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ORIGINAL CONTRIBUTION

Choline supply of preterm infants: assessment of dietary intake and pathophysiological considerations

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Abstract
Background Choline forms the head group of phosphatidylcholines, comprising 40–50 % of cellular membranes and 70–95 % of phospholipids in surfactant, bile, and lipoproteins. Moreover, choline serves as the precursor of acetylcholine and is important for brain differentiation and function. While accepted as essential for fetal and neonatal development, its role in preterm infant nutrition has not yet gained much attention.
Methods The adequate intake of choline of preterm infants was estimated from international recommendations for infants, children, and adults. Choline intake relative to other nutrients was determined retrospectively in all inborn infants below 1,000 g (extremely low birth weight) or below 28 weeks gestational age, admitted to our department in 2006 and 2007 ($N = 93$).
Results Estimation of adequate intake showed that children with 290 g body weight need more choline than those with 1,200 g (31.4 and 25.2 mg/kg/day, respectively). Day-by-day variability was high for all nutrient intakes including choline. In contrast to the continuous intrauterine choline delivery, median supply reached a plateau at d11 (21.7 mg/kg/day; 25th/75th percentile: 19.6; 23.9). Individual choline supply at d0–d1 and d2–d3 was <10 mg/kg/day in 100 and 69 % of infants, respectively. Furthermore, intakes <10 mg/kg/day were frequently observed beyond day 11. Median adequate intakes (27.4 mg/kg/day at 735 g body weight) were achieved in <2 %.
Conclusions Nutritional intake of choline in this cohort of preterm infants was frequently less than the estimated adequate intake, with particular shortage until postnatal d10. Because choline is important for brain development, future studies are needed to investigate the effects of adequate nutritional choline intake on long-term neurodevelopment in VLBW infants.

Keywords Choline deficiency · Enteral nutrition · Parenteral nutrition · Preterm infants

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Introduction

Choline is an essential component of phosphatidylcholine (PC) and sphingomyelin, both phospholipids comprising about 50 % of all cellular membranes in vertebrates including humans. Also, PC is the major phospholipid in many secretions, like lung surfactant, bile, and lipoproteins, comprising 80–95 % of secreted phospholipids [1–3]. Additionally, choline is present in the central and peripheral nervous system, where it serves for the synthesis of acetylcholine and is important for brain development [4–6]. Finally, after oxidation of choline to betaine, choline serves as a methyl donator for the formation of methionine from homocysteine. After activation to *S*-adenosyl-methionine, this component is the methyl donator for the endogenous synthesis of PC from phosphatidylethanolamine in the liver via phosphatidylethanolamine-*N*-methyl transferase (PEMT), for the synthesis of creatine, and for many other methylation reactions [7, 8]. Choline, methionine, and folate metabolism interact, as methionine can also be formed from homocysteine in a cobalamine- and folate-dependent manner by methionine synthase [9]. In essence, however, choline is a dietary component essential

for normal cell function and development of all organs [6, 10].

Choline influences fetal and postnatal stem cell proliferation and apoptosis in the brain and improves neural tube development. In later life, fetal/perinatal deficiency induces disorders in memory and in the function of liver, kidneys, and pancreas [for review see: 11]. Principally, choline concentrations in fetal plasma and amniotic fluid are 3–10 times higher than in plasma of pregnant women, due to an active, directed transport through the placenta, where choline is stored as acetylcholine to maintain a constant supply of choline to fetal plasma [11–14]. As a consequence, maternal stores of PC are progressively depleted during pregnancy in spite of increased choline generation via the hepatic PEMT reaction. Consequently, nutritional choline needs are increased during pregnancy [15]. According to the essential nature of choline for neuronal and parenchymal development and homeostasis, the National Academy of Sciences has provided recommendations on adequate daily intake, which are higher for pregnant (450 mg/day) and lactating (550 mg/day) than for other premenopausal women (400–425 mg/day). Recommended weight-adjusted doses are also higher for infants of 0–6 months (18 mg/kg/day or 125 mg/day) than for adult males (550 mg/day = 8.34 mg/kg/day) [16, 17]. Supposedly, choline needs of preterm infants are even higher because of their exceptionally high growth rate [18].

For this study, we estimated the adequate intake of choline for preterm infants from recommendations issued by the National Academy of Sciences [16] and determined the effective supply of choline, folic acid, and methionine as well as of macronutrients via enteral and parenteral nutrition in extremely low-birth-weight (ELBW; <1,000 g) or low-gestational-age (<28 weeks) infants during a 2-year period.

Methods

Ethics committee approval is not required in Germany for this type of retrospective observational study intended for quality assurance.

Estimation of adequate intake

Weight-adjusted choline demand during growth was calculated according to published age-dependent recommendations for adequate intake of choline in infants, children, and adolescents from 0 to 18 years [11, 16], based on the calculations of human milk content for 0–6-month-old infants, prevention of alanine amino transferase (ALT) increases in adults, and extrapolations for children according to the equation adequate $\text{intake}_{\text{child}} = \text{adequate intake}_{\text{adult}} \times F$, where $F = (\text{weight}_{\text{child}}/\text{weight}_{\text{adult}})^{0.75} \times (1 + \text{growth factor})$ [19]. Data were combined with median postnatal body weight according to age of Western European populations [17] (Table 1). From the resulting logarithmic function of growth-dependent adequate intake of choline, that of preterm infants was calculated according to body weight at birth, as the initial postnatal period was assumed to be the most vulnerable during development [20]. For folate, adequate intake of preterm infants (35 μg/kg/day) was retrieved from recommendations by the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition [21].

Study population

We evaluated data from all 96 inborn preterm infants consecutively admitted in 2006–2007 who had either a birth weight <1,000 g or a gestational age (GA) at birth <28 weeks (Table 2). Three infants were excluded as they had died within 72 h after birth. Data were collected from d0 ($N = 93$) until d98 ($N = 31$) or to discharge home, transfer, or death ($N = 62$).

Unit guidelines for enteral and parenteral nutrition

During the study period, enteral nutrition (breast milk or preterm infant formula) was commenced on day 1 of life at 10–15 ml/kg/day and advanced by 15–20 ml/kg/day. Expressed breast milk was collected from the infant's own mother and administered via gavage or bottle. Breast feeding was introduced during the last 1–2 weeks before discharge, and intake was then determined by subtracting pre- from postfeeding weight at each feed. Because breast milk does not meet the requirements [43] of rapidly growing preterm infants, breast milk fortification was initiated as soon as 150 ml/kg/day was tolerated. Whereas parenteral glucose (starting with 6–7 g/kg/day) and amino acid infusions (starting with 2–2.5 g/kg/day) were commenced on the first day of life to complement enteral feeds, parenteral lipid (20 % fat emulsion; Table 3)- and fat-soluble vitamin emulsions were generally started on day 3 of life, but only if enteral feeding advancements were slower than expected. Every infant who received parenteral lipids received at least 4 ml/kg/day of the fat-soluble vitamin emulsion. During periods of severe systemic inflammatory response (e.g., sepsis, necrotizing enterocolitis), parenteral lipid emulsions were reduced or even temporarily withheld. These nutritional practices were unchanged during the 2-year observational period.

Determination of exact nutrient intake

Patient files were analyzed for nutritional intake. Concentrations of total energy, protein/amino acids, fat,

Table 1 Postnatal body weights and adequate intakes of choline

Postnatal age	Choline recommended [16] (mg/day)	Mean body weight according to [17] (kg)	Choline recommended on weight basis (mg/kg b.w./day)
0–6 months	125	6.12 at 3 months	18 [16][a]
6–12 months	150	9.51 at 9 months	17 [16][a]
1–3 years	200	13.46 at 2 years	14.86[b]
4–8 years	250	21.81 at 6 years	11.46[b]
9–13 years	375	36.56 at 11 years	10.26[b]
14–18 years	550	65.94 at 16 years	8.34[b]

Data were taken from the literature [16, 17]. They were either directly adopted ([a]) or used to calculate adequate intakes of choline on a weight basis from combination of refs. [16, 17] ([b]). These data are the basis to estimate adequate intake levels of the study cohort of preterm infants by semilogarithmic extrapolation (see "Results"; Fig. 1)

Table 2 Patient characteristics

Number of infants admitted[a]	96
No. of infants evaluated	93
Birth weight (g) (mean ± SD) (range)[b]	758 ± 202 (290–1,200)
Gestational age at birth (weeks) (mean ± SD) (range)[b]	26.5 ± 2.2 (23.0–32.0)
No. of males/females[b]	42/51
No. of infants who never received parenteral amino acids[b]	0
No. of infants who never received parenteral lipids[b]	8
No. of infants who received <200 ml expressed breast milk[b]	15
Duration of data collection (days) (mean ± SD) (range)	77 ± 24 (7–98)

[a] Fulfilling the inclusion criteria: birth year 2006 or 2007. Birth weight <1,000 g or gestational age ≤28 weeks. Inborn

[b] Of those evaluated

Table 3 Macro- and micronutrient content of enteral and parenteral nutrition components during the study period

	Preterm human milk[a] (per 100 ml)	Preterm formula[b] (per 100 ml)	Human milk fortifier[b] (per 5 g)	Amino acid solution[b] (per 1 ml)	Lipid emulsion[a] (per 1 ml)	Fat-soluble vitamin emulsion[b] (per 1 ml)	Water-soluble vitamin solution[b] (per 1 ml)	Glucose solution[b] (per 1 g)
Protein (g)	1.41	2.3	1	0.1	0	0	0	0
Fat (g)	3.9	4.2	0.02	0	0.2	0.1	0	0
Carbohydrates (g)	6.6	8.6	0.515	0	0	0	0	1
Choline (mg)	13.7	12.4	0.58	0	1.1[c]	1.664[c]	0	0
Methionine (mg)	30	50	23	4.3	0	0	0	0
Folic acid (μg)	4.3	56	50	0	0	0	40	0
Energy (kcal)	67	80	18	0.41	1.9	0.95	0	4.1
Energy (kJ)	280	335	75	1.7	8	4	0	17

[a] According to [20, 21]

[b] Data according to manufacturer

[c] Phosphatidylcholine as an emulsifier was the only choline source here. Mean values of human milk contents were retrieved from mothers of moderately preterm infants (27–35 weeks gestational age) at postnatal days 1–31 for folic acid, and day 27–32 for choline [20, 21]. The preterm infant formula was designed for all preterm infants <2,500 g birth weight. The parenteral nutrition components were specifically designed neither for preterm infants nor for term neonates, but also for older children and adults. The preterm infant formula was designed for all preterm infants <2,500 g birth weight. According to current recommendations [41], this formula contains more protein and energy (i.e., fat and carbohydrates) than preterm human milk. According to the available information, choline concentration was similar for human milk and preterm formula—although the individual choline-containing compounds may differ [20]

carbohydrates, choline, folate, and methionine were calculated, based on the manufacturer's information on the preterm infant formula and parenteral nutrition components used in the nursery at that time (preterm formula: BEBA-FN®, Nestlé Nutrition, Frankfurt, Germany; multicomponent human milk fortifier: FM85, Nestlé Nutrition;

Vitalipid Infant and Clinoleic, Baxter Deutschland GmbH, Unterschleißheim, Germany; Aminoven 10 % Kabi, Fresenius, Bad Homburg, Germany), and the literature available for breast milk constituents [21, 22]. The concentrations of the macro- and micronutrients of interest in each nutritional component are depicted in Table 3. Exact daily oral and parenteral intake of formula, mother's milk, fortifier, and parenteral nutrition was recorded, and the amount of individual nutrients was calculated using a purpose-written Excel-based macro. Supplied amounts of total energy were calculated from energy density of the macronutrients, which was 17 kJ/g for carbohydrates and proteins/amino acids and 39 kJ/g for fat. Potential variations in enteral absorption were not taken into account.

Statistical analysis

Data are provided as medians and 25th/75th percentiles. Where appropriate, minimum and maximum values are indicated as well to demonstrate the broad range of nutrient supply. Calculation of regression curves was performed using Excel 2002 (Microsoft Corporation, Redmont, WA, USA). Statistical analysis was performed using InStat 3.0 (GraphPad Software, Inc., La Jolla, CA, USA). Significance was accepted at $P < 0.05$.

Results

The recommended adequate intakes of choline, ranging from 8.34 mg/kg/day at 16 years to 18 mg/kg/day at 3 months of age, were related to the respective median body weights from 6.1 kg at 3 months up to 66 kg at 16 years (Table 1). This resulted in a logarithmic function of $y = -4.3458 \times \mathrm{Ln}(x) + 26.02$ ($R^2 = 0.9699$) (Fig. 1, diamonds and solid line), where adequate intake is inversely related to body weight. Here y is the adequate intake for choline in mg/kg/day, and x equals body weight in kilogram. The estimated adequate intake values for choline supply in preterm infants are indicated as filled squares and a dashed line. Employing this function for the preterm infants included in this study resulted in adequate intake values increasing from 25.2 to 31.4 mg mg/kg/day for infants from 1,200 g down to 290 g body weight. As median birth weight of the study cohort was 735 g, the estimated median adequate intake was 27.4 mg/kg/day (Fig. 1).

Patient characteristics are displayed in Table 2. Of the 93 patients, 67 were below both 28 (range, 23.14–27.14) weeks GA and 1,000 (290–995) g birth weight; 20 infants were between 28.14 and 32.14 weeks GA, weighing 650–995 g at birth. These patients fulfilled the definition of being ELBW infants, whereas 6 infants of

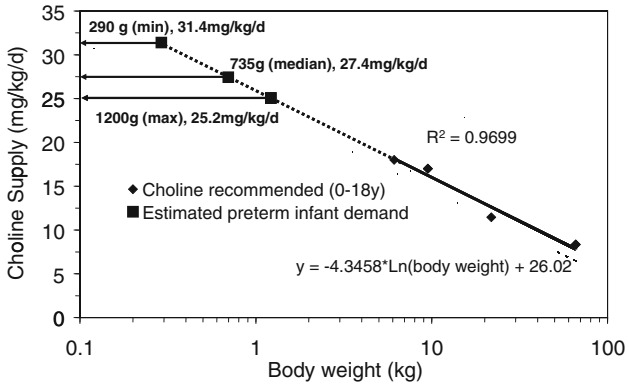

Fig. 1 Estimation of adequate choline intake relative to body weight. Data points are taken from the dietary recommendations on adequate intake of choline/day for humans from 0 to 18 years of age [11] relative to the median body weight in Western communities [17, 20]. *Filled diamonds* and *solid lines* indicate postnatal adequate intake values. Estimated adequate intake from best fit logarithmic function is indicated as *dashed line* and *solid squares*

26.14–27.14 weeks GA had birth weights from 1,010 to 1,200 g, therefore only fulfilling the characteristics of being very low birth weight (VLBW). Median hospital stay in our center was 86 days (7–187 days; 25th/75th percentile: 59, 108 days). Parenteral nutrition was administered starting on day 1 to complement enteral feeds according to unit guidelines, and occasionally later because of gastrointestinal complications. All 93 patients received parenteral amino acids, whereas only 85 received parenteral fat.

Choline

Choline supply strongly correlated with that of triglycerides (Fig. 2a, b). As parenteral choline essentially comprises lipid-bound choline (PC; see Table 3), which is not cleaved prior to absorption, and bypasses the portal circulation, enteral and parenteral supply is shown separately. Parenteral (phosphatidyl)choline was a major contributor to choline supply during the first week of life (Fig. 2a). Later on, parenteral choline was only occasionally administered via parenteral fat and fat-soluble vitamins, that is, when enteral feeding had to be restricted (see "Unit guidelines for enteral and parenteral nutrition"). Whereas median values for parenteral supply were zero from d7 onward, enteral choline supply increased linearly from d0 to d7 onward, reaching a median level of 21.7 mg/kg/day (25th–75th percentile, 19.6–23.9 mg/kg/day) at d11. Notably, median choline intake was below the estimated median adequate intake of 27.4 mg/kg/day throughout. While maximum values of enteral choline occasionally reached this value beyond d11, minimum daily choline supply was frequently zero (Fig. 2a). Data beyond d28 did not differ from those found between d11 and d28 (not shown).

Like parenteral choline, median parenteral fat peaked at d5 and had returned to zero by d7 (Fig. 2b). Median choline-to-fat ratio was 10.7 mg/g; 25th–75th percentile, 8.9–16.6 mg/g. The high interquartile range in this ratio reflected the daily differences in concomitant fat-soluble vitamin supplementation, with high concentration of PC as an emulsifier, but little triglyceride content (Table 3). Enteral fat increased in a linear fashion from d0 to d7 (Fig. 2b), as did enteral choline (Fig. 2a), and reached a plateau of 6.6 (5.91–7.03) g/kg/day from d11 onward. One gram of enteral fat equaled 3.3 mg enteral choline, with narrow ranges for choline/fat ratio (25th–75th percentile, 3.0–3.6 mg/g). Minimum values occasionally were zero (like those for choline) if enteral feeding restrictions had been deemed necessary.

Relation of choline supply to gestational age and birth weight

As shown in Fig. 1, the estimated adequate intake increases at lower body weight. However, in clinical reality the opposite was found for actual choline intake. Figure 3a demonstrates for the first 11 days after birth that choline supply was positively correlated with birth weight and GA (Fig. 3b), reflecting the difficulties in rapidly establishing enteral feeding in more immature infants. However, in spite of these significant correlations, the interindividual range of choline intake at any given birth weight or GA exceeded by far the differences in median supply between the most immature and more mature infants.

Variability in choline supply

As intrauterine nutrition is characterized by a constant nutrient supply, we further analyzed the individual day-by-day variability in supply to assess whether critical shortage in choline supply exceeding 24 h occurred under intensive care conditions. During initial total parenteral nutrition, no free choline was administered, as parenteral nutrients only contained PC (Table 3). Whereas the proportion of patients with critical shortage below 10 mg/kg/day continuously decreased, daily choline intakes of ≥27.4 mg/kg/day were reached in only 1–2 % of patients beyond postnatal d11 (Fig. 4a). In agreement with the data shown in Fig. 3, there was a trend toward more delayed enteral feeding below 500 g birth weight. However, this only refers to 6 infants (not shown). During treatment, choline supply was interrupted whenever enteral nutrition was not appropriately increased, reduced, or even discontinued. As exemplarily shown in Fig. 4b for three patients, this frequently happened due to impaired feeding tolerance, necrotizing enterocolitis, intestinal perforations, acute abdomen and sepsis, or other reasons like meconium plug, coprostasis, and in perioperative periods. In patient no. 53, choline supply was continuously increasing with increasing enteral feeds. However, equilibrium was delayed due to initial feeding intolerance. Patient no. 73 quickly reached

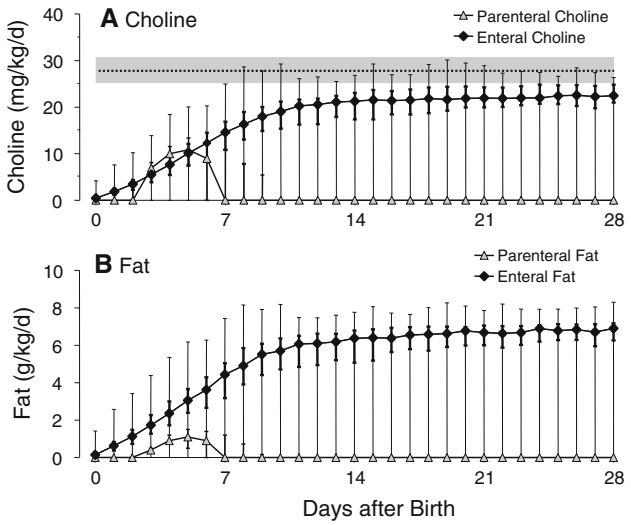

Fig. 2 Supply of preterm infants with enteral and parenteral choline (**a**) and fat (**b**). Data were calculated as described in "Methods" and are indicated as medians. *Thick bars* represent the 25th and 75th percentile, and *thin bars* represent minimum and maximum of a total of 93 (one case of death at d27) patients. The *dashed line* in **a** defines the median estimated adequate choline intake of the study group (27.4 mg/kg/day), whereas the *gray box* indicates estimated values for 1,200 g (25.2 mg/kg/day) and 290 g (31.4 mg/kg/day) birth weight

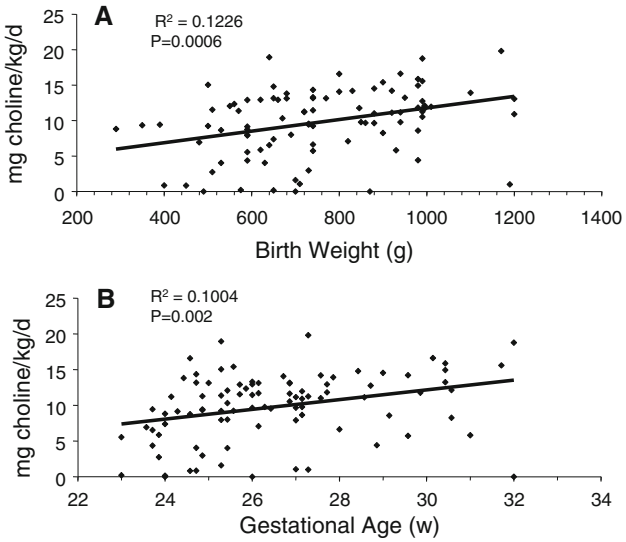

Fig. 3 Enteral choline supply of preterm infants in relation to birth weight (**a**) and gestational age (**b**). Data on median enteral choline supply from d0 to d11 were calculated from total nutrient intakes of the 93 patients indicated in Fig. 2. From these values median intakes, linear regression, and statistical significance were calculated as described in "Methods"

equilibrium values. This was, however, not maintained due to necrotizing enterocolitis with perforation of the small intestine, followed by a slow increase that reached adequate intake after d28 (not shown). In patient no. 82, daily choline supply followed median values up to d6/d7, but then decreased twice to very low values. This was due to an episode of acute abdomen (d8–d11), with need for enterostomy and decompression of impacted stools, and a second decrease to 5 mg/kg/day at d18/19 due to an episode of feeding intolerance during *Staphylococcus haemolyticus* sepsis.

Other macronutrients relative to choline supply

To relate choline to the overall supply with nutrients, Fig. 5a describes the total energy (which is the sum of protein, fat, and carbohydrates) via enteral and parenteral delivery. Initially, a major part of total energy was from parenteral supply, reaching its maximum on d3–4, while after 10 days enteral nutrition was generally established. These time curves were similar for the supply with parenteral and enteral protein (Fig. 5b), which as a macronutrient is important to development, and as a progenitor of exogenous methionine for the synthesis of activated methyl groups. Total carbohydrates reached their plateau on d5 (not shown), with 11.5 g/kg/day (25th–75th percentile, 10.4–12.7 mg/kg/day). Summing up parenteral and enteral values, a median total energy supply of at least 500 kJ/kg/day was achieved by d12 (median, 512 kJ/kg/day; 25th–75th percentile, 438–546). For total protein/amino acid, 3 g/kg/day was already surmounted on d2 (median, 3.7 g/kg/day; 25th–75th percentile, 3.0–3.6) (not shown). The rather small range of energy and protein supply was due to the standardized nutritional protocol in place in the unit. Nevertheless, enteral supply of macronutrients was frequently zero, and this was only partly compensated for by parenteral nutrition. This is shown by the minimal values of total energy supply, which were frequently below 0.4 mJ/kg/day and below 4 g protein/kg/day as their median values (Fig. 5a, b).

Choline-related nutrients

Other choline-related nutrients, like folate and enteral methionine, reached their plateau after 2 weeks, with median values of 89.7 mg/kg/day (25th–75th percentile, 74.7–99.0) for folate and 85.5 (75.6–92.5) mg/kg/day for methionine. Median values for parenteral folate and methionine were zero from d8 and d10 onward. A median value of 35 mg/kg/day folate, which is its recommended supply [43], was already achieved by d3, via initial high parenteral and subsequent enteral supply (Fig. 6a, b). Similar time courses of supply were found for methionine

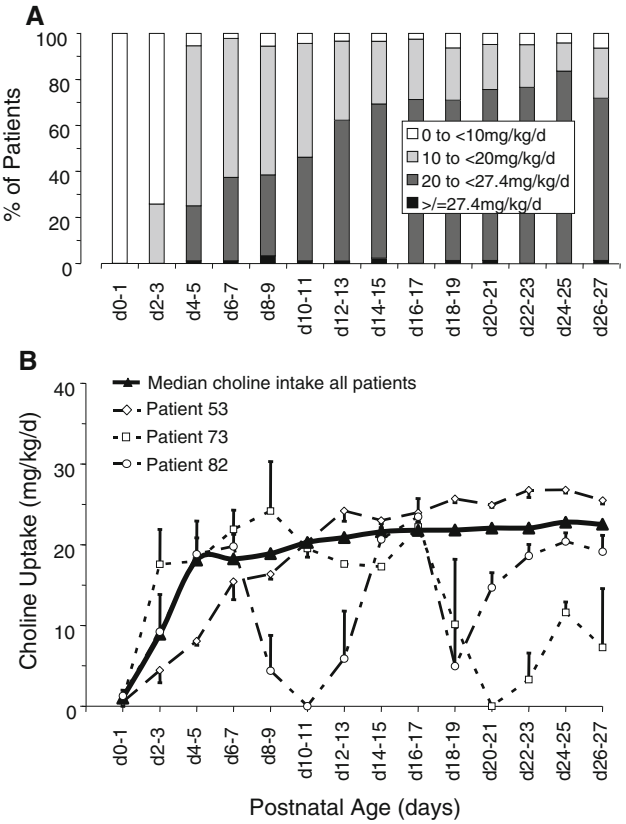

Fig. 4 Incidence of choline intake below adequate intake (**a**) and mean daily choline supply of individual representative patients (**b**) from d0 to d27. **a** Proportion of infants with choline supply of 0 to <10, 10 to <20, 20 to <27.4, ≥27.4 mg/kg/day based on all evaluated infants from d0 ($N = 93$) to d42 ($N = 88$). **b** Choline supply of individual patients from d0 to d27. Data are mean value and range of 2 consecutive days; *closed triangles* and *solid line* indicate median values of the whole cohort. Patient no. 53 (*open diamonds*): female, 26 weeks + 2 days gestational age, 940 g birth weight. Mechanically ventilated for 5 days, on continuous positive airway pressure (CPAP) for 64 days, supplemental oxygen for 77 days; delayed tolerance of enteral feeding advancements. Patient no. 73 (*open squares*): female, 24 weeks + 6 days gestational age, 390 g birth weight. Mechanically ventilated for 23 days, CPAP for 90 days, supplemental oxygen for 77 days; necrotizing enterocolitis with intestinal perforation on d18. Patient no. 82 (*open circles*): female, 30 weeks + 1 day gestational age, 940 g birth weight, 30 days CPAP with supplemental oxygen for 30 days, episode of acute abdomen at d9, feeding intolerance at d18/19 during *Staph. haemolyticus* sepsis

(Fig. 6c, d), for which adequate intake values are unknown, as a result of parenteral amino acid and enteral protein administration (Table 3). However, minimum individual daily intakes of folate and methionine were frequently zero during postnatal intensive care (Fig. 6a–d).

Discussion

This retrospective analysis demonstrates that the nutritional reality during neonatal intensive care does not match the

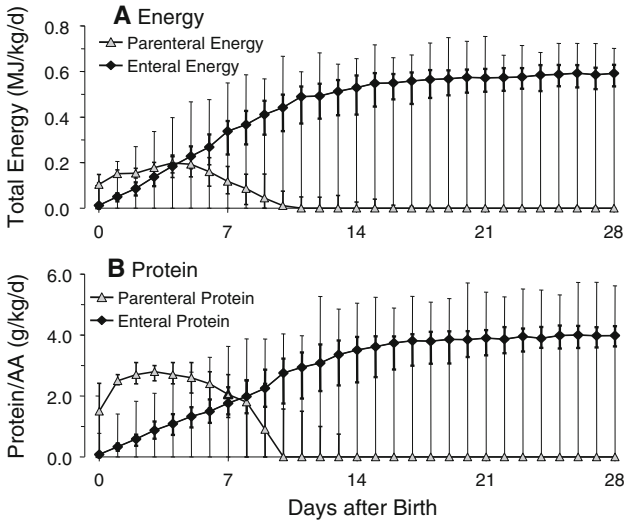

Fig. 5 Supply of hospitalized preterm infants with total energy (**a**) and protein/amino acids (**b**). Data were calculated as described in "Methods" and are indicated as medians. *Thick bars* represent the 25th and 75th percentile, and *thin bars* represent minimum and maximum of 93 (one case of death at d27) patients. Data are indicated as medians

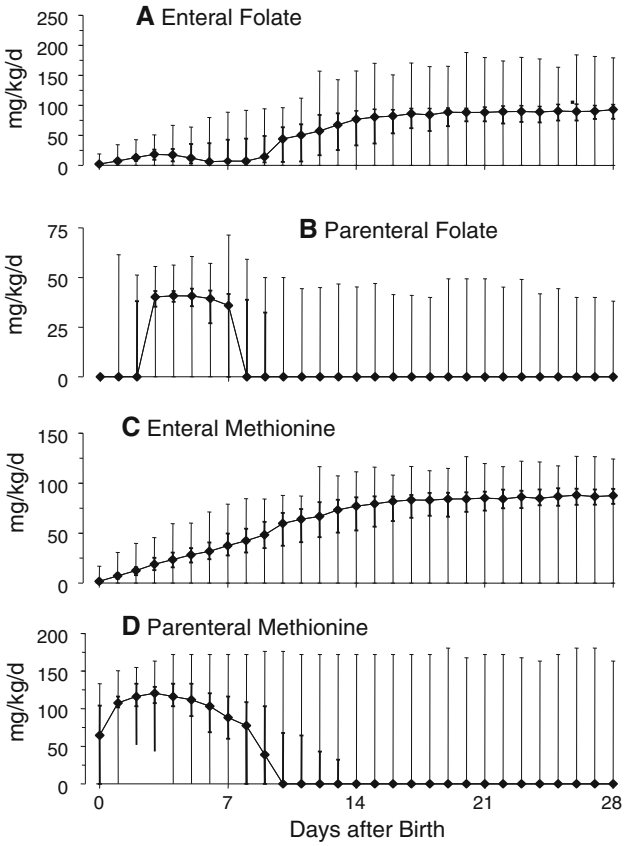

Fig. 6 Supply of hospitalized preterm infants with methionine and folate. Enteral (**a**, **c**) and parenteral (**b**, **d**) supply of folate (**a**, **b**) and methionine (**c**, **d**) is indicated as *median* values. *Thick bars* represent the 25th and 75th percentile, whereas *thin bars* represent minimum and maximum values of 93 (one case of death at d27) patients. Data are indicated as medians

intrauterine situation of a continuous, physiologic supply with choline. In contrast, whereas neonatal intensive care should continuously meet all nutritional needs of the rapidly growing preterm infant, our findings show that choline intake was below the adequate intake in these infants. This was caused by an initial delay in enteral feeding, and by inter- and intra-individual dietary variations later on. The importance of choline in fetal growth is highlighted by the threefold to fourfold enrichment of choline in fetal (36 µmol/l) compared with maternal plasma (10–12 µmol/l) [14]. This is consistent with our estimated adequate intake of preterm infants, which is about 3–4 times higher than that of adults, ranging from 25.2 to 31.4 mg/kg/day in our study group [6]. The rapid decrease in plasma concentration to one-third of this value after term birth and the rapid clearance of deuterium-labeled choline from plasma [23] indicate that tissues rapidly take up all choline available. Nevertheless, plasma choline remains above >14 µmol/l for 1–2 years in term infants, a prerequisite of increased choline uptake into brain and other tissues [18, 24]. Extracellular choline concentrations depend on intake and correlate with the rate of PC and acetylcholine synthesis in cells [18, 25–27]. The reason for this is the high constant of Michaelis of low-affinity choline transporters in plasma membranes [26, 28, 29]. Therefore, temporarily and even more so continuously insufficient choline supply may curtail organ growth and differentiation in preterm infants, thereby potentially contributing to the neurocognitive impairment seen in this population [6, 11].

The need for choline is related to organ growth, differentiation, and signaling [6, 10, 11, 15, 16]. Just the liver by itself, as a central organ of metabolism, grows by 125 g between 24 and 40 weeks GA [30]. As the PC content of liver tissue increases toward 20 µmol/g [31], the liver's choline pool alone increases by more than 260 mg, not taking into account this organ's specific role in supplying the brain and other organs with choline and polyunsaturated PC [18, 32]. Although the exact need of choline is unknown in preterm infants, our data are interpreted in the context of our estimated adequate intake of choline of (at least) 25 mg/kg/day as extrapolated from the adequate intake values for term infants, children, and adolescents recommended by the National Academy of Sciences [16]. For choline, epidemiological studies on "estimated average requirement" (EAR), which defines the median amount sufficient for 50 % of a population, and "recommended dietary allowances" (RDA), defining sufficient supply of more than 95 % of individuals, do not exist. Adequate intake values are used as the guide when data are

insufficient to establish an RDA [16]. They are recommended average daily nutrient intakes, based on experimentally derived or observed mean intake levels by apparently healthy people. The published adequate intake values for choline in humans from term delivery to 18 years were derived from choline content in human milk, and intakes that prevented elevations in liver enzymes in adults [16, 19]. Differences in the parameters used to assess calculation of deficiency will affect adequate intake values. Recent studies suggest that not all individuals on a diet below adequate intake will develop clinical symptoms. On the other hand, recommended adequate intake values may be too low for at least 20 % of adults, depending on gender, age, and genetic background [18, 33]. Therefore, supply below adequate intake values does not necessarily define choline deficiency in an individual, whereas in others, this may be critical. In contrast, administration even above adequate intake does not necessarily indicate sufficient supply, as for this the duration and continuation of supply must also be taken into account. In essence, the lower the intake relative to adequate intake value is, the higher the probability of choline deficiency is [19, 34].

The uncertainty about choline requirements of preterm infants is reflected by the vague recommendation of the ESPGHAN Committee on Nutrition [43], suggesting a range of 8–55 mg/kg/day of enteral choline supply. The lower limit of this recommendation is even below 50 % of the adequate intake of term-born infants during 0–6 months of age (18 mg/kg/day), which is based on choline supply via human milk [16, 19]. The upper limit of 55 mg/kg/day, far beyond our estimation of 25 mg/kg/day, indicates that toxicity is not expected even during high-dose choline supplementation. Our estimation of an adequate intake of at least 25 mg/kg/day is further supported by experiments in adults using deuterium-labeled plasma choline [23, 32]. Assuming for simplicity that choline is freely diffusible into cells proportional to the extracellular concentration (in reality it is actively enriched in cells and readily phosphorylated for PC and acetylcholine synthesis [14, 27]), total choline pool in an adult of 70 kg birth weight, 60 % body water, and 10 µmol/l choline in plasma is 420 µmol (43.7 mg). Its turnover of 3 h [23, 32] therefore means a need of at least 350 mg/24 h equaling 5.0 mg/kg/day, which is very close to the adequate intake of 8 mg/kg/day [16]. For a fetus of 1,000 g birth weight comprising 85 % body water [35, 36], a physiological choline concentration of 36 µmol/l means a total choline pool of 30.6 µmol/kg birth weight. Assumed that choline metabolism in fetuses is as effective as in adults, as evidenced by metabolic studies [32, 37, 38], this would result in a turnover and calculated demand of 245 µmol/kg/day (25.46 mg/kg/day), consistent with our estimated adequate intake (Fig. 1). However, because of a potentially more rapid choline turnover and additional urinary and fecal losses [39], the "true adequate intake" of preterm infants may still be considerably higher. Further studies are, therefore, required to determine choline metabolism in preterm infants [32].

Choline supply, in the preterm infants investigated here, primarily depended on postnatal age and on oral fat intake and correlated with the increasing supply of other nutrients given orally. In fact, median enteral intake increased steadily from zero until reaching a plateau of 21.7 mg/kg/day from postnatal d11 onward. Intakes of 25 mg/kg/day or more, however, were reached in <20 % of infants despite sufficient total energy supply. In contrast to formula diet, choline concentration of breast milk is variable [24]. We employed mean values as we were unable to perform day-by-day biochemical analyses of individual milk samples. Consequently, actual minimum and maximum choline intake during breast milk feeding may differ to some degree from calculated values. Nevertheless, the observation that choline supply of preterm infants is inappropriate during the first 10 days after delivery and frequently thereafter due to interruption of oral feeding cannot be questioned based on the variable choline content of human milk. Decreases in continuous choline supply, as shown for individual patients in Fig. 4b, were a result of discontinued enteral alimentation. Figure 4b does not take into account parenteral glucose, amino acids, lipids, and lipid-soluble vitamins, which either contained no choline or choline only in the form of PC as an emulsifier. In spite of the higher choline-to-fat ratio in parenteral compared to enteral fat, choline deficiency-associated steatosis is not prevented by PC in parenteral lipids, but only by supplementation with free choline [40]. In essence, daily choline supply across patients varied largely, often by more than one order of magnitude, which is in contrast to the constant supply to the growing fetus in utero [6, 11, 13]. Whether this choline undernutrition results in decreased postnatal plasma choline concentrations in preterm infants compared to cord blood from postmenstrual age-matched fetuses/newborns is currently addressed by our group in an ongoing study.

Theoretically, insufficient choline supply can be compensated by endogenous choline synthesis: In liver, PC can be formed via stepwise methylation of phosphatidylethanolamine (PE) via the PE methyl transferase (PEMT) pathway, requiring activated methyl groups in the form of *S*-adenosylmethionine (SAMe) [41]. SAMe can be derived from exogenous methionine via enteral protein or parenteral amino acid supply. Moreover, methionine can be synthesized from homocysteine by methionine synthase, requiring 5-methyltetrahydrofolate and vitamin B12. Finally, it can be formed by the betaine homocysteine *S*-methyltransferase (BHMT) reaction, requiring betaine from choline degradation [32]. Unlike enteral methionine,

parenteral methionine is preferentially transaminated in peripheral tissues rather than providing methyl groups for hepatic choline synthesis [42, 43]. Because of the combined deficiency in enteral choline and methionine supply during both the first 14 days of life and all subsequent periods of discontinued enteral nutrition, it seems unlikely that choline deficiency can be compensated by endogenous synthesis. Furthermore, genetic variability in folate metabolism and the BHMT reaction may affect choline availability despite apparently sufficient folate intake [44–46]. Finally, it is unclear whether the PEMT pathway is sufficiently active in the growing liver of preterm infants, as in fetal rats it is not [47]. PEMT in mature hepatocytes is under control of estrogens, but estrogen concentrations decrease by 99 % as soon as the feto–placento–maternal unit is disrupted [7, 48]. Consequently, it seems unlikely that choline deficiency can be compensated by endogenous synthesis.

In summary, sufficient choline supply is essential for normal development. Although the exact need of choline is unknown in preterm infants, it is certainly higher than in adults. We estimate that at least 25 mg/kg b.w./day should be delivered continuously to the infant to meet adequate intake, probably even more in ELBW infants. Particularly during the first 2 weeks of life, but also during later periods of inadequate enteral nutrition, choline intake is critically below this value. However, as no RDA and EAR values are available for choline, follow-up studies on the consequences of low choline supply on neurodevelopment are necessary.

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