Medium-Chain Triglycerides in Infant Formulas and their Relation to Plasma Ketone Body Concentrations

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ABSTRACT. A mild ketosis is known to prevail in the mother, fetus, and newborn infant during the 3rd trimester and in the early neonatal period. It has been shown that during an equivalent period in the rat ketone bodies are readily oxidized and serve as key substrates for lipogenesis in brain. Since medium-chain triglycerides are known to be ketogenic, preterm infants may benefit from dietary medium-chain triglycerides beyond the point of enhanced fat absorption. Our objective was to determine the ketogenic response in preterm infants (gestational age: 33 ± 0.8 wk) fed three different isocaloric formulas by measuring the concentrations of 3-hydroxybutyrate and acetoacetate in the plasma of these infants. At the time of entrance to the study the infants were receiving 110 kcal/kg/24 h. Study I (11 infants): the infants were fed sequentially in the order; PM 60/40 (PM), Special Care Formula (SCF), and Similac 20 (SIM). In SCF greater than 50% of the fat consists of medium-chain length fatty acids while PM and SIM contain about 25%. The concentration of 3-hydroxybutyrate in plasma was significantly higher when infants were fed SCF than PM and SIM [0.14 ± 0.03 , 0.06 ± 0.01 , and 0.05 ± 0.01 mM, respectively ($p < 0.01$)]. Study II (12 infants); the infants were fed SCF, then SIM, or the reverse. The concentration of acetoacetate in plasma was 0.05 ± 0.01 and 0.03 ± 0.01 mM when infants were fed SCF and SIM, respectively ($0.1 > p > 0.05$). The concentrations of 3-hydroxybutyrate in plasma were similar to those measured in study I for the respective formulas. The increased plasma levels of 3-hydroxybutyrate and total ketone bodies when SCF was fed indicate that SCF promotes a mild ketosis in preterm infants similar to that reported in breast-fed term infants. (*Pediatr Res* 20: 338–341, 1986)

Abbreviations

AcAc, acetoacetate
3HB, 3-hydroxybutyrate
MCT, medium-chain triglycerides
PM, PM 60/40
SCF, Similac Special Care Formula
SIM, Similac 20

The past decade has witnessed a major change in our concept of ketone bodies from a limited role as alternative oxidative substrates in muscle and brain to a role as key substrates for tissue respiration and lipid synthesis. Recent studies with animal models (1–4) and in human infants (5–9) suggest that the ketone bodies, AcAc and 3HB, may be particularly important in the developing infant. Several investigators have demonstrated that AcAc and 3HB, in addition to serving as general oxidative substrates, are preferentially incorporated into sterols and fatty acids in the neonatal rat brain (1, 2, 10). This occurs during the “brain growth spurt” when cell proliferation and myelination are major developmental events. In the human myelination is most active during the 3rd trimester and throughout the 1st yr of life (11, 12). It has also been demonstrated in both the rat (13–15) and human (6, 8, 16) that the uptake of ketone bodies into brain is linearly related to the arterial concentration.

The concentrations of the ketone bodies in blood are elevated above typical adult levels both in the fetus during the later half of gestation (8, 17) and in the breast-fed term infant (18). These data warrant considering that metabolically stable preterm infants should have a mild ketosis of similar magnitude to that of the fetus at the close of the 3rd trimester. A recent report by Lucas *et al.* (18) has shown that the circulating concentrations of ketone bodies in the formula-fed term infant are significantly lower than in the breast-fed term infant. Currently, formulas developed for preterm infants contain significant amounts of MCT to increase fat digestibility (19). Since MCT are known to be ketogenic (20), premature infants may benefit from dietary MCT beyond that of enhanced fat absorption. The objective of this study was to compare plasma ketone body concentrations in infants fed three different formulas, one of which contains a significant amount of MCT.

MATERIALS AND METHODS

Twenty-three appropriate for gestational age, preterm infants with gestational ages between 30 to 35 wk were entered into the study after informed consent was obtained. The infants were healthy and on full oral feedings, taking at least 110 kcal/kg/24 h. Two studies were performed.

Study I. Eleven infants were fed sequentially with 3 isocaloric (20 cal/30 ml) formulas: (i) PM, (ii) SCF, and (iii) SIM. The differences in the composition of the three formulas used in this study in comparison with human milk are shown in Table 1. MCT accounts for more than 50% of the fat content of SCF. For the study, the infants were given each formula for a period of at least 96 h. At the end of this stabilization period, samples for assay of plasma ketone bodies were obtained 2 h after a feeding and the plasma stored at -20°C until analysis. Plasma ketone

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bodies, 3HB and AcAc, were assayed according to the method of Webber with a modification of the methods described by Williamson *et al.* and Persson (4, 9, 21, 22).

It was later determined that when plasma samples were stored at -20°C , degradation of acetoacetate occurred. Therefore, samples of plasma from rat pups were collected, pooled, and aliquoted. The plasma was frozen within 1 h after collection and stored at three different temperatures: at -20°C in a Whirlpool Upright freezer, at -50°C in a Revco Ultralow Chest Type Freezer, and at -196°C in liquid nitrogen, as well as assayed for acetoacetate content without prior freezing. The plasma samples were stored frozen and after 4, 7, 14, 21, 28 and 42 days the concentration of acetoacetate was determined. The results of these determinations are shown in Figure 1. After 30 days of storage at -20 or -50°C there was greater than a 50% loss in AcAc content. Since plasma samples from infants in study I had been stored at -20°C for variable periods of time prior to

determining concentrations of acetoacetate, the data are unreliable and have not been reported.

Mean body weight, gestational age, and caloric intake of the infants at birth and while they were on the different formulas are shown in Table 2A.

Study II. Study II was designed both to permit accurate determinations of acetoacetate in preterm infants under two of the three feeding conditions described above and to confirm in part the results of study I. Twelve infants were evaluated; mean body weight, gestational age, and caloric intake of the infants at birth and while they were on the different formulas are shown in Table 2B. Plasma samples were frozen immediately and stored in liquid nitrogen for no more than 6 wk prior to analysis. Only two formulas were used sequentially, SIM and SCF. This protocol also allowed more flexibility in terms of permitting earlier discharge of the infants from the hospital.

RESULTS

Study I. Figure 2 shows the plasma concentrations of 3HB for infants when they were fed PM, SCF, and SIM. The plasma concentrations of 3HB during the PM and SIM feeding periods were similar. However the mean plasma concentrations of 3HB were approximately 2-fold higher when infants were fed SCF (paired Student's *t* test, $p < 0.01$). In addition the variability in the plasma levels of 3HB was greater when infants were fed SCF (range: 0.049 to 0.322 mM) compared to PM (range: 0.034 to 0.126 mM) and to SIM (range: 0.030 to 0.093 mM).

Study II. The differences in the plasma concentration of 3HB and AcAc in infants fed SIM and SCF formulas are shown in Figure 3. As in study I, the mean plasma concentrations of 3HB was significantly higher ($p < 0.01$) during SCF than during SIM feeding and again the variability in the plasma levels of 3HB was much greater when infants were fed SCF (range: 0.072 to 0.380 mM) compared to SIM (range: 0.038 to 0.099 mM). The values and ranges for 3HB were similar to those obtained in study I for the respective formulas. Concentrations of plasma AcAc in infants on SCF and SIM showed a trend similar to 3HB. The mean

Table 1. Composition of human milk and formula

	Human milk	PM	SCF	SIM 20
A. Macronutrient composition of human milk and formula				
Protein				
g/liter	11	15.8	18.3	15.5
% cal	6%	9%	11%	9%
Source		Cow's milk (whey 60%)	Cow's milk (whey 60%)	Cow's milk
Fat				
g/liter	45	37.6	36.7	36.1
% cal	55%	50%	47%	48%
Source		Coconut and corn oil	MCT, corn and coconut oil	Coconut and soy oil
Carbohydrate				
g/liter	71	68.8	71.7	72.3
% cal	39%	41%	42%	43%
Source		Lactose	Lactose and corn syrup	Lactose
B. Fatty acid composition of human milk and formula (wt%)				
8:0		3.4	28.1	2.8
10:0	1.0	2.8	14.1	2.4
12:0	4.6	24.2	10.3	19.4
14:0	5.9	10.2	4.6	8.5
16:0	21.6	11.0	6.6	9.6
18:0	7.5	4.8	2.5	4.6
18:1	37.0	16.1	10.7	14.8
18:2	14.8	26.9	22.5	32.6
18:3		0.3	0.7	4.8
18:3 + 20:1	1.5			
20:0	0.2	0.2	0.2	0.4

Fig. 1. Differences in the decrement of AcAc concentrations in plasma samples over time under varying storage conditions.

Table 2. Characteristics of infants in two studies (mean $\pm$ 1 SD)

	Birth	PM	SCF	SIM 20
A. Study I. 11 infants				
Wt (g)	1311.8 $\pm$ 216.2	1450.0 $\pm$ 216.2	1594.5 $\pm$ 164.2	1769.1 $\pm$ 184.9
Gestational age (wk)	31.0 $\pm$ 2.4	33.9 $\pm$ 2.7	34.8 $\pm$ 2.6	35.8 $\pm$ 2.6
Oral intake (kcal/kg/24 hr)		115.0 $\pm$ 5.5	119.4 $\pm$ 7.2	123.1 $\pm$ 6.0
B. Study II. 12 Infants				
Wt (g)	1337.9 $\pm$ 143.8		1641.0 $\pm$ 158.4	1595.0 $\pm$ 146.6
Gestational age (wk)	31.4 $\pm$ 1.9		34.8 $\pm$ 1.5	34.3 $\pm$ 1.35
Oral intake (kcal/kg/24 hr)			118.7 $\pm$ 6.7	117.6 $\pm$ 6.7

Fig. 2. Plasma concentrations of 3HB for infants fed PM, SCF, and SIM 20. The mean plasma concentration of 3HB was higher during SCF feeding than PM and SIM ($p < 0.01$).

Fig. 3. Plasma concentrations of 3HB and AcAc for infants fed SIM 20 and SCF. The mean concentration of 3HB was significantly higher when infants were fed SCF ($p < 0.01$). Mean concentration of AcAc showed the same trend but did not reach statistical significance ($0.1 > p > 0.05$).

concentration in plasma AcAc was slightly higher, although not significantly at $p < 0.05$, when infants were fed SCF compared to SIM. As with 3HB the variability in plasma levels of AcAc was greater when infants were fed SCF (range: 0.024 to 0.110 mM) compared to SIM (range: 0.007 to 0.057 mM).

DISCUSSION

Our study shows that feeding a milk formula containing MCT as a major component of the milk fat produces a blood ketone

body concentration comparable to that of term infants who have been breast-fed (18). Whether equivalent conditions can be obtained with MCT in amounts lower than 50% of the fat in milk remains to be determined. The fatty acids of medium-chain length are octanoic, decanoic, and lauric acid. In SCF fatty acids of medium-chain length comprise 52% of the total fat, with 42% as octanoic acid and decanoic acid from MCT and 10% as lauric acid from coconut oil. On the other hand, PM and SIM contain no MCT and are rich in lauric acid from coconut oil at 24.2 and 19.4% of the total fat, respectively. It is not clear from these data whether the fatty acids in MCT are more ketogenic than lauric acid or if the optimal concentration of fatty acids of medium-chain length for promoting a mild ketosis in premature infants lies between 25 and 50% of the total fat in formula. This is an important consideration since the metabolism of the fatty acids of medium-chain length at 50% of milk fat results in the excretion of dicarboxylic acids and other hydroxyacids and their derivatives in the urine of both premature and term infants (23, 24). This may suggest that the pathways for the oxidation of medium-chain length fatty acids are overwhelmed by the substrate load, leading to their incomplete catabolism and then disposal as urinary acids. The concentrations are small since less than 2 to 3% of a dietary load of MCT at 50% of the total fat is excreted as urinary dicarboxylic acids and hydroxyacids (25). Nevertheless, it would seem appropriate to adjust the proportion of fatty acids provided as MCT in milk fat to an amount associated with a minimal excretion of urinary dicarboxylic acid by-products while maintaining appropriate concentrations of ketone bodies in blood. Support for this recommendation can be found in reports on metabolic conditions which prevail in children with inherited defects in the oxidation of medium chain length fatty acids (26–29).

A pronounced increase in the concentration of 3HB in blood was observed in both studies when the formula was changed from either PM or SIM to SCF. Concentrations of acetoacetate did not change significantly. These observations are very similar to the observations made by Lucas *et al.* (18) who showed that the concentration of 3HB, but not acetoacetate, was significantly higher in blood of 6-day-old term infants who were breast-fed when compared to formula-fed infants.

Since SCF contains 42% of the milk fat as MCT and because their medium-chain length fatty acids are readily metabolized to ketone bodies, we anticipated a more substantial increase in the concentrations of acetoacetate and 3HB than was found when the infants were fed SCF. To explain our observation it may be that the NADH status of the mitochondria is high, and this is reflected by the decreased ratio of [acetoacetate]:[3-hydroxybutyrate] (18, 30, 31). In addition, the steady state concentration of acetoacetate may not increase because utilization equals the capacity for acetoacetate production. Although oxidation of lipids and synthesis of ketone bodies may be enhanced in infants fed SCF, the appropriate quantity of MCT or medium-chain length fatty acids in infant formulas necessary to promote normal growth has not been established.

As stated earlier ketone bodies, *i.e.* 3HB and AcAc, are readily available substrates for respiration and lipogenesis in the fetus. Although the mechanism of ketone body transfer across the human placenta has not been clearly defined, their concentrations in the fetus have been shown to be closely related to those in maternal plasma (17, 30–32). Paired umbilical venous-arterial concentrations have been measured in the fetuses of mothers whose total plasma ketone bodies were 0.2–1.4 mM during early and advanced stages of labor (17). It was concluded from these studies that a positive maternofetal gradient exists. Thus net fetal uptake and transfetal utilization of ketone bodies appears to take place.

The mitochondria and cytosol have been found to be rich in enzymes for the metabolism of ketone bodies in both animal and human studies (3, 7, 10, 33, 34). Recently, homogenous primary cultures of each of the major cell types in developing rat brain have been prepared (2). Studies on the metabolic

capacity of these cells show that neurons, astrocytes and oligodendrocytes all have the capacity to use ketone bodies for respiration and that their capacity to use ketone bodies is much greater than their capacity to use glucose for this function. In humans, the cerebral capacity to oxidize ketone bodies is higher in neonates than in older infants. Also, the capacity of the brains of neonates and infants to remove and oxidize ketone bodies is five and four times higher than in brains of adults at a given arterial concentration (6, 13, 14, 16). The development of this capacity to utilize ketone body parallels the development of lipogenesis and myelination of the central nervous system—an essential step in the maturation process.

Another significant observation with respect to the metabolic requirements of the preterm infant comes from a recent paper by Bitman *et al.* (35). They report that fat in breast milk from mothers of very low birth weight infants has higher concentrations of fatty acids of medium-chain length than fat in milk from mothers of larger birth weight preterm infants, and that fat in milk from both these groups has higher concentrations of fatty acids of medium-chain length than breast milk from mothers of term infants. This difference persists until 42 days of age (35).

As we begin to appreciate the central role for ketone bodies as oxidative substrates and as precursors for lipids in the growing infant, the capacity of formulas to provide an acceptable concentration of ketone bodies in infants fed exclusively formula needs to be evaluated. At the same time, however, the potential for adverse effects due to excessive dietary amounts of MCT should also be taken into consideration. Future studies should delineate an optimal amount of MCT in formulas to achieve an appropriate ketosis with minimal production of unusual urinary dicarboxylic acids.

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