THE USE OF MEDIUM-CHAIN TRIGLYCERIDES IN PRETERM INFANTS

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THE USE OF MEDIUM-CHAIN TRIGLYCERIDES IN PRETERM INFANTS

(HET GEBRUIK VAN MIDDEL-KETEN VETZUREN BIJ PRETERME PASGEBORENEN)

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Aan mijn ouders

Voor Annemarie

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CHAPTER I

INTRODUCTION

Approximately 7% of all infants in western countries have a birth weight below 2500 gram, which is the definition of a low birth weight (LBW) infant. The survival of LBW infants, particularly that of very low birth weight (VLBW) infants with a birth weight below 1500 gram (approximately 0.6% of all live births), has improved dramatically over the past decades.

Most of these infants are fed special "preterm" formulas. These formulas differ in several ways in their compositions, reflecting unanswered questions regarding the optimal composition of such feedings. After a few optimistic reports on the beneficial effects of the addition of medium-chain triglyceride (MCT) oil to special preterm formulas, most manufacturers have included this "artificial foodstuff" in their formulas. However, more recent clinical studies have shown contradictory results while our understanding of the specific metabolism of MCTs in the preterm neonate is still insufficient. With the increasing survival of VLBW infants, the possible effects of optimal nutrition on final outcome and quality of life of survivors become increasingly more important. Some twenty years after the introduction of MCTs in preterm formulas there is still no common opinion on the benefits of its use. Even so, we feed many infants this artificially prepared food fat in amounts that are much higher than human breast milk would ever provide.

In this chapter several issues regarding basal understanding of MCT metabolism and possible clinical benefits will be addressed. At the end of the chapter specific questions and hypotheses will be formulated.

Historical background of MCT[1]

In the early 1950s V.K. Babayan, research organic chemist for the Drew Chemical Corporation, started experimenting with the fatty acids of eight and ten carbon atoms that were isolated by distillation as an early fraction during the

[1]Source: Medium Chain Triglycerides, John R. Senior (ed.), Univ. of Pennsylvania Press, 1968: Introductory remarks by the chairman.

processing of coconut oil, in order to reesterify them into a triglyceride mixture. According to a well known anecdote, he was stimulated to do so by Mr. Drew himself who noticed the piling up of these fractions as a "waste product" of the manufacturing of long-chain triglycerides from coconut oil and challenged his biochemist to develop a "fatless fat". The story also suggests that the challenge to devise an oil that would provide a satisfying diet without the risk of excess weight accumulation was inspired by Mr. Drew's well known tendency towards corpulence. Babayan developed a new manufacturing process where free fatty acids were liberated from coconut oil by steam hydrolysis, doubly distilled, and the most volatile fraction containing the 8- and 10-carbon length fatty acids was used for the reesterification of glycerol, carried out with a finely divided zinc catalyst.

The first trial of MCTs in animals supposedly occurred in 1955 in the laboratory of H. Kaunitz at Columbia, revealing that rats could consume approximately twice as much MCT as lard and maintain equal weight, without toxicity. Soon, the first three studies of MCT administration to humans [1-3] initiated other studies to find applications for the clinical use of MCTs in human medicine.

In **table 1** the fatty acid composition of three "normal" sources of fat providing predominantly long-chain fatty acids (LCFAs), (soy oil, milk fat, and olive oil) are compared with coconut oil and MCT oil. It is evident that coconut oil is much higher in fatty acids with a chain length of less than sixteen carbon atoms than the first three fat sources. Furthermore, the "MCT oil", which is distilled from the latter, contains in its turn fatty acids with an average chain length much shorter than coconut oil itself.

METABOLISM OF MCT

Physicochemical properties

A typical, commercially available "MCT oil" is made up of a mixture of C6:0 (1 to 2%), C8:0 (65 to 75%), C10:0 (25 to 35%), and C12:0 (1 to 2%) fatty acids, obtained by the hydrolysis of coconut oil followed by the fractionation of the fatty acids. The medium-chain fatty acids (MCFAs) are subsequently esterified again with glycerol to form the triacylglycerols [4].

Although it is obvious that the vast majority of the "MCT oil" is formed by MCFAs with chain length of eight- and ten carbon atoms (typically over 96%), some

Table 1
Typical composition of some food fats (weight%).
Only fatty acids that are present in significant amounts are mentioned.

Source	Soy oil	olive oil	milk fat	coconut oil	MCT oil
C6:0			5.5	0.5	1.0
C8:0			1.5	7.0	61
C10:0			3.0	6.0	37
C12:0			3.5	46	1.0
C14:0			11	19	
C16:0	11	11	28	9.5	
C16:1		1.5	2.5		
C18:0	4.0	2.4	10	3.0	
C18:1	22	72	25	7.5	
C18:2	55	8.0	3.0	2.0	
C18:3	7.5	1.0	0.5		

authors consider MCFAs to be all fatty acids with a chain length ranging from C6:0 to C12:0. Others reserve the term MCFAs specifically for C8:0 and C10:0. As C12:0 and C14:0 take in many aspects of their metabolism an intermediate position between LCFAs and MCFAs the separate term "intermediate-chain fatty acids" (ICFAs) is warranted. Also in this thesis we will reserve the term MCFA for fatty acids with a chain length of 8 and 10 carbon atoms. As an illustration **figure 1** shows the appearance of the different fatty acids in the portal blood *versus* the thoracic duct after a feed [5]. From this it is evident that C12:0 cannot be considered a MCFA, as (in contrast to C8:0 and C10:0) transport after absorption is predominantly in the thoracic duct lymph, much more like the LCFAs. C6:0 can be considered a short chain fatty acid (SCFA) or a MCFA, merely depending on exactly what behavior during certain chemical processes or what specificity of certain enzyme systems is deemed important by the author. The issue is not that

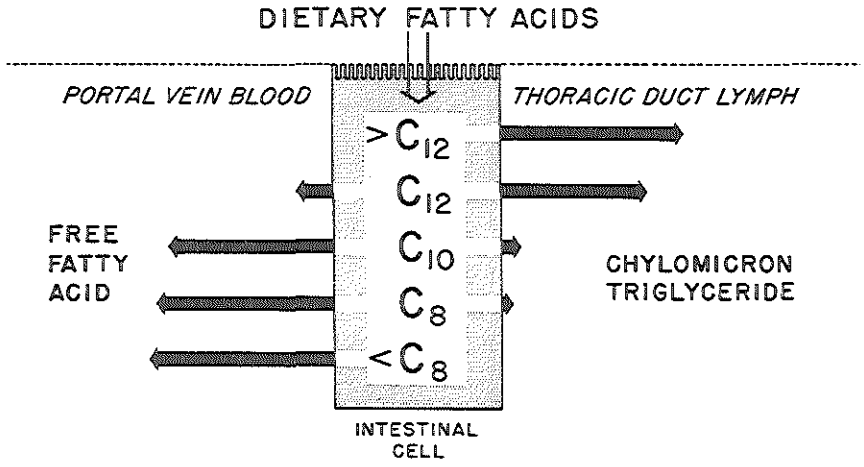

Fig. 1
Scheme of fatty acid transport following intestinal absorption.
(reproduced with permission from Medium Chain Triglycerides, Penns. Univ. Press 1968.)

relevant to this thesis, as the level of C6:0 in food fats is always extremely low.

The melting point of the MCFAs is much lower (C8:0, 16.7°C; C10:0, 31.3°C) than that of the long-chain fatty acids (LCFAs) (*e.g.* C16:0, 63.1°C; C18:1, 33.3°C). As a consequence MCFAs, but also the MCTs, are liquid at room temperature. Due to their smaller molecular size MCFAs are more soluble in water: octanoate for instance is about hundred times more soluble in water at 20°C than is palmitate (680 mg/l for C8:0 *versus* 7.2 mg/l for C16:0). The fact that MCFAs are relatively weak acids and are highly ionized at neutral PH contributes to their solubility in biological fluids. These different properties have profound effects on several aspects of metabolism, which differs greatly from the "regular" food fats, which are LCTs.

Absorption

Probably due to their smaller molecular weight and higher solubility MCTs are hydrolysed faster and more completely by pancreatic lipase than LCTs. In the case of mixed triacylglycerols the MCFAs are liberated preferentially. The products of MCT hydrolysis are absorbed much faster than LCTs, virtually at the same rate as glucose [6]. Due to their rapid and complete hydrolysis MCTs are absorbed mainly

as free fatty acids. There is evidence that in case of bile salt and/or pancreatic lipase deficiency, a part of the MCTs can be absorbed as intact triacylglycerols [7]. Earlier studies [8] found also evidence *in vivo* in rats for absorption of unhydrolyzed trioctanoin and the existence of an intestinal lipase in the enterocyte hydrolyzing subsequently all medium-chain acylglycerols into MCFAs and glycerol.

In the intestinal cells, unlike LCFAs, MCFAs are probably not incorporated to a significant extent into triacylglycerols again for transport as lymphatic chylomicrons (see **figure 1**). Instead, MCFAs are taken up in the portal system and transported as such to the liver, either bound to albumin or in free fatty acid form [9].

Hepatic metabolism

Once in the hepatocyte, MCFAs are not significantly incorporated into the lipids synthesized by the hepatic tissue [10]. They cross the double mitochondrial membrane very rapidly and, unlike the LCFAs, they probably do not require the presence of carnitine [10]. In the mitochondrion, MCFAs are acylated by an octanoyl-CoA synthetase, after which a rapid and supposedly complete β-oxidation occurs [11-13]. The acetyl-CoA formed can partially enter the Krebs cycle, but generally a surplus of acetyl-CoA will be formed. This non-oxidized acetyl-CoA is predominantly redirected towards the formation of ketone bodies which are utilized in the peripheral tissue.

Extra-hepatic metabolism

Except for the utilization of ketone bodies, the extra-hepatic metabolism of MCT is limited because of their rapid portal absorption and hepatic oxidation. Bach and Babayan [13] postulated that there might be only significant metabolism by extra hepatic tissues when MCTs are supplied intravenously.

In general it is accepted that extra hepatic tissues do not incorporate MCFAs to a significant extent. Sarda et al. [14] using direct transmethylation of fatty acids, have argued that up to 12% of the ingested MCFA are incorporated in the subcutaneous fat of infants receiving MCT-containing formulas. However, they included lauric acid in the definition of MCT and this fatty acid was responsible for the majority of the MCFAs stored in adipose tissue. We argued before that C12:0 is either in an intermediate position between MCFAs and LCFAs or belongs to the LCFAs, but behaves metabolically clearly distinct from the MCFAs. In a comment from two of the leading authorities in the field, the same point of view is taken [15].

CLINICAL USE

For almost 40 years, use have been made of the special properties of MCTs in human medicine, particularly in cases where the digestion, absorption or transport of usual dietary fats is disturbed. Successful use of MCTs has been reported in patients with the following disorders: [ref. 13 and references therein]

- *disorders of lipid digestion,* including:
  - major resections of esophagus or stomach
  - biliary atresia, obstructive jaundice and primary biliary cirrhosis
  - blind-loop syndrome
  - pancreatitis and pancreatectomy
  - cystic fibrosis
- *disorders of lipid absorption,* including:
  - massive resection of small intestine
  - celiac disease, Whipple's disease and Crohn's disease
  - enteritis and gluten enteropathy
  - tropical or idiopathic sprues
- *disorders of lipid transport,* including:
  - congenital β-lipoprotein deficiency and other states of deficient chylomicron synthesis
  - intestinal lymphangiectasia and other lymphatic disorders due to engorgement
  - chyluria, chylous ascites, chylothorax and other lymphatic disorders due to leakage

However for many of these indications for the use of MCTs, data are scarce or contradictory.

MCTs have also been used in several other disorders not related to fat absorption, like cholesterol-related cholelithiasis, ketogenic diet as anticonvulsive therapy, hyperalimentation and obesity control [13]. The fact that MCTs have been advocated for so many purposes, including for instance both for parenteral hyperalimentation and as an aid in obesity control, is more a sign of its popularity than a proof of its merits. As these indications for MCTs fall far beyond the scope of this thesis we will refrain from discussing them any further at this point.

USE OF MCT IN PRETERM INFANTS

Introduction

Preterm infants often show significant fat malabsorption compared with term infants and older children. There are several physiological limitations to the digestion and absorption of fat blends in prematures which are related to immaturity, the most identified causes being a lower bile salt secretion and a lower pancreatic lipase secretion. Duodenal bile acid secretions are often well below critical micellar concentrations in preterm infants [16,17]. Watkins et al. have shown that this is due to a reduction in bile acid pool size as well as synthesis rate, both of which are reduced to 30-50% of the values found at term [18].

Preterm infants also have a reduced pancreatic response to secretin and cholecystokinin [19], low lipase activity in duodenal fluids [20,21] and increased concentrations of duodenal triglycerides [20].

Recent evidence suggests that the newborn probably depends on several alternative mechanisms for efficient fat absorption. Lingual and gastric lipases - which are biochemically difficult to distinguish- appear before 26 weeks of gestation and have high activity at birth [22]. The products of intragastric lipolysis (fatty acids and monoglycerides) can theoretically compensate for low bile salt levels by emulsifying the lipid mixture.

The infant fed (non-pasteurized) breast milk benefits also from the "bile salt stimulated lipase" of human milk, but this enzyme is outside the scope of this thesis dealing about formula fed infants and thus will only be mentioned here.

A higher rate of hydrolysis of MCT as compared to LCT has been described for all lipases, but this is probably primarily an *in vitro* phenomenon, as a consequence of the higher solubility in water of MCTs and thus accessibility by the enzyme. For most events no reliable *in vivo* data are available. Hamosh et al. [23] showed in a group of premature infants with equally good absorption of high- or low MCT formula, that intragastric hydrolysis was 20% for MCT *versus* 16% for LCT formula, a difference that was not statistically significant.

Fat provides the major source of energy for growing premature infants. In human milk about 50% of the energy comes from fat, and in commercial formulas fat provides 40% to 50% of the energy. It has been known for a long time that the low birth weight infant digests and absorbs poorly the predominantly saturated triglycerides of cow's milk. The recognition of the magnitude of this fat

malabsorption led to the use of low-fat formulas for feeding premature infants in the 1940s and 1950s. By 1960, after it was recognized that (poly-)unsaturated long-chain triglycerides from vegetable oils were much better absorbed than were cow's milk fats, such fats were used in commercial formulas for feeding premature infants.

Soon after the development of MCT oils it was recognized that the nutrition of premature infants could be an important clinical application of this artificial food stuff.

Theoretically, one of the greatest benefits could be expected in the orally fed preterm infant. After the first study of Yamashita et al. [24], several studies have appeared where the effects on growth of different levels of MCT in formulas for low birth weight infants have been compared. Results have been very inconsistent.

Beneficial effects

Studies on growth and nitrogen retention

In the first 10 years after the introduction of MCTs in premature infant feeding several studies were published showing more or less positive effects of the addition of MCT to premature formulas [24-27]. It was found that fat balance significantly improved when using MCT in all studies and in all but one study [26], growth was also better with MCT. In two studies [26,27] nitrogen balance was studied and it was found that the latter improved significantly with MCTs in some subgroups, although remarkably enough not in the ones where improved growth was found. These positive reports have attributed certainly to the fact that since then more and more special premature formulas have been developed that contained -and most formulas do so until today- up to 50% of fat as MCTs.

Later studies could only report on a relatively small improvement on fat absorption without an effect on growth [28,29] or on a total absence of any effect on energy absorption [30,31] or fat absorption [23]. For the sake of completeness it must be noted that the group of Jensen et al. [29] have published their data on fat balance in more detail in a different publication [32] and that Hamosh et al. recently published a report similar to the one mentioned earlier, describing essentially the same results [33].

Studies on mineral absorption

Administering a mineral supply to the orally fed preterm neonate so that intrauterine accretion rates can be approached is quite difficult. Several studies have shown the existence of a certain correlation between the fecal excretion of fat and minerals, in particular calcium [34]. In 1978 Tantibhedhyangkul performed the first study on the effects of MCT on mineral metabolism, reporting a significant improvement in calcium absorption with MCTs [35]. Two later studies [28,29] did not report on any significant improvement in the mineral absorption rates with MCTs. One important observation however is that in the study of Okamoto et al. calcium, magnesium and nitrogen absorption all showed a very clear tendency to improve on MCT feeding. A relatively small number of patients was investigated and it can be estimated from their data that p values must have been almost significant. Thus it can be concluded that data are contradictory in this aspect of the use of MCTs.

Effects on ketones

In 1986 Wu et al. [36] reported that in preterm infants, like in adults, a mild ketosis could be provoked by feeding a formula containing 50% of fat as MCT. He found that levels of 3-hydroxybutyrate but not acetoacetate were increased, a pattern similar to the one observed by Lucas et al. [37] in breast-fed term infants in a comparative study of two groups of neonates that were either breast- or formula fed. Wu et al. suggested that there could be a beneficial effect in the preterm infant of the well known ketogenic properties of MCTs, because of the presumed central role of ketone bodies in brain lipid synthesis and as alternative oxidative substrates.

On the other hand, Brooke mentioned that MCT replacement of dietary fat at two levels of energy intake in preterm infants was associated with a tendency towards acidosis [30]. He ascribed the higher incidence of metabolic acidosis (which was not statistically significant) to the "potentially undesired ketogenic effect of MCT". Until now, both points of view have not been established as clinically relevant, but the fact that the ketogenic properties of MCTs are explained in such diverse ways proves how little is known about both the metabolism of ketone bodies and MCTs in the preterm neonate.

Potentially detrimental effects

Effects on gastric emptying

Several authors [38], reporting an increased incidence of lactobezoars in preterm infants since the introduction of special premature infant formulas, have attributed this to the change in fat source from LCTs to MCTs, based on studies in adults that showed delayed gastric emptying in some instances [39,40]. Using 99m-technetium sulphur colloid, Sidebottom et al. [41] could not show any difference in gastric emptying time in preterm infants between formulas containing 14% or 50% of the lipids as MCTs.

Dicarboxylic aciduria

The medium-chain dicarboxylic acids sebacic, suberic, and adipic acid are produced in the cytoplasm by ω-oxidation of medium-chain fatty acids. This is normally a minor pathway and only small amounts of dicarboxylic acids are excreted in the urine. In 1980 Mortensen et al. [42] drew attention to dicarboxylic aciduria in infants on MCT feeding, leading to confusion in the diagnosis of inherited defects of the β-oxidation pathway. One year later it was established that (ω-1)-hydroxy-monocarboxylic acids also were increased in (preterm) infants fed MCTs [43]. An increased excretion of both organic acids during MCT feeding was confirmed in a comparative study between two formulas fed to preterm infants containing either 4% or 54% MCTs [44]. In the same year Henderson et al. [45] also drew attention to the problem and suggested that further studies were warranted to exclude toxic effects of dicarboxylic acids, as Passi et al. showed an antimitochondrial effect of dicarboxylic acids in topical cream used for the treatment of melanocytic skin tumors [46]. However, no further evidence has been provided on the issue until today.

In terms of energy balance the excretory loss of the measured organic acids is negligible (*i.e.* $<1\%$ of energy intake) in all studies, and the clinical significance of these findings remain to be established.

Gastrointestinal disturbances

In the study of Okamoto et al. [28], the majority of the infants fed one of the two MCT-containing formulas developed transient gastrointestinal symptoms like vomiting, bilious or blood-containing gastric aspirates, abdominal distension, or loose stools. Although no statistics were given, it can be deduced from their data

that the incidence of these gastrointestinal disturbances was certainly significantly higher in the MCT groups. In two other studies remarks were made on a possibly higher incidence of loose stools [24,27] and "mild to moderate" abdominal distension [27]. However, with current MCT formulas this phenomenon seems to be uncommon in clinical practice and several other studies specifically reported on either a good gastrointestinal tolerance [47], or no difference in stool frequency or vomit count between MCT- and LCT formula groups [48]. All other comparative studies do not comment on any remarkable difference in gastrointestinal tolerance between formulas containing high- or low amounts of MCTs.

Inhibition of Δ6-desaturase

Carnielli et al. [49] reported on lower levels of Poly-Unsaturated Fatty Acids (PUFAs) during MCT feeding as compared with mothers own milk. However, it is more likely that these lower levels of PUFAs with MCT feeding are related to the formula feeding per se and not specifically to MCTs, as levels of the longer-chain PUFAs are known to be much higher in human milk than in formulas, regardless whether the fat source is MCT or LCT.

SUMMARY

Although it is clear that in some studies certain clinical benefits of MCT formula have been reported, the existing evidence is insufficient to support the view that MCT is definitely indicated for (certain groups of) premature infants. Fat absorption is increased in some infants on MCT formula, but not consistently so. Growth and nitrogen retention have also been reported to improve with MCT, and afterwards several studies specifically reported to have found no advantages in this regard.

The discrepancy between increased energy absorption on one hand and the absence of increased weight gain on the other hand renders suspicion about the metabolic fate of MCTs. Keeping in mind the fact that in an overfeeding design in animals a lower food efficiency (the original aim of MCTs!) was found, it seems likely that increased heat production with MCTs can play a role in these findings in humans. An increased heat production can be explained in several ways: Several authors assume an "obligatory oxidation of MCTs", because much higher oxidation

rates than with LCTs have been measured in animals and in adults, whereas storage as MCTs is very limited compared with LCTs. If MCTs are indeed oxidized preferably compared to LCTs, one could imagine a situation where heat production is increased because of ongoing oxidation of other substrates like LCTs. In other words, metabolic control falls short in the case of MCTs, compared to the normal tight regulation of substrate oxidation as in the "Randle cycle" of glucose and fatty acids where oxidation of one substrate decreases the oxidation of the other.

Another possible cause for a lower food efficiency with MCTs is a high rate of lipogenesis, which is an energy requiring process. As MCTs are hardly stored as such, MCFAs that are not oxidized, have to be converted into LCFAs before storage. Available data suggests that the mechanism of this conversion during MCT feeding is predominantly *de novo* lipogenesis. A similar situation occurs if MCTs decrease glucose oxidation while glycogen reserves have already been saturated, as excess carbohydrates also have to be converted into LCFAs before storage.

Regarding mineral balance, and especially calcium balance which is probably the most important for the preterm infant, available data show contradictory results and evidence is insufficient to say if-, and under what circumstances, mineral balance improves with MCT feeding.

Finally, although several potentially adverse effects of MCTs have been reported, the fact that observations have not been consistent eventhough MCTs have been included in many preterm formulas for several years, probably indicates that there are no major disadvantages of MCTs. Of course the ultimate proof would lie in a very large study where the influence of MCT feeding on long-term follow up with regard to growth, lipid profiles, bone density and even neurological outcome is being studied. However, before studying such a large number of infants more clear short term benefits must have been established.

From the available data the following questions were formulated:

1- What is the reason for the observed discrepancy between improved fat balance and the absence of increased weight gain in MCT fed prematures, more specifically, does MCT feeding increase metabolic rate?
2- If food efficiency is lowered by an increased metabolic rate, is this caused by increased lipogenesis during MCT feeding or by an increased total fat oxidation due to the obligatory oxidation of MCFAs?

3- What is the fate of the MCFAs, oxidation or conversion into other substrates?
4- What is the composition of new tissue during growth when feeding the VLBW infant an MCT formula compared to an LCT formula?
5- Does the calcium balance improve with MCT feeding in the stable VLBW infant, and are there any signs of mineral deficiency on LCT formula?
6- Are the benefits of MCTs more pronounced in the nutritional management of the very small and very young premature infant?

These central questions and some related issues are dealt with in the following chapters, after which a general discusion follows in **chapter 9** and where possible, a synthesis of new knowledge and proposition for further research is formulated. Finally, the results of the studies are briefly summarized in **chapter 10.**

In **Chapter 2** we describe the fat and protein balances in two groups of clinically stable and growing VLBW infants fed either a high- or a low MCT formula. Balances were combined with indirect calorimetry in order to be able to calculate the composition of the new tissues laid down on these diets. In this way we would be able to answer question 1 as well as 4.

In **Chapter 3** we describe mineral balances and relevant hormone levels in the same groups of relatively healthy prematures in order to answer question number 5. Mineral absorption rates are also correlated with data from Chapter 2.

In **Chapter 4** questions 2 and 3 are addressed, by investigating the oxidation rate of MCT with stable isotopes, using [1-^{13}C]-octanoate as a tracer, arguing that a complete oxidation of MCTs (as found in fasted individuals) would point towards an obligatory oxidation, whereas an incomplete oxidation would indicate the need for conversion into other substances and probably increased lipogenesis from MCT-derived acetyl-CoA. The percentage of substrate oxidized also informes us on the metabolic fate of MCTs in the preterm infant.

In **Chapter 5** question 2 is (partially) addressed by investigating the effects of MCT on glucose metabolism in two groups of preterm neonates. A stable isotope labeling technique is used to investigate glucose oxidation, production, storage and recycling. Combined with simultaneously performed indirect calorimetry, calculations are performed for the rate of lipogenesis from glucose with MCT *versus* LCT feedings.

In **Chapter 6** we use a newly developed high-sensitivity determination of the enrichment of fatty acids after administration of the MCFA octanoic acid labeled with a ^{13}C stable isotope. The on-line coupling of a gas chromatograph to an isotope-ratio-mass spectrometer is being employed (GC-IRMS) for the *in vivo* investigation of chain elongation from MCT, relating specifically to questions 3 and 2.

In **Chapter 7** using newly developed equations we elaborate on our earlier findings on octanoate oxidation. Using GC-IRMS measurements after oral and intravenous tracer administration the splanchnic and peripheral oxidation rates of octanoate are investigated separately.

In **Chapter 8** the last of our studies is described, even though it could also have been the "first chapter" of a new set of investigations. We studied extremely-low-birth-weight infants in order to answer (partially) question 6. Too little is known about the specific needs of these infants due to the fact that they are often "too ill to be studied". Using a non-invasive diaper collection and anthropometry we have tried to gain a first insight into the usefulness of MCT in this particular patient group.

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CHAPTER II

COMPARISON OF TWO PRETERM FORMULAS WITH OR WITHOUT ADDITION OF MEDIUM-CHAIN TRIGLYCERIDES (MCTs) I: EFFECTS ON NITROGEN AND FAT BALANCE AND BODY COMPOSITION CHANGES.

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SUMMARY

Medium-chain triglycerides (MCTs) are included in the fat blend of several preterm formulas because of their complete absorption and rapid oxidation. The effects of two different fat blend compositions on nitrogen and fat balances and macronutrient oxidation were investigated in 28 healthy very-low-birth weight infants at four weeks of age. A preterm formula with a traditional corn oil/MCT blend, containing 38% MCTs (MCT group) was compared to a new fat blend, designed to resemble human milk more, containing 6% MCTs (LCT group). There were no differences in nitrogen absorption or in excretion. Median nitrogen retention was 74% (MCT) *vs.* 71% (LCT) of intake. Fat absorption was higher ($p < 0.05$) in the MCT group (88%) *vs.* 79% in the LCT group (median values). MCTs did not stimulate fat oxidation as measured by indirect calorimetry, so fat deposition was also higher on the MCT formula. As weight gain was not different between groups, the percentage of weight gain consisting of fat accretion was significantly ($p < 0.005$) higher with the MCT formula (24% *vs.* 21%). On the other hand, there was no increase in percent protein accretion (both 15% of weight gain). We conclude that the existence of a slightly lower fat absorption in the healthy growing neonate fed a LCT formula as compared with a MCT formula does not impair growth or

nitrogen retention, but merely induces a slight decrease in the high relative fat accretion encountered in the preterm neonate.

INTRODUCTION

The fat blend of most special formulas for preterm infants consists of 30- to 50% medium-chain triglycerides (MCTs), the triglycerides of C8:0 and C10:0 fatty acids, primarily in order to enhance fat absorption. The clinical benefit, however, of adding MCT oil to preterm formula has been questioned by some authors [1,2], pointing out the lack of proof regarding the advantages on one hand, and the increased incidence of gastrointestinal symptoms and dicarboxylic- and ω-1-hydroxy fatty acid excretion in urine as a sign of increased ω-oxidation on the other hand.

Although initial reports described striking improvements in fat absorption [3-6], later reports described only marginally improved fat absorption [1,2] or no difference in fat- [7] or energy- [8,9] absorption. Also, the initially described improved weight gain [4-6] or nitrogen retention [3,4] has not been confirmed in other studies [1,2,8-10].

From the finding of increased fat absorption without improved weight gain in some of the studies [1-3] one might expect that infants fed a MCT-containing formula in some instances either have an increased energy expenditure or lay down more fat.

We compared a corn oil based formula containing 38% of fat as MCT to a new formula with a fat blend that more closely resembles human milk and to which no MCT was added, in 28 very-low-birth weight infants. Results from indirect calorimetric measurements and from nitrogen and fat balances were combined to calculate macronutrient accretion rates.

PATIENTS AND METHODS

Subjects

Twenty-eight patients were randomized before the introduction of oral feedings to receive either a preterm formula in which 40% of the fat consists of MCTs (MCT group, $n = 15$) or a formula with no MCTs added (LCT group, $n = 13$).

Only infants with a birth weight of less than 1600 g, initially admitted to the neonatal intensive care unit of the Academic Hospital Rotterdam/Sophia Children's Hospital were included in the study. Infants were eligible for inclusion if they were free from major metabolic problems or congenital malformations and if they were clinically stable breathing room air at the introduction of oral feedings. All infants received total parenteral nutrition (TPN) during their first postnatal week. This protocol has been shown to be an important tool in the prevention of necrotizing enterocolitis [11]. At day 7, enteral feedings were gradually introduced by continuous nasogastric gavage feeding until a full intake of 150 ml/kg/d was reached at postnatal day 16-19. The composition of both feedings is given in table 1.

Table 1
Composition of the two formulas (per 100 ml)

	MCT	LCT
Energy content (kcal)	83.2	83.7
Carbohydrates (g)	8.0	8.0
- lactose/maltodextrins	50/50	50/50
Protein (g)	2.19	2.23
- casein/whey	40/60	40/60
Fat (g)	4.51	4.50
- Fatty acid composition (%)		
C4:0	-	0.4
C6:0	1.2	0.2
C8:0	22.8	3.0
C10:0	14.0	2.4
C12:0	2.0	16.9
C14:0	-	7.4
C16:0	6.4	9.8
C16:1	0.3	0.4
C18:0	1.6	3.3
C18:1	19.1	35.5
C18:2	31.7	17.0
C18:3	0.3	1.7
C20:0	0.3	0.3
C20:1	0.3	0.2

Both formulas were specially manufactured by Nutricia (Zoetermeer, The Netherlands) with a completely equal composition, except for their fat blend. The batch of MCT formula in this study contained 38% medium chain fatty acids (MCFAs) in the fat blend. The MCFAs in the MCT formula are present as triacylglycerols consisting of nearly exclusively MCFA. The 6% MCFA in the LCT formula derived from coconut oil, are predominantly present in the 3-position of the triacylglycerols with lauric acid (C12:0) at the 2-position and C14:0 and C16:0 at the 1-position.

One and the same batch from each formula was used during the study. For each batch, concentrations of nutrients were measured and gross energy intake was calculated using appropriate conversion factors. The caloric value of a mixture of lactose (value taken from ref. 12) and dextrins (value taken from ref. 13) was assumed to be 4.03 kcal/g, whereas the values for the fat blends were calculated to be 9.02 and 9.12 kcal/g for MCT and LCT respectively [12]. A value of 4.704 kcal/g was assumed for food proteins, assuming urea, ammonia and creatinine to be the end products in a 90:5:5 ratio [12].

A 72 hr balance period was performed at least 3 days after the infant had received full oral feedings, and a second study was done another 3-5 days later. Indirect calorimetry was performed on the second day of both 3 day balance periods.

For each patient, an individual standard deviation score (SDS) was determined, defined as the actual birth weight of the infant minus the mean birth weight for that gestational age, divided by one standard deviation for that gestation according to the growth curves of Usher and McLean [14]. The gestational age was determined by medical history, ultrasound data and Ballard score [15].

The mean birth weight, gestational age, SDS, and study weight of the patient groups are described in **table 2.**

Table 2
Characteristics of the two groups studied

	Birth weight (grams)	Gestational age (weeks)	SDS	Study weight (grams)	Study day (days)
MCT (n=15)	1129 ± 218	31 ± 1.9	-2.1 ± 1.9	1488 ± 270	26 ± 5
LCT (n=12)	1271 ± 165	32 ± 1.8	-1.9 ± 1.5	1629 ± 217	29 ± 5

Results presented as mean ± 1 SD, with non of the differences significant.

Weight gain was calculated from the growth during the 4th week of life as difference in weight between days 28 and 21 divided by the average weight during this period. One infant in the LCT group was excluded from the original group and is not presented in this study as will be discussed in the Results section. Infants were only included after obtaining informed consent from at least one of the parents. The study protocol was approved by the medical ethical committee of the hospital.

Methods

Indirect calorimetry

Metabolic rate and substrate utilization were measured in a closed circuit indirect calorimeter. Continuous measurements were performed for 6-8 hrs, as previously described [16]. Briefly, an air mixture devoid of carbon dioxide enters the incubator. In the air leaving the incubator the CO_2 concentration is measured at one side of a differential infrared meter (Unor 6N, Maihak, Hamburg, Germany), whereafter all carbon dioxide is filtered out by a soda-lime filter. Then CO_2 is injected again into the airflow by a mass flow injector system at such a rate that the same concentration of CO_2 is measured at the other side of the differential meter, where the air flow has returned via a loop in the system. The amount of carbon dioxide injected into the system equals the carbon dioxide production of the infant. Thereafter, all CO_2 is filtered by a second filter. The amount of oxygen consumed by the infant is equal to the amount of oxygen that has to be injected into the system to keep the oxygen tension constant, as measured by polarographic oxygen cells (type 6223771, Beckman, La Brea, USA) in the system and in a reference vessel. In order to compensate for changes in air pressure, the reference vessel is connected to the flow circuit by means of a capillary. Oxygen consumption and carbon dioxide production are measured with an accuracy of 0.2 ml/min; the respiratory quotient (RQ) during calibration with butane was within 2% of the theoretical value.

Urine was collected in plastic bags over periods of three days. The urinary nitrogen concentration of a pooled sample was determined by combustion in an automatic nitrogen analyzer (ANA 1400; Carlo Erba, Italy).

The metabolic rate and protein, fat, and carbohydrate oxidation (utilization) was calculated from nitrogen excretion, VO_2, and VCO_2 using the tables of Lusk [17]. The term utilization is used in accordance with Frayn [18], indicating that in the presence of metabolic processes such as lipogenesis, the apparent rate of

carbohydrate oxidation is the sum of the rates of utilization for oxidation and for lipogenesis. Likewise, fat utilization represents fat oxidation minus fat formed from glucose through lipogenesis. By using the term utilization instead of oxidation, it becomes more clear that, for instance, fat storage, calculated as metabolizable fat intake minus fat utilization, also includes the amount of fat derived from lipogenesis. The correct way to calculate fat accretion, therefore, is as the difference between fat absorption and fat utilization. Assuming the fat accretion to be equal to energy retention ignores accretion of carbohydrate and therefore would not be correct.

Balance study

The feces nitrogen concentration of freeze-dried aliquots of samples pooled over the three day periods were determined by combustion in the automatic nitrogen analyzer. Fat excretion in feces was determined using a modification of the Jeejeebhoy method [19], with twice as much hydrochloric acid added. In a few randomly chosen samples (n = 9), the total energy excreted per gram of dry weight of feces was checked also by combustion in a adiabatic calorimeter (IKA C-4000, Janke & Kunkel, Staufen, Germany). This value was compared with a value calculated from nitrogen and fat excretion, assuming a caloric value of 9.25 kcal/g for fat and 5.65 kcal/g for protein [9].

Statistics

Data are presented as mean $\pm$ 1 SD. In cases of apparent nonnormality, data are presented as median with interquartile range (25-75%) in parentheses. Differences between the MCT and the LCT groups were tested using Wilcoxon's rank-sum (Mann-Whitney two-sample) test. Differences in body composition changes between groups and the reference fetus were tested using Wilcoxon's matched sample sign test. Correlations given were calculated by simple linear regression analysis and confirmed with the non-parametric rank correlation test of Spearman. To separate the effects of fecal weight and fecal fat on fecal nitrogen excretion, multiple linear regression analysis was performed.

A value of $p < 0.05$ was considered statistically significant.

RESULTS

There were no significant differences between the two study groups in any of the parameters described in table 2. The study weight and age at the time of the study described here are the mean values of the two balance periods. As the two balance periods and the two calorimetric measurements showed no trend in any of the examined parameters, all results presented are the average values of the two periods. One infant in the LCT group was excluded because of a technical defect of the calorimeter during that period from this study, but his balance data are reported elsewhere [20].

The results of the fat and nitrogen balances are described in table 3.

There were no significant differences in any of the parameters regarding nitrogen metabolism. Nitrogen losses in stool tended to be higher in the LCT group, but not significantly so ($p = 0.12$). Fat intake -like nitrogen intake- was not different between groups. However, fat losses in stool were significantly greater in

Table 3
Results from nitrogen and fat balances in the two study groups

	MCT ($n = 15$)	LCT ($n = 13$)	p
Nitrogen (mg/kg/day)			
Intake	525 ± 18	532 ± 17	NS
Stools	53 (40 - 76)	72 (54 - 99)	NS
Urine	84 (67 - 94)	77 (67 - 93)	NS
% Absorption	90 (86 - 92)	86 (81 - 90)	NS
Retention	386 (372 - 402)	380 (336 - 403)	NS
% Retention	74 (71 - 76)	71 (63 - 75)	NS
Total fat (g/kg/day)			
Intake	6.8 ± 0.35	6.8 ± 0.30	NS
Stools	0.79 (0.73 - 0.99)	1.4 (1.1 - 2.0)	< 0.005
% Absorption	88 (85 - 89)	79 (70 - 83)	< 0.02
Absorption	6.0 (5.8 - 6.0)	5.4 (4.8 - 5.6)	< 0.02

Results presented as mean ± 1 SD, or median with interquartile range in case of apparent nonnormality. NS, not significant.

the LCT group, and hence the % absorption and the absolute retention of fat were significantly lower in the LCT group. Fecal nitrogen excretion was correlated with fecal fat excretion ($r = 0.51, p < 0.01$), but also with fecal weight ($r = 0.69, p < 0.00005$). Indeed, when fecal fat was entered into the multiple linear regression model for fecal nitrogen after entering the fecal weight, there was no increase in R^2 (both were 0.50), as a measure of the percentage of the variability in fecal nitrogen explained by the entered variables.

In itself, fecal output (g/kg/d) also tended to be higher in the LCT group, although this did not reach statistical significance (2.6 ± 0.84 *vs.* 3.6 ± 1.4 g/kg/d, MCT *vs.* LCT, $p = 0.06$).

The total energy excreted per gram of dry weight of feces as measured by bomb

Table 4

Results of calorimetric measurements in the two study groups

	MCT ($n = 15$)	LCT ($n = 13$)	p
Nonprotein $\dot{V}O_2$ (ml/kg/day)	8.0 ± 0.5	8.0 ± 0.6	NS
Nonprotein $\dot{V}O_2$ (ml/kg/day)	7.4 ± 0.5	7.3 ± 0.5	NS
Nonprotein RQ	0.93 ± 0.032	0.91 ± 0.033	NS
Carbohydrate intake (g/kg/day)	12.1 ± 0.4	12.0 ± 0.4	NS
Carbohydrate utilization (g/kg/day)	11.1 ± 1.6	10.4 ± 1.6	NS
Carbohydrate storage (g/kg/day)	1.0 ± 0.3*	1.6 ± 1.6*	NS
Fat absorption (g/kg/day)	6.0 (5.8 - 6.0)	5.4 (4.8 - 5.6)	<0.02
Fat utilization (g/kg/day)	2.0 (1.1 - 2.2)	2.2 (1.2 - 2.7)	NS
Fat storage (g/kg/day)	4.3 ± 0.7	3.2 ± 0.9	<0.005
Metabolic rate (kcal/kg/day)	62 ± 3.5	62 ± 3.8	NS

Results presented as mean ± 1 SD, or median with interquartile range.

* Significantly different from zero at $p < 0.01$ by t test.

calorimetry was 98 ± 18% of the value calculated from nitrogen and fat data in the MCT group (n = 5) and 102 ± 9% in the LCT group (n = 4).

Results of indirect calorimetry and the calculated fat and carbohydrate storage using these results are presented in **table 4**. There were no significant differences in any of the parameters between both groups regarding indirect calorimetry. Therefore, fat storage was significantly higher in the MCT group. The metabolic rate correlated inversely (**fig. 1**) with the SDS at birth as a measure of intrauterine growth retardation (metabolic rate (kcal/kg/d)= 60 - 1.3 x SDS, $p < 0.0005$). Using the Spearman test, a significance level of $p < 0.001$ was found.

Anthropometric parameters are described in **table 5**. Again, none of the measured parameters showed any statistically significant difference between the MCT and the LCT group. **Figure 2** shows that the weight gain was correlated with the nonprotein RQ (weight gain (g/kg/d)= -14 + 33 x nonprotein RQ, $p < 0.05$ both Pearson's correlation coefficient and Spearman's rho). The weight gain was not correlated with metabolic rate, energy losses in stools, or a combination of these parameters.

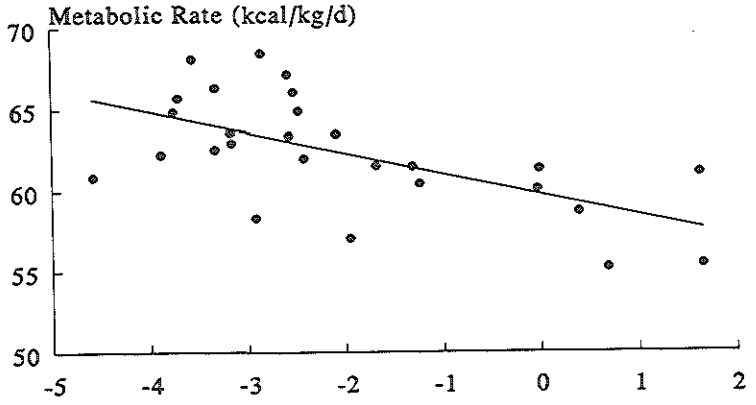

Fig. 1
Relationship between SD score of weight for gestational age as a measure of intrauterine growth retardation and metabolic rate during the study period: Y = 60 - 1.3X.

Table 5
Anthropometric parameters of the two study groups

	MCT (n = 15)	LCT (n = 12)	p
Weight gain (g/kg/day)	16.6 ± 2.42	16.1 ± 3.23	NS
Length gain (cm/week)	0.87 ± 0.47	1.1 ± 0.42	NS
Head circumference gain (cm/week)	1.2 ± 0.56	1.0 ± 0.27	NS

Results presented as mean ± 1 SD, with no significant differences.

Combining the results of tables 3, 4, and 5 the percentage of weight gain consisting of fat and protein retention was calculated. **Figure** 3 compares the calculated proportional fat and protein content of the daily weight gain (median values) from the two formulas with the intrauterine values at postconceptional age 35-36 weeks according to the data of Ziegler et al. [21].

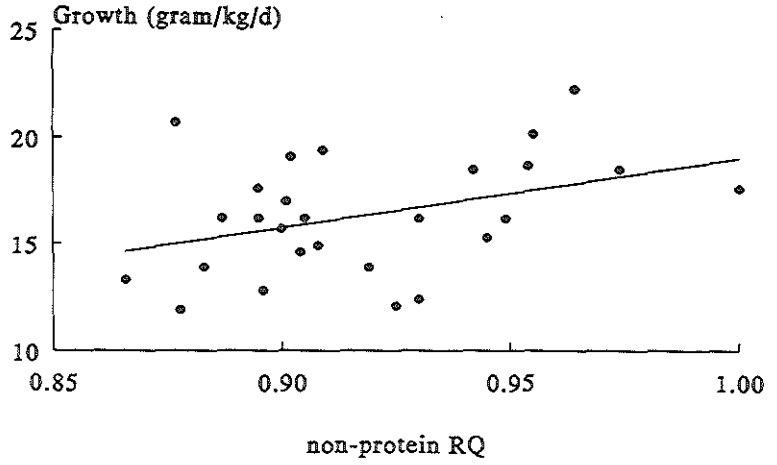

Fig. 2
Relationship between the nonprotein Respiratory Quotient and weight gain during the fourth week of life: Y = -14 + 33X.

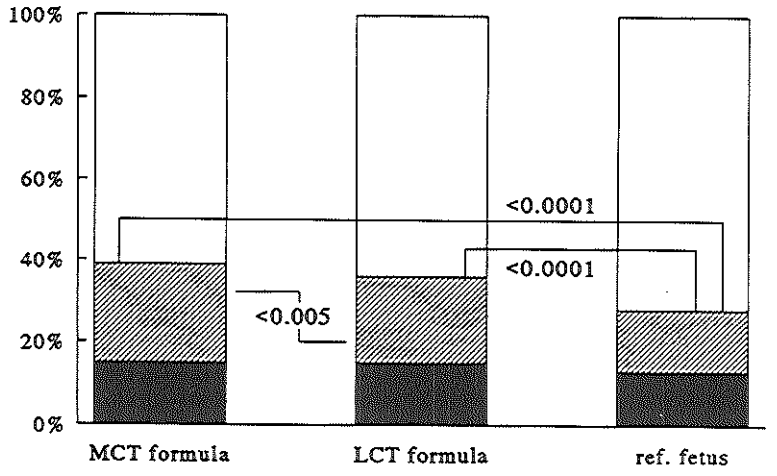

Fig. 3
Accretion of fat and protein as a percentage of total weight gain in the two study groups compared with the reference fetus. Black bars, protein; striped bars fat; white bars, other.

The percent fat accretion was significantly ($p < 0.005$) higher in the MCT group (24% (range of 23-28%) *vs.* 21% (range of 17-22%)), whereas the % protein retention was equal in both groups (15% (range of 14-16%) *vs.* 15% (range of 12-18%)). The fat accretion percentage was higher in both groups than in the reference fetus ($p < 0.0001$); protein accretion was not significantly different from the reference fetus in both groups. In a multiple regression analysis, the SDS at birth as a measure of growth retardation did not enter the equation significantly both alone and in combination with the feeding group.

DISCUSSION

For the last 15 years, most preterm infant formulas available have contained MCT oil in order to enhance fat absorption and to promote weight gain. Some promote the use of MCTs in order to provide "a readily available source of energy", although this seems to be a rather vague concept when administering (semi)-continuous feeds to the preterm neonate. Concern has been raised about the side effects of such an unphysiological fat intake in terms of fat deposition spectrum [22],

and in view of the existence of limited pathways for metabolism besides oxidation and hence dicarboxylic- and ω-1-hydroxy fatty aciduria [23]. Several investigators have reported an increased incidence of gastrointestinal symptoms with MCT feeding [1,2,4]. The European Society of Paediatric Gastroenterology and Nutrition (ESPGAN) has been proclaiming cautiousness but only advised an upper limit of MCT intake of 40% of fat calories [24]. In this study, we compared two formulas differing only in their fat composition. The MCT formula is based on a corn oil/MCT oil blend as incorporated in one of the commercial formulas in The Netherlands from the 1970s until 1988. The LCT formula is based on a new fat blend, designed to be closer to breast milk, while still being sufficiently absorbed. Consequently, the new formula contains an amount of α-linolenic acid in line with the recent ESPGAN recommendations [24], accepting the essentiality of the ω-3 series of the polyunsaturated fatty acids next to that of the ω-6 series. The amount of linoleic acid was lowered also according to the ESPGAN guidelines and to obtain a physiologic ratio between the ω-6 and the ω-3 series of the essential fatty acids. Compared with human milk [22], the LCT blend contains less palmitic acid (C16:0) but more lauric acid (C12:0). The former fatty acid is known to be absorbed less well from infant formulas than from human milk. This phenomenon is attributed to the preferential esterification of palmitic acid at the 2-position of the glycerol molecule, whereas in vegetable oils it is primarily situated at the 1- and 3- positions within the triglycerides. The levels of the MCFAs C8:0 and C10:0 were about eight times less than that in the MCT formula and approached levels normally found in human milk.

This study shows that there are no differences in nitrogen losses in stool or urine between the two groups. In addition, although fecal nitrogen and fat excretion were correlated, fecal output (in g/kg/d) alone was the best predictor for fecal nitrogen excretion. Thus, we cannot conclude that fecal nitrogen is influenced by fat excretion, as variability in intestinal transit rate might have caused the variability in fecal weight and the variability in fat and nitrogen excretion.

Infants on LCT feeding excreted significantly more fat in the feces. If we examine the fatty acid composition of the two fat blends again (table 1), we can see that besides the fact that MCT is replaced by C12:0 and C14:0, there are also significant differences in C18:1 and C18:2 between the formulas. However, several studies [25,26] have shown a difference of no more than 2% in the relative absorption rates of these fatty acids; we can assume that the difference in fat absorption found is related to the MCT content of the two different formulas. The

extra loss of fat (median value 0.6 gram/kg/d) causes an extra loss of about 5-6 kcal in the LCT group. Although some energy can be lost during MCT feeding in the urine as metabolites of the MCFAs, this does not contribute significantly to the energy balance [23]. Fat utilization was not higher in the MCT group, and hence fat deposition was greater in the MCT-fed infants. As there were no differences in growth rate, relative fat deposition was higher in the MCT group. This can explain the findings of increased fat absorption without increased weight gain in some groups that were studied [1-3]. Remarkable is the fact that MCT-fed infants did not show a higher fat-utilization; in fact, their mean nonprotein RQ was higher than in the LCT-fed infants, although not significantly so. The fat utilization as measured by indirect calorimetry, represents oxidation minus lipogenesis from glucose [18]. Thus, a preferential oxidation of MCT could still have taken place, but must have been counterbalanced by increased lipogenesis from glucose.

It has been suggested [9], that the disparity between reports of equal energy absorption [8,9] on MCT and LCT using bomb calorimetry and other studies reporting higher fat absorption [1,2] was partly due to underestimation of MCT losses with fat extraction methods, even with the Jeejeebhoy method [19], which has been designed to measure fecal fat content of MCT diets. Our data on bomb calorimetry in a limited number of samples do not support this suggestion, as total energy calculated from fat- and nitrogen data was not different from the measured total energy excretion in both groups. The finding that energy losses can be explained completely by protein and fat excretion is in agreement with negligible carbohydrate losses in preterm infants [27].

The relation found between metabolic rate and the SDS at birth allows us to calculate the mean difference in metabolic rate between appropriate for gestational age (AGA) and small for gestational age (SGA) infants: as the mean SDS in our study is -0.19 for AGA infants and -3.12 for SGA infants, and the coefficient of the regression equation is -1.6, the average metabolic rate of SGA infants is nearly 4 kcal/kg/d higher than that of AGA infants, confirming our earlier findings in this patient population [28]. This study also confirms another, remarkable finding reported by us before [29] in an entirely different group of patients, namely the existence of a correlation between the nonprotein RQ and weight gain without the presence of such a correlation between metabolic rate and/or energy losses and growth. In a very recent publication, a similar observation was made concerning the relationship between RQ and weight gain in obese adults [30]. However, the exact underlying mechanisms regarding this observation are not yet understood.

Our study is the first to show that the percent fat accretion on MCT formula is higher without a beneficial effect on nitrogen accretion, weight gain or linear growth. The percent fat accretion on LCT formula is closer to the observed intrauterine fat accretion. There is no evidence yet from human studies that a high fat accretion in preterm neonates can have adverse effects in later life. From a purely theoretical standpoint, it seems reasonable to strive for a fat accretion closest to the intrauterine fat accretion, as long as there is no negative influence of this caloric restriction on linear growth or nitrogen accretion. The percentage of weight gain consisting of fat accretion is still higher than intrauterine in the LCT group and the question remains whether we still give too many calories to healthy preterm infants.

In conclusion, the study presented indicates that MCTs have no advantage in the routine feeding regimen of otherwise healthy, growing preterm infants. The small increase in fat absorption and hence energy retention that results from it in the orally fed, older preterm infant does not promote nitrogen retention, but only increases fat deposition. Further studies are needed to examine whether MCTs have a place in the treatment of younger and more immature preterm infants.

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CHAPTER III

COMPARISON OF TWO PRETERM FORMULAS WITH OR WITHOUT ADDITION OF MEDIUM-CHAIN TRIGLYCERIDES (MCTs) II: EFFECTS ON MINERAL BALANCE.

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SUMMARY

Medium-chain triglycerides (MCTs) are included in the fat blend of several preterm formulas because of their complete absorption and rapid oxidation. The effects of two different fat blend compositions on calcium (Ca), phosphorus (P), and magnesium (Mg) balances and plasma levels and on plasma levels of parathyroid hormone (PTH), alkaline phosphatase (AP), and 1,25-dihydroxyvitamin D (1,25-$(OH)_2D$) were investigated in 28 healthy very-low-birth weight infants at four weeks of age. A preterm formula with a traditional corn oil/MCT blend containing 38% MCTs (MCT group) was compared to a new fat blend, designed to resemble human milk more, containing 6% MCTs (LCT group). There was a higher absorption of Ca in the MCT group (73% *vs.* 60%, $p < 0.005$), and an equal absorption of P (both 92%). The excretion of Ca correlated with the excretion of fat ($p < 0.00005$). The LCT group showed a higher median PTH level (MCT: 2.1 pmol/l, LCT: 4.7 pmol/l, $p < 0.01$) and a higher urinary P excretion ($p < 0.001$). Mg absorption was also lower with LCT, but retention of Mg exceeded intrauterine values in both groups. Mineral plasma levels were in the normal range in both groups. AP was not different between groups and in the upper part of the reference range, whereas 1,25-$(OH)_2D$ levels were above the normal range and also not different between groups.

We conclude that with the LCT formula, Ca absorption is slightly lower than with the MCT formula, whereas P absorption is unaffected. The resulting imbalance in absorption is compensated for by an increased urinary excretion of P. We conclude that current recommendations for the maximum Ca/P ratio in preterm formulas (2:1) might be too low in formulas containing only LCT.

INTRODUCTION

The fat blend of most preterm formulas available consist of 30- and 50% fat as medium-chain triglycerides (MCTs) in order to enhance fat absorption. In an earlier study in 28 very-low-birth weight infants [1], we compared a formula containing 38% of fat as MCTs to a new formula with a fat blend that more closely resembles human milk and to which no MCTs was added. Fat and nitrogen balance, energy substrate utilization, and body composition were investigated and a slightly higher fat absorption and deposition were found in the infants fed the MCT-containing formula. It is known that a relatively high fecal excretion of fat correlates with the presence of increased amounts of minerals in the feces, in particular Ca [2]. Reports on the effect of MCT oil in preterm formula on mineral absorption are contradictory [3,4]. Therefore, we investigated the effects of the presence of MCTs in an infant formula on Ca, P and Mg balances and plasma levels of these minerals as well as those for AP, 1,25-$(OH)_2$D and PTH.

PATIENTS AND METHODS

Subjects

Twenty-eight patients were randomized before the introduction of oral feedings to receive either a preterm formula in which 40% of the fat consists of MCTs (MCT group, $n = 15$) or a formula with no MCTs added (LCT group, $n = 13$).

Only infants with a birth weight of less than 1600 g, initially admitted to the neonatal intensive care unit of the Academic Hospital Rotterdam/Sophia Children's Hospital, were included in the study. Infants were only eligible for inclusion if they were free from major metabolic problems or congenital malformations and if they were clinically stable breathing room air at the introduction of oral feedings. All

infants received routine total parenteral nutrition during their first postnatal week, containing 40 mg of Ca and 20 mg of P/kg/day. This protocol has been shown to be an important tool in the prevention of necrotizing enterocolitis [5].

At day 7, enteral feedings were gradually introduced by continuous nasogastric gavage feeding until a full intake of 150 ml/kg/day was reached at days 16-19. The macronutrient composition of the formulas is described in table 1. The composition of the fat blend has been described in more detail earlier [1]. Both formulas were especially manufactured by Nutricia (Zoetermeer, The Netherlands) with a completely equal composition, except for their fat blend. One and the same batch from each formula was used during the study.

Table 1
Composition of the two formulas (per 100 ml)

	MCT	LCT
Energy content (kcal)	80	80
Carbohydrates (g)	8	8
- lactose/maltodextrins	50/50	50/50
Protein (g)	2.2	2.2
- casein/whey	40/60	40/60
Fat (g)	4.5	4.5
- % MCFA	38%	6%
Calcium (mg)		
Freshly prepared	100	100
Formula administered	80	79
Phosphate (mg)		
Freshly prepared	51	52
Formula administered	43	44
Magnesium (mg)		
Freshly prepared	9	9
Formula administered	9	9

Feedings were prepared daily by adding demineralized water to the powdered formula. After arrival on the ward, 2-hr dosages were aspirated into syringes and stored in a refrigerator until feeding. The syringes were emptied at 10-15 minutes intervals over the two hours. Each infant received supplemental vitamin D up to a total of 600 IU/day. No infant was withdrawn from the study once the test feedings were introduced.

A 72 hr balance period was performed at least 3 days after the infant had received full oral feedings, and a second balance study was performed another 3-5 days later. Blood samples were obtained by venipuncture during the balance periods for determination of Ca, P, Mg, AP, 1,25-$(OH)_2$D, and PTH.

To determine the intake of minerals, samples were taken from freshly prepared formula, from the bottles before aspiration into syringes and from simulated nasogastric tube feeding. The routine nursery preparation of the formula resulted in some sedimentation of Ca and P due to delay in aspiration into syringes and lack of shaking. As tests of the resulting concentrations of minerals proved to be reproducible, and concentrations were equally lowered in both study groups, no attempts were made to alter this feeding routine during the study. The resulting Ca and P concentrations are described in table 1. No significant further loss occurred with the administration of the feeding through the nasogastric tube.

For each patient, an individual standard deviation score (SDS) of weight at birth for gestational age was determined, as described before [1]. Mean birth weight, gestational age, SDS and study weight of the patient groups are described in table 2. Infants were only included after obtaining informed consent from at least one of the parents. The study protocol was approved by the medical ethical committee of the hospital.

Table 2
Characteristics of the two groups studied

	Birth weight (grams)	Gestational age (weeks)	SDS	Study weight (grams)	Study day (days)
MCT (n = 15)	1129 ± 219	31 ± 1.9	-2.1 ± 1.9	1488 ± 270	26 ± 5
LCT (n = 13)	1290 ± 173	32 ± 1.7	-1.8 ± 1.5	1639 ± 211	28 ± 5

Results presented as mean ± 1 SD, with non of the differences significant

Methods

Balance study

Urine and stools were collected separately and stored at -20°C until assayed. All glassware was acid soaked for at least 24 hrs and rinsed six times with demineralized water (Milli-Q system/Millipore). The total amount of feces collected during the three day balance period was weighed, homogenized and a small sample of the homogenate was freeze-dried. An aliquot was destroyed by heating for three hrs at 300°C in 5 ml of a concentrated HNO_3/concentrated H_2SO_4 mixture (2:1). Ca and Mg were measured with an atomic absorption spectrophotometer (Perkin-Elmer 2380). The total urine volume was measured and urinary Ca and Mg were also measured by atomic absorption spectrophotometry. Phosphate was measured by a colorimetric method [6]. In all cases, matrix effects were eliminated by means of the standard addition method. Urine and feces nitrogen concentrations of freeze-dried aliquots of a pooled sample were determined by combustion in an automatic nitrogen analyzer (ANA 1400, Carlo Erba, Italy).

Fat excretion was determined using a modification of the Jeejeebhoy method [7], with twice as much hydrochloric acid added.

Blood samples were collected in heparinized tubes. After separation, plasma samples were stored at -80°C until assayed. Plasma Ca and AP were determined using commercially available analysis kits (Hoffman-La Roche, France and Boehringer, Germany respectively). Plasma inorganic phosphate was determined by the same method as described for urine. Plasma PTH(1-84) was determined with the PTH-kit of Medgenix Diagnostics SA (Belgium). Intra- and interassay variation was 2.4 and 6.8%, respectively (n = 20). 1,25-$(OH)_2$D was determined with a commercial kit (Incstar, Minn, USA), with extraction over a Sep-pak C_{18}- cartridge (Waters, Millipore corp., Mass, USA). Intra- and interassay variation in 12 samples was 6.4 and 4.7% respectively. PTH and 1,25-$(OH)_2$D assays were performed at the laboratory of Dr. W.H.L. Hackeng (Rotterdam, The Netherlands).

Statistics

Data are presented as mean $\pm$ 1 SD. In case of apparent nonnormality, data are presented as the median with interquartile range (25-75%) in parentheses. Differences between the MCT and the LCT groups were tested using the Mann-Whitney U test. Correlations given were calculated by simple linear regression analysis and confirmed with the non-parametric rank correlation test of Spearman.

To separate the effects of fecal weight and fecal fat on fecal mineral excretion, multiple linear regression analysis was performed. A value of $p < 0.05$ was considered statistically significant.

RESULTS

There were no significant differences in mean birth weight, gestational age, SDS, study weight and average day of the balance periods between the two groups, as is shown in **table 2**.

As no trend was detected in the results of the first and the second balance period, the two values were averaged for each patient. All results presented here were calculated from these averaged values.

Results of the mineral balance studies are presented in **table 3**. In both groups the mean intake volume (expressed as ml/kg/day) was identical and because the mineral concentrations in the two formulas were also identical, the intake of minerals in both study groups was highly comparable. As the fecal losses of Ca were significantly higher in the LCT group, and the urinary losses were very small in both groups, the absorption and retention of Ca was significantly lower in the LCT group.

P losses in stools and hence the absorption percentages of P were the same in both groups. However, due to a significantly higher urinary phosphate excretion in the LCT group, the retention and percentage retention of phosphate was lower in the LCT group.

The Mg balances followed the same pattern as the Ca balance, showing a significantly higher fecal loss in the LCT group and hence a lower percent absorption. Also, urinary excretion was not different and retention of Mg was significantly higher in the MCT group. All correlations that will be presented are calculated using data from both feeding groups. There was a significant correlation between urinary Ca and Mg excretion ($r = 0.69$, $p < 0.0001$; **fig. 1**).

As reported earlier, fat absorption was significantly higher in the MCT group (88 % *vs.* 79% in the LCT group, $p < 0.02$), but the median nitrogen retention was equal in both groups (386 and 380 mg/kg/day) [1].

Table 3
Results of calcium, phosphate, and magnesium balances in the two study groups

	MCT (n=15)	LCT (n=13)	p
Intake (ml/kg/day)	150 ± 5	150 ± 4	NS
Calcium (mg/kg/day)			
Intake	121 ± 4	119 ± 4	NS
Stools	33 (29 - 43)	49 (43 - 58)	<0.02
Urine	1.6 (0.65 - 2.4)	0.92 (0.43 - 1.31)	NS
% Absorption	73 (64 - 76)	60 (51 - 63)	<0.005
Retention	86 (77 - 89)	70 (59 - 75)	<0.005
% Retention	71 (64 - 74)	59 (50 - 63)	<0.005
Phosphate (mg/kg/day)			
Intake	64 ± 3	65 ± 3	NS
Stools	5.4 (3.8 - 6.3)	5.4 (4.3 - 6.6)	NS
Urine	3.9 (0.85 - 7.1)	12 (9.0 - 15)	<0.001
% Absorption	92 (90 - 94)	92 (90 - 93)	NS
Retention	54 (51 - 59)	48 (46 - 50)	<0.002
% Retention	85 (79 - 92)	73 (70 - 77)	<0.001
Magnesium (mg/kg/day)			
Intake	13 ± 1	13 ± 1	NS
Stools	2.9 (2.5 - 3.9)	4.0 (3.2 - 4.8)	<0.05
Urine	0.37 (0.20 - 0.69)	0.32 (0.11 - 0.53)	NS
% Absorption	78 (71 - 82)	69 (63 - 76)	<0.02
Retention	10 (9.0 - 10)	8.7 (7.6 - 9.5)	<0.005
% Retention	75 (67 - 77)	68 (59 - 74)	<0.05

Results presented as mean ± 1 SD, or median with interquartile range in the case of apparent nonnormality. NS, not significant.

The fecal excretion of Ca was correlated with the excretion of fat (**fig. 2**). In a multiple regression model, fecal fat alone was the best independent variable (compared to fecal weight alone, or a combination with fecal weight) for fecal Ca ($r = 0.69$, $p < 0.00005$). The same goes for fecal Mg ($r = 0.68$, $p < 0.0001$) but not for fecal phosphate or nitrogen excretion.

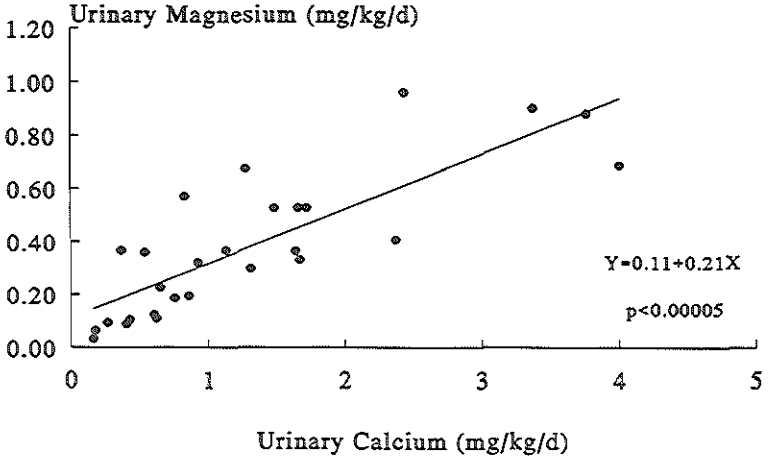

Fig. 1
Relationship between urinary excretion of magnesium and calcium

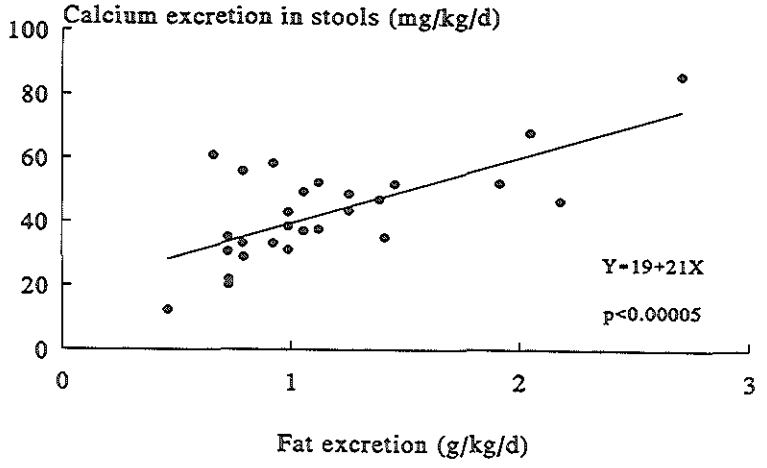

Fig. 2
Relationship between fecal excretion of fat and calcium

The plasma levels of Ca and phosphate at the time of the study (**table 4**) were well within normal ranges and not different between the groups. AP was not significantly different between groups and at the upper part of the normal range for neonates (80-280 IU/l). 1,25-$(OH)_2$D was also not significantly different between groups; both groups showed levels above our adult reference range (36-118 pmol/L). PTH levels in the LCT group were higher ($p < 0.01$) than in the MCT group, and also higher than the adult reference range (0.3-4.0 pmol/l). PTH was significantly correlated with urinary P excretion (**fig. 3**). However, no correlation was found between Ca levels and PTH levels. A weak but significant correlation was found between the Ca/P ratio (weight/weight) after absorption (amount of Ca absorbed/ amount of P absorbed) and the urinary phosphate excretion ($r = 0.43$, $p < 0.05$; **fig. 4**). Due to this excretion of phosphate, the Ca/P ratio (median and interquartile range) of the retained minerals (amount of Ca retained/amount of P retained) became 1.4 (1.2-1.5) in the LCT group whereas the Ca/P ratio of the absorbed minerals in that group was 1.1 (1.0-1.3). In the MCT group, the ratio of the absorbed minerals was 1.5 (1.3-1.6) and the ratio of the retained minerals was 1.6 (1.4-1.7). This phenomenon is depicted graphically in **fig. 5**, combined with the data about mineral concentrations that were also used for **table 1**.

Table 4

Plasma values during the study period in the two study groups

	MCT (n=15)	LCT (n=13)	p
Calcium (mmol/l)	2.4 ± 0.12	2.4 ± 0.081	NS
Phosphate (mmol/l)	2.2 ± 0.29	2.3 ± 0.21	NS
Magnesium (mmol/l)	0.89 ± 0.12	0.82 ± 0.06	NS
Alkaline phosphatase (IU/l)	256 (218 - 321)	288 (270 - 327)	NS
1,25-$(OH)_2$D (pmol/l)	216 (173 - 249)	233 (216 - 293)	NS
Parathyroid hormone (pmol/l)	2.1 (1.1 - 5)	4.7 (4.0 - 6.7)	<0.01

Results presented as mean ± 1 SD, or median with interquartile range in the case of apparent nonnormality. NS, not significant.

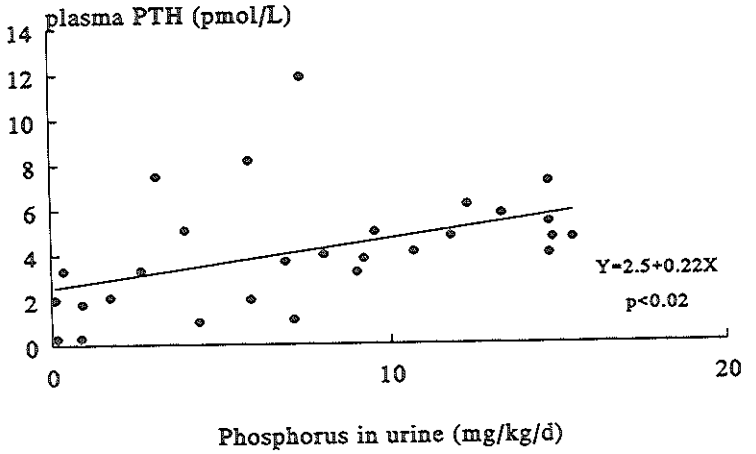

Fig. 3
Relationship between parathyroid hormone levels in plasma and urinary excretion of phosphorus

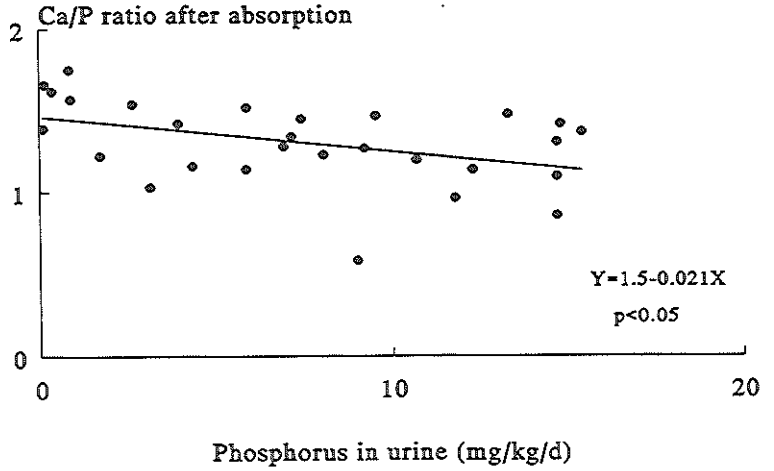

Fig. 4
Relationship between the Ca/P ratio of the absorbed minerals and urinary excretion of phosphorus

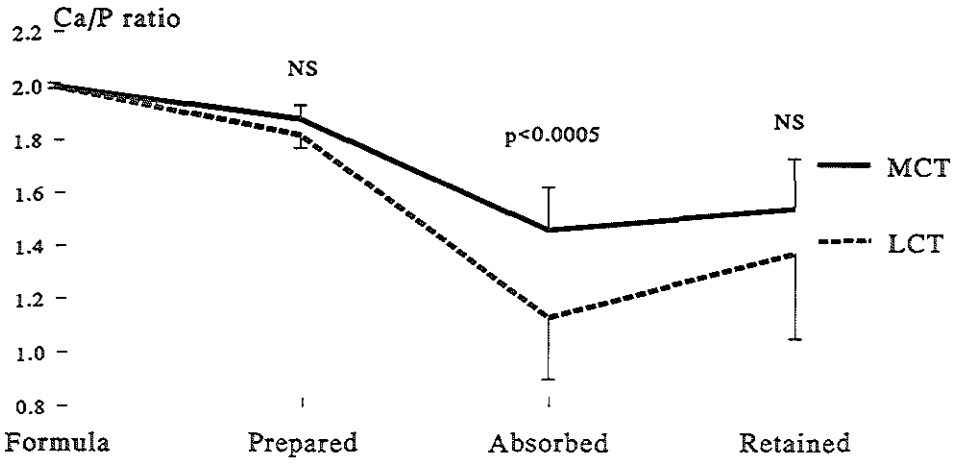

Fig. 5

Representation of changes in the Ca/P ratio as they occur during the feeding (decrease after preparation of the formulas) and in the body (further decrease through fecal Ca loss and increase through urinary P excretion, both especially in the LCT group)

DISCUSSION

This study confirms an earlier report of increased Ca and Mg absorption with MCT-containing preterm formula [3]. The difference in Ca and Mg absorption was much less compared with this earlier study, probably because fat absorption differed more in that study group [8]. We also could confirm the existence of a correlation between fecal fat excretion and Ca excretion [2]. The absolute retention of Ca and P was below the intrauterine accretion rate, although the intakes of Ca and P were within the recommended range [9].

Administering a mineral supply large enough so that intrauterine accretion rates can really be achieved is quite difficult. Several studies showed that larger Ca and phosphate intakes can be achieved by experimental formulas [10,11] or by separate supplementation of Ca salts [12]. Reports of a formula with a high Ca content [4,13] that is in stable solution have recently been questioned [14].

Even so, the fact remains that overt bone disease can probably be prevented by

relatively low amounts of supplementation [11], as is also shown in our results with AP levels that are only marginally higher than values of term neonates. The fact that 1,25-$(OH)_2D$ levels are above the adult range, might indicate that there is still a need for more Ca and P in both study groups. On the other hand, the European Society of Paediatric Gastroenterology & Nutrition (ESPGAN) has recently recommended not to exceed a level of intake of 140 mg Ca/100 kcal, considering the possible complications of very high Ca intakes and the lack of evidence that such high concentrations are beneficial [9]. Further study is needed to define mineral needs by measuring resulting bone mineralization in vivo and combining this with accurately performed balance studies.

This study clearly shows that with LCT fat, Ca absorption is impaired whereas P absorption is not. The Ca/P ratio of 2:1 in the formulas was lowered to about 1.8:1 in the formulas because of sedimentation of preferentially Ca in both formulas. More importantly, the Ca/P ratio was significantly lower in the infants fed LCT after absorption than in the MCT group (**fig. 5**). Our results indicate that LCT-fed infants probably have a relatively low Ca/P ratio after absorption, which is then increased by increasing P excretion in the urine, mediated by an increased PTH level. The resulting ratio of the retained minerals is not significantly different any longer between groups. If we assume that the mechanism of adjusting the P excretion in urine in order to retain the correct ratio of minerals is indeed mature in these infants, then the fact that MCT-fed infants raised their Ca/P ratio of 1.5:1 after absorption only to 1.6:1, without showing large excretion in the urine of one of the two minerals, might indicate that this level is close to the ideal situation. This would be in agreement with a reported ratio for Ca and P accretion of 1.6:1 during a postconceptional age of 35-36 weeks [15]. It also means that if formulas are designed with LCT fat (or with a high Ca content, most often also causing a relative decrease in Ca absorption), Ca/P ratios might be needed that are above the recommended maximum ratio of 2:1 [9,16]. Several experimental formulas with a Ca/P ratio higher than 2:1 have been used before [10,17], although no fat balances were reported. Taking into account the absorption rates of Ca and P for the LCT formula, we can estimate that the ratio for Ca and P upon dietary intake should be around 2.3:1 to obtain an absorption at a 1.6:1 ratio, or even 2.5:1 if there would be a relatively higher sedimentation rate of Ca, like in our study. However, because the fat balance might worsen if too much Ca is added and also because some infants have high Ca absorption rates and also need sufficient P, it seems prudent at the moment to try to improve the solubility of calcium salts in premature formula first.

The amount of Mg in both formulas was slightly above the recommended intake [16] and retention rate appeared to be higher than the intrauterine retention in both groups. The intake of Mg from most standard formulas ranges from 12-30 mg/kg/day and may be as much as threefold greater than the highest suggested daily intake [16]. As Mg levels in plasma were within the normal range, the significance of this remains to be established.

In conclusion, the most important difference between formulas designed for feeding preterm infants containing only LCT fat blends or containing 60% LCT/ 40% MCT blends is the slightly lower Ca absorption rate with the LCT formula. This causes a Ca/P imbalance, inducing a compensating increased urinary excretion of P. Although a Ca/P ratio slightly above 2:1 might be needed in LCT-containing preterm formulas, it seems prudent to first investigate whether the Ca source can be improved in LCT formulas.

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CHAPTER IV

QUANTITATION OF OXIDATION OF MEDIUM-CHAIN TRIGLYCERIDES IN PRETERM INFANTS.

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SUMMARY

Medium-chain triglycerides (MCTs), with a chain-length of eight and ten carbon atoms form up to 50% of the total fat content in some preterm infant formulas. In 20 small preterm infants (birth weight: 1153 ± 227 g; mean ± S.D.) fed a special formula containing 40% MCTs, a primed constant oral infusion study of [1-^{13}C]-potassium octanoate was conducted to quantify the oxidation of MCTs. A plateau in ^{13}C enrichment in breath CO_2 was reached in all patients within 1-3 hrs. Simultaneously, substrate utilization was measured using a closed system indirect calorimeter. No significant difference was found between appropriate for gestational age (AGA, n = 8) and small for gestational age (SGA, n = 12) infants in the percentage of the administered tracer that was oxidized (44.9 ± 9.1% *vs.* 48.5 ± 11.0%). In all patients, the recovery was calculated to be 47.1 ± 10.2%, which is less than previously estimated and corresponds to a mean MCT oxidation of 1.26 ± 0.27 g/kg/day. With indirect calorimetry, a total fat oxidation of 1.42 ± 0.84 g/kg/day in AGA and 2.00 ± 0.85 g/kg/day in SGA infants was found, indicating that MCTs accounted for around 85% of the total fat oxidation in AGA *versus* 65% in SGA infants.

INTRODUCTION

The optimal composition of the oral feeding solution for a preterm infant remains a matter of debate. Milk from mothers who delivered preterm, although containing more protein and minerals than milk from mothers who delivered at term, may not be sufficient to prevent deficiencies or to produce weight gain equivalent to the intrauterine growth curve [1,2].
The various formulas specially designed for preterm infants differ considerably, reflecting unanswered questions regarding the optimal composition of such formulas.

Some preterm infant formulas contain up to 50% of the fat as MCTs, intended to enhance fat absorption and to provide a readily available energy source. Improved weight gain and retention of fat, protein, and calcium have been described with MCT-containing formulas [3,4]. No reliable data exist regarding the percentage of orally administered MCTs that is oxidized by the preterm infant. The objectives of this study were to quantify octanoic acid oxidation as an indication of MCT oxidation and to measure utilization of other substrates in preterm infants receiving a formula containing 40% of fat as MCTs.

MATERIALS AND METHODS

Subjects

Infants with a birth weight of less than 1600 g were included in the study after they were on full oral feedings, gaining weight, and clinically stable breathing room air. Twenty patients entered the study, eight patients appropriate for gestational age (AGA), group I, and twelve patients small for gestational age (SGA), group II.

AGA was defined as a birth weight within 2 SD of the mean for gestation, according to the growth curves of Usher and McLean [5]. SGA was defined as a birth weight more than 2 SD below the mean. Gestational age was determined by medical history and Ballard score [6]. All infants were initially admitted to the neonatal intensive care unit of the Academic Hospital Rotterdam/Sophia Children's Hospital, five in group I and six in group II had been ventilated for the respiratory distress syndrome in the neonatal period. Mean body weight, gestational age and age at the time of study are shown in table 1.

Table 1
Clinical parameters of infant groups studied

	Birth weight (g)	Gestational age (wk)	Age when studied (d)	Weight when studied (g)
Total (n = 20)	1153 ± 227	31.0 ± 1.7	28 ± 6	1508 ± 313
AGA (n = 8)	1362 ± 170	30.3 ± 1.7	28 ± 7	1761 ± 243
SGA (n = 12)	1014 ± 134	31.4 ± 1.6	28 ± 6	1340 ± 233
	$p < 0.001$	NS	NS	$p < 0.005$

Values expressed as mean ± 1 SD

Methods

Feeding regimen

All infants received a formula feeding (Nenatal, Nutricia, Zoetermeer, The Netherlands) in which 40% of the fat is provided as MCTs, from the day oral feedings were started, usually at day 7. The composition of the formula is given in table 2. In our ward very-low-birth weight (VLBW) infants routinely are fed by continuous nasogastric gavage. Infants were studied at least three days after they had reached the maximum intake of 150 ml/kg/day of the formula. During the studies, the gavage feeding was administered using a syringe pump (Perfusor Secura FT, Braun, Melsungen, FRG).

Study procedure

The study protocol was approved by the Human Subject Review Committee of the Academic Hospital Rotterdam and Erasmus University. Written informed consent was obtained from the parents.

The study design was as follows. In the 4th postnatal week urine was collected for 72 hrs. In the middle of this collection period, indirect calorimetry was performed for a 6- to 8-hr period. During indirect calorimetry, a primed constant infusion of [1-^{13}C]-potassium octanoate was given.

Table 2
Composition Nenatal 07 (Nutricia, The Netherlands) per 100 ml*

Energy	80 kcal
Protein	2.2 g
Carbohydrates	8.0 g
Lactose	4.0 g
Dextrine maltose	4.0 g
Fat	4.5 g
MCT	
C8:0	1.1 g
C10:0	0.7 g
LCT	
C16:0	0.3 g
C18:0	0.1 g
C18:1	0.9 g
C18:2	1.4 g
Sodium	23 mg
Potassium	60 mg
Calcium	100 mg
Phosphorus	50 mg

*Composition before September 1988.

Indirect calorimetry

Metabolic rate and substrate utilization were measured by a closed circuit indirect calorimeter. Continuous measurements were performed for 6-8 hrs, as previously described [7]. Briefly, an air mixture devoid of carbon dioxide enters the incubator. In a sample of the air leaving the incubator the CO_2 concentration is measured, whereafter all carbon dioxide is filtered out by a soda-lime filter. The CO_2 is injected again into the airflow by a mass flow injector system until the same concentration of CO_2 is measured by the infrared meter (Unor 6N, Maihak, Hamburg, FRG). The amount of carbon dioxide injected into the system equals the carbon dioxide production of the infant. Thereafter all CO_2 is filtered by a second

filter. The amount of oxygen consumed by the infant is equal to the amount of oxygen that has to be injected into the system to keep the oxygen tension constant, as measured by polarographic oxygen cells (type 6223771 Beckman, Fullerton, Ca., USA) in the system and in a reference vessel. To compensate for changes in air-pressure, the reference vessel is connected to the flow circuit by means of a capillary. Oxygen consumption and carbon dioxide production are measured with an accuracy of 0.2 ml/min, the RQ during calibration with butane was within 2% of the theoretical value. Urine was collected in plastic bags over a period of three days. Urinary nitrogen concentration of a pooled sample was determined by combustion in an automatic nitrogen analyzer (ANA 1400; Carlo Erba, Milano, Italy) by the Dumas procedure [8].

Measurement of octanoate oxidation

Octanoic acid oxidation was measured using [1-^{13}C]-potassium octanoate. The labeled octanoic acid (99% purity, Sigma Chemical Co., St. Louis, USA) was converted into its potassium salt by means of titration with KOH in our laboratory and diluted in sterile water. To determine the natural background of ^{13}C, three baseline breath samples were collected during 15 minutes each before starting the isotope infusion. The bicarbonate pool was then primed with 6.9 μmol.kg^{-1} of a 6.9 mmol/l solution of $NaH^{13}CO_3$ (80% purity, Merck Isotopes, Montreal, Canada) as previously described [9]. At the same time, a [1-^{13}C]-octanoate priming dose of 2 mg/kg was given, followed by a constant infusion of 2 mg/kg/hr for 6-8 hrs. All isotopes were administered through a separate nasogastric tube. The delivery rate of the pump during the study was measured by dividing the difference in weight of the syringe before and after the experiment by the duration of the experiment to determine the actual quantity of [1-^{13}C]-octanoate administered. Once the isotope infusion was started, 15 minutes CO_2 breath collections were made every hour for 4 hours, and every 30 minutes for 2-4 more hours. Breath CO_2 was collected by passing a sample of the air leaving the incubator through an all glass spiral condensor, containing 10 ml of a fresh 1 mol/l NaOH solution. Samples were transferred to Vacutainers and stored at -20°C until analysis. Trapping of CO_2 was shown to be complete during 15 minutes at a flow of approximately 10 ml/min and hence isotopic fractionation was prevented. In a study conducted without the administration of isotopes, a shift in natural background over time was ruled out, as could be expected during a continuous feeding regime.

Isotopic Ratio Mass Spectrometric Analysis

Respiratory carbon dioxide was liberated at $< 10^{-3}$ torr by adding 85% phosphoric acid to the $NaHCO_3$ solution. Pure CO_2 was collected in a small glass container in liquid nitrogen whereas the water is trapped in a methanol dry ice bath. The glass container with pure CO_2 was connected to a VG Sira 10 isotope ratio mass spectrometer (Vacuum Generators, Winsford, Cheshire, UK). Results of ^{13}C abundance of both baseline and plateau were calculated as APE/reference CO_2 tank standard sample. Isotopic plateau was defined by eyeballing as the absence of a change in abundance with time.

Calculations

The percentage of $^{13}CO_2$ recovery was calculated as follows:

1) $^{13}CO_2$ production (μmol/kg/hr) =

$$\frac{\dot{V}CO_2 \times 60}{22.4} \times \frac{H}{100} \times \frac{100}{C} \times 1000$$

$\dot{V}CO_2$ = carbon dioxide production (STPD) in ml/kg/min; H = plateau height $^{13}CO_2$ above baseline in APE; C = correction factor for $^{13}CO_2$ retention in the bicarbonate pool, calculated from the energy intake of the infant [9].

2) ^{13}C administered (μmol/kg/hr) = [1-^{13}C]-octanoate (μmol/kg/hr) x 0.99
0.99 = purity of [1-^{13}C]-octanoate

3) ^{13}C recovery = $$\frac{^{13}CO_2 \text{ production}}{^{13}CO_2 \text{ administered}} \times 100\%$$

4) The percentage ^{13}C recovery was considered to be equal to the percentage of the tracer that was oxidized.

5) Nonprotein $\dot{V}O_2$ and nonprotein $\dot{V}CO_2$ were derived from $\dot{V}O_2$ and $\dot{V}CO_2$ by correcting the values for the protein oxidation, calculated as 6.25 x the timed urinary nitrogen-excretion.

6. Metabolic rate and partition of the nonprotein macronutrients were calculated

from nonprotein RQ and nonprotein $\dot{V}O_2$ using the tables of Lusk [10].

7. Statistical analysis of the differences between the results of the two groups was conducted using the Mann-Whitney *U* test.

RESULTS

The energy and substrate intake of the infants is shown in table 3. There were no differences in intake between SGA and AGA infants. The results of indirect calorimetry are shown in table 4. The metabolic rate was significantly higher in the SGA infants ($p < 0.005$) per kilogram bodyweight, as described earlier [11], probably due to a higher fat oxidation (2.00 ± 0.85 *versus* 1.42 ± 0.84 g/kg/day), although the difference in fat oxidation did not reach statistical significance.
The production of $^{13}CO_2$ in breath rose in the first hour in all patients and a plateau was reached in all patients within 1-3 hrs. An example of a characteristic study is shown in figure 1. The results of the isotopic studies are shown in table 5. These results show a $^{13}CO_2$ recovery for AGA and SGA infants of 44.9% and 48.5% respectively. The difference between the two groups is not significant, and the mean $^{13}CO_2$ recovery of the labeled octanoate in all patients is 47% with a SD of 10% and a range of 32 to 64%.

Table 3
Substrate and total caloric intake of infant groups

	Intake (kcal/kg/day)	Total fat intake (g/kg/day)	MCT intake (g/kg/day)	Glucose intake (g/kg/day)
Total ($n = 20$)	120 ± 4	6.71 ± 0.20	2.69 ± 0.08	11.23 ± 0.41
AGA ($n = 8$)	119 ± 2	6.71 ± 0.10	2.68 ± 0.04	11.19 ± 0.16
SGA ($n = 12$)	120 ± 5	6.73 ± 0.26	2.73 ± 0.10	11.26 ± 0.52
	NS	NS	NS	NS

Values expressed as mean ± 1 SD

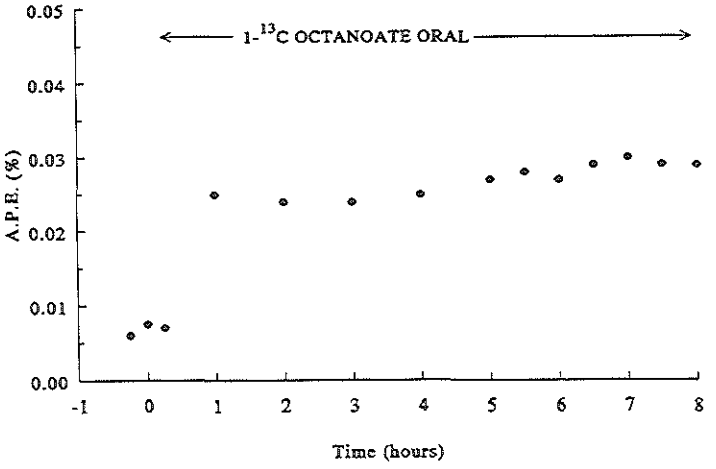

Fig. 1.
Time course of $^{13}CO_2$ enrichment in breath after a primed constant oral infusion of [1-^{13}C]-octanoate in one patient. Three baseline breath samples were taken before the isotope administration.

Table 4
Results of indirect calorimetric measurements

	Total (n = 20)	AGA (n = 8)	SGA (n = 12)	p
$\dot{V}O_2$ (ml/kg/min)	8.34 ± 0.64	7.90 ± 0.35	8.63 ± 0.63	<0.01
$\dot{V}CO_2$ (ml/kg/min)	7.58 ± 0.58	7.23 ± 0.38	7.81 ± 0.58	NS
RQ	0.91 ± 0.03	0.92 ± 0.03	0.91 ± 0.03	NS
Metabolic rate (kcal/kg/day)	61.7 ± 4.5	58.4 ± 2.4	63.6 ± 4.5	<0.005
Fat utilization (g/kg/day)	1.77 ± 0.87	1.42 ± 0.84	2.00 ± 0.85	NS
Glucose utilization (g/kg/day)	10.77 ± 1.91	10.77 ± 1.95	10.77 ± 1.97	NS

Values expressed as mean ± 1 SD

Table 5
Results of stable isotopic measurements

	Recovery (%)	MCT oxidation (g/kg/day)
Total (n = 20)	47.1 ± 10.2	1.26 ± 0.27
AGA (n = 8)	44.9 ± 9.1	1.20 ± 0.24
SGA (n = 12)	48.5 ± 11.0	1.30 ± 0.29
	NS	NS

Values expressed as mean ± 1 SD

DISCUSSION

Premature infants have a relative deficiency of pancreatic lipase and bile salts, causing loss of part of the administered lipids in the feces [12,13]. Orally administered MCTs are absorbed mainly as free fatty acids, because their intraluminal hydrolysis is rapid and almost complete. Recently it was shown that marked hydrolysis occurs in the stomach of the preterm neonate [14]. MCTs can also be absorbed in part as triglycerides [15]. In most cases a total of more than 99 % are absorbed [3,16,17]. If absorption as a triglyceride takes place, hydrolysis by an intestinal lipase will follow within the enterocyte [15].

Unlike long-chain triglycerides (LCTs), the medium-chain fatty acids (MCFAs) derived from MCTs are not reesterified, so all MCTs will leave the intestinal cells as MCFAs and reach the liver via the portal vein as free fatty acids in solution in the plasma or bound to albumin [18].

To study the metabolism of MCTs, sodium-^{13}C-octanoate has been used as a tracer in adults [19]. Octanoate can be regarded as a tracer representative of trioctanoin metabolism, considering the fact that both the free fatty acid and triglyceride with a chain length of eight carbons will enter at one stage the free fatty acid pool in the intestinal cells.

MCTs have been included in preterm infant formulas because of their high rate of absorption. They are also referred to as a readily available source of energy,

implying a rapid and almost complete oxidation of MCTs [15,20,21]. Even the existence of an obligatory oxidation of MCFAs with increased heat production has been suggested [22,23]. In this study, we found that in both AGA and SGA infants an average of no more than 47% of the administered octanoate was oxidized, with a range of 32 to 64%.

In a study using the bolus technique and ^{13}C labeled trioctanoin as a tracer in a small number of preterm infants, a cumulative oxidation of 27% of the tracer was found [24]. Recent studies showed that MCTs might have a tendency to adhere to the feeding tube, due to its hydrophobic properties [25]. If part of the trioctanoin used as a tracer adheres to tubing during the experiment, this can give falsely low results on recovery of the tracer. By using the water-soluble potassium salt of octanoic acid we minimized the risk that tracer would adhere to the tubing. This might explain the fact that we found a higher oxidation rate, although the bolus technique is not fully comparable to the constant infusion technique. When discussing the physiological significance of our findings we will have to consider the further steps in the metabolic pathways followed by MCTs.

In the liver, MCFAs readily cross the membrane into the mitochondria, where they are activated and broken down into acetyl-CoA. This acetyl-CoA can enter the tricarboxylic acid cycle and, under the conditions of our study, will be detected as labeled CO_2 after oxidation. When there is excess acetyl CoA, a major pathway is formation of ketone bodies; in fact, MCFAs are known to be ketogenic [26]. Feeding of a MCT-containing formula for four days promotes a mild ketosis in preterm infants [27]. These ketone bodies will be quickly utilized peripherally, especially after a period of adaptation [28]. Because the conversion to ketones is a rapid process [29] most of the excreted $^{13}CO_2$ due to the oxidation of ketone bodies derived from MCTs will also contribute to the ^{13}C enrichment in expired air, thus being included in our calculation.

One of the conditions that have to be fulfilled to make this assumption is that the pool of ketones remains constant, which means that the concentration of ketone bodies in the plasma should not change during the experiment. We think this was the case during these studies, because these infants were on the same intake of the MCT formula for at least three days before starting the study, and were in a clinical stable condition. Hence, there is no reason to assume that in the groups studied an increase in the ketone body pool during the experiment could have been responsible for retention of the label.

We conclude that both ways of immediate oxidative disposal of MCFAs,

entering the Krebs cycle directly or via the formation of ketone bodies, are included in the values for oxidation of MCTs we found in this study. Following this line of reasoning, about half of the MCTs in this feeding regimen follows other pathways than oxidation. Urinary loss of dicarboxylic acids that can exist during MCT feeding probably accounts for less than 1% of the dietary intake of MCTs in preterm infants [30].

Furthermore, MCFAs are not to a significant amount activated by coenzyme A in the extramitochondrial space to participate directly in triglyceride and phospholipid synthesis [31]. Accordingly storage of octanoate as a triglyceride in adipose tissue does not seem to be of great importance [32-34]. Apparently, MCFAs have to be converted into long-chain fatty acids (LCFAs) before they can be stored as LCTs in the body fat tissue. Probably the most important route quantitatively for this is the breakdown of MCTs into acetyl-CoA, whereafter LCTs are formed through *de novo* lipogenesis [35].

The relatively low oxidation rate we found is consistent with the findings that breakdown of MCFAs and lipogenesis occur sequentially during MCT feeding [36]. The oxidation rates we found might indicate that in these patients lipogenesis is an important pathway for MCTs, whereas fitting less well with the assumption of an obligatory oxidation as a cause of the enhanced thermogenesis that is sometimes encountered on a MCT diet [22,23]. If we assume a mean absorption of 85% of the LCTs in these patients [16,17], MCTs would account for around 45% of the absorbed fatty acids. Combining the mean value for total fat oxidation we obtained by indirect calorimetry with results from the isotopic studies, we find that oxidation of MCTs accounts for 65% of the fat oxidation in SGA and 85% in AGA infants. Apparently there is a preferential oxidation of MCTs, but whether this aspect of MCT metabolism is advantageous remains to be proven.

Remarkably, although SGA infants have a significantly higher metabolic rate, we found no increase in the MCT oxidation suggesting there might be a limit to the amount of MCTs that can be completely oxidized by the small preterm infant.

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CHAPTER V

DECREASED GLUCOSE OXIDATION IN PRETERM INFANTS FED A FORMULA CONTAINING MEDIUM-CHAIN TRIGLYCERIDES.

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SUMMARY

Several formulas for preterm infants contain medium-chain triglycerides (MCTs) to enhance fat absorption. Although fat absorption with MCTs was slightly higher in several studies in preterm infants, a beneficial effect on growth has only been reported in one publication. We hypothesized that when part of the fat blend of preterm formula is substituted by MCT oil, this might lead to a different metabolic pattern in which, due to the preferential oxidation of MCTs, an increase in lipogenesis from glucose could lead to an increase in metabolic rate. To study the impact of MCTs on glucose metabolism, 18 preterm infants were randomized to receive either a MCT or a long-chain triglyceride (LCT) formula, containing 38- and 6%-by-weight medium-chain fatty acids (MCFAs), respectively, in their fat blend. At 4 weeks of age, the metabolic rate, substrate utilization, glucose kinetics, and oxidation were measured by indirect respiratory calorimetry in combination with a constant-rate oral infusion of [U-^{13}C]-glucose. The "true" rate of appearance of glucose (Ra "true") was measured from the dilution of the uniformly labelled (m+6) species of infused tracer whereas "apparent" rate of appearance of glucose (Ra "apparent") was measured from the dilution of infused tracer C (carbon). The latter was measured by an on-line combustion method using a gas chromatograph-isotope

ratio mass spectrometer. At a carbohydrate intake of 8.4 mg/kg/min, total utilization of carbohydrate was equal in both groups at 7.6 mg/kg/min. However, glucose oxidation, as measured by the appearance of ^{13}C in breath CO_2 was significantly lower in the MCT group (4.5 ± 0.83 *vs.* 5.7 ± 0.67 mg/kg/min, MCT *vs.* LCT). Therefore, it can be assumed that a larger proportion of the glucose intake in the MCT group was used in the nonoxidative pathway, *e.g.* synthesis of fat as compared with the LCT group. There was no difference in the rate of endogenous glucose production and glucose C recycling between the two groups. In addition, the metabolic rate calculated from the rate of oxygen consumption was also similar (59 ± 5 *vs.* 62 ± 4 kcal/kg/d, MCT *vs.* LCT). We conclude that, in preterm infants fed a formula containing 38% MCTs in their fat blend, glucose oxidation is significantly decreased whereas lipogenesis is probably increased. These findings may provide a mechanism for the so-called metabolic inefficiency of MCTs.

INTRODUCTION

The fat blend of several special formulas for preterm infants consists of 30% to 50% medium-chain triglycerides (MCTs) to enhance fat absorption. In several studies in which MCT-containing fat blends were used in preterm infants, increased fat absorption without improved weight gain was reported [1-3]; thus, one might expect that infants fed a MCT-containing formula either have an increased energy expenditure or lay down relatively more fat per gram new tissue, resulting in a higher energy cost of growth. We hypothesized that an increased energy expenditure, which has been described before in animals and human adults during overfeeding with MCTs [4-7], explains the lack of increase in growth rate at the higher energy absorption in preterm infants fed MCT formulas. We further speculated that an increased metabolic rate might result from an increased lipogenesis with ongoing fat oxidation, as MCT feeding is known to be associated with increased lipogenesis. In a previous study [8], using the same methods as in the present article, we showed a higher metabolic rate in parenterally fed infants receiving only glucose as their nonprotein energy source compared with infants receiving glucose and lipids. Moreover, we showed strong evidence that the higher metabolic rate was due to the conversion of glucose into fat, resulting in a lower food efficiency. To elucidate the mechanism of the apparently lower food efficiency

in MCT feeding, we compared the effects on glucose metabolism and metabolic rate in two groups of very low birth weight infants fed formulas with different MCT content.

PATIENTS AND METHODS

Subjects

Eighteen infants with a birth weight of less than 1600 g were included in the study. Infants were eligible for inclusion if they were free from major metabolic problems and congenital malformations and if they were clinically stable and breathing room air at the introduction of oral feedings. At one week of age, after permission was obtained from the parents, the infants were randomly assigned to receive either a MCT or a LCT formula, with nine infants entering each group. Enteral feedings were gradually introduced from day 7 by continuous nasogastric tube feeding until a full intake of 150 ml/kg/day was reached. Both formulas were especially manufactured by Nutricia (Zoetermeer, The Netherlands) with a composition that was completely equal, except for their fat blend. The batch of MCT formula in this study contained 38% MCFAs, derived from MCT oil, and the batch of LCT formula 6% derived from coconut oil. Both provided 8 g carbohydrates (lactose/polycose 50/50), 2.2 g protein and 6.8 g fat/100 ml of prepared formula. Similar formulas are commercially available and recommended for preterm infants. One and the same batch from each formula was used during the study. The study was performed at least 3 days after the infant had received full oral feedings. The median day of study was 22 in the MCT and 24 in the LCT group; the total range was 19 to 37. Patient characteristics and average intakes are described in **table 1**. The study protocol was approved by the medical ethical committee of the Erasmus University Academic Hospital/Sophia Childrens Hospital, Rotterdam, The Netherlands.

Methods

Indirect calorimetry

Metabolic rate and substrate utilization were measured in a closed-circuit indirect calorimeter. Continuous measurements were performed for 6 to 7 hrs, as

Table 1
Patient characteristics

	MCT	LCT
Birth weight (kg)	1.2 ± 0.24	1.2 ± 0.16
Gestational age (wks)	31 ± 1	32 ± 2
Study weight (kg)	1.5 ± 0.27	1.6 ± 0.21
Caloric intake (kcal/kg/d)	126 ± 3	126 ± 2
Carbohydrate intake (mg/kg/min)	8.4 ± 0.21	8.4 ± 0.10
Fat intake (mg/kg/min)	4.7 ± 0.12	4.7 ± 0.06
Protein intake (mg/kg/min)	2.3 ± 0.06	2.3 ± 0.30
Weight gain (g/kg/d)	16 ± 4	16 ± 3

previously described in detail [9]. During the study period, feeding was continued by a syringe pump at the same rate as during the three days before the study day. Urine was collected in plastic bags over periods of three days. Urinary nitrogen concentration of a pooled sample was determined by combustion in an automatic nitrogen analyzer (ANA 1400 Carlo Erba, Milano, Italy).

Glucose oxidation study

During the calorimetry study, a stable isotope study was performed using uniformly labeled [U-^{13}C]-glucose (Merck Isotopes, Montreal, Canada; 98.9% ^{13}C) to quantitate the oxidation of glucose.

To determine the natural background abundance of ^{13}C in expiratory air, three baseline breath samples were collected during 10 minutes each before starting the isotope infusion. The bicarbonate pool was then primed with 6.9 μmol/kg of a 6.9 mmol/l solution of $NaH^{13}CO_3$ (80% purity, Merck Isotopes) and a [U-^{13}C]-glucose priming dose of 2.7 μmol/kg was given, followed by a constant infusion of 2.7 μmol/kg/hr for 6 to 7 hrs. All isotopes were administered through a separate nasogastric tube. Once the isotope infusion was started, 10-min CO_2 breath collections were made every hour for 4 hours, and every 15 minutes for 2 to 3 more hours. Breath CO_2 was collected by passing a sample of the air leaving the calorimeter

through an all-glass spiral condenser, containing 10 ml of a fresh 1 mol/l NaOH solution. Samples were transferred to Vacutainers (Becton Dickinson, Etten-Leur, The Netherlands) and stored at -20°C until analysis. Trapping of CO_2 was shown to be complete for at least 15 minutes at the sample flow used.

In a study conducted without the administration of isotopes, a shift in natural background over time was ruled out, as could be expected during a continuous feeding regime.

Mass spectrometric analysis

Breath CO_2: Respiratory CO_2 trapped in NaOH was liberated at $< 10^{-3}$ torr by adding 85% phosphoric acid to the $NaHCO_3$ solution. CO_2 was collected by cryogenic distillation and analyzed on a VG SIRA 10 isotope ratio mass spectrometer (Vacuum Generators, Winsford, Cheshire, UK). Results of ^{13}C abundance of both baseline and plateau were calculated as atom % excess (APE) relative to the reference CO_2 tank standard sample. Isotopic plateau during the final part of the study was confirmed by simple regression analysis on the last five data points. **Fig. 1** shows an example of the curves of enrichment obtained in expiratory CO_2.

Blood glucose: Immediately before and after the study, blood was obtained by heel-stick from a warmed heel. After separation, plasma was stored at -80°C for analysis of the isotopic enrichment of plasma glucose: 1) in uniformly labeled species, *i.e.* (m+6); and 2) for the overall enrichment of glucose C by combustion using a gas chromatograph-isotope ratio mass spectrometer (GC-IRMS).

The (m+6) enrichment of glucose was measured by chemical ionization gas chromatography-mass spectrometry (GC-MS) of the aldononitrile-pentacetate derivative of plasma glucose with selective ion monitoring of m/z 328 and 334, as described before [11].

The ^{13}C enrichment of glucose C was also measured with a new technique, the on-line combustion using a GC-IRMS (Isochrom-III, VG, Middlewich, Cheshire, UK) [12]. With this technique, the effluent of the GC, containing the same pentacetate derivative of plasma glucose, is combusted on-line in a catalytic furnace, and the resulting CO_2 is driven by a helium flow into the ion source of a standard IRMS after passing through a water trap. In this way, the tedious manual isolation of plasma glucose before combustion to CO_2 necessary for the determination of ^{13}C enrichment of glucose as the precursor for glucose oxidation [13] is circumvented.

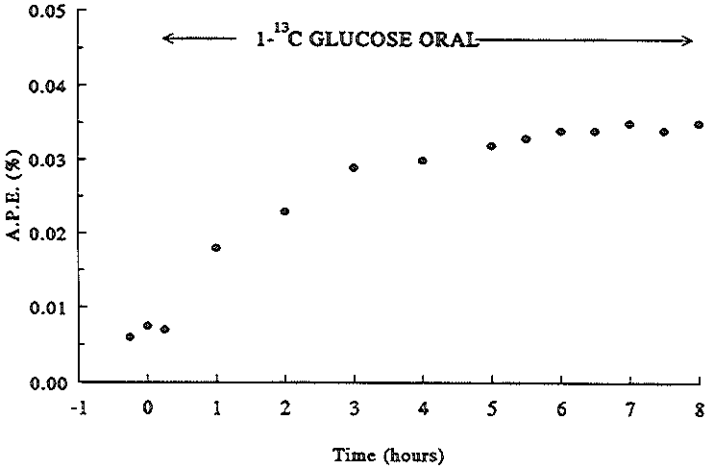

Fig. 1
$^{13}CO_2$ enrichment in expiratory air during a primed constant oral tracer infusion of [U-^{13}C]-glucose in one infant.

Calculations

Metabolic rate was calculated using the approach of Lusk [10], assuming an RQ of 1.00 for glucose and 0.72 for fat:

$$np\dot{V}O_2 = \dot{V}O_2 - (N \times 6.25 \times 0.9963) \quad (1)$$

where $np\dot{V}O_2$ = nonprotein O_2 consumption in ml/kg/min, $\dot{V}O_2$ = total O_2 consumption in ml/kg/min, N = urinary nitrogen in mg/kg/min, and 0.9663 = ml O_2 consumed/mg protein oxidized

$$npRQ = \frac{\dot{V}CO_2 - (N \times 6.25 \times 0.7739)}{\dot{V}O_2 - (N \times 6.25 \times 0.9963)} \quad (2)$$

where $\dot{V}CO_2$ = CO_2 production in ml/kg/min, and 0.7739 = ml CO_2 produced/mg protein oxidized

$$MR = 1.44 \times \left[4.702 + \frac{npRQ - 0.72}{0.28} \times 0.345\right] \times np\dot{V}O_2 + energy \quad (3)$$

from protein oxidation

where MR = metabolic rate (kcal/kg/day), 1.44 = conversion factor for ml/min to l/day, 4.702 = caloric equivalent of 1 l O_2 at a np RQ of 0.72, and 0.345 = difference between caloric equivalents of 1 l O_2 at np RQ of 1 and 0.72

The percentages of the O_2 consumed by carbohydrate or fat are calculated according to the following formulas:

$$\% \ Carbohydrate = \frac{npRQ - 0.72}{0.28} \tag{4}$$

$$\% \ Fat = \frac{1 - npRQ}{0.28} \tag{5}$$

The term carbohydrate utilization is used in accordance with Frayn [14], arguing that the rate of carbohydrate oxidation as measured with indirect calorimetry is the net result of glucose oxidation, lipogenesis from glucose, and gluconeogenesis from amino acids. At a high caloric intake when gluconeogenesis is low and lipogenesis can be high, the total carbohydrate "balance" or utilization as measured by indirect calorimetry is equal to the sum of the rate of true carbohydrate oxidation (c_o) and the rate of conversion of carbohydrate to fat (c_f), or $c = c_o + c_f$.

For this reason, the difference between carbohydrate utilization from indirect calorimetry and true carbohydrate oxidation measured independently, *e.g.* by isotopes, can give an indication of the rate at which the process of lipogenesis is occurring [8].

The fraction of infused tracer appearing in respiratory CO_2 is calculated according to the following formula:

$$Fraction \ of \ infused \ tracer \ in \ CO_2 = \frac{\dot{V}CO_2 \ x \ IE_{CO2}}{0.989 \ x \ F \ x \ 6 \ x \ c} \tag{6}$$

where $\dot{V}CO_2$ is the rate of CO_2 production, IE_{CO2} is the increase in isotopic enrichment of CO_2 at plateau value, 0.989 is the infused tracer enrichment, F is the tracer infusion rate, 6 is the number of C atoms/glucose molecule, and c is the correction factor for CO_2 retention in the bicarbonate pool. Instead of taking a fixed fraction of 0.81 for c, we calculated it individually according to the regression equation we described in an earlier publication [15]:

$$c(\%) = 64.2 + intake(kcal/kg/d) \ x \ 0.1667 \tag{7}$$

The "true" Ra of glucose in the plasma pool was calculated from the dilution of the (m+6) species of the labeled glucose.

$$Ra\ true = \left[\frac{E_i}{E_p} - 1\right] x\ F \qquad (8)$$

where E_i = (m+6) enrichment of infused tracer = $(0.989)^6$, E_p = (m+6) enrichment of plasma glucose, and F = rate of infusion of the tracer

This is considered a measure of true Ra of glucose because the opportunity for a uniformly labelled (m+6) species to recycle back via Cori cycle is considered to be negligible [16].

The "apparent" rate of glucose appearance in the blood was calculated from the enrichment of glucose C measured by the combustion method using the same dilution equation. In this case E_i = 0.989, *i.e.* the ^{13}C enrichment of infused tracer, and E_p = the ^{13}C enrichment of blood glucose C. It should be recognized that ^{13}C enrichment of blood glucose C measured by the combustion method includes the recycled carbon, and therefore will result in a lower rate of glucose appearance (Ra "apparent") as compared with the true Ra.

$$Glucose\ oxidation = Ra\ apparent\ x\ fraction\ of\ infused\ tracer\ in\ CO_2\ (Eq.\ 6) \qquad (9)$$

Glucose production was calculated by subtracting the glucose intake (calculated from the individual intakes of the continuously fed formula) from the rate of glucose appearance obtained by isotope dilution, Ra true. Glucose intake was calculated from the glucose part of lactose plus the glucose within the glucose polymers, assuming there is complete intestinal hydrolysis and absorption of complex carbohydrates in preterm infants, as has been shown before [17]. The galactose was not included in the glucose intake. If galactose is converted into glucose by the liver, it will be measured as glucose production upon release. The calculation also assumes there is no significant first-pass extraction of glucose in the splanchnic area of the (labeled) glucose. A validation study in five orally fed preterm infants was performed where we measured (m+6)-glucose enrichment with both an oral and an intravenous tracer infusion protocol two days apart. **Fig. 2** shows the enrichment in plasma obtained after infusion of [U-^{13}C]-glucose in five orally fed preterm infants (represented by

individual lines). The enrichment obtained by i.v. infusion would be expected to be higher than the enrichment obtained by oral infusion of tracer if there had been a significant amount of label retained in the liver, but in this case was 98 ± 10% of the "oral enrichment". This result indicates that all (labeled) glucose passes the liver into the circulation and no significant amount is being extracted on the first pass. These results are in accordance with studies in adults [18] showing an *initial* splanchnic extraction of glucose between 2.4 and 8%.

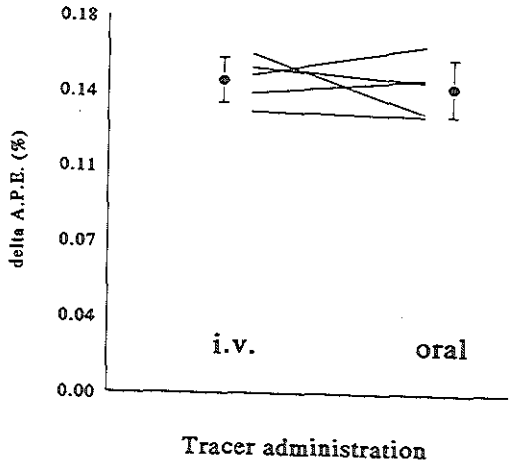

Fig. 2
Rise in (m+6)-glucose enrichment after continuous infusion of [U-^{13}C]-glucose in five patients. Labeled glucose was infused i.v. on one day and orally two days later. Individual patients are indicated by *individual* lines, average enrichment oral *vs.* i.v. shows no trend towards one method (see text).

$$Recycling = Ra\ true - Ra\ apparent \qquad (10)$$

The recycling measured in this way is the amount of glucose recycled from three carbon intermediates like lactate through the Cori cycle, as discussed above.

$$\textit{net glucose storage} = \textit{carbohydrate intake} - \textit{carbohydrate utilization from calorimetry} \quad (11)$$

Statistics

Data are presented as mean $\pm$ 1 SD. Differences between the MCT and the LCT group were tested using Wilcoxon's rank-sum (Mann-Whitney two-sample) test. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

There were no significant differences between the two study groups in any of the parameters described in table 1.

Results of the glucose studies are described in table 2. Carbohydrate intake was equal at 8.4 mg/kg/min in both groups, and carbohydrate utilization was 7.6 mg/kg/min in both groups. In both groups, a small but significant amount of glucose was being stored, but this was not different between the two feeding groups. There were also no differences in fat utilization or metabolic rate as measured with indirect calorimetry. The Ra of glucose calculated at the two different ways used here yielded very similar results, and hence the difference between the two tracer methods -which is expected to represent recycling through three carbon fragments- was not different from zero. The glucose production was not significantly different between groups and not significantly different from the galactose intake, which was 2.1 mg/kg/min in both groups.

A statistically significant difference between the two groups in glucose oxidation was observed: 4.5 mg/kg/min in the MCT group *versus* 5.7 in the LCT group ($p < 0.01$). The difference between glucose oxidation measured with stable isotopes and total glucose utilization from calorimetry, as a measure of lipogenesis, was significantly higher in the MCT-fed group, 3.0 *versus* 1.9 mg/kg/min in the LCT group ($p < 0.05$).

Table 2
Glucose metabolism.

		MCT	LCT	p
Intake				
Carbohydrate intake	[1]	8.4 ± 0.21	8.4 ± 0.10	NS
Glucose intake	[2]	6.3 ± 0.16	6.3 ± 0.08	NS
Calorimetry				
Glucose utilization	[3]	7.5 ± 0.96	7.6 ± 0.87	NS
Fat utilization		0.97 ± 0.59	1.24 ± 0.53	NS
Metabolic rate (kcal/kg/d)		59 ± 5	62 ± 4	NS
^{13}C-glucose				
Ra "true"	[4]	9.0 ± 1.0	9.0 ± 0.83	NS
Ra "apparent"	[5]	9.1 ± 1.0	8.7 ± 0.90	NS
Glucose oxidation	[6]	4.5 ± 0.83	5.7 ± 0.67	<0.01
Calculated variables				
Glucose production	[4]-[2]	2.79 ± 0.90	2.38 ± 0.97	NS
Recycling	[4]-[5]	-0.06 ± 0.69^{ns}	0.25 ± 0.83^{ns}	NS
"Lipogenesis"	[3]-[6]	3.0 ± 1.3	1.9 ± 1.1	<0.05
Net storage	[1]-[3]	0.85 ± 1.0$^{<0.05}$	0.78 ± 0.78$^{<0.05}$	NS

All values in mg/kg/min, except metabolic rate in kcal/kg/d. Values between brackets are arbitrarily designated numbers, for use in the calculated variables.
NS: comparison between groups not significantly different.
<0.01 or <0.05, comparison between groups significantly different at $p < 0.01$ or 0.05
^{ns}: value in itself not significantly different from zero.
$^{<0.05}$: value in itself significantly different at $p < 0.05$.

DISCUSSION

Our initial hypothesis that MCT-fed infants would have a higher metabolic rate, to explain that their growth is often not higher even though their metabolizable energy intake is higher, could not be substantiated in this study.

In an earlier study, we showed that infants fed a MCT-containing formula

absorbed an extra amount of approximately 5-6 kcal/kg/d of fat [19]. A remarkable result in the present study was the decrease of 1.1 mg/kg/min of glucose oxidation in the MCT group, which means a decrease of over 6 kcal/kg/d in the energy derived from direct glucose oxidation. This strongly suggests that MCTs very specifically decrease glucose oxidation in the well-fed preterm infant. In contrast with this we found carbohydrate utilization from calorimetric data to be virtually identical.

Body stores of glycogen in adults can probably rise to a maximum of about 100 g [20], equivalent to an estimated maximum in the neonate of around 2 g/kg total body stores. As the infants have been and are continuously fed high caloric feedings that provide 12 g/kg/d of carbohydrates, it can be expected that glycogen stores are filled. Galactose presented to the liver in that case can not lead to net glycogen accretion. This is consistent with our finding that glucose production rate was not significantly different from the galactose intake. Also, in a recent publication [21], Spedale et al. have measured *in vivo* a preferential first-pass hepatic uptake for galactose and the absence of a net hepatic uptake of glucose in the postabsorptive newborn lamb, confirming the idea that galactose is taken up preferentially by the newborn lamb, with a net hepatic glucose output approximately equal to the galactose uptake in the postabsorptive state.

Glucose probably cannot be stored as glycogen to a significant extent after a period of continuous delivery of 120 kcal per kg/day as argued before, and it seems likely that the nonoxidized glucose will be shuttled into lipogenesis. The increased difference between stable isotope and calorimetric measurement of glucose metabolism in the MCT group also points at an increased rate of lipogenesis from glucose, as we have argued before [8,22]. Indirect calorimetry measures utilization of fat and carbohydrates where the amount converted ultimately into CO_2 is the entity measured and the pathway taken does not influence the total stoichiometry. For instance, ketones formed from fat and consequently oxidized have the same effect on stoichiometry as direct fat oxidation, and carbohydrate conversion into fat with subsequent fat oxidation results in "measurement" as carbohydrate oxidation. Thus, in some states of excess energy supply where lipogenesis is occurring with ongoing fat oxidation, RQ might not rise above 1 (no net lipogenesis), but still, the carbohydrate utilization obtained by the calorimetric measurement overestimates the real glucose oxidation. As Frayn [14] showed that (in the absence of some other processes like ketogenesis with urinary loss of ketone bodies) calorimetry quantitatively overestimates glucose oxidation by the amount of glucose converted

into fat, we can estimate from the significantly increased difference between the two techniques that an extra 1.1 mg/kg/min of glucose was converted into fat in the MCT group (table 2). For the absolute amount of fat oxidized to be determined, an estimate should be made of what part of the difference between glucose oxidation and glucose utilization already existing in the LCT group was due to lipogenesis and what amount could be ascribed to other causes. One such cause would be the underestimation of glucose oxidation by the tracer technique due to intracellular oxidation of glycogen that did not enter the plasma pool and hence made no contribution to the measured glucose production [13]. Although fairly confident about increased lipogenesis as the cause of the increased discrepancy between the two measurements of glucose metabolism in the comparison MCT *vs.* LCT, we feel that it would be too speculative to suppose that the difference between stable isotopes and calorimetry can always be quantitatively converted into values for lipogenesis.

The finding in a recent study [23] of a significant difference between isotopically determined glucose oxidation and indirect calorimetry values during hyperinsulinemic euglycemia, but not during saline infusion, confirms the idea that both methods can yield identical results in the fasting state, whereas a difference attributable to lipogenesis is present during hyperinsulinemia.

In one study, most relevant to ours [13], the effect of elevated Free Fatty Acid (FFA) levels on glucose oxidation was studied using the same two techniques as ours. It was concluded that FFA lowers carbohydrate utilization as measured with calorimetry, whereas plasma [U-^{13}C]-glucose oxidation was not influenced. It was concluded that FFA probably lowered intracellular glycogen oxidation under those particular circumstances. Although there was no effect of FFA on plasma glucose oxidation in the latter study even though there was an additional caloric intake due to the infusion of an intravenous fat emulsion, in the present study [U-^{13}C]-glucose oxidation was significantly decreased with isocaloric replacement of part of the LCTs in the fat blend by MCTs, again pointing at a very specific effect of MCTs. The presence of carbons with chain lengths < 16 is negligible in the fat emulsion used, and, to our knowledge no reports have been published on the effect of MCTs on isotopically measured glucose oxidation *in vivo*.

Apart from the original indication of fat malabsorption, MCT supplementation is currently being recommended for a variety of purposes [24], including seemingly contradictory objectives like (parenteral) hyperalimentation and obesity control. The literature does not definitively analyze the benefits of MCT administration.

Moreover, all trials have been performed comparing MCTs with LCTs. In light of our study showing a more pronounced effect of MCTs than LCTs on plasma glucose oxidation, reconfirming the conclusion of the carbohydrate-like behavior of MCTs as suggested 25 years ago [25], it might be more realistic to also compare MCT administration to simply additional carbohydrates administration. Obviously, these considerations do not apply to specific indications like general malabsorption.

The overestimation of glucose oxidation obtained with calorimetry in our study shows that at least part of the lipogenesis with MCT feeding occurs from glucose in preterm infants on regular preterm MCT formula. In an earlier study, similar indirect evidence of increased lipogenesis was found to be associated with a decreased food efficiency (higher metabolic rate at equal energy intake) in the glucose-only parenteral feeding regimen [8]. Although not confirmed in the present study, an increased metabolic rate during overfeeding with MCTs has been described in animals and adult human volunteers [4-7]. In these cases, the observed increase in metabolic rate has been ascribed either to ketogenesis [4], obligatory oxidation [5,6], or lipogenesis from MCTs [7].

In summary, we propose that the following metabolic picture can be derived from our results: In preterm infants receiving an energy intake of 120 kcal/kg/d providing 8.4 mg/kg/min carbohydrates, approximately 10% of the carbohydrate intake is stored or converted nonoxidatively in this period of rapid growth ($\approx$1.6%/d). Another two thirds of the glucose in the LCT group is directly oxidized, whereas the remaining glucose is either oxidized intracellularly coming directly from glycogen or converted to fat. When MCTs are replacing part of the LCT fat in the diet, a significant decrease in the glucose oxidation is observed, but as carbohydrate balance is strictly regulated this does not show in the carbohydrate utilization measured with calorimetry. This observation indicates that the extra glucose not oxidized in the MCT group is probably converted into fat, which influences the calorimetric measurement and not the direct glucose oxidation measurement. In this way, an inefficient consequence of the supposed "obligatory oxidation" of MCTs could exist, as lipogenesis occurs together with ongoing fat oxidation. However, in our study design, this did not lead to a higher total metabolic rate.

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CHAPTER VI

CONVERSION OF OCTANOIC ACID INTO LONG-CHAIN SATURATED FATTY ACIDS IN PREMATURE INFANTS FED A FORMULA CONTAINING MEDIUM-CHAIN TRIGLYCERIDES.

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SUMMARY

A large number of very-low-birth weight (VLBW) infants are fed formulas containing medium-chain triglycerides (MCTs) to enhance fat and calcium absorption. Studies are available on the intestinal absorption of MCTs, which is nearly complete, but uncertainties exist on the metabolic fate of octanoic acid, the major component of MCTs. Oxidation accounts for approximately 50% of the dietary intake and losses as dicarboxylic acids in the urine are negligible. As direct storage in adipose tissue is limited, conversion to long chain fatty acids (LCFAs) is likely to be an important route. To study the nonoxidative metabolism of MCTs five preterm infants fed a standard premature formula containing 35 wt% MCTs (54 mol% medium-chain fatty acids (MCFAs) of which 35 mol% octanoic acid) were studied at 4 weeks of age when on full oral intake and receiving on average 130 kcal/kg. The study consisted of an oral primed constant rate infusion of [^{13}C]-octanoate and the measurement of the ^{13}C enrichment of individual fatty acids in plasma triglycerides by a highly sensitive on-line combustion method using a gas chromatograph-isotope ratio mass spectrometer (GC-IRMS). We observed a significant incorporation of the dietary [^{13}C]-octanoic acid in plasma triglycerides (10.0 $\pm$ 4.5% of the enrichment of the diet). A noticeable incorporation of the label

was detected in myristic and palmitic acids (4.6 ± 2.5 and 7.8 ± 4.1% of the octanoic enrichment of the diet). When the absolute amount of the fatty acids was studied with conventional gas-chromatography, the plasma triglyceride fatty acid profile differed markedly from the diet. When expressed as mol percent of the total amount of fatty acids, octanoic and decanoic acids were only 7.3% and 32% respectively in plasma compared to the mol percent of total fatty acids in the diet and myristic and palmitic acids were higher by 225% and 343% compared with the mol percent composition of the diet. Our findings demonstrate for the first time *in vivo*, the conversion of octanoic acid into long-chain saturated fatty acids. The latter, apart from being metabolically inefficient could be responsible for the high levels of long-chain saturated and mono-unsaturated fatty acids found in subjects fed MCT-containing diets and may interfere with the metabolism of other fatty acids.

INTRODUCTION

Medium-chain triglycerides (MCTs) are added to the fat blend of several preterm formulas to promote intestinal absorption of fat and calcium [1,2]. However, scanty data are available on the metabolic fate of the medium-chain fatty acids (MCFAs) in the small preterm human neonate. After intestinal absorption, MCFAs could be oxidized, deposited as such, or possibly converted into other fatty acids. Studies in animals and human adults show evidence of almost complete oxidation and minor tissue deposition of dietary MCFAs [3]. Nevertheless the oxidation of octanoic acid in premature infants was found to be only 50% of the dietary intake [4]. Thus, as oxidation in preterm infants apparently is not complete, tissue deposition or metabolic interconversion should represent important pathways. Octanoic and decanoic acids (the major fatty acids of MCTs) were found to be about 5% in weight of the fatty acids in adipose tissue of newborn infants fed MCTs [5]. This deposition does not yet explain the incomplete oxidation. Elimination of MCFAs as hydroxy fatty- and dicarboxylic acids is a well described pathway [6] but it is negligible from the point of view of energy and carbon losses. Conversion of MCFAs into long-chain fatty acids could be a significant nonoxidative route. This process was described in animal studies [7,8]. It has been hypothesized to occur in human adults fed MCTs [9] because of the observation of unexpectedly high levels of palmitic acid in plasma triglycerides (TG). This latter finding point towards an

increased palmitate synthesis during MCT feeding. No information is available to date on the nonoxidative fate of MCFAs in the small premature infants.
The objective of the present study was to investigate whether the conversion of MCFAs into LCFAs does occur in the human newborn infant fed MCTs. This was achieved with stable isotope technology and high sensitivity tracer detection, using gas chromatography-isotope ratio mass spectrometry (GC-IRMS).

PATIENTS AND METHODS

Subjects

Five premature infants (birth weight 1.5 ± 0.12 kg and gestational age 31.8 ± 1.7 weeks), free of major congenital or acquired diseases were studied at 26 ± 6 days postpartum when on full oral feeds for at least 10 days. All infants were fed a MCT formula. Body weight at the time of the study was 1.82 ± 0.12 kg. Individual data are reported in table 1.

Table 1.
Patients Characteristics

Patient no.	Birth weight (kg)	Gestational age (wk)	Age at study (days)	Weight at Study (kg)	Energy Intake (Kcal/kg/day)	Fat Intake (g/kg/d)	Octanoic acid intake (g/kg/d)
1	1.33	33	28	1.82	122	6.3	1.3
2	1.37	34	20	1.77	132	6.6	1.5
3	1.63	32	32	1.95	149	7.7	1.6
4	1.57	31	33	1.92	124	6.0	1.7
5	1.59	29	19	1.63	132	6.8	1.4
Mean	**1.50**	**31.8**	**26.4**	**1.82**	**132**	**6.7**	**1.5**
SD	0.14	1.9	6.6	0.13	11	0.6	0.2

Feeding regimen

All infants were fed a formula (Mellin O, Star™ Italia) in which 35 % of the fat (wt/wt) is provided as MCTs. This amount corresponds to 54% mol percent (mol/mol) of the total fat content (octanoic acid is 35 mol%, decanoic acid 19 mol%). All infants were on full oral feeding and they were all gaining weight at the time of the study. The composition of the preterm formula is given in table 2. All infants were fed during the studies *via* an oro-gastric tube and gavage feeding was administered using a syringe pump.

Table 2.
Composition of Mellin 0 (Star™, Italy) per 100 ml formula

Energy		76 kcal
Protein		2.02 g
Carbohydrates		8.32 g
Lactose		5.43 g
Dextrine maltose		2.89 g
Fat		3.75 g
MCT	C8	22.7 wt% (34.9 mol%)
	C10	15.0 wt% (19.3 mol%)
LCT	C16	10.7 wt% (9.7 mol%)
	C18	4.4 wt% (3.4 mol%)
	C18:1	18.0 wt% (14.1 mol%)
	C18:2	17.0 wt% (13.5 mol%)
	C18:3	1.7 wt% (1.4 mol%)
Sodium		25 mg
Potassium		75 mg
Calcium		72 mg
Phosphorus		40 mg

Study procedure

Informed consent was obtained from the parents of the infants studied. The study consisted of a primed constant oral infusion of either 1-$^{13}C_1$ or 1,2,3,4-$^{13}C_4$ octanoate. The isotope infusion was administered *via* a second small diameter oro-gastric tube and infused by a syringe pump. The exact amount of the isotope solution administered was verified by weighing the syringe before and after the 6h constant infusion. The amount of tracer infused, when converted into labeled ^{13}C carbons contained in the tracer, varied from 0.2 to 0.5 μmol/kg/min ^{13}C atoms. Venous blood samples for the determination of the fatty acid composition of plasma TG and their isotopic enrichment were collected by venipuncture before the infusion and again at 6 hours of isotope administration. The concentration and enrichment of the isotope tracer solution was checked by gas chromatography (GC) and by gas chromatography mass spectrometry (GC-MS).

Methods

Sample preparation

Blood was collected in EDTA-containing Vacutainers and centrifuged at 4°C for 10 minutes at 1400 g. The plasma was collected and stored at -70°C. Plasma lipids were extracted according to Folch [10]. Trinonanoin and tripentadecanoin were added as internal standard to 100 μl of plasma. Then 1.9 ml chloroform/methanol (2:1, v/v) was added and the tube was vigorously shaken. After addition of 0.4 ml 0.9% NaCl solution the mixture was again shaken and centrifuged during 10 minutes at 2500 g and the lower phase was collected. An additional extraction was performed with 2 ml chloroform. The extracts were pooled and dried under nitrogen at 45°C. The lipids were dissolved in 0.10 ml chloroform/methanol (2:1, v/v) and applied on a Kieselgel 60 thin layer plate using a Camag Linomat IV (Merck, Darmstadt, Germany). The TLC-plate was developed with hexane/diethylether/acetic acid (80/20/1, v/v/v). After drying the plate the lipids were visualized by spraying with Rhodamine-G and detection under UV-light. The lipid fractions were scraped off with a scalpel and collected in glass tubes. Methylation was performed as follows: 1 ml 3 M dry HCl in methanol was added to the tubes and after flushing with nitrogen the tubes were capped and incubated during 60 minutes at 95°C. After cooling, HCl was neutralized by adding 0.8 ml 1.5 M K_2CO_3. The methylesters were extracted with 2 ml pentane and the pentane phase was transferred into an injection vial and

stored at -20°C. Before analysis the vial was placed on ice and the pentane phase was concentrated under nitrogen until a volume of about 0.1 ml.

Quantitation of fatty acids in plasma triglycerides

The separation and identification of fatty acid methyl esters from plasma triglycerides was performed in a gas chromatograph (GC) (Hewlett Packard 5890 II) equipped with a fused silica column (Supelcowax 10, 60m x 0.25 mm ID, 0.25 μm film thickness), a flame ionization detector (260°C) and a split-splitless injector used in splitless mode. The GC was operated with the following temperature program: 80°C initially for 4 min, thereafter 25°C per minute until 205°C and held at this temperature for 13 min. The temperature was increased again at 0.3°C per minute until 225°C. Helium was used as a carrier gas (2 ml/min) and peak areas were calculated by HP-Chem station software using nonanoic acid and pentadecanoic acid as internal standards. Fatty acids were identified by comparing retention times with known standards (Nu Chek Prep, Elysian, Minnesota, USA). All reagents were analytical grade.

Isotopic enrichment of plasma fatty acids

Methods and instrumentation

The GC-IRMS system used was the newly developed Isochrom III (VG Isotech, Middlewich, Cheshire, UK). Briefly, it consists of a Hewlett-Packard 5890 Series II gas chromatograph, connected with a combustion interface to a VG-Optima isotope ratio mass spectrometer IRMS. The GC-column was a low bleeding 25 meter SGE fused capillary column with an internal diameter of 0.25 mm and 0.11 μm HT-5 liquid phase. The injector temperature was 260°C. The oven temperature was held during 3 minutes at 45°C and ramped with 50°C/min to 120°C for 2 minutes, thereafter the oven temperature was programmed with 3°C/min to 270°C. During solvent elution the effluent of the column was directed towards the flame ionization detector and after a timed interval the column effluent was directed towards the combustion interface where the compounds are combusted to CO_2 and H_2O. The water was trapped in a liquid nitrogen cooled metal coil held at -100°C, while the CO_2 was introduced into the IRMS. A representative example of the tracing obtained is shown in **figure 1**.

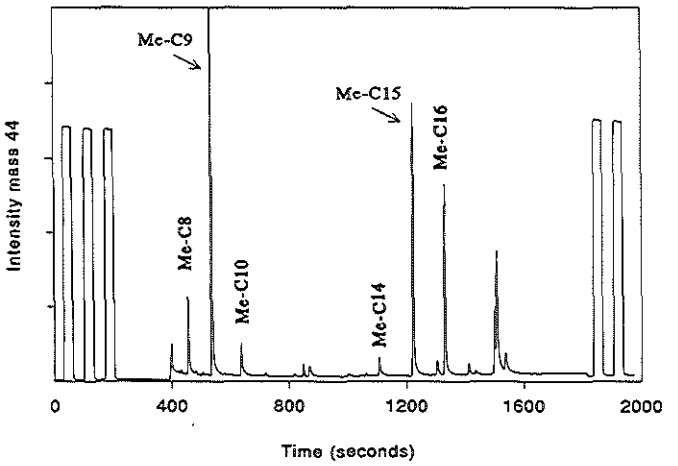

Fig. 1

GC-IRMS tracing of a free fatty acid spectrum (mass 44 signal only), including octanoate itself and several of the measured longer-chain saturated fatty acids.

Linearity of the system.

To test if the measured ratio was independent of the pressure in the ion source we introduced different amounts of CO_2 gas into the mass spectrometer using reference gas pulses both introduced directly or *via* the GC column. While the peak heights for mass 44 ranged from 1E-10 to 5E-9 Ampere, indicating a 50-fold difference in in the amount of CO_2 reaching the ion source, the measured ratio showed no tendency to increase or decrease and was very constant, the mean atom % being 1.1167 with a standard deviation of only 0.0002 atom % for 10 analyses.

Calibration curve.

A calibration curve was constructed by adding different amounts of enriched [1-^{13}C]-octanoate to naturally enriched octanoate. After methylation with 3 M dry HCl in ethanol the formed methylesters were analyzed. The linear regression line ($Y = Ax + b$) was calculated with the δ_{PDB} on the Y-axis and the mol % excess (MPE) on the X-axis (**figure 2**). The MPE was calculated from the weighed amounts of [1-^{13}C]-octanoate added to weighed amounts of unlabeled octanoate. As 1 molecule of methyloctanate consists of 9 atoms, the atom % excess will be one ninth of the mol % excess.

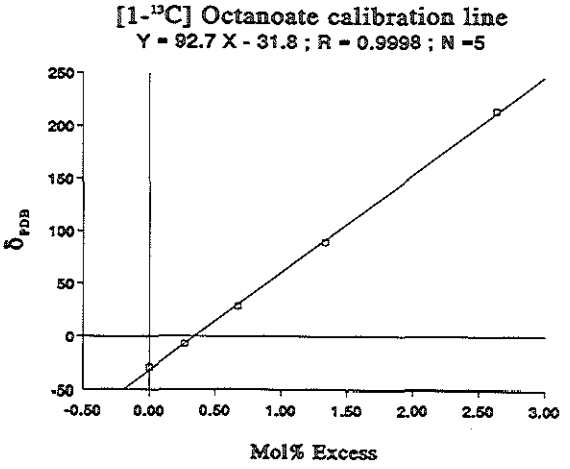

Fig. 2
Calibration curve obtained by spiking naturally enriched octanoic acid with different amounts of [1-^{13}C]-octanoic acid.

Practical detection limit and standard deviation.

According to the manufacturer the system could analyze a minimum of 0.5 nmol of a C10 compound (220 ng carbondioxide) with a standard deviation of 0.0003 atom %. We modified the open split interface, by placing a small length of fused silica capillary (length 30 cm x 0.32 mm internal diameter), so preserving the same pressure in the combustion tube, while reducing the helium outflow and hence lowering the GC-effluent dilution. Also the capillary between the open split interface and the watertrap was replaced with a shorter capillary, with a smaller diameter. In this way the residence time of the compound and peakwidth broadening were reduced. After this modification we were able to analyze 0.09 nmol methyloctanoate (38 ng CO_2) with a standard deviation of 0.0002 atom % (n = 5) or 32 pmol methyloctanoate with a standard deviation of 0.0008 atom % (n = 3).
Over 4 months we prepared plasma samples for lipid analysis with tripentadecanoylglyceride as internal standard. By analyzing the internal standard as methylpentadecanoate we found for 28 analysis a mean δ PDB value of -27.0 $\pm$ 0.4. This corresponds with a mean of 1.0940 $\pm$ 0.0004 atom %. Because in biomedical stable isotope work we are dealing with delta values (basal value subtracted from measured value) the standard deviation becomes $\sqrt{2}sd^2$ or 0.0006 atom %. Thus, the minimum

detection limit of our system will be 0.0017 atom % excess, assuming that a signal should be at least 3 times as high as the standard deviation. For quantitative work a safe margin would be 20 x sd = 0.011 atom percent excess.

Calculations

Results obtained from the instrumentation are based on the ^{13}C enrichment of the CO_2 of the combusted sample and are expressed in atom percent excess (APE) compared to a reference CO_2 gas of known mass 45/44 ratio. The enrichments of the CO_2 deriving from the combustion of the fatty acids methyl esters (FAMEs) at baseline and at 6 hour infusion are used to calculate the increase in enrichment (in APE) in individual fatty acids. No attempt was made to correct the enrichments of the FAMEs for the relatively minor contribution of the methyl group added during the transesterification of the fatty acids.

RESULTS

Individual data for energy, fat and octanoic acid intakes are reported in **table 1**. Total fat intake and the octanoic acid intake averaged 6.6 ± 0.6 and 1.5 ± 0.2 g/kg/day respectively. In **figure 3** a comparison is made between the fatty acid profiles of the formula and that of the plasma TG of the patients studied. Medium chain fatty acids (C8:0 + C10:0) represent almost 55 mole % of dietary fat but are much lower in plasma TG. Conversely, palmitic and stearic acid, the most abundant saturated fatty acids are higher by a factor of 3 and 1.5 respectively, *e.g.*, palmitic acid was 9.7 mol% (or 10.7 wt%) in the fat of the formula and 32 mol% in plasma TG. The ^{13}C enrichment of saturated fatty acids in plasma TG is reported in **table 3**. Patients 1 through 3 received 1-$^{13}C_1$ octanoate and patients 4 and 5 received 1,2,3,4-$^{13}C_4$ octanoate. The calculated C8:0 enrichment of the diet ranged from 0.4 to 1.4 APE, depending on the relative infusion rates of tracer and feeding, averaging 0.8 APE. Average C8:0 enrichment in plasma was only 0.08 APE, being 10.0 ± 4.5 % of the enrichment of the diet octanoate. Of the longer-chain saturated fatty acids measured, decanoic and dodecanoic acids were not significantly enriched while myristic and palmitic acids show average enrichments of 0.04 and 0.07 APE, respectively 4.6 ± 2.5 and 7.8 ± 4.1% of the octanoic enrichment of the diet. The latter two values were on average 56 and 96% of the enrichment of octanoic acid.

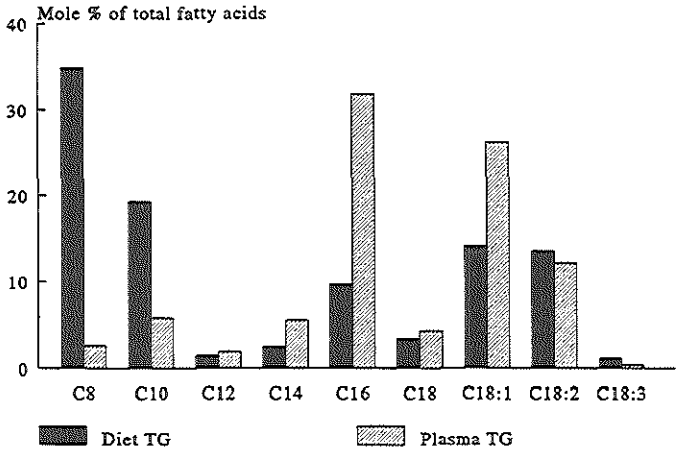

Fig. 3
Comparison between the fatty acid composition of the premature formula (mean values of 3 determination) and the plasma triglycerides (mean values of the 5 patients).

Table 3. Isotopic enrichment of plasma triglyceride saturated fatty acids (APE). Diet enrichment was calculated from the known intake of tracer and diet.

	diet	Triglycerides				
Patient no.	Octanoic C8:0	Octanoic (Caprylic) C8:0	Decanoic (Capric) C10:0	Dodecanoic (Lauric) C12:0	Tetradecanoic (Myristic) C14:0	Hexadecanoic (Palmitic) C16:0
1	0.398	0.041	0.001	n.d.	0.010	0.018
2	0.682	0.087	0.001	0.005	0.059	0.098
3	0.673	0.108	-0.002	0.002	0.017	0.028
4	1.006	0.057	0.000	0.004	0.059	0.101
5	1.421	0.093	-0.002	n.d.	0.068	0.115
Mean	**0.836**	**0.077**	**0.000**	**0.004**	**0.043**	**0.072**
SD	0.392	0.027	0.002	0.002	0.027	0.045

DISCUSSION

With the use of stable isotope technology we present in this paper direct evidence of incorporation of dietary octanoic acid into longer-chain fatty acids in the plasma TG fraction, as we could demonstrate a significant ^{13}C carbon atom enrichment in the longer-chain saturated fatty acids myristic and palmitic acid after administration of [^{13}C]-labeled octanoate. The latter finding indicates that synthesis of saturated long chain fatty acids from MCFAs does occur in premature infants fed MCT-containing formulas.

The incorporation of labeled octanoic acid into plasma TG could have occurred both in chylomicron TG and in very-low-density lipoprotein (VLDL) TG. Unfortunately, we were not able to perform lipoprotein analysis in this study.

Although the majority of MCFAs is absorbed and transported to the liver *via* the portal system [11,12] it has been described that a minor portion is incorporated into triglycerides synthesized in enterocytes and into chylomicrons [7,13]. If triglycerides from intestinal origin were the only circulating TG we would expect octanoic acid to have the same ^{13}C enrichment as the diet. The enrichment of octanoic acid in plasma triglycerides was on average 10% of the enrichment of the diet thus indicating that a dilution of the tracer must have occurred. Since the major site for triglyceride synthesis is the liver, the majority of the circulating TG octanoate was probably incorporated in very low density lipoproteins in the liver. The question of the origin of the unlabeled octanoate, is difficult to answer. Incomplete β-oxidation of long chain fatty acids (chain shortening) in the liver or in the gut, MCFA production from intestinal bacteria, liver/adipose tissue cycling of octanoic acid and incomplete lipogenesis from glucose could be potential sources of unlabeled octanoic acid.

Data obtained from adult patients with liver cirrhosis given radio labeled long-chain fatty acids suggest that under fasting conditions 60% to 80% of the serum octanoate was obtained from incomplete β-oxidation of long-chain fatty acids and that 20% to 40% may be formed de novo [14]. The concentration of octanoic acid in adipose tissue of infants fed formulas containing MCTs when measured with new techniques is not negligible [5] as previously thought, even though the storage is still much lower with C8:0 than with LCFAs or even with the "intermediate-chain fatty acids" C12:0 and C14:0.

Studies indicate that octanoic and decanoic acid -unlike fatty acids with chain length of 12 and more- are not a good substrate for phospholipid synthesis [15,16],

and are only incorporated very slowly into hepatic TG *via* 1,2-diacyl-*sn*-glycerol acyltransferases [16]. Even so, at the moment we cannot conclude whether the octanoic acid incorporation into liver triglycerides could be the result of an overload of the liver capacity for oxidation or chain elongation of octanoate.

Palmitic acid in plasma TG of our patients was on average more then 3 times as high as in the formula fat blend. Since plasma TG fatty acids are generally known to reflect the dietary fatty acid pattern, this difference depicted in figure 3 is remarkable. From this fatty acid pattern it can be argued that de novo fatty acid synthesis may have occurred. This finding parallels the data from Hill et al., during overfeeding studies in adults fed MCTs [9]. In the present study we give direct evidence that saturated fatty acids in plasma TG can be derived from octanoic acid. This finding may (partially) explain the high levels of saturated fatty acids found in our five patients. No significant enrichment was detected in decanoic and dodecanoic fatty acids while the average enrichments of C14:0 and C16:0 were 57% and 96.7% of octanoic acid enrichment.

In the present work we cannot provide information on the possible biochemical mechanism involved in the transfer of carbon atoms from octanoic acid into myristic and palmitic acids. Especially since the enrichment in palmitate is just as high as the enrichment in octanoate in TG (and even higher in one patient), we can only conclude that TG octanoate is probably not a good representative of the precursor pool for palmitate synthesis, *i.e.* octanoate in TG probably underwent significant dilution at some stage in its metabolism after its conversion into long-chain saturates. Thus, we don't want to speculate on the fractional synthetic rate of palmitate synthesis from octanoate and data in table 3 should only be considered qualitatively. The site of palmitic acid synthesis from octanoic could be the intestinal mucosa or the liver. Incorporation of octanoic acid derived radioactivity has been demonstrated in fatty acids with chain length greater than 12 carbon atoms in intestinal slices [7]. Chain elongation of long chain fatty acids is also described in the small intestine [17]. Furthermore, octanoic chain elongation occurs efficiently in liver mitochondria [18]. Synthesis of LCFAs from octanoic acid should be considered an energetically costly activity and it has been speculated that it could account for a substantial portion of the increased energy expenditure reported in adults with excess MCT feeding [19,20].

There are similarities between the changes in blood lipids produced by MCT feeding and those reported with feeding high carbohydrate diets. Although we have demonstrated that lipogenesis from octanoate does occur in our patients we did not

measure lipogenesis from glucose. A glucose sparing effect of MCTs was described in a similar group of preterm infants fed MCT formula [21]. It is conceivable that a decrease in carbohydrate oxidation in nutritionally replenished patients as is the case in the growing premature infant fed 120-130 kcal/kg/d could be associated with enhanced lipogenesis from glucose, also leading to increased saturated fatty acid levels.

We did not measure the ^{13}C enrichment in oleic acid, however, the absolute levels of the mono-unsaturated oleic acid were also almost two-fold higher in the plasma TG fatty acid spectrum compared to the diet. It is tempting to hypothesize that this fatty acid could also be endogenously produced *via* lipogenesis and desaturation by the Δ9-desaturase [22]. Leveille et al. [8], in their rat studies and Hill et al. [9] in studies in human adults have similarly noticed an increase in mono-unsaturated fatty acids. This may indicate that the fatty acid desaturases may also be involved in processing of the metabolic derivatives of MCFAs. In a recent study [23] it was shown in newborn piglets that feeding MCTs *vs.* coconut oil resulted in higher C16:0 and C18:1 levels in all lipids, concomitantly with a decrease in tissue phospholipid arachidonic acid (C20:4n-6), an essential longer-chain poly-unsaturated fatty acid thought to be important for growth and development [24].

Our data suggest that the elevated saturated fatty acids found in preterm infants fed MCTs were derived, at least partially, from octanoic acid. Further studies using lipoprotein analysis and appropriate isotopic modelling could give insight into the mechanisms involved and the possible influence of MCT diets on (poly-unsaturated) fatty acid metabolism.

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CHAPTER VII

ESTIMATION OF SPLANCHNIC (HEPATIC) AND PERIPHERAL OXIDATION OF OCTANOIC ACID IN PRETERM INFANTS USING DUAL ISOTOPE ADMINISTRATION.

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SUMMARY

Several formulas intended for premature infant feeding contain medium-chain triglyceride (MCT) oil, supplying medium-chain fatty acids (MCFAs), to enhance fat absorption. These fatty acids of eight and ten carbon atoms were assumed to be almost completely oxidized as capacity for storage as such is limited. Doubt has been expressed on the ability of the premature infant for β-oxidation of these MCFAs, as the excretion of dicarboxylic acids is known to increase during MCT feeding. The oxidation of octanoate as a tracer of MCFA metabolism in three premature infants was studied using a dual isotope approach with determination of plasma [1-^{13}C]-octanoate enrichment by the novel technique of GC-IRMS. The recovery of label after both intravenous and intragastric administration was 34 $\pm$ 3% of the intake. The fractional extraction of the liver during the initial splanchnic passage was high, 79 $\pm$ 8%. The part of the infused octanoate passing through the splanchnic bed, underwent a very significant systemic dilution, the "production" of octanoate being 1.4 $\pm$ 0.5 gram per kg per day, compared to an intake of 1.5 $\pm$ 0.2 g/kg/d. Calculated oxidation percentages in the liver (or splanchnic tissue) and the peripheral tissues were quite similar, 34 $\pm$ 7% vs. 34 $\pm$ 2%. The "total" octanoate oxidation that could be quantified using this new model was 66 $\pm$ 6% of the

octanoate intake. We conclude that the oxidation of MCFAs, although higher than reported earlier was not complete in preterm infants.

INTRODUCTION

Special formulas intended for the needs of prematurely born infants contain up to 80% by weight of the fatty acids as medium-chain fatty acids (MCFAs), with a chain length of eight and ten carbon atoms. This socalled "MCT oil", which is reesterified from hydrolyzed and fractionated coconut oil, is added to preterm formulas to enhance fat absorption and to provide a "readily available source of energy". Storage of octanoate or decanoate does not seem to be a major route in the disposal of these MCFAs [1-3], and it is generally assumed that oxidation of MCFAs is nearly complete [4-6]. In the premature infant, the excretion of dicarboxylic acids is enhanced when fed a 40% MCT formula [7], leading to questions on the maximum percentage of MCT that can be β-oxidized by the preterm infant. However, no methods were available to quantitate *in vivo* the whole-body oxidation of MCFAs. In an earlier study we measured the oxidation of orally administered [1-^{13}C]-potassium-octanoate, and found that an average of 47% of the administered tracer was recovered in expiratory air [8]. We argued that there was not much dilution in the precursor compartment, due to the fact that very low stores of C8:0 had been described, and thus estimated the oxidation of C8:0 to be approximately 50 %. This number, although higher than the oxidation rate for normal fat blends in preterms as calculated from indirect calorimetry, is still much lower than the supposedly complete oxidation of MCTs. From these results we concluded that a very significant part of the MCFAs administered is not oxidized by the preterm infant. Furthermore, because storage as part of triacylglycerols in adipose tissue is minimal, almost half of the administered MCT has to undergo chain elongation, probably *de novo* lipogenesis, before the body can store this excess of MCFAs.

However these conclusions were made under the assumption of a negligible production -and hence isotope dilution- of octanoate. To validate the conclusions we studied C8:0 oxidation in three infants administering the tracer on two separate occasions either intravenously (iv) or intragastrically (ig).

PATIENTS AND METHODS

Subjects

All children were admitted to the neonatal intensive care unit (NICU) of the Padua University Hospital, Padua, Italy. Infants with a birth weight below 1600 gram were included in the study if they had been on full oral feedings for at least ten days and still had a patent i.v. catheter. This relatively rare occurrence on a NICU was present when the infant was doing clinically well, but was receiving intravenous antibiotics because of suspected or proven infection. In all cases the clinical suspicion of invasive infection was not present any more and cultures were negative, but the "minimal course" of the antibiotic treatment was still taking place. The amount of formula feeding was decided upon by the responsible clinician. After obtaining permission by at least one of the parents, the first study was undertaken with intravenous administration of the tracer and oral administration of the formula feeding. Two days later, a similar study was undertaken but now with oral administration of both tracer and formula feeding. The sequence of tracer administration mode was not randomized, because in virtually all cases the infants were on the last day(s) of their antibiotic course, once they had been selected and permission was obtained from the parents. As it was regarded unethical to leave the infusion catheter in for study purposes only, we decided to study first the i.v. administration.

Mean birth weight was 1.4 $\pm$ 0.16 kg, mean gestational age was 33 $\pm$ 1.5 weeks. The weight of the infants during the study is described in **table 1.**

Feeding regimen

Infants received a formula (Mellin O, Star^{TM}, Italy) containing 3.75% fat, of which 38 % consisted of MCFA, supplying 0.85 gram of C8:0 per 100 ml formula. Infants were studied at least 10 d after attaining a full oral intake. The decision if the "full" oral intake should provide the standard 150 ml/kg/day, or a higher amount in case of a relatively low growth rate, was made by the responsible clinician. However, care was taken that the amount of feeding did not change between measurements 1 and 2.

During the studies the oral feeding was administered continuously by a syringe pump, whereas normally the feeding was administered in a semi-continuous fashion by hand.

Methods

Indirect calorimetry

During the study period with infusion of the tracer, the infant was nursed in an incubator which contained a custom-made ventilated hood calorimeter. $\dot{V}CO_2$ was measured using an infrared diapherometer (Biomedin^{TM}, Padua, Italy). Calibration was checked by combustion of butane, the resulting respiratory quotient (RQ) being within 2% of the theoretical value.

Measurement of [1-^{13}C]-octanoate recovery

Measurement of octanoate oxidation was essentially the same as described before [8]. One minor difference was that in the present study, the octanoic acid tracer was administered as the sodium salt in stead of the potassium salt.

Labeled [1-^{13}C]- sodium octanoate (99% enriched, MSD isotopes, Montreal, Canada) was dissolved in sterile water at the hospital pharmacy and passed through a 0.22 μm filter into 10 ml vials, which were then sterilized by autoclavation at 120°C. In order to determine the natural background of ^{13}C, three baseline breath samples were collected during 15 minutes each before starting the isotope infusion. The bicarbonate pool was then primed with 6.9 μmol.kg^{-1} of a 6.9 mmol/l solution of $NaH^{13}CO_3$ (80% enriched, Merck Isotopes). At the same time, a [1-^{13}C]-octanoate priming dose of 2 mg/kg was given, followed by a constant infusion of 2 mg/kg/hr for 6-8 hrs. In two infants (B and C in **table 1**), double doses of labeled bicarbonate and octanoate were used. The delivery rate of the pump during the study was measured by dividing the difference in weight of the syringe before and after the experiment by the duration of the experiment in order to determine the actual quantity of [1-^{13}C]-octanoate administered. Once the isotope infusion was started, 15-min CO_2 breath collections were made every hour for 7 hours. Before and after the isotope infusion, 100 μl blood samples were obtained by capillary heelstick from a warm heel. Breath CO_2 was collected by passing a sample of the air leaving the incubator through an all glass spiral condenser, containing 10 ml of a fresh 1 molar NaOH solution. Samples were transferred to Vacutainers and stored at -20°C until analysis. 2 days after the i.v. administration of the label, the study was repeated using oral administration of label under exactly identical circumstances.

Isotopic Ratio Mass Spectrometric Analysis of CO_2

Respiratory carbon dioxide was liberated at $< 10^{-3}$ torr by adding phosphoric acid 85% to the $NaHCO_3$ solution. Pure CO_2 was collected in a small glass container in liquid nitrogen while the water was trapped in a methanol/dry ice bath. The glass container with pure CO_2 was connected to a VG Sira 10 isotope ratio mass spectrometer (Vacuum Generators, Middlewich, Cheshire, UK). Results of ^{13}C abundance of both baseline and plateau levels were calculated as APE/reference CO_2 tank standard sample.

Isotopic enrichment of plasma fatty acids

The analysis of the isotopic enrichment of the plasma fatty acids was performed as described in detail earlier [9], the only difference being that the free fatty acid fraction was analyzed in this study.

Calculations

The percentage $^{13}CO_2$ recovery of infused label was

$$recovery = \frac{\dot{V}CO_2 \; x \; \Delta E_{CO2}}{E_i \; x \; F \; x \; c} \; x \; 100\% \qquad (1)$$

where $\dot{V}CO_2$ is the amount of expired CO_2 in micromoles CO_2 per kilogram per minute corrected to standard conditions, E_{CO2} is the expired CO_2 atom percent enrichment, E_i is the enrichment (mole percent excess, MPE) of the tracer infusate and F is [1-^{13}C]-octanoate infusion rate in μmoles per kilogram per minute. c is the correction factor (as a fraction) for $^{13}CO_2$ retention in the bicarbonate pool, calculated individually from the energy intake of the infant [10], where the correction factor c (%) = 64.2 + 0.1667 x caloric intake (kcal/kg/d).

Because a significant retention in the splanchnic bed of the enterally administered tracer was expected, systemic octanoate fluxes were calculated using the enrichment of octanoate in plasma during i.v. tracer infusion only. The systemic octanoate flux (octanoate Ra_{sys}, μmol/kg/min) is

$$Octanoate \; Ra_{sys} = \left[\frac{E_i}{E_p} - 1 \right] \; x \; F \qquad (2)$$

where F is the [1-^{13}C]-octanoate infusion rate (μmol/kg/min) during the i.v. tracer study and E_i and E_p are the [1-^{13}C]-octanoate enrichment in the infusate and in plasma during the i.v. tracer administration (Mole Percent Enrichment), respectively.

The fraction of this systemic flux derived from the dietary octanoate was calculated by the standard precursor-product relationship:

$$\textit{fraction of plasma octanoate from diet} = \frac{\textit{plasma } [^{13}C]\textit{Octanoate(ig) MPE}}{\textit{diet } [^{13}C]\textit{Octanoate MPE}} \quad (3)$$

where [1-^{13}C]octanoate MPE is the ^{13}C enrichment of plasma (after intragastric tracer administration) and in the diet respectively. The enrichment of the diet was calculated from the known intakes of tracer and formula octanoate.

Therefore, the actual Ra of octanoate derived from the diet in the plasma space after splanchnic extraction (Ra_{diet}, μmol/kg/min) was

$$Ra_{diet} = Ra_{sys} \times \textit{fraction of plasma octanoate from diet} \quad (4)$$

The fraction of dietary octanoate extracted on its first splanchnic passage f was

$$f = \frac{\textit{Octanoate intake} - Ra_{diet}}{\textit{Octanoate intake}} \quad (5)$$

where octanoate intake was calculated from the amount of formula administered and the known concentration of octanoate in the formula. It should be mentioned that these calculations are made under the assumption that intermediary pools are at steady state (likely if CO_2 enrichments already have reached plateau) and if absorption of the tracer and dietary C8:0 is complete (very likely for C8:0, the absorption of which in prematures has been shown to be close to 100% (*e.g.*, [11])

Endogenous octanoate Ra (Ra_{endo}, μmol/kg/min) is therefore

$$Ra_{endo} = Ra_{sys} - Ra_{diet} \quad (6)$$

Calculation of octanoate oxidation

So far, dual isotope approach calculations have been used, similar to ones used before to investigate metabolism of glucose (*e.g.*, [12]) and leucine [13]. Mostly, a combination of a carbon-labeled and a deuterium labeled tracer (or their radioactive counterparts) are used for intragastric and intravenous tracer infusion. Naturally, in these cases only the carbon labeled tracer can be used to calculate oxidation rates, and both the intragastric and the intravenous route have been used for the carbon label. There is probably not much objection against using the two routes interchangeably in the case of protein or carbohydrate oxidation, as in the case of glucose initial splanchnic extraction is quite low, and in the case of leucine the intracellular precursor enrichment of its keto-acid (α-KICA) can be used in the equation. Also, oxidation of both glucose and leucine occurs predominantly in the peripheral tissues (*i.e.* muscle)

As the fractional extraction of octanoate is thought to be higher than several other metabolites, and a large fraction of the oxidation is supposed to occur in the liver, knowledge of the two recovery rates of carbon-labeled tracers administered by different routes is necessary to calculate more exactly both the splanchnic and the peripheral oxidation rate of octanoate. As, to the best of our knowledge, no such calculations have been published, we derived the following equations solving the oxidation term in parameters directly measured when two carbon labeled tracers are used.

The recovery of the tracer delivered intravenously equals the percentage of oxidation in the peripheral tissues.

$$recovery(iv) = ox\%(p) \tag{7}$$

where recovery(iv) is the recovery (in percent) of $^{13}CO_2$ during the intravenous tracer administration calculated according to ***Equation 1***, and ox%(p) is the percentage of the labeled (and unlabeled) octanoate appearing in the plasma pool that is oxidized by the peripheral tissues.

The recovery of label during intragastric tracer administration (rec(ig)) will include a part that is derived from the oxidation of octanoate taken up by the splanchnic bed and the oxidation of the label that has bypassed the liver (and other splanchnic tissues) and is oxidized in the peripheral tissues

$$recovery\,(ig) = f\ x\ ox\,\%\,(s) + (1 - f)\ x\ ox\,\%\,(p) \quad (8)$$

where recovery(ig) is the recovery in percent of $^{13}CO_2$ during the intragastric tracer administration calculated according to ***Equation 1***, and ox%(s) is the percentage of the labeled (and unlabeled) octanoate oxidized by the splanchnic bed.

The "total" oxidation of octanoate, assuming that unlabeled octanoate is handled in the same way as labeled octanoate will equal

$$Oxidation = I\ x\ f\ x\ ox\,\%\,(s) + Ra_{sys}\ x\ ox\,\%\,(p) \quad (9)$$

where Ra_{sys} is derived in ***Equation 2***, and I is the total intake of (unlabeled) octanoate. Using ***Equations 7*** and ***8*** the latter can also be written as

$$Oxidation = I\ (rec\,(ig) - (1 - f)\ x\ rec\,(iv)) + Ra_{sys}\ x\ rec\,(iv) \quad (10)$$

The oxidation rate calculated earlier [8], assuming no endogenous production, was obtained by multiplying the intake of octanoate by the recovery of the (orally administered) tracer, or: Oxidation = Intake x rec(ig). Comparing this with ***Equation 10***, it is clear that the total oxidation will be higher, the difference being

$$I\ x\ (-1 + f)\ x\ rec\,(iv) + Ra_{sys}\ x\ rec\,(iv) \quad (11)$$

or

$$rec\,(iv)\ x\ (Ra_{sys} - (1 - f)\ x\ I) \quad (12)$$

the latter term being only zero when the endogenous production is zero (then Ra_{sys} in plasma will equal I x (1-f), the latter being the amount passing through the liver).

Even when the intragastric recovery equals the intravenous recovery, it can only be concluded that the ox%(p) and ox%(s) are equal in that case, as it follows then according to ***Equations 7*** and ***8***, ox%(p) = f x ox%(s) + (1-f) x ox%(p), or f x ox%(p) = f x ox%(s)

Thus, the "total" oxidation of octanoate can be calculated using ***Equations 9 and 10***, while using all information available (recovery and plasma dilution, both

after i.v. and i.g. tracer administration) is necessary to calculate the true oxidation rate, as there are no fixed relationships between these four variables.

RESULTS

In all experiments a plateau was obtained after the 4th hr of the study as assessed by the lack of a significant slope in the breath enrichment by linear regression analysis. For sake of simplicity we took the average of the four delta values obtained during the last 3 hrs of the study as the plateau value. An example of two curves obtained in the same patient on the two occasions is presented in figure 1. Basal enrichments of the oral studies, which were always performed as the second in row, were not significantly higher than basal enrichments observed during the i.v. studies (data not shown).

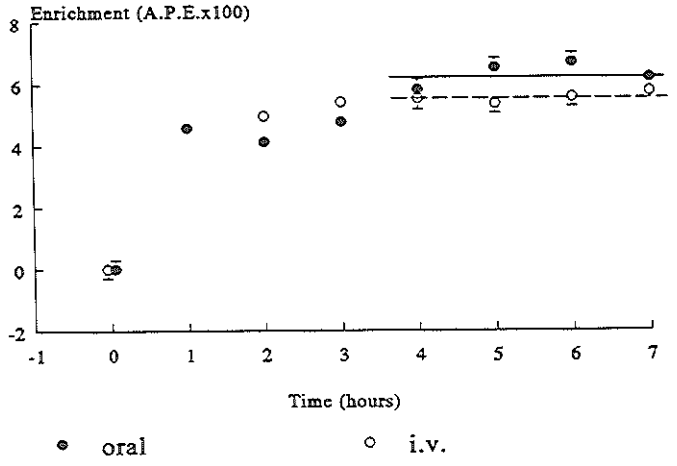

Fig. 1
Time course of $^{13}CO_2$ enrichment in breath after a primed constant oral infusion of [1-^{13}C]-octanoate in one patient after either intragastric (oral) or intravenous (i.v.) tracer administration.

Individual results of this study are described in **table 1**. Recovery of infused tracer in breath was 34 ± 3%, with a range between 28 - 37%. This is slightly lower than we have reported before in somewhat younger preterm infants [8].
I.v. and oral tracer administration yielded almost identical results. The ratio of the calculated recoveries on the two experimental protocols was close to 1 (**table 1**). Still, as argued earlier, this doesn't necessarily indicate that total oxidation can be calculated without knowledge of the endogenous production, as this is not necessarily zero when i.v. and i.g. recovery are equal.

The derived variables regarding the metabolism of octanoate, calculated according to the equations presented earlier, are shown as individual patient data in **table 2.**

Although calculations were performed in micromoles per kg per day, we found it easier to discuss the numbers in mg per kg per day, hence to obtain the original numbers one should divide the numbers by 207.36 (= 144*1440/1000).

To illustrate the results more clearly, approximate "average" values of **table 2** have been taken and are presented in **figure 2.**

Table 2
Calculated parameters of octanoate metabolism.

Patient	A	B	C
octanoate intake (mg/kg/d)	1395	1690	1440
Ra_{sys} (mg/kg/d)	1722	2473	1187
fraction octanoate from milk	0.18	0.18	0.15
splanchnic extraction *f*	0.77	0.73	0.88
Ra_{diet} (mg/kg/d)	309	440	176
Ra_{endo} (mg/kg/d)	1366	1934	914
oxidation % splanchnic (%)	37	26	38
oxidation % periphery (%)	35	35	32
"total" octanoate oxidation (%)	69	69	60

Table 1 Patient characteristics and stable isotopic measurements.

Patient, mode of infusion	Weight (kg)	Isotope infusion (μmol/kg/min)	Formula intake (ml/kg/d)	Recovery (%)	ratio i.v./oral	MPE plasma
A iv	1.70	0.22	169	35	1.05	2.69
A ig	1.82	0.21	158	36		0.58
B iv	1.86	0.47	203	35	0.81	4.01
B ig	1.95	0.45	194	28		1.01
C iv	1.58	0.47	162	32	1.17	8.21
C ig	1.77	0.45	176	37		0.94
	1.8 ± 0.1			34 ± 3	1.01 ± 0.18	

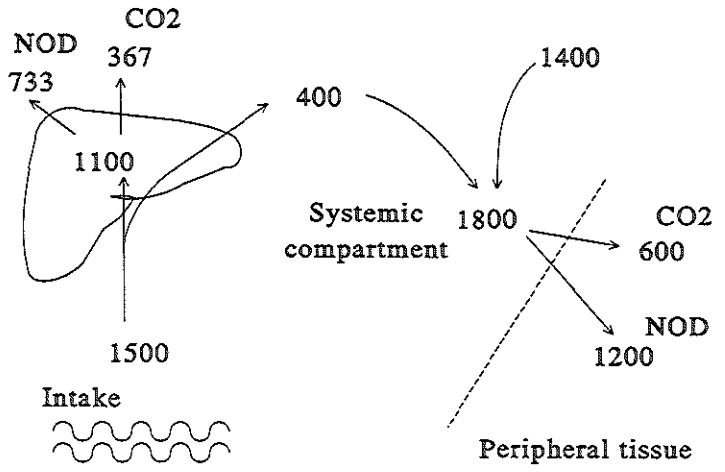

Fig. 2 Approximate model of octanoate metabolism, based on averages of the measured parameters in the three patients, with rounded off numbers. NOD = Non-oxidative disposal. 1500 = Intake of octanoate, extraction% f = 73%, ox% in the splanchnic bed = 1/3. 400 = Ra(diet), 1400 = Ra(endo) and 1800 = Ra(sys).
Ox% in the peripheral tissues = 1/3. The recovery of an intragastrically administered tracer in this example would have been also 1/3 ((1500 x 0.73 x 1/3 + 1500 x 0.27 x 1/3)/1500), but due to the endogenous production the total oxidation is actually higher i.e. 64% of the intake (1500 x 0.73 x 1/3 + 1800 x 1/3)/1500.

DISCUSSION

In this study we report oxidation rates of infused [1-^{13}C]-labeled octanoate (C8:0), as a representative tracer of MCFA metabolism. We have used the oral administration of tracer octanoate before, to investigate some peculiarities of MCFA oxidation in preterm infants (*i.e.*, possible excess intake of MCFAs and consequently less than complete oxidation and redirection towards other pathways). In the latter study however we assumed that the oral tracer route was applicable to this kind of study, as it is similar to the route taken by the fed formula. We implicitly assumed that, because body stores of C8:0 are negligible, endogenous production was negligible.

With the developed set of equations details of octanoate metabolism can be investigated further. Some of the calculated variables fit well with what had been predicted from perfusion experiments, for instance the high fractional extraction by the splanchnic tissue or liver that we calculated, is indeed higher than described for other substrates studied more extensively like glucose or amino acids. Also, the direct oxidation of a fairly large part of the octanoate by the splanchnic bed is consistent with the known characteristics of octanoate. The total oxidation of octanoate, although higher than calculated earlier and much higher than described for most other substrates, especially LCFAs, is not complete. It is generally assumed that MCFAs constitute a "rapidly available source of energy" that is quickly and completely oxidized or converted into ketone bodies and subsequently oxidized.

If C8:0 (and C10:0) would not be completely oxidized, the theoretical metabolic advantage of MCTs (apart from a high absorption rate) would be decreased, as they probably have to be converted into LCFAs before significant storage in the body can take place.

Problems could arise however if a significant part of the administered octanoate is oxidized peripherally after having passed the liver, especially if the enrichment of octanoate is being diluted peripherally by endogenous production of (unlabeled) octanoate. In that case the precursor enrichment for the peripheral oxidation would be lower and the oxidation measured by oral tracer administration underestimates the total oxidation. We therefore performed a validation study with intravenous and oral administration of tracer during exactly identical circumstances.

The model was constructed, assuming that significant oxidation could occur in the peripheral tissues, as several authors (*e.g.*, [14]) have shown that the percentage of octanoate oxidized is not significantly lowered after (functional) hepatectomy in rats. Results of the study showed that recovery of labeled octanoate was approximately 1/3 after both oral and intravenous tracer administration, pointing indeed towards a capacity of the peripheral tissues to oxidize octanoate. However, as

argued before, this does not indicate that the "true oxidation" is not underestimated when only intragastric tracer is infused. It also shows that -unlike what is generally done with proteins and carbohydrates- it should be carefully considered by what route the carbon-labeled tracer used for measuring oxidation should be given in case of dual tracer experiments. The enrichment in plasma after administration of tracer both intragastrically and intravenously was measured, and results indicated a very significant endogenous rate of appearance of octanoate as the enrichment of [1-^{13}C]-octanoate in plasma during oral tracer infusion was much lower than the enrichment in the administered feeding. Thus, the part of the administered tracer that escapes the first pass extraction by the liver is diluted several fold by octanoate present elsewhere in the body entering the systemic compartment, thereby explaining also the relatively low recovery rates sometimes encountered during bolus tracer experiments (*e.g.*, [15]). The question where this unlabeled octanoate originates from is difficult to answer. In stead of regarding this as production of octanoate by peripheral tissues, it could also be that octanoate just interchanges very rapidly between the intracellular- and the plasma compartment. For instance, octanoic acid is said to be independent of carnitine for its transport via the mitochondrial membrane, at least in certain tissues [16]. Also, data obtained from adult patients with liver cirrhosis and given radio-labeled long chain fatty acids suggest that under fasting conditions 60% to 80% of the serum octanoate was obtained from incomplete β-oxidation of long chain fatty acids [17].

Radiolabeled studies in animals and in vitro studies have shown that a large part of labeled octanoate is being oxidized or present as water-soluble products in the hepatic vein [4]. However, this doesn't necessarily contradict our findings, as in our study hepatic extraction was very efficient being around 75%, so a relatively minor part passes the liver. However, in this study, due to the development of new and very sensitive measurement techniques, we were able to show that the part of the tracer passing the liver is also oxidized peripherally. Since it is assumed that the unlabeled octanoate behaves the same as its labeled counterpart, the endogenous production that dilutes the labeled octanoate in the plasma space also has to undergo this oxidation, leading to a much higher oxidation rate than the one calculated without this additional information.

Oxidation rate calculated using this new approach was 64%. At the moment it is difficult to explain why the recovery during intragastric tracer administration, in this study is lower than found earlier [8].

The somewhat older preterm infant studied here, is often receiving MCT enriched formulas to approximately 40 weeks postconception, whereas we have now shown that a significant part of the MCFAs is not being oxidized as was assumed earlier. The biological significance of this however remains to be established. Also

the fact that so much octanoate is produced peripherally and hence also taken up again by the peripheral tissues (as oxidation is only 33%), probably indicates that there is quite a large capacity of disposal of octanoate in the peripheral tissues.

Whether the excretion of dicarboxylic acids that is known to occur with increasing intake of MCFAs, is related to a relative overload of MCFAs that cannot be oxidized via the normal route is not certain. However using this new method of quantitation of oxidation, more insight can be gained into the regulation of octanoate oxidation during high intakes of MCFAs.

In this study we have developed a new technique of quantifying *in vivo* whole-body oxidation of octanoate and shown that healthy prematures oxidized approximately two thirds of the oral load of MCFAs.

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CHAPTER VIII

EFFECTS OF MEDIUM-CHAIN TRIGLYCERIDE FEEDING IN EXTREMELY-LOW-BIRTH WEIGHT INFANTS.

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SUMMARY

Most of the formulas available for preterm infants include medium-chain triglycerides (MCTs), which contain fatty acids with chain length of eight and ten carbon atoms, in order to improve fat absorption. Theoretically, the improvement in fat and calcium absorption during MCT feeding might be more pronounced in very small preterm infants, especially during their first weeks after birth.

Extremely-low-birth weight (ELBW) infants weighing under 1000 grams at birth were randomized to receive a formula containing either 5% (LCT formula) or 33% (MCT formula) of fat as MCTs. Anthropometric measurements during the first month of life were performed together with three periods of fat and calcium balances. Seven infants in both groups were studied; birth weight (876 $\pm$ 48 *vs.* 898 $\pm$ 80 gram, MCT *vs.* LCT; mean $\pm$ 1 S.D.) and gestational age (29.7 $\pm$ 2.5 *vs.* 29.3 $\pm$ 2.4 wks) were comparable between groups. Overall formula tolerance was good and caloric intake was comparable between groups, approximately 110 kcal/kg/day during week 2, and 120 kcal/kg/day during wks 3 and 4. Percent fat absorption was significantly higher with MCTs (average 84 $\pm$ 3%) than with LCTs (average 76 $\pm$ 6%, $p < 0.01$). Calcium absorption was highly variable and not significantly different between groups (average 55 $\pm$ 13% *vs.* 46 $\pm$ 14%, MCT *vs.* LCT, $p = 0.24$). There was no clear evolution over time in fat- or calcium absorption, possibly related to the cautious feeding regimen with graded introduction

of formula during the second week of life. Although fat absorption was significantly higher with MCTs, there were no differences in increments in standard deviation scores for weight gain (-0.41 ± 0.58 *vs.* -0.36 ± 0.66, $p = 0.9$) and head circumference gain (+0.57 ± 0.91 *vs.* +0.33 ± 0.34, $p = 0.5$). Weight gain expressed per kg was extremely variable at the age of two weeks, but consistently around 20 g/kg/d in both groups during weeks 3 and 4. We conclude that the known small beneficial influence on intestinal absorption of fat and calcium with MCT formula compared with current LCT formulas containing coconut oil are present to a similar magnitude in ELBW infants during the first month after birth as reported earlier in older preterm infants.

INTRODUCTION

The majority of the commercially available formulas intended for the prematurely born infant contain medium-chain triglycerides (MCTs), the triglycerides of C8:0 and C10:0 fatty acids, primarily in order to enhance fat and mineral absorption. The clinical benefit however of adding MCT oil to preterm formula has been questioned. Initial optimistic reports were not followed by more evidence on the beneficial effects, whereas there is still a lack of knowledge on the metabolism of this artificially synthesized food stuff.

The improved weight gain on MCT feeding described initially [1-3], has not been confirmed in other studies [4-8]. These studies were performed in preterm infants with a birth weight between 1200 and 2000 grams. In only one study [9], smaller premature infants with an average birth weight around 1000 gram were studied, but the postnatal age was variable with the smallest infants being studied as late as six weeks after birth.

In an earlier study in 28 very low birth weight infants [10], we compared a formula containing 38% of fat as MCTs, to a new formula with a fat blend which more closely resembled human milk with addition of coconut oil and not of MCT oil. Fat and nitrogen balance, energy substrate utilization, and body composition were investigated and only a slightly higher fat absorption and -deposition were found in the infants fed the MCT-containing formula, without any affect on linear growth or weight gain.

Most of the physiological limitations to fat absorption and digestion in the preterm neonate are related to immaturity and some authors have shown a linear correlation between gestational age or postconceptional age and fat absorption under certain conditions [11].

We therefore hypothesized that the major beneficial effects of MCTs were most likely to be present when feeding sick and/or very small premature infants during their first weeks after birth. The scarcity of data on effects of MCTs in these infants is probably related to the fact that they are often considered "too ill to study", even though an appropriate caloric intake might be critical, especially in this group of patients.

To investigate the possible beneficial effects of MCT feeding we randomized 30 extremely-low-birth weight (ELBW) infants, weighing less then 1000 grams at birth, to receive either a formula containing 5% medium-chain fatty acids (MCFAs) by weight (Long Chain Triglyceride (LCT) formula) or a standard MCT formula containing 33% MCFAs. Anthropometric measurements were made during the first month of life together with three periods of 72-hours fat- and calcium balances.

PATIENTS AND METHODS

Subjects

All infants with a birth weight between 750 and 1000 grams that were initially admitted to the neonatal intensive care unit (NICU) of the Sophia Children's Hospital between november '91 and december '92 were considered eligible for the study.

During the first week after birth, in the absence of an immediate life threatening condition and exclusion criteria like congenital disorders or inborn errors of metabolism, permission was sought from at least one of the parents. At that time infants were receiving total parenteral nutrition (TPN) as is routinely done in ELBW infants in our ward [12].

Permission for the study was obtained for 30 infants. Eight of these infants were withdrawn in an early stage (four because of decision of the mother at a later stage to feed with mothers own milk, three because of transfer to another hospital and one surgical intervention). 22 infants proceeded with the study, of which a total of eight infants were withdrawn during the study. Three of these infants were withdrawn by

the attending physician because of need for fluid restriction due to patent ductus arteriosus, unexplained neonatal hypertension, and cardiac failure. One infant was withdrawn because he was prescribed a topical ointment for Candida infection of the perineal region which might influence by its high fat content the fat measurements in stools. One infant was withdrawn because of blood-containing stools and gastric aspirates probably secondary to a coagulation disorder.

Three infants were excluded by the attending physician for reasons (possibly) related to the feedings: In two infants fed the MCT formula the daily residuals were more than 25% of the daily amount, fed for more than one day, and one infant fed LCT formula developed a necrotizing enterocolitis.

Thus, a total of fourteen infants completed the study, seven infants in both groups. The composition of the fat blends of the MCT and LCT formula used is given in **table 1.** Fat concentration was 4.20 g/100 ml in the LCT formula and 4.31 g/100 ml in the MCT formula and calcium concentration was 80 mg per 100 ml in both formulas (measured by us after simulated gavage feeding).

Table 1 Composition of formula fat blends (wt%)

	MCT formula	LCT formula
C6:0	0.13	0.26
C8:0	19	2.9
C10:0	14	2.3
C12:0	0.48	18
C14:0	0.33	7.4
C16:0	5.5	8.6
C16:1	0.14	0.14
C18:0	2.6	3.5
C18:1	31	30
C18:2	23	22
C18:3	1.9	1.8

Both formulas were prepared in one batch by Nutricia, Zoetermeer, The Netherlands. The composition of the LCT formula was comparable to most of the current commercially available special formulas for premature infants, containing coconut oil and no MCT oil.

After randomization, oral feedings were gradually introduced, as is routinely done in our ward, following the scheme in **table 2.**

Table 2 Feeding schedule

AA; Amino Acids, 1:1 signifies the dilution with 5% dextrose.

Day	Glucose iv ml/kg/d	AA iv ml/kg/d	Fat iv ml/kg/d	Volume iv ml/kg/d	Formula ml/kg/d	Energy kcal/kg/d
1	60	–	–	60	–	22
2	62	12	6	80	–	41
3	64	24	12	100	–	61
4	84	24	12	120	–	68
5	104	24	12	140	–	76
6	124	24	12	160	–	83
7	124	24	12	160	–	83
8	104	24	12	140	20 (1:1)	84
9	84	24	12	120	40 (1:1)	84
10	64	24	12	100	60 (1:1)	85
11	82	12	6	100	60	97
12	62	12	6	80	80	105
13	42	12	6	60	100	114
14	22	12	6	40	120	123
15	20	–	–	20	140	119
> 16	–	–	–	–	160	128

Infants were fed on a 24-hour schedule through nasogastric gavage feeding using syringes with 2-hr dosages. Semi-continuous feeding was achieved by nursing staff administering a small volume approximately every 15 min, or by use of gravity with an opened syringe in the upright position in case of artificial ventilation. Gastric residuals were checked every 4 hrs. Any gastric residuals were returned to the infant, but the volume was subtracted from the following 2-hr dosage of formula, thus leading to a lower intake of formula. Feedings were withheld, or progression was delayed, in cases where over 25% of the formula prescribed was not fed to the infants due to gastric retention. A delay of maximal one day in following the study design was tolerated before exclusion from the study.

Methods

Balance studies

At day 12, on the second day of receiving undiluted formula, a carmine-red marker was administered. The first passage of colored feces (usually the next day) was included in the collection period and from that moment on records were kept of the amount of feeding prescribed, and the actual volume administered due to gastric residuals in order to be able to calculate the caloric intake. 72 hours after the first marker, a second carmine-red marker was administered. All feces were collected until the second period of colored feces, usually on day 16, the latter being excluded from the total feces collection. Feces was collected on pre-weighed diapers and immediately put at -20 °C. Results of this balance period are referred to as period I.

Similar balance studies were done starting on day 19 (period II) and on day 26 (period III). An overview of the study design is given in figure 1. Weight, skinfold thickness, and head circumference were recorded from the first day of the balance studies (day 12) to day 29. Weight gain was also calculated using the birth weight as a starting point.

Z-scores for weight and head circumference, indicating the standard deviations below or above the mean for the postconceptional age, were calculated using the data of Usher and McLean [13]. The latter was done by calculating the exact post-conceptional age for each time-point and by linear interpolation of the available data of weekly intervals of gestational age. Weight was measured to the nearest 5 gram, and head circumference was measured using individual measuring tapes kept with

each infant during its stay on the ward. Skinfolds were measured after one minute compression at three sites (subscapular, mid-thigh and triceps area) with a Harpender caliper. Measurements were made by the same research nurse in all infants, and the c.v. for 10 measurements on one day was approximately 4%. Length gain was also measured, but not analyzed any further as this measurement proved to be highly variable.

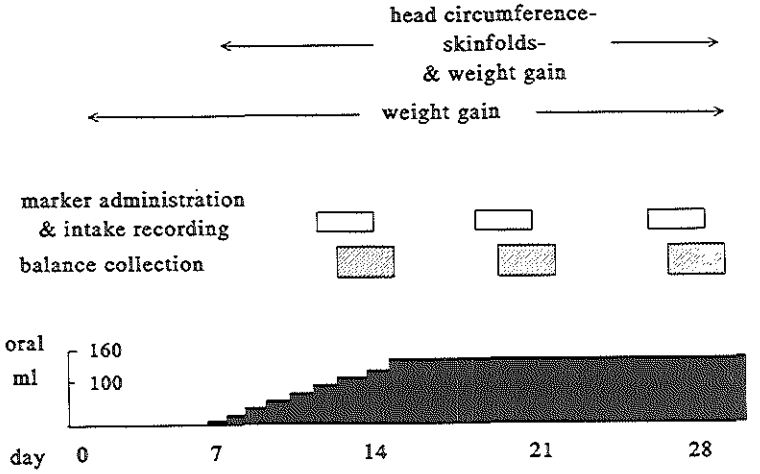

Fig. 1 Study design

Determinations in feces

Fat excretion in feces was determined using a modified Jeejeebhoy method [10]. Calcium excretion in feces was measured by atomic absorption spectrophotometry as described before [14].

Statistics

Data are presented as mean ± 1 SD. Differences between the MCT and the LCT feeding group were tested using one way ANnalysis Of VAriance (ANOVA). Gestational age was included as a continuous variable in a two-way ANOVA design with continuous variables from the Stata™ statistical software package for the analysis of increments in anthropometry. Since there was no significant time effect, as checked by linear regression on the three balance data points, data were treated as

repeated measures, and tested with ANOVA with gestational age as a covariate using the SPSS^{TM} statistical software package for the PC.

RESULTS

Balance data

Formula tolerance was good in the infants who completed the study. All infants, except the three discussed in the methods section, could progress according to the feeding schedule. Caloric intake, although slightly lower than intended, was followed according to the schedule in all infants (with maximal one day delay). The small difference between the actual and the intended intake of formula, was caused by delay in increasing the daily amount appropriate for weight on the ward and was not due to intolerance. Formula intake was not different between groups (**table 3**), although during the final days of the study it tended to be slightly lower on the LCT group. Gastric retention was low, and decreased from an average 8 ml/kg/d to 2 ml/kg/d from weeks two to four (**table 3**). Retention of formula in gastric aspirates was not significantly different between the two feeding groups.

Results of the fat and calcium balances are presented in **table 3**. There was no relation between postnatal age (or balance period) and fat absorption. This was confirmed by simple linear regression analysis on the three different time periods in both groups. Fat absorption in the MCT group was significantly higher during the second and the third balance period. As there was no time related effect on fat absorption, data were analyzed with repeated measures ANOVA for feeding effect. A p value of 0.007 was found for the combined data. The latter significance value did not change when gestational age was included as a covariate. Also, when the arcsin transformation was performed to "correct" for the fact that percentages were compared, significance value was still 0.027.

On the other hand, calcium absorption was not different in any of the three periods, although it tended to be higher on MCTs. Regression analysis on individual data showed the absence of a time effect also with calcium (coefficients for the regression not significantly different from zero; 0.000 ± 0.076 for MCT and 0.027 ± 0.126 for LCT), even though the averages seemed to increase with time. But even when repeated measurements ANOVA was performed on the calcium data

there was no significant difference between groups in the percentage of calcium absorbed.

The difference in metabolizable energy intake between feeding groups was relatively small and only significant during period II (table 3).

Anthropometry

Results of anthropometric measurements are described in table 4. Initial measurements were made on day of birth only for weight, whereas initial values for head circumference and skinfolds thickness were obtained on day 12.
All variables were similar on MCT and LCT feeding. The difference between

Table 3
Intake and absorption data

	Period			
	I	II	III	
Caloric intake				kcal/kg/d
-MCT	107 ± 12	123 ± 4	115 ± 3	
-LCT	107 ± 10	122 ± 6	121 ± 7	
Gastric retention				*ml/kg/d*
-MCT	8 ± 6	3 ± 3	2 ± 1	
-LCT	7 ± 5	3 ± 4	1 ± 2	
Metabolizable				kcal/kg/d
intake -MCT	84 ± 9	104 ± 6	107 ± 7	
-LCT	87 ± 12	110 ± 5	108 ± 4	
		p<0.05		
Fat absorption				percent
-MCT	85 ± 8	81 ± 5	86 ± 6	
-LCT	79 ± 10	71 ± 7	77 ± 8	Combined:
		p<0.01	p<0.02	$p < 0.01$
Ca absorption				percent
-MCT	50 ± 30	57 ± 20	59 ± 19	
-LCT	46 ± 20	42 ± 14	51 ± 20	Combined:
				$p = 0.24$

groups in total weight gain was not statistically significant ($p = 0.3$). With ANOVA a significant effect of gestational age was noted, but the p value for the comparison of feeding groups remained 0.3.

If weight gain was expressed as gram per kg per day, variable results were obtained in the first two weeks of life, showing no significant differences between groups (MCT 10 ± 9 *vs.* LCT 0 ± 15 g/kg/d). In the latter two weeks of the study, with most infants recovering from their critical illness, rates of weight gain were more stable with 22 ± 10 *vs.* 20 ± 10 g/kg/d at three weeks of life and 15 ± 11 *vs.* 22 ± 10 g/kg/d (MCT *vs.* LCT) at four weeks of life, values which were all not significantly different between groups.

Table 4
Anthropometric data.

	start	day 29	increment	*p* value of factor	
				feeding	gest age
Weight	(day 0)				
-MCT	876 ± 48	1269 ± 193	393 ± 168		
-LCT	898 ± 80	1201 ± 116	304 ± 110	.3	.03
Head circumf.	(day 12)				
-MCT	26 ± 1.1	29 ± 1.9	2.6 ± 0.94		
-LCT	26 ± 1.3	28 ± 1.3	2.3 ± 0.21	.5	.1
average skinfolds	(day 12)				
-MCT	0.22 ± 0.02	0.27 ± 0.06	0.052 ± .046		
-LCT	0.18 ± 0.02	0.24 ± 0.04	0.058 ± .039	.6	.05

Similar results were obtained when the increment in anthropometric variables was expressed as z-scores (table 5), thus correcting for differences in distribution of gestational age among study groups. No significant differences between the MCT and LCT feeding groups were found in any of the parameters (table 5).
Using one-way ANOVA, p values of 0.9 for weight z-score increments (both from birth to day 29 and from day 12 to day 29) and 0.5 for incremental z-score on head circumference were found.

Table 5
Z-scores of standard deviations on anthropometric data.
All comparisons not significantly different.

	day 0	day 12	day 29	Δd29-d12	Δd29-0
Weight					
-MCT	-2.2 ± 0.43	-2.5 ± 0.66	-2.9 ± 1.0	-0.41 ± 0.58	-0.70 ± 0.71
-LCT	-2.0 ± 0.54	-2.4 ± 0.47	-2.8 ± 1.0	-0.36 ± 0.66	-0.77 ± 0.68
Head circ.					
-MCT		-3.0 ± 0.80	-2.4 ± 0.87	0.57 ± 0.91	
-LCT		-3.1 ± 0.71	-2.8 ± 0.53	0.33 ± 0.34	

DISCUSSION

With the increasing survival of extremely-low-birth weight infants, providing adequate feeding for this patient group becomes increasingly important. Although a number of studies have reported on the optimal feeding of preterm infants, little relates specifically to the feeding of those, weighing under 1000 gram at birth. Factors that account for this lack of knowledge may include the high morbidity, and the small numbers in individual centers.

Several studies have focussed on the use of MCTs in formulas especially adapted for the preterm neonate, but none of these studies have reported on the absorption of MCT formulas by ELBW infants during their first four weeks of life.

In this study we have compared the effects of MCT *versus* LCT feeding in the young and tiny premature, because we expected that the largest differences would be obtained in this vulnerable group of patients. Formula tolerance was comparable between the two feeding groups. After one week of TPN, actual caloric intake corrected for gastric retention reached approximately 110 kcal/kg/d at the end of the second week and approximately 120 kcal/kg/d during the last two weeks of the study. Although there were two cases of feeding intolerance in the MCT group and one case of necrotizing enterocolitis in the LCT group, we cannot comment on side effects attributable to the feedings as numbers are clearly too small to draw any conclusions.

Weight gain was quite acceptable in these children after the establishment of full oral feedings, with a median of 20 gram/kg/d being in the upper part of the intrauterine weight gain curves. MCTs showed no advantage over LCTs in terms of weight gain. When expressed as relative standard deviation score, or z-score, all anthropometric measurements were similar between MCT and LCT feeding groups. The use of this score "corrects" for differences in (distribution of) gestational age between groups. Interestingly, z-scores of weight gain show small negative values, whereas z-score increments for head circumference were positive, probably pointing towards a relative brain-sparing effect. It is not clear if the absence of an increase in z-score for weight indicates that catch-up growth cannot be achieved in the first month in ELBW infants, or that caloric intake is still not optimal during the first two weeks.

Fat absorption was higher with MCTs showing results comparable to the ones measured earlier in older preterm infants [10]. Although the intake was slightly lower at the end of the fourth week for MCTs, it is clear that in the third and fourth week of life an advantage of approximately 6 kcal/kg/d in energy absorption is achieved by MCTs. This could lead to an extra weight gain of roughly 2 g/kg/d, as the energy cost of growth is approximately 3 kcal/gram [15,16]. Whether this is clinically relevant in this group of infants with a satisfactory growth rate especially during the third and fourth week of life is unclear. However, in several clinical situations, *e.g.* when fluid restriction is indicated, the use of MCTs might have advantages that have to be weighed against the possible side-effects.

In this small group of infants, calcium absorption was not significantly different between groups, even not when measurements were combined. Even so, the mean absorption of calcium was slightly higher in all three periods in the MCT group and probably a larger group of infants would show that calcium absorption is also improved by MCT feeding in the ELBW infant. Due to the relatively large variance, a fairly large number of infants however will need to be studied, using the data obtained here we can calculate that approximately 120 infants would have to be studied, using the present relative standard deviation when studying calcium absorption at only one time point. The large variance in the calcium absorption data is in our view probably not due to the analytical variance, which is quite small compared to the biological variance. It can also not be fully explained by variance inherently part of measurements using fecal collections, due to variation in transit time, as the variance in the calcium data seems larger than the variance in fat absorption which is subject to the same interfering factor(s). The influence of MCT

feeding on calcium and fat absorption is however relatively small, and future studies designed to prove a possible benefit of MCTs on calcium absorption certainly will have to include measurements on outcome variables like bone densitometry to ensure that a real benefit is present.

One remarkable finding was the absence of a significant relation between absorption of nutrients and postnatal age. This finding is in agreement with the findings of Hamosh et al. [9] in a slightly different population of preterm infants of different postnatal ages. One explanation in our study could be that the formula load was small in this study during the first two weeks of life, posing less stress on the absorptive mechanisms and leading to absorption rates similar to the ones later in life when 120 - 130 kcal were fed orally per kg per day. In other words, the cautious feeding regimen might have prevented significant malabsorption with both formulas.

We conclude that in a relatively small group of extremely-low-birth-weight infants minor beneficial effects were present of the addition of medium-chain triglycerides to specialized preterm formulas. The larger differences found earlier between MCT and LCT formulas even in the more mature preterm infant, have disappeared when a comparison is made with an LCT formula containing larger amounts of lauric (C12:0) and myristic (C14:0) acid, derived from coconut oil, than in the earlier days. Furthermore, with current formulas, differences between LCTs and MCTs are not more pronounced during the first weeks after birth.

The present study shows that the intestinal absorption of fat and calcium in extremely-low-birth weight infants is not enhanced by MCTs to a larger extent than it is in the older and larger preterm infant, even not during the first weeks of life. The effect on outcome variables therefore will have to be investigated in larger groups of infants. Nevertheless, in a limited number of patients with a more pronounced malabsorption, the use of MCTs can be considered, although the possible side effects are not yet fully understood.

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CHAPTER IX

GENERAL DISCUSSION

Advances in perinatal medicine have resulted in an increased survival of very-low-birth weight infants (VLBW; birth weight < 1500 gram) and extremely-low-birth weight infants (ELBW; birth weight < 1000 gram), with some infants as small as 500 gram surviving. The increased survival is probably due to improvements in the treatment of ventilatory and circulatory problems, but also to improvements in nutritional care.

Between 22 and 32 weeks, the fetus doubles its body weight approximately every five weeks, and this rapid intrauterine growth is often hard to maintain in the prematurely born. Several factors contribute to the decreased growth in the prematurely born infant, one of them being the existence of "physiological" limitations in the digestion and absorption of nutrients, especially in relation to fat, probably due to a relative deficiency of pancreatic lipase and bile salts.

Approximately 50% of the nonprotein energy in human milk and formula is provided by fat. Furthermore, most of the energy lost in excreta consists of fat, whereas carbohydrate absorption is always above 95%, even in the smallest infants during the first weeks of life.

Medium-chain triglycerides (MCTs), which are manufactured from glycerol and medium-chain fatty acids (MCFAs) with a chain length of eight and ten carbon atoms, are less dependent on sufficient levels of pancreatic lipase and bile salts than the long-chain fatty acids (LCFAs) with chain lengths of sixteen and eighteen atoms that dominate in our regular food fats. After a few optimistic reports showing improvements in fat, mineral, and nitrogen absorption, the clinical benefits of addition of MCT oil to preterm formulas could not be confirmed. The latter could be related to the fact that fat blends in general have changed, where predominantly saturated fats from cow's milk were replaced by fats from vegetable oils.

Several studies have reported on an improved fat absorption with MCT-containing formulas, without any beneficial effect on weight gain. As overfeeding studies in animals as well as adult human volunteers showed evidence for an increase in metabolic rate during MCT feeding, we studied heat production and body composition in preterm infants fed either a MCT or a LCT formula (Chapter 1). An improvement in fat balance was noted in the MCT group, but there was no increase in weight gain or nitrogen retention. However, in this study the absence of increased weight gain in the presence

of a higher metabolizable energy intake was not due to an increased metabolic rate in the MCT group as we had hypothesized. In contrast, a significantly higher percent of new tissue was composed of fat in the MCT group, as could be calculated from individual weight gain related to the fat balance.

Theoretically, the benefits of MCT formula should be most pronounced in the smallest infants during their first weeks after birth, as the fat malabsorption has been shown to be related to immaturity. In fact, some authors have described a linear relation between fat absorption and gestational or postconceptional age. Remarkably, in the ELBW infant fed MCT formula during his first weeks after birth, we could not find an indication that fat absorption was improved to a larger extent than in the older preterm infant (Chapter 8). Growth was very similar in these groups, whether fed a MCT formula, or a LCT formula containing coconut oil. It can be estimated from our data that if there are significant advantages of MCT on weight gain or linear growth in these infants, a very large number of infants would need to be studied to prove this. The absence of major differences even in these tiny prematures is possibly related also to the use of a cautious feeding regimen and/or the use of an LCT formula containing coconut oil and "high oleic safflower oil", with low levels of palmitic and stearic acids.

In conclusion, there is a slight improvement in fat balance when using MCTs, leading to an increase in metabolizable energy intake of around 5-6 kcal/kg/day compared to LCT formula. Theoretically, this might lead to an additional weight gain of aproximately 2 grams per kg per day in the MCT group, but in both age groups studied this difference was not detectable. The latter could be due to the fact that groups are too small to detect such a small difference, or to the fact that the percent fat in new tissue is indeed higher with MCT feeding.

From chemical analysis of human fetal material it has been calculated that the mean accumulation of calcium and phosphorus is about 130 and 75 mg respectively per kg per day between 26 and 36 weeks of gestation. At the moment, preterm formulas as well as human milk are unable to provide enough of these minerals to achieve these retention rates. Even so, it has been shown that overt bone disease in the preterm infant can be prevented with calcium intakes much lower than the theoretical intrauterine accretion [1]. A significant improvement in calcium absorption using MCT formula has been reported [2], but in that study the differences in long-chain saturated fatty acid intake between high MCT and control feeding were also very pronounced. As later studies concluded that there was no improvement in mineral absorption with MCT, we investigated mineral metabolism comparing a current LCT formula with addition of coconut oil to a MCT containing formula (Chapter 3).

Our data do show a significant enhancement of calcium absorption with MCTs. In the LCT group there were signs of an imbalance between calcium and phosphorus absorption, with increased PTH levels in plasma and high urinary phosphorus levels. However, calcium, as well as alkaline phosphatase and 1,25-$(OH)_2$-vitamin-D levels were not different between groups. Still there is evidence that the amount of calcium absorbed was relatively low compared with the amount of phosphorus absorbed in the LCT group. The most logical way to resolve this would be addition of calcium to the formula. However, the latter is technically difficult and not without risks, regarding negative effects on fat balance and increased risk of bolus obstruction.

A remarkable finding in the group of ELBW infants was that the difference in calcium absorption between MCT and LCT formula was also not more pronounced in the tiny premature infant during the first weeks after birth (Chapter 8).

In conclusion, if technical difficulties prohibit the development of new formulas with higher availability of calcium, there is a small but definite advantage of MCT feeding with regard to intestinal absorption of calcium.

It is generally assumed that MCFAs are absorbed and oxidized (almost) completely when fed enterally and are not present in significant amounts in adipose tissue. It is not known whether there is a maximum in the amount of MCFAs that can be readily oxidized by the preterm infant, nor whether the increased excretion of dicarboxylic acids and other side-effects of MCT feeding are related to the ability to oxidize MCFAs. Therefore, the determination of oxidation rates of MCFAs under different circumstances might provide important information. Using octanoate labeled with the stable isotope ^{13}C ([1-^{13}C]-octanoate), we found oxidation rates corresponding to approximately 50% of the infused MCFA (Chapter 4). This confirmed the postulated preferential oxidation of MCFAs also in the preterm infant. However, more than 50% of the infused tracer was not oxidized and we speculated that a significant part of the MCTs fed had to be chain-elongated before storage in adipose tissue was possible. This would be an energy-requiring process, confirming the "carbohydrate-like" behavior of MCTs.

In a further study, we investigated the splanchnic metabolism of octanoate using a dual isotope approach, and derived appropriate equations to compare splanchnic and peripheral oxidation rates (Chapter 7). The dual isotope approach consisted of administering [1-^{13}C]-octanoate *via* nasogastric route on one day, and under exactly the same circumstances intravenously the other day. Splanchnic first-pass extraction was 78% for octanoate, which is much higher than for amino acids or glucose, thus being consistent with previous estimations from animal studies. About one-third of this

extracted octanoate was directly oxidized. The significant dilution of the intravenously administered tracer in the systemic circulation is possibly due to exchange of tracer with intracellular pools of octanoate coming from ongoing β-oxidation of fatty acids. Such exchange has been described in human adults using radiolabeled tracers, but was not quantitated before [3]. Thus, about one-third of the ingested MCFAs is directly oxidized in the splanchnic bed, and a similar part of the MCFAs entering the systemic compartment, coming mainly from other sources than the diet, is oxidized in the peripheral tissues. At the moment it is not clear whether the sum of splanchnic and peripheral octanoate oxidation is a good representative of the total amount of MCFAs oxidized, as it might include part of the β-oxidation of LCFAs.

In conclusion, further isotopic modeling is needed to validate the calculations used, as it is not sure at the moment whether the dilution of tracer in the systemic pool represents endogenous production entering the plasma pool and hence, we cannot be certain that the sum of the estimated splanchnic and peripheral oxidation truly represents the whole-body oxidation.

A high activity of lipogenic enzymes with MCT feeding as compared with LCT feeding has been found by several authors [*e.g.*, 4], and recently it was found that this was independent of insulin status [5]. Using a combination of indirect calorimetry and stable isotopes, we found that MCT decreased glucose oxidation as compared to LCT, but had no influence on total carbohydrate utilization (Chapter 5). Consequently, lipogenesis from glucose is probably higher during MCT feeding. This fact points again at the "carbohydrate-like" behaviour of MCT, oxidation being high enough to supply excess acetyl-CoA and inhibit the oxidation of glucose. In conclusion, we have provided indirect evidence that lipogenesis is increased during MCT feeding and thus it can be speculated that increased levels of (saturated) LCFAs found during MCT feeding are not only the result of chain elongation from MCT, but also due to increased lipogenesis from glucose.

The increase in levels of myristic, palmitic, and oleic acids in plasma on MCT feeding compared to the diet given was confirmed in preterm infants using traditional gas chromatography. Using a new stable isotope methodology we were able to show *in vivo* the conversion of labeled octanoate into the LCFAs myristic and palmitic acid (Chapter 6). Although the data cannot be interpreted quantitatively, it is clear that chain elongation of octanoate (either directly or *via* lipogenesis from acetyl-CoA units) does occur. In conclusion, we found an increase in the plasma levels of C16:0, C18:0, and C18:1 compared to the MCT containing diet, and using stable isotopes we have shown that this is at least partially caused by the conversion of MCFAs into nonessential

longer chain fatty acids. In a recent study [6] it was shown in newborn piglets that feeding MCTs *vs.* coconut oil resulted in higher C16:0 and C18:1 levels in all lipids, concomitantly with a decrease in tissue phospholipid arachidonic acid (C20:4n-6), an essential longer-chain poly-unsaturated fatty acid. Although this could be an important disadvantage of MCT feeding, quantitative measurements are necessary to investigate this matter further, using lipoprotein separation and appropriate modelling.

In conclusion, we have shown that there is a consistent enhancement of fat absorption with MCT formulas in the preterm neonate. The tolerance of MCT formula was good and no side-effects were noticed. However, except for a slight increase in the percent fat accretion in new tissue, there are no beneficial effects detectable regarding growth. Calcium absorption is also increased on MCT feeding, and this might be of advantage to the preterm population at large, regarding the fact that current calcium intakes are only marginally sufficient with either human milk or formula feeding.

On the other hand, our studies emphasize the peculiarities of the metabolism of MCTs, which is different from the LCTs which constitute the conventional food fats. The direct oxidation of MCTs is much higher than with LCTs, pointing at a preferential oxidation. This is in accordance with our finding that glucose oxidation is significantly decreased on MCT feeding, and lipogenesis from glucose is probably increased. However, as oxidation was found to be incomplete, MCFAs have to be stored, probably after conversion into LCFAs. Our data show that a direct conversion of octanoate into longer-chain fatty acids does occur in the preterm infant. Conversion of the unoxidized glucose as well as MCFAs into LCFAs requires energy and can lead to higher levels of saturated and monounsaturated fatty acids. In our studies, only the higher absolute levels of saturated fatty acids in plasma were confirmed, while we found no differences in metabolic rate between MCT and LCT groups. The explanation could be that the differences are very small and that the demonstration of it requires larger study groups, or that an increased heat production only occurs at a higher level of energy intake. The influence of MCFAs on saturated fatty acids and PUFA metabolism was recently confirmed in newborn piglets and requires further attention.

Although some authors have argued that milk from mothers who delivered preterm contains significant amounts of MCTs, this is not the case for the "true MCTs" with chain length of eight and ten carbon atoms [7]. In fact the level of fatty acids with a chain length less than 12 carbon atoms just rises from about 1- to 1.5 % in human milk with preterm delivery [8], still an insignificant portion of the energy provided when breast feeding the preterm infant.

Regarding the fact that human milk does not contain significant amounts of

MCFAs, and the proven effect of MCTs on metabolism of other substrates like glucose and (saturated) fatty acids, it seems prudent to continuously reevaluate the indications for such "artificial" foodstuff. As clinical benefits are relatively small, even in the very tiny preterm infant during the first weeks after birth, the data presented in this thesis do not support the use of MCTs in the routine nutritional management of the preterm infant.

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CHAPTER X

SUMMARY

For many years, medium-chain triglycerides (MCTs), with a chain length of eight and ten carbon atoms, have been included in preterm infant formulas. MCTs are manufactured by reesterification of medium-chain fatty acids (MCFAs) from coconut oil, and their intestinal absorption is assumed to be nearly complete, in contrast with "regular" long-chain triglycerides (LCTs). There is however no consensus on the exact benefit of their routine use in the preterm neonate.

Chapter 1 summarizes the history of the development of MCTs, and the possible clinical indications for adults. The knowledge regarding the use of MCTs in premature infants is discussed, together with possible side effects of MCTs.

Chapter 2 describes a study comparing MCT and LCT formulas in one month old preterm infants, using balance and indirect calorimetry techniques. Fat balance was improved with MCT, but the main result was a higher percent fat in the newly formed tissue, with no changes in linear growth or weight gain.

Chapter 3 describes the results in the same study groups of calcium, phosphate, and magnesium balances, and levels of several calciotropic factors. With MCT feeding, calcium retention was higher and parathyroid hormone levels were lower, but plasma levels of calcium, alkaline phosphatase, and 1,25 dihydroxy-vitamin-D were not significantly different between groups.

Chapter 4 describes the measurement of the oxidation of MCFAs in preterm infants, using orally administered octanoate labeled with the stable carbon isotope ^{13}C, and collection of breath to determine the ^{13}C enrichment in CO_2. On average 47% of the orally administered tracer was oxidized, a value much lower than reported in some studies in animals or adult human volunteers.

Chapter 5 describes a comparative study between MCT and LCT regarding glucose metabolism. Using ^{13}C-labeled glucose, evidence was found that glucose oxidation is lower with MCT feeding, whereas lipogenesis from glucose is probably increased.

Chapter 6 describes the measurement of conversion of octanoate *in vivo* into longer-chain fatty acids, using high-sensitivity gas chromatography-isotope ratio mass spectrometry (GC-IRMS). A significant enrichment of myristic and palmitic acids is found, confirming one of the proposed mechanisms of non-oxidative disposal of MCTs.

Chapter 7 describes the measurement of splanchnic oxidation of octanoate as a tracer representative for MCFAs, using a dual isotope tracer approach in preterm infants. There is indeed a high first-pass splanchnic extraction and oxidation of octanoate from the feeding. However, the "systemic" rate of appearance of octanoate is high, probably due to exchange with octanoate derived from partial β-oxidation of long-chain fatty acids, leading to difficulties estimating the "true" oxidation rate of MCFAs.

Chapter 8 describes a balance study, performed during the first month of life in extremely-low-birth weight (ELBW) infants, with a birth weight below 1000 grams. The differences found in this particular patient group between MCT and LCT feeding with regard to intestinal absorption of fat and calcium, are not more pronounced than those in the somewhat larger preterm infants that were studied in chapters 2 and 3.

SAMENVATTING

Al geruime tijd worden middel-keten triglyceriden (medium-chain triglycerides, MCTs), triglyceriden met ketenlengte van acht en tien koolstofatomen, toegepast in de voeding voor te vroeg geboren kinderen. Deze MCTs, gefabriceerd door verestering van middel-keten vetzuren (medium-chain fatty acids, MCFAs) uit kokosnootolie, worden in tegenstelling tot de "normale" lang-keten vetzuren waarschijnlijk vrijwel volledig opgenomen in de darm. De literatuur is echter niet eenduidig over de werkelijke voordelen van de routinematige toevoeging van MCTs aan de voeding voor de preterm geboren neonaat.

Hoofdstuk 1 vat de geschiedenis samen van de ontwikkeling van MCTs, en de eventuele klinische indicaties voor gebruik bij volwassenen. De literatuur over het gebruik van MCTs bij prematuur geborenen wordt besproken, inclusief de mogelijke bijwerkingen van MCTs.

Hoofdstuk 2 beschrijft een studie waarin MCT en LCT ("long-chain triglyceride") voedingen worden vergeleken bij ongeveer één maand oude preterm geborenen, met behulp van balanstechnieken en indirecte calorimetrie. De vetbalans was sterker positief in de MCT groep, maar dit resulteerde slechts in een hoger vetpercentage van het nieuw gevormd weefsel, terwijl er geen verschil tussen de groepen viel aan te tonen in lengtegroei of gewichtstoename.

Hoofdstuk 3 beschrijft de resultaten in dezelfde studiegroepen van balansstudies van calcium, fosfaat en magnesium en de plasma spiegels van enkele calciotrope factoren. De retentie van calcium was hoger, en de parathyroid hormoon spiegel was lager tijdens MCT voeding. Echter, er waren geen verschillen tussen de beide groepen in de plasma spiegels van calcium, alkalische fosfatase of 1,25-dihydroxy-vitamine-D.

Hoofdstuk 4 beschrijft de meting van de oxidatie van MCFAs in preterm geborenen, gebruikmakend van de orale toediening van octanoaat gelabeled met ^{13}C (een stabiel koolstof isotoop), waarbij de uitademingslucht werd opgevangen om de ^{13}C-verrijking in CO_2 te bepalen. Gemiddeld werd 47% van de oraal toegediende tracer geoxideerd. Deze waarde is veel lager dan gewoonlijk gevonden wordt in proefdieren

of volwassen vrijwilligers.

Hoofdstuk 5 beschrijft een vergelijkende studie tussen MCT en LCT voeding wat betreft het metabolisme van glucose. Met gebruikmaking van stabiele isotopen, werd gevonden dat de glucose oxidatie lager is tijdens MCT voeding, terwijl lipogenese vanuit glucose waarschijnlijk is verhoogd.

Hoofdstuk 6 beschrijft de meting van de omzetting van octanoaat *in vivo* tot langere-keten vetzuren, met gebruikmaking van een zeer gevoelige gaschromatograaf-isotopen-ratio massa-spectrometer (GC-IRMS). Er werd een significante ^{13}C-verrijking in myristinezuur en palmitinezuur gevonden, wat één van de voorgestelde mechanismen van verwerking door het lichaam van niet-geoxideerde MCTs bevestigt.

Hoofdstuk 7 beschrijft de meting van de splanchnische oxidatie van octanoaat, als een representatieve tracer voor MCFAs, onderzocht door de tracer zowel oraal als intraveneus toe te dienen. Er is inderdaad een grote extractie van octanoaat tijdens de eerste passage door het splanchnische bed en een deel van de octanoaat afkomstig uit de voeding wordt hier direct geoxideerd. De "systemische" verdunning door ongelabeled octanoaat is echter hoog, waarschijnlijk door uitwisseling met octanoaat afkomstig van β-oxidatie van langere vetzuren, waardoor het moeilijk wordt de "ware" oxidatiesnelheid te schatten.

Hoofdstuk 8 beschrijft tenslotte een balansstudie die gedaan werd in de eerste maand na de geboorte in preterme pasgeborenen met een geboortegewicht onder de 1000 gram (extremely-low-birth weight, ELBW). De verschillen die in deze speciale patiëntengroep werden gevonden tussen MCT en LCT voeding wat betreft de opname in de darm van vet en calcium, zijn niet groter dan welke worden gevonden in oudere preterme pasgeborenen zoals bestudeerd in hoofdstuk 2 en 3.

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ABSTRACTS

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