in Human Milk and Hi-Poly groups, but tended to increase progressively in the Hi-Mono group. LDL-cholesterol levels tended to rise progressively only in the Hi-Mono group, while HDL-cholesterol concentrations declined progressively across all three groups (time effect $P < 0.001$). Thus, changes in total cholesterol do not necessarily reflect longitudinal changes in the individual lipoprotein fractions, and changes of LDL-cholesterol and HDL-cholesterol over the first year appeared to be independent of the dietary fatty acid and cholesterol. These trends occurred with a concomitant gradual decrease (approximately 10%) in fat content of the diet.

In summary, there are several trends over the first year of life observed across these cohorts. After 12 months of predefined diet, total cholesterol, LDL-cholesterol, and HDL-cholesterol were significantly lower ($P < 0.05$) in the Hi-Poly formula group. Earlier in the course of this year-long program (at 4 and 9 months), no recognizable differences were evident between these two low-cholesterol formula groups. After 4 months, nonetheless, both the Hi-Poly and Hi-Mono groups had lower total and LDL-cholesterol serum responses than the Human Milk group (whose diet closely paralleled the Hi-Mono fatty acid profile). With continued high-compliance feedings from 4 to 12 months, although the Hi-Mono group showed a trend for gradual increase in the total and LDL-cholesterol, all lipid fractions of this Hi-Mono group were

no different by 12 months from those of the Human Milk group. In group comparisons, there were no significant differences in HDL-cholesterol levels at the 4- and 9-month intervals.

An important question about formula diets for infants is whether enrichment in polyunsaturated fatty acids is advisable, as the current observations on the lipoprotein fractions raise the possibility that they could have a broad influence. Over the PUFA/MUFA range of 0.3 to 2.1 in these groups, HDL-cholesterol concentrations were lower in the Hi-Poly cohort. Thus, the present study does not confirm short-term and long-term safety of high intakes of polyunsaturated fatty acids in infant formulas, but it underscores the need for additional investigation of this potentially important issue.

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