Plasma Cholesterol and Lathosterol Levels in Term Infants in the Early Neonatal Period

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ABSTRACT. Plasma cholesterol concentrations increase after birth. Whether this is due to increased cholesterol synthesis has not been reported. Additionally, it is not known if formulas, which lack cholesterol, result in higher rates of cholesterol synthesis than feeding breast milk. To address this, plasma lathosterol (quantitated by gas chromatography), a potential indicator of cholesterol synthesis, was measured in term infants at birth (cord) and 4 d of age fed either formula or breast milk ($n = 6$ each), normal adults ($n = 6$) at the expected nadir and peak of the diurnal rhythm (1700 h and 0830 h), and cholestyramine-treated hypercholesterolemic adults ($n = 6$). Plasma cholesterol and apo B increased, and apo AI (measured by immunoprecipitation) did not change over the first 4 d of life. The increase in plasma cholesterol was greater in the formula-fed infants compared with breast-fed infants. The plasma lathosterol concentrations ($\mu\text{mol/L}$) and the lathosterol:cholesterol ratios ($10^2 \times \text{mmol lathosterol/mol cholesterol}$) decreased from birth to 4 d of age by 12 and 36%, respectively, in the breast-fed infants and by 20 and 46%, respectively, in formula-fed infants. The infant plasma lathosterol concentrations, however, were not different from normal adult levels [1700 h: 5.50 ± 1.52 (SD); 0830 h: 6.18 ± 1.80], but were lower than those of cholestyramine-treated adults (20.48 ± 13.41). Sterol ratios of infants were higher than those of normal adults (1700 h: 112.5 ± 26.0 ; 0830 h: 129.4 ± 42.2), but not different from those of cholestyramine-treated adults (322.9 ± 168.4). These data show that the evaluation in plasma cholesterol between birth and d 4 of age is not accompanied by increased plasma lathosterol levels, suggesting no increase in cholesterol synthesis. Further, infants fed formula do not have greater plasma lathosterol levels than breast-fed infants. (*Pediatr Res* 31: 396–400, 1992)

Abbreviations

HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A
glc, gas-liquid chromatography

In several mammalian species studied, plasma cholesterol levels increase after birth and fall at the approximate time of weaning (1–4). Humans, too, exhibit a postnatal increase in plasma cholesterol levels, and work by Potter (5) shows that this

occurs by 5 d postpartum. Thereafter, plasma cholesterol levels continue to increase until 3–4 y of age, decrease at adolescence, and then again rise slowly throughout life (6). Several hypotheses have been offered to explain the early postnatal increase in plasma cholesterol. These include the start of oral feeding (7–9), change(s) in hormonal milieu (8, 9), a decrease in LDL receptor number (10), and reduced adrenal uptake of lipoproteins (11).

One possible mechanism for the postnatal elevation of plasma cholesterol is increased cholesterol synthesis. Studies in the rat, however, have shown that cholesterol synthesis, as measured by the hepatic activity of the rate-limiting enzyme HMG CoA reductase (EC 1.1.1.34), decreases after birth (12), and some evidence of possible cholesterol synthesis inhibitors in milk have been published (12, 13). These data have been extrapolated to humans (5) to suggest that this is a universal postnatal phenomenon, but to the best of our knowledge cholesterol synthesis has been measured in only the human fetus (14, 15), not the newborn.

Cholesterol synthesis is commonly assessed *in vitro* by assay of the activity of HMG CoA reductase (16). Alternative methods more appropriate for the human, which do not require liver biopsy, are based on incorporation of deuterated water into cholesterol (17) or assay of plasma mevalonate (18). These involve inappropriately large blood samples for newborn studies and/or laborious methodology. Farkkila and Miettinen (19) have described a method for assessment of cholesterol synthesis *in vivo* by direct assay of serum sterol using glc. Quantities of lathosterol (free and esterified), a sterol precursor of cholesterol, are correlated with the activity of hepatic HMG CoA reductase in human liver (20) and with whole-body cholesterol synthesis as measured by balance studies (21). It is thought that total lathosterol leaks from the cell into the plasma at a rate proportional to the synthesis of cholesterol (21). Lathosterol is present in plasma at concentrations 1000-fold less than cholesterol.

The objective of this study was to determine whether or not the postnatal elevation in plasma cholesterol levels is coincident with enhanced cholesterol synthesis, as indicated by plasma lathosterol levels. Plasma lathosterol concentrations were determined in term infants at birth and 4 d of age. Infants fed breast milk and formula have comparable plasma cholesterol levels during the first week of life (22), breast milk providing approximately 20 mg/dL cholesterol (23) and formula containing no cholesterol. Therefore, these studies also determined whether or not additional cholesterol synthesis, as indicated by plasma cholesterol, was required to maintain plasma cholesterol levels in infants fed formula.

MATERIALS AND METHODS

Infants. Twelve single-birth term infants born by elective cesarean section at Grace Hospital, Vancouver, B.C., were recruited for this study. This population, chosen to avoid possible effects of labor and delivery on cord plasma cholesterol levels (5, 24–26), had a mean gestational age of 38.7 ± 0.8 (SD) wk and birth

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weight of 3372 ± 440 g. None of the mothers had diabetes of any origin or hypertension, and all infants were apparently medically and metabolically normal. The mother's choice to breast-feed or use infant formula was made freely without the advice of anyone involved in this study. The infants were fed *ad libitum* at approximately 3-h intervals, and their weight was recorded daily. Six of the infants were fed exclusively with formula; five were fed only Enfalac (Mead Johnson, Belleville, Ontario, Canada), and one infant was fed Enfalac for 48 h and then Prosoybee (Mead Johnson). The other six infants were breast-fed; three infants had one to five supplementary feedings of Enfalac (totalling 20, 60, and 140 mL) during the first 48 h. All of the breast-fed infants were Caucasian, and, of the infants fed formula, one was Caucasian and five were Oriental. There were equal numbers of males and females in each group.

Blood Collection. At birth, the umbilical cord was clamped and blood (1 mL) was collected by puncture of the umbilical vein within 2 min of delivery. Blood (0.5 mL) was sampled at 4 d of age (92–103 h, between 0815 and 1545 h) by venipuncture of a vein on the dorsum of the hand. Plasma was obtained after centrifugation at 3000 rpm, 15 min, 4°C, and stored frozen at –70°C within 4 h of collection.

Adult Reference Populations. Normal, healthy, Caucasian male adults were recruited for this study if they met the following criteria: they were nonsmokers, had no history of heart disease, were not taking medications, were not drinking more than four cups of coffee/d, were within 1 SD of their ideal body weight, and ate a typical North American diet as verified by a 24-h food intake record. The final six participants had a mean age of 29.3 ± 3.8 (SD) y, weighed 69.2 ± 9.3 kg, and were 179.2 ± 7.1 cm tall. Blood was sampled by venipuncture before meals at 1700 h (± 25 min) and 0830 h (± 20 min) the next morning. These sampling times represent the nadir and peak, respectively, of the cholesterol synthesis diurnal cycle as measured by deuterium incorporation (17). Plasma was prepared and stored as described above.

Blood was sampled by venipuncture (0500 and 1000 h), from six familial hypercholesterolemia patients from the Lipid Clinic of University Hospital, Shaughnessy Site, Vancouver, B.C. Patients were undergoing p.o. therapy with cholestyramine resin (Questran; Bristol Laboratories, Belleville, Ontario, Canada), a bile acid sequestrant known to increase cholesterol synthesis and plasma lathosterol concentrations (20, 27). The patients included two women and four men ranging in age from 16 to 72 y. The dose of cholestyramine resin ranged from 4 to 16 g/d over a duration of 1–5 y, affecting a reduction of plasma LDL-cholesterol levels of 1.5–2.6 mmol/L. All plasma samples were prepared and stored as above.

Sterol Analyses. Plasma cholesterol levels were determined using an enzymatic kit (Diagnostic Chemicals, Ltd., Charlotte-town, Prince Edward Island, Canada), and quality control serum (Sigma Chemical Co., St. Louis, MO) was included in each assay. The interassay coefficient of variation was $< 2\%$. Plasma lathosterol was extracted (19) and quantitated by glc (20) as previously described with minor modification. In brief, 5 α -cholestane (internal standard, Sigma Chemical Co.) was added to 50–100 μ L of plasma and saponified with 50% KOH and methanol (6:94, vol/vol) for 1 h at 80°C. This was extracted three times with petroleum ether, dried, and silylated with hexamethyldisilane:trimethylchlorosilane:dimethylformamide, 20:2:5 vol/vol/vol (Pierce, Rockford, IL), for 5 min at room temperature. The sterols were then injected into a Varian (Varian Canada Ltd., Georgetown, Ontario, Canada) 3400 glc equipped with flame ionization detection and a Varian 401 data system. Separation was achieved on an RTx-1, 25 m capillary column, 0.25 mm internal diameter, 0.25 μ m thickness (Restek Corp., Bellefonte, PA). Samples were injected at 80°C, and, after remaining at this temperature for 1 min, the oven temperature was programmed to increase to 120°C (20°C/min), hold for 7 min, rise to 249°C (20°C/min), hold for 15 min, rise to 269°C (20°C/min), and hold

for 20 min. The oven was then heated to 320°C (20°C/min) for 5 min before subsequent analyses. The injector and detector were set at 300°C and 320°C, respectively. The carrier gas (helium) flow rate was 1.25 mL/min with the inlet splitter set at 100:1 and the relays programmed to come on at 0.7 min after the run start.

Lathosterol peak identification was confirmed using an authentic standard (Sigma Chemical Co.). The interassay coefficient of variation was 11%. As done previously (19), lathosterol was reported as μ mol/L and as the ratio of lathosterol:cholesterol ($10^2 \times$ mmol lathosterol/mol cholesterol).

Apolipoprotein Analyses. Plasma apo AI and B were measured in the frozen plasma samples by immunoprecipitation (Beckman Array Protein System, Palo Alto, CA). The interassay coefficient of variation was $\leq 8\%$ for both assays.

Statistical Analysis. *Infants.* Group and time effects and their joint effect on the data were examined using a multivariate analytic approach [Roy's greatest root (28)] to the repeated measures design. When there was evidence of an interaction between group and time on an effect, group effects were examined separately at each time using *t* tests and, similarly, paired *t* tests were used to examine the time effects for each group.

Adults. One-sided, paired *t* tests were used to compare normal adult data at 1700 and 0830 h. A series of two-sided *t* tests were used to compare infant with normal adult values, and one-sided *t* tests were used to compare both infant and normal adult values with cholestyramine-treated patient data.

Ethics. Study protocol was approved by the clinical screening committees for research and other studies involving human subjects of the University of British Columbia, British Columbia Children's and Grace Hospitals. Informed consent was obtained from all volunteers and parents to allow their infant's participation.

RESULTS AND DISCUSSION

Plasma cholesterol and apolipoproteins. The increase in plasma cholesterol levels by approximately 40% over the first 4 d of life found in this study (Table 1) is consistent with the results of others (5, 9, 22, 29). However, the higher cholesterol levels in both cord and d-4 plasma of the formula group than in the breast milk group ($p = 0.05$) differ from previous reports of no differences at 3 d (22) and 5 d (5) of life. Separate statistical analyses of the cord plasma cholesterol revealed no significant racial differences ($p = 0.71$). We, along with others (30), have observed that by 6 wk of age breast-fed infants have higher plasma cholesterol levels than their counterparts fed formula (unpublished observations).

Results of the apolipoprotein analyses indicate that the rise in cholesterol level was associated with VLDL and LDL rather than HDL. Others (9, 29) have reported that cholesterol is fairly equally distributed between HDL and LDL in cord blood, but after birth LDL predominates. In agreement with previous findings, we found (Table 1) no change in apo AI concentrations between cord and d-4 samples (5, 9, 22, 29) but an increase of approximately 66% in apo B levels (9, 22, 29).

Plasma lathosterol concentrations. Values obtained for the adult population are similar to those reported in three recent studies (19, 31, 32). As observed previously (20, 27), cholestyramine treatment of hypercholesterolemic patients (Table 2) resulted in a marked elevation in plasma lathosterol levels and the lathosterol:cholesterol ratio above normal adult values, thus validating current methodology.

Deuterated water analysis (17) and glc assay of methyl sterols (33) have shown a diurnal cycle in cholesterol synthesis in adults, but it is not known whether lathosterol levels show periodicity. We found that plasma lathosterol levels may be an index of this phenomenon, plasma concentrations of lathosterol at 0830 h being greater than at 1700 h ($p = 0.04$, Table 2).

Plasma lathosterol concentrations decreased from birth to d 4

Table 1. Cholesterol, apoproteins, and lathosterol values in neonatal plasma [mean $\pm$ SD (n)]

	Diet*	Cord	Day 4	<i>p</i> values		
				Group $\times$ time	Group	Time
Cholesterol (mmol/L)	BM	1.63 $\pm$ 0.21 (6)	2.22 $\pm$ 0.21 (6)	0.4	0.05	0.0001
	F	1.78 $\pm$ 0.34 (6)	2.56 $\pm$ 0.23 (6)			
Apo AI (g/L)	BM	0.63 $\pm$ 0.10 (6)	0.61 $\pm$ 0.10 (6)	0.05		
	F	0.67 $\pm$ 0.10 (6)	0.73 $\pm$ 0.12 (6)			
Apo B (g/L)	BM	0.18 $\pm$ 0.03 (6)	0.31 $\pm$ 0.07 (6)	0.8	0.8	0.0001
	F	0.18 $\pm$ 0.03 (6)	0.30 $\pm$ 0.09 (6)			
Lathosterol (μ mol/L)	BM	5.69 $\pm$ 0.52 (6)	4.99 $\pm$ 1.40 (6)	0.4	0.07	0.03
	F	7.09 $\pm$ 1.42 (6)	5.64 $\pm$ 0.91 (6)			
Lathosterol/cholesterol ($10^2 \times$ mmol lathosterol)/mol cholesterol	BM	352 $\pm$ 56 (6)	226 $\pm$ 74 (6)	0.3	0.4	0.0003
	F	404 $\pm$ 97 (6)	220 $\pm$ 24 (6)			

* BM, breast milk; F, formula diet group.

Table 2. Plasma cholesterol and lathosterol values in adult reference populations [mean $\pm$ SD (n) and (range)]

	Normal adult males		Cholestyramine-treated patients
	1700 h	0830 h	
Cholesterol (mmol/L)	4.85 $\pm$ 0.33 (6) (4.58–5.33)	4.85 $\pm$ 0.52 (6) (4.24–5.66)	6.09 $\pm$ 0.89 (6) (4.60–7.29)
Lathosterol (μ mol/L)	5.50 $\pm$ 1.52 (6)*† (3.39–7.24)	6.18 $\pm$ 1.80 (6)*† (3.72–8.09)	20.48 $\pm$ 13.41 (6)†‡ (10.47–43.47)
Lathosterol/cholesterol ($10^2 \times$ mmol lathosterol)/mol cholesterol	112.5 $\pm$ 26.0 (6)†§ (73–140)	129.4 $\pm$ 42.2 (6)†§ (78–177)	322.9 $\pm$ 168.4 (6)† (170–595)

* Normal adults, 1700 vs 0830 h, $p = 0.04$.

† Normal vs cholestyramine-treated adults, $p = 0.02$.

‡ Infants (cord or d 4) (see Table 1) vs cholestyramine-treated adults, $p = 0.02$.

§ Normal adults vs cord, (see Table 1) $p < 0.0001$.

|| Normal adults vs d 4 (see Table 1) infants, $p = 0.002$ – 0.0002 .

(Table 1), suggesting that the postnatal increase in plasma cholesterol levels is not coincident with enhanced cholesterol synthesis. Conceivably, the high levels of cholesterol may repress its synthesis via the LDL receptor pathway. The infants had not regained their birth weight by 4 d of age [95.3 ± 4.7 (SD)% of their birth weight]. A potential decrease in cholesterol synthesis at this time could be explained by limiting acetyl CoA levels for synthetic pathways due to negative energy balance during the establishment of enteral feeding in the first days after birth.

It is not known whether the newborn human has an established diurnal cycle of cholesterol synthesis by 4 d of age. To contrast values with adult levels, cord and neonatal blood samples were collected over a short time span and compared with the adult values at both 1700 and 0830 h. Inasmuch as no differences between formula and breast milk groups were apparent, these data were pooled before being compared with adult values. No differences were found between neonatal and adult lathosterol concentrations at either adult extreme.

Plasma lathosterol:cholesterol ratios. Plasma lathosterol:cholesterol ratios were higher in cord versus d-4 samples (Table 1) and in neonatal versus adult samples (Table 2). This is likely due to the lower plasma cholesterol levels in the cord and neonatal samples, respectively. The plasma lathosterol:cholesterol ratio found in the cholestyramine-treated patients was higher than at both normal adult sampling times but comparable to those found in neonatal samples. The observation that precursor levels are the same, yet product is reduced, leads one to speculate that there is an increased demand for, or turnover of, plasma cholesterol in the rapidly growing neonate. The similar ratios in neonatal and cholestyramine-treated patient plasma is likely a reflection of both lower plasma cholesterol and lathosterol in the neonates.

Effect of breast- versus formula-feeding on plasma lathosterol levels. The plasma lathosterol concentrations of infants were not influenced by diet (Table 1). It was hypothesized that the lack of

cholesterol in the formula would stimulate additional cholesterol synthesis and hence result in increased plasma lathosterol levels in the infants fed formula.

Plasma lathosterol as an indicator of cholesterol synthesis in the neonate. The validity of using plasma lathosterol as an indicator of cholesterol synthesis rates in the newborn has not been tested. At present, this would not be feasible, inasmuch as it would require a liver biopsy for the measurement of HMG CoA reductase activity. In addition, stable isotope methodology requires more extensive blood sampling and an interval of time to measure isotope incorporation and would thus be unsuitable for single point determinations, *i.e.* birth and 4 d of age. Finally, plasma mevalonate analysis would also require verification for use in the neonate. In support of the lathosterol methodology, our data from preterm infants (34) show elevated plasma lathosterol levels coincident with administration of 10% Intralipid, which is predicted to be a stimulant of cholesterol synthesis (35).

Hypotheses to explain postnatal hypercholesterolemia. Onset of feeding. Although it seems that the cholesterol content of breast milk is not responsible for the postnatal elevation in plasma cholesterol levels, other dietary components may trigger this effect. Fat-feeding itself increases plasma cholesterol levels (36), and, theoretically, the reason for this could be increased cholesterol synthesis. Our data, however, indicate that there is no increase in the plasma lathosterol concentration, at least during the first 4 d of life. Breast milk contains saturated fatty acids, which, when compared with polyunsaturated fatty acids, are known to induce hypercholesterolemia in adults (37). Infant formulas, in contrast, are relatively enriched in polyunsaturated fatty acids and contain few long-chain saturated fatty acids. Manipulation of diet fat type in older infants (3–16 mo) shows that plasma cholesterol levels can be influenced by the degree of fat saturation in the same way as in adults (38). We, however, did not see different plasma lathosterol levels between breast-fed infants and those fed formula. Thus, it seems unlikely that the

type of fat fed to infants had a significant effect on liver cholesterol synthesis in the first days of feeding.

Maturation of regulation of cholesterol homeostasis. LDL receptors are present on most cells and regulate uptake of intracellular cholesterol (39). Brown and Goldstein (39) have presented the hypothesis that there are relatively more LDL receptors at birth than in the adult (therefore, higher plasma cholesterol levels in the latter).

Supportive evidence for this theory was provided by Rudling and Peterson (10), who found greater *in vitro* LDL receptor binding in fetal (low plasma cholesterol levels) than postnatal (higher plasma cholesterol levels) tissue homogenates. Additionally, in our laboratory, ligand blotting has been recently used to demonstrate greater LDL receptor mass in fetal and neonatal than in adult liver membranes (40). Contrary to this, in humans, Andersen and Johansen (41) report that the number of LDL receptors in cultured cord blood lymphocytes is not different from that in adult control lymphocytes. One might suspect that this reflects an up-regulation that may occur during culture.

There is much evidence that the LDL receptor is fully mature and functional in both the fetus and the newborn (41–45). Most of this data is the result of the measurement of cholesterol synthesis in cultured cells and its ability to be stimulated and suppressed. This has been done with cord lymphocytes (40), amniotic fluid cells (42, 43), newborn foreskin cells (42, 44), and fetal hepatocytes (45). Given that the LDL receptor is already functional in the fetus, it seems plausible that changes in activity and/or mass of the receptor at birth may be the result of postnatal elevation of plasma cholesterol levels rather than its cause.

Involution of fetal zone of adrenal. The fetal zone of the human adrenal has a high requirement for cholesterol for production of many steroids (11). There is considerable evidence that the human fetal adrenal preferentially uses LDL cholesterol (11) acquired via the classical LDL receptor pathway (46) as the substrate for steroidogenesis. Theoretically, when the fetal zone of the adrenal regresses at birth, the demand for LDL cholesterol decreases and plasma levels increase (11). This theory can explain a number of observations: the increase in LDL at birth due to a rapid decrease in LDL clearance, the use of a unique lipoprotein (HDL_e) in human fetal life (47) to substitute functionally for LDL in delivering cholesterol to cells, and elevated postnatal plasma cholesterol levels without increasing cholesterol synthesis.

Conclusions. Although the etiology of early postnatal elevation of plasma cholesterol levels in humans is unproven, data from this study suggest that increased lathosterol synthesis is not part of the mechanism. If, as in the adult, plasma lathosterol levels reflect cholesterol synthesis, our data dispel the hypothesis that initiation of a high-fat diet at birth stimulates cholesterol synthesis. It also seems that if the LDL receptor function is fully developed at birth, changes in receptor activity and/or mass may result from, rather than evoke, the elevation of plasma cholesterol levels in the first few days after birth. Theories forwarded by others describing the involution of the fetal zone of the adrenal are consistent with the results of this study. If not adrenal fetal zone atrophy, another mechanism independent of increased cholesterol synthesis likely accounts for the phenomenon of elevated plasma cholesterol levels immediately after birth.

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