ELSEVIER

Bioavailability of choline and choline esters from milk in rat pups

Wei-Ling Cheng, Minnie Q. Holmes-McNary, Mei-Heng Mar, Eric L. Lien,* and Steven H. Zeisel

*Department of Nutrition, School of Public Health, School of Medicine, CB No. 7400, University of North Carolina, Chapel Hill, NC USA; and *Wyeth-Ayerst Research, P.O. Box 8299, Philadelphia, PA, USA*

Previously, we had shown that human milk and infant formulas contained choline, phosphocholine (PCho), glycerophosphocholine (GPCho), and phosphatidylcholine (PtdCho). The relative bioavailability of these choline-containing compounds in milk has not previously been studied. Using a rat pup model, infant formula (S.M.A.™, Wyeth-Ayerst) containing either [^{14}C-methyl]-choline chloride (^{14}C-Cho) [^{14}C-methyl]-PCho, [^{14}C-methyl]-GPCho), or [L-α-dipalmitoyl-^{14}C-methyl]-PtdCho was fed intragastrically by a single intubation into 15-day-old postnatal rat pups. Label from the water-soluble metabolites of choline (choline, phosphocholine, and glycerophosphocholine) appeared rapidly within blood and liver, reaching peak levels within 1 to 5 hr, and label in brain continued to increase for more than 24 hr. Label from the lipid soluble metabolite, phosphatidylcholine, took much longer to appear in blood and liver (5 to 8 hr) and label remained elevated in blood for at least 24 hr. Label in brain continued to increase for more than 24 hr, but always remained lower than that attained after treatment with the labeled water-soluble choline metabolites. The liver is a major storage site for choline metabolites, and provides a sensitive indicator of dietary choline status. In liver, a large portion of the label derived from the water-soluble choline metabolites was in the form of betaine at 4 hr post dose. At the same time, most of the PtdCho-derived label was still present as PtdCho in liver. At 24 hr after dose, most of the label derived from choline and PCho in liver was present as betaine (85%) and PtdCho (15%), label derived from GPCho was found as betaine (54%), PtdCho (15%), PCho (11%), GPCho (2%), and choline (18%). Label derived from PtdCho was found as betaine (13%) PCho (2%), and PtdCho (85%). We conclude that 15-day-old postnatal rat pups can absorb the various choline compounds in milk. Choline and PCho appear to be essentially identical in their absorption and metabolic fate. GPCho and PtdCho have different rates of absorption and/or metabolism. Thus, we conclude that there are significant differences in bioavailability, tissue uptake and metabolism among the choline compounds that are present in milk. (J. Nutr. Biochem. 7:457–464, 1996.)

Keywords: rat; milk; infant formula; intubation; choline; phosphocholine; glycerophosphocholine; phosphatidylcholine

Introduction

Choline is an essential nutrient for many mammalian species.[1] It is a precursor for the biosynthesis of the phospholipids phosphatidylcholine (PtdCho), lysophosphatidylcholine, sphingomyelin (SM), and choline plasmalogens, which are all essential constituents of membranes (*Figure 1*).[2] In particular, PtdCho is the predominant phospholipid (>50%) in most mammalian membranes and disaturated PtdCho is the major active component of lung surfactant.[3] PtdCho is also essential for secretion of very-low-density-lipoprotein (VLDL) by liver.[4,5] Choline is needed to make acetylcholine, an important neurotransmitter influencing the function of the brain, heart, muscle, adrenal gland, gastrointestinal tract, and many other organs.[6,7] Choline also is the major source of methyl-groups in the diet; its metabolite, betaine, participates in the methylation of homocysteine to form methionine.[6] For many mammals, ingestion of a diet deficient in choline has major consequences, including fatty infiltra-

Address reprint requests to Steven H. Zeisel, M.D., Ph.D., at Department of Nutrition, School of Public Health, School of Medicine, CB No. 7400, University of North Carolina, Chapel Hill, NC 27599-7400, USA
Received February 9, 1996; accepted May 15, 1996.

© Elsevier Science Inc. 1996
655 Avenue of the Americas, New York, NY 10010

0955-2863/96/$15.00
PII S0955-2863(96)00079-4

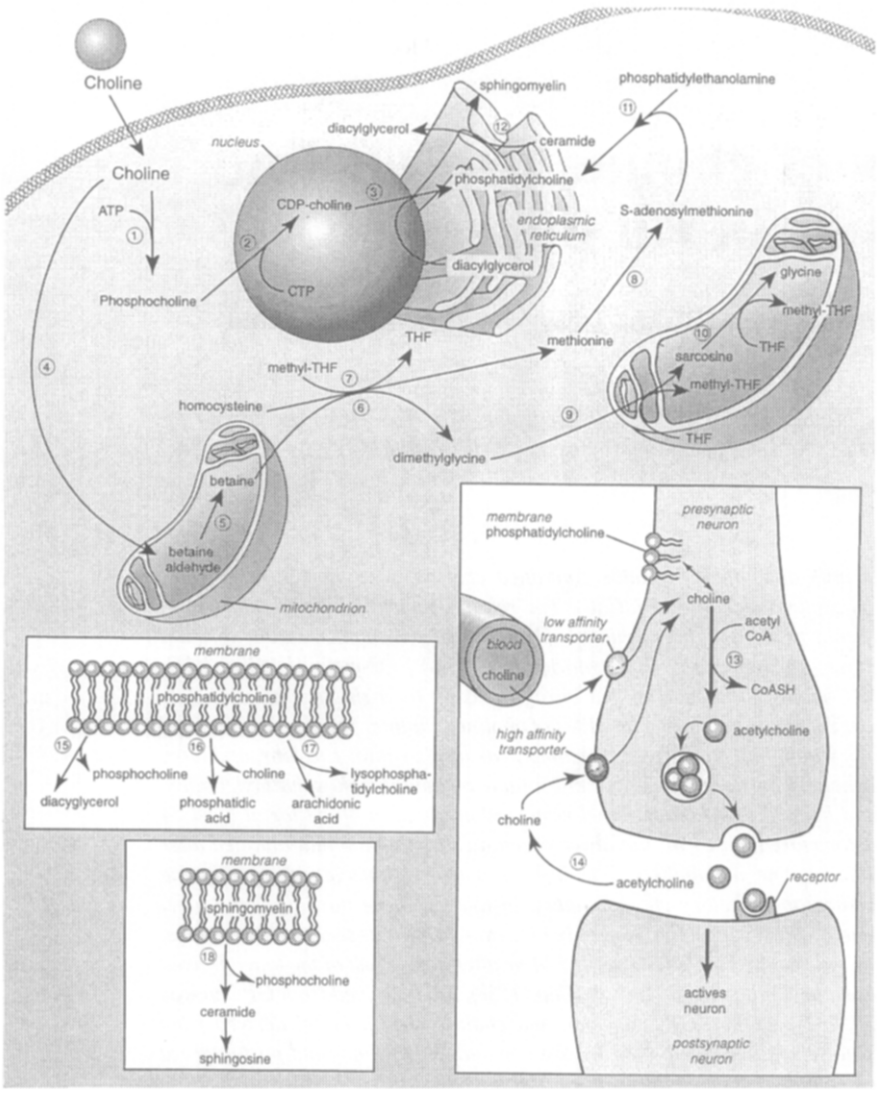

Figure 1 Choline-containing compounds of physiologic importance. The major choline compounds in milk are derived from the metabolism of choline. These pathways exist in mammary gland, liver, and many other tissues.

tion of the liver, renal dysfunction, and spontaneous carcinogenesis.[6]

The neonatal mammal requires especially large amounts of choline to sustain growth as well as for normal maintenance of tissue mass.[8] Much of the choline needed by the newborn is derived from its only source of food, milk, which contains a high concentration of this quaternary amine.[8,9] Human milk contains choline in the form of choline, phosphocholine (PCho), glycerophosphocholine (GPCho), SM, and PtdCho.[10] In rat milk, choline and PtdCho are present in amounts similar to those previously reported in human milk.[9] In addition, rat milk contains greater amounts of GPCho (almost 75% of the total choline moiety in milk) and PCho than were detected in human milk.[10] Infant formulas also contained choline and choline-containing compounds[9] but significantly vary (between formulas, and between formulas and milk) in the distribution of choline and choline-compounds present.

Because concentrations of choline and choline metabolites vary in milk and infant formula, we were interested in determining the relative bioavailability of choline and choline esters using a rat pup model.

Methods and materials

Infant formula and radioactive isotopes

S.M.A.™ (Wyeth-Ayerst, Philadelphia, PA USA) infant formula in liquid concentrated form was purchased locally. It was diluted according to package instructions with distilled water. [^{14}C-methyl]-Choline chloride (203.5 × 10^7 Bq/mmol; 55 mCi/mmol), [^{14}C-methyl]-phosphocholine (223.9 × 10^7 Bq/mmol; 50 mCi/mmol), and [L-α-dipalmitoyl-^{14}C-methyl]-phosphatidylcholine (580.9 × 10^7 Bq/mmol; 157 mCi/mmol) were obtained from New England Nuclear, Boston, MA USA). [^{14}C-methyl]-Glycerophosphocholine was synthesized from [L-α-dipalmitoyl-^{14}C-methyl]-phosphatidylcholine using a method as previously described.[11]

Chemicals

High-pressure liquid chromatography (HPLC) grade methanol was obtained from Mallinckrodt Specialty Chemicals Co., Paris, Kentucky USA. HPLC grade chloroform and ScintiSafe 30% scintillation fluid were obtained from Fisher Scientific, Fair Lawn, NJ USA. Sodium chloride crystals were obtained from EM Science, Gibbstown, NJ USA.

Animals

Timed-pregnant Sprague-Dawley rats (Charles River Breeding Laboratories, Raleigh, NC USA) were received on day 16 of gestation. All rats were housed in individual opaque polyethylene cages with stainless steel tops. Animals were maintained in an air-conditioned environment at a temperature of 24°C and were exposed to a 12-hr light cycle, 0600 to 1800 hr. AIN-76A semi-purified diet containing 1.1 g choline chloride/kg diet (Dyets, Inc., Bethlehem, PA) and water were provided ad libitum. Birth was regarded as day 0 and 1 day after birth was postnatal day 1 (PND1). On PND1 pups were cross-fostered among the dams in a randomized block design. On PND14, pups were sexed by visual observation of the anal-genital distance. Neonatal rats used in the study were kept with lactating dams up to the day of the experiment. On PND15, rat pups were separated from their dams and placed in a warm environment (approximately 30°C) for 3 hr. Then pups were fed infant formula, labeled with approximately 15,725 Bq of either ^{14}C-Cho, ^{14}C-PCho, ^{14}C-GPCho, or ^{14}C-PtdCho, delivered directly into the pup's stomach in a volume equivalent to 1.5% (v/w) of pup body weight.[12,13] Pups were placed in a cage with a lactating dam (6 pups/dam) until sacrificed (0 min, 30 min, 1, 2, 4, 8, 12, and 24 hr after intubation). Animal use was approved by the Committee on Use of Animal Subjects in Research of the University of North Carolina at Chapel Hill.

Collection and analysis of stomach contents

Stomachs were removed and solid contents were squeezed out, then the stomachs were rinsed with 1 mL of cold 0.9% NaCl solution. Rinsed contents were combined with solid contents, weighed, and stored at −80°C. At the time of analysis, stomach contents were disrupted using a sonicator (Heat Systems-Ultrasonics Inc., Model W-225R Cell Disruptor, Plainview, NJ USA; 50% day cycle-setting 2, 10 sec) in a cold 0.9% NaCl solution. Radioactivity was measured by scintillation spectrophotometry (Model 1410, Wallace Inc., Gaithersburg, MD USA) using 500 μL aliquots of sonicate in 5 mL scintillation fluid. Concentrations of choline-containing compounds in stomach contents were determined. Contents were homogenized (Tekmar, Model TR-10 Tissumizer, Cincinnati, OH USA) in an extraction solution of cold methanol/chloroform (2:1, v/v) and incubated at −20°C overnight for extraction. After incubation, homogenates were mixed and subjected to centrifugation at 4°C for 5 min at 3,000 rpm (Sorvall Instruments, Model RC-3B Refrigerated Centrifuge, Wilmington, DE USA). The residue was resuspended in 1 mL methanol-chloroform-water (2:1:0.8, v/v), mixed, and again subjected to centrifugation at 3,000 rpm for 5 min. The supernatant was aspirated and combined with the first supernatant, and 1 mL each of chloroform and water was added. This was mixed and spun at 1,000 rpm for 5 min 4°C. The aqueous phase was dried in the Speed-Vac (Savant Instruments, Farmingdale, NY USA) and water-soluble choline-containing metabolites (betaine, choline, PCho, and GPCho) were separated using HPLC and concentrations assayed using gas chromatography/mass spectrometry (GC/MS).[14] The organic phase was dried in the Speed-Vac and lipid-soluble choline-containing metabolites (PtdCho, lysoPtdCho, and SM) were separated using thin layer chromatography and concentrations assayed using GC/MS.[14]

Collection and analysis of blood

At each time point, pups were lightly anesthetized with ether. A mid-line incision was made on the chest and blood was collected by cardiac puncture into heparinized syringes and immediately placed on ice. Radioactivity was measured by scintillation spectrophotometry using 50 μL aliquots of blood in 5 mL scintillation fluid. The remaining blood samples were stored at −80°C.

Collection and analysis of tissues

Livers, brains, stomachs, and intestines were removed, weighed, frozen in liquid N_2, and stored at −80°C. At the time of analysis, tissue samples were homogenized (Tekmar, Model TR-10 Tissumizer, Cincinnati, OH USA) in an extraction solution of cold methanol/chloroform (2:1 v/v) and incubated at −20°C overnight for extraction. After incubation, homogenates were mixed and centrifuged at 4°C for 5 min at 3,000 rpm. Overnight, 500 μL aliquots of supernatant were evaporated to dryness. After drying, 5 mL of scintillation fluid were added to the vials and the contents were sonicated to dissolve the residues. Radioactivity was determined using scintillation spectrophotometry.

The remaining liver supernatant was saved. The residue was resuspended in 1 mL methanol-chloroform-water (2:1:0.8, v/v), mixed, and again subjected to centrifugation at 3,000 rpm for 5 min. The supernatant was aspirated and combined with the first supernatant, and 1 mL each of chloroform and water were added. This was mixed and spun at 1,000 rpm for 5 min at 4°C. The aqueous phase was dried in the Speed-Vac and water-soluble choline-containing metabolites (betaine, choline, PCho, and GPCho) were separated using HPLC and radioactivity determined with on-line radiometric detection.[14] The organic phase was dried in the Speed-Vac and lipid-soluble choline-containing metabolites (PtdCho and SM) were separated using thin-layer chromatography and radiolabel determined using scintillation spectrophotometry.[14]

Calculations

In all studies we attempted to administer approximately the same amount of radiolabel. The measured DPM were adjusted so that they reflected a standard administered dose of 15,725 Bq rather than the measured Bq. We calculated Bq per tissue using the measured total tissue volumes. For blood we assumed that rat pups had 43 mL blood per kg body weight.[15]

Statistical analysis

Data were analyzed using a general linear model (GLM) with the differences of means multivariate analysis of variance (MANOVA) to compare the differences of means across four treatment groups adjusted for time and for the response (appearance/disappearance). Graph tests were used for calculating the area under the curve and GLM and Dunn T tests were also used to test the significance of the graph test.[16]

Results

Label was diluted in SMA infant formula that contained endogenous choline (700 μM), PCho (120 μM), GPCho (800 μM), and PtdCho (90 μM). Just after oro-gastric intubation (time 0), label was present in stomach contents in the form it was administered (100% as choline by our assay). Because no significant differences between male and female pups were observed, the average values of both sexes were calculated. The specific activity in rat pup stomach contents (containing administered formula plus residual rat milk) of (^{14}C-Cho) was 46 Bq/nmole; of [^{14}C-methyl]-PCho was 260 Bq/nmole; of [^{14}C-methyl]GPCho was 39 Bq/nmole; or of [L-α-dipalmitoyl-^{14}C-methyl]-PtdCho was 201 Bq/nmole.

We characterized the disappearance of radiolabel from

stomach, its sequential appearance in the GI tract, liver, blood and brain (*Figures 2–6*). Disappearance of choline-, PCho-, and GPCho-derived labels from stomach contents were similar (*Figure 2*). There was a 3 fold decrease in all three labels at 30 min post-intubation, and at 4 hr post-intubation there was essentially no label present in the stomach contents. Disappearance of PtdCho-derived label in stomach contents was slower than for the other three labels ($P < 0.01$). It took 8 hr before we observed a 3 fold decrease in PtdCho-derived label and more than 12 hr before this label disappeared from the stomach contents. In the GI tract, choline-, and GPCho-, PtdCho-derived labels were similar and the total area under the curves are significantly different from the PCho-derived label total area under the curve.

The labeled choline-compounds were absorbed and distributed to the gastrointestinal tract, liver, blood, and brain of the rat pups (*Figures 3–6*). The appearance and disappearance of choline-, GPCho-, and PtdCho-derived labels in the cells of the intestinal tract were similar (*Figure 3*). PCho-derived label appeared and disappeared more rapidly than did the other labels ($P < 0.01$). In the liver, PCho appeared and disappeared more rapidly than did the other three labels (*Figure 4*) ($P < 0.01$). Label from all of the water-soluble forms of choline reached maximum levels within 4 hr, PCho did so by 1 hr. PtdCho appeared much

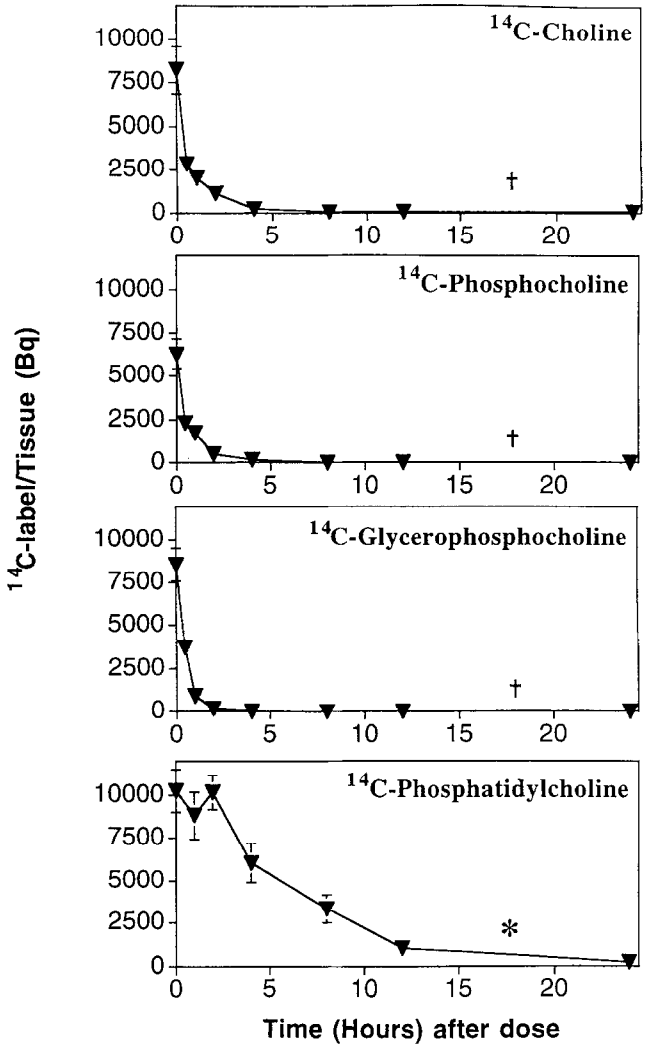

Figure 2 Appearance/disappearance of ^{14}C-Label in the stomach contents of 15-day-old rat pups. Rat pups, 15 days of age, were fed one time by oro-gastric intubation with infant formula containing either ^{14}C-radiolabeled choline or ^{14}C-phosphocholine or ^{14}C-glycerophosphocholine or ^{14}C-phosphatidylcholine (approximately 16,000 Bq). Rat stomach contents were collected and analyzed at the indicated times as described under "Methods and materials". Data are expressed as mean Bq per stomach contents ± SEM for n = 6) rat pups/point. Points without standard error bars have errors that were smaller than the symbol size. Curves marked with different symbols (†, *) differ in areas under the curves ($P < 0.01$). Graph tests were used for calculating the area under the curve using a general linear model with the differences of means MANOVA to compare the differences of means across four treatment groups adjusted for time of the response (appearance/disappearance).

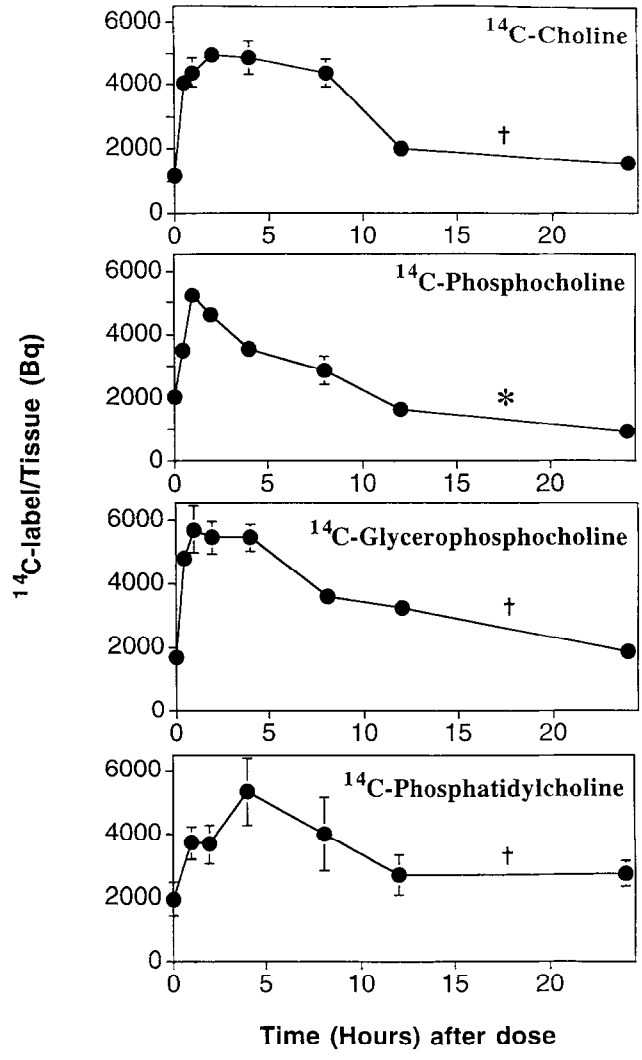

Figure 3 Appearance/disappearance of ^{14}C-Label in the gastrointestinal tissue of 15-day-old rat pups. Rat pups were treated as described in Figure 2. Rat stomach tissue (washed) and intestines were collected and analyzed at the indicated times as described in Methods. Data are expressed as mean Bq per tissue ± SEM for n = 6 rat pups/point. Points without standard error bars have errors that were smaller than the symbol size. Curves marked with different symbols (†, *) differ in areas under the curves ($P < 0.01$) as described in Figure 2.

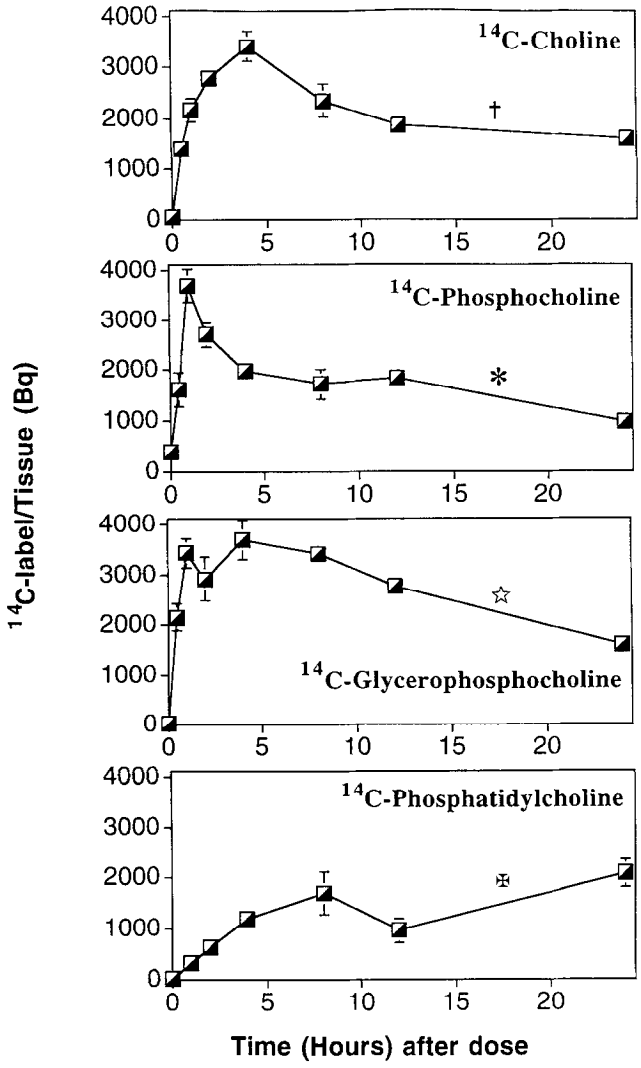

Figure 4 Appearance/disappearance of ^{14}C-Label in the liver tissue of 15-day-old rat pups. Rat pups were treated as described in Figure legend 2. Rat livers were collected and analyzed at the indicated times as described under "Methods and materials". Data are expressed as mean Bq per liver + SEM for $n = 6$ rat pups/point. Points without standard error bars have errors that were smaller than the symbol size. Curves marked with different symbols (†, ✱, ☆ ⊕) differ in areas under the curves ($P < 0.01$) as described in Figure 2.

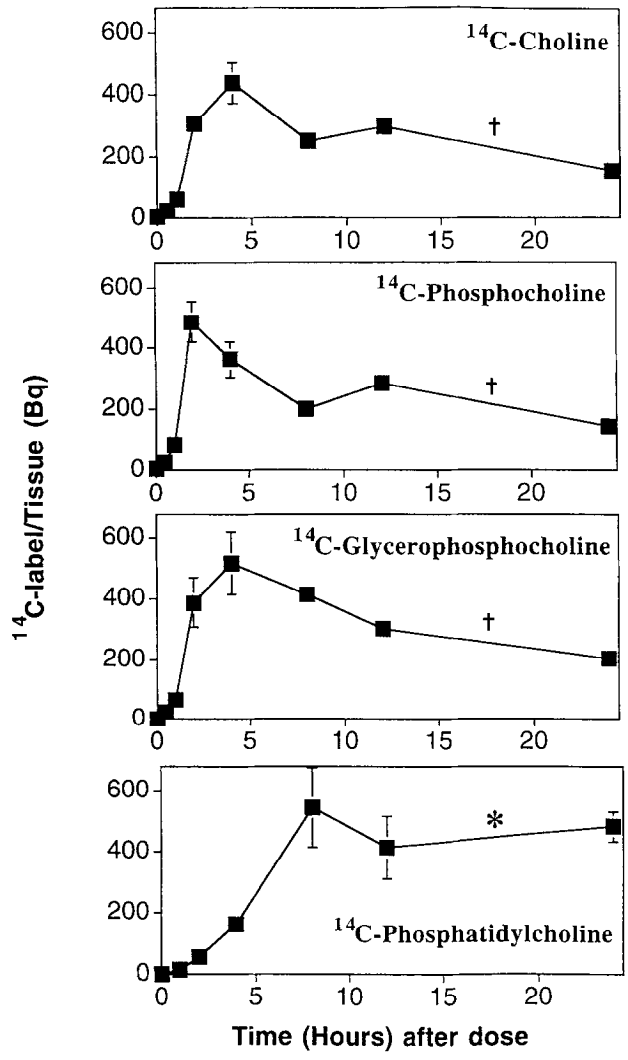

Figure 5 Appearance/disappearance of ^{14}C-Label in the blood (total volume) of 15-day-old rat pups. Rat pups were treated as described in Figure 2. Rat blood was collected and analyzed at the indicated times as described under "Methods and materials". Data are expressed as mean Bq per total blood volume ± SEM for $n = 6$ rat pups/point. Points without standard error bars have errors that were smaller than the symbol size. Curves marked with different symbols (†, ✱) differ in areas under the curves ($P < 0.01$) as described in Figure 2.

more slowly than did the other labels ($P < 0.01$), and was still accumulating at 24 hr post dose. In the liver, the total area under the curves was significantly different ($P < 0.01$) for each derived label over the time periods studied.

In rat pups' blood, both choline- and GPCho-derived labels increased and peaked at 4 hr post-intubation, whereas PCho-derived label increased and peaked at 2 hr post-intubation (*Figure 5*). All three labels achieved approximately the same peak accumulation of label in blood. After peaking, choline- and PCho-derived labels gradually decreased during the 24 hr post-intubation period studied. The GPCho-derived label, behaved similarly, though at 24 hr there was approximately 25% more GPCho-derived label than there was of the choline- and PCho-derived labels. PtdCho-derived label in blood appeared more slowly than did the other labels ($P < 0.01$), but it also disappeared much more slowly, so that at 24 hr PtdCho-derived label remained near peak levels and there was 2.4 to 3.2 times as much PtdCho-derived label in blood as there was of any of the other labels.

In rat pups' brain, choline- and GPCho-derived labels increased at similar rates and the maximum concentrations in brain were similar during the 24 hr post treatment (*Figure 6*). PCho-derived label achieved amounts of label in brain that were approximately 12% greater than achieved by choline-derived label ($P < 0.01$). PtdCho-derived label increased at a much slower rate than the other three labels over 24 hr, and achieved amounts of label in brain that were approximately 12% lower than amounts achieved by choline-derived label ($P < 0.01$). In rat pups' brain, the total

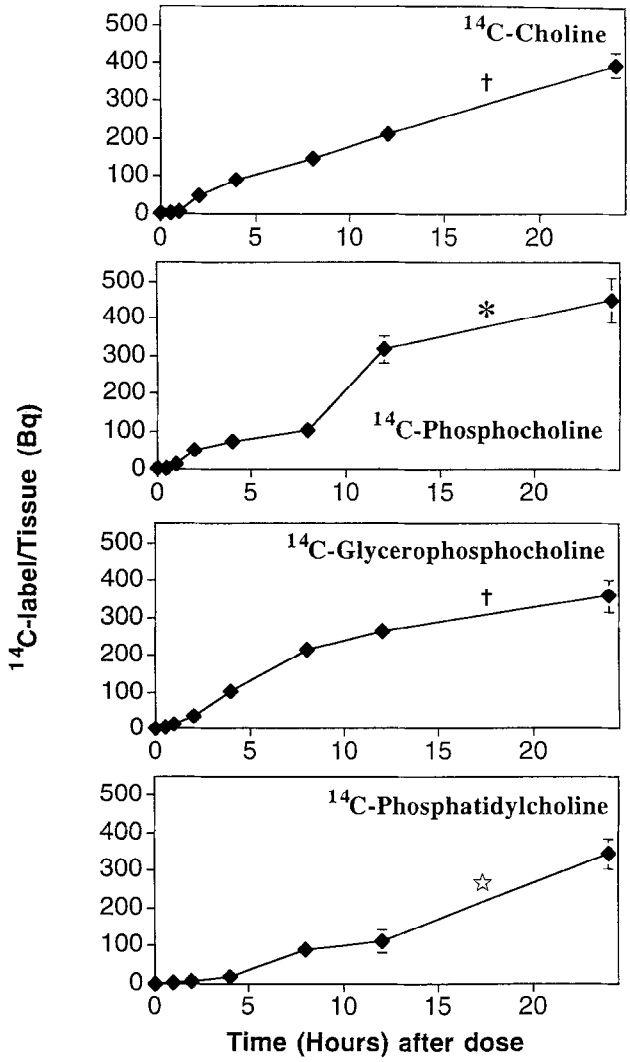

Figure 6 Appearance/disappearance of ^{14}C-Label in the brain tissue of 15-day-old rat pups. Rat pups were treated as described in Figure 2. Rat brains were collected and analyzed at the indicated times as described under "Methods and materials". Data are expressed as mean Bq per brain ± SEM for $n = 6$ rat pups/point. Points without standard error bars have errors that were smaller than the symbol size. Curve marked with different symbols (†, ☆) differ in areas under the curves ($P < 0.01$); phosphocholine curve differs from choline and glycerophosphocholine curves by $P < 0.05$) (✱), as described in Figure 2.

area under the curve for PCho- and GPCho-, and PtdCho-derived labels were significantly different from the total area under the curve for the choline-derived label ($P < 0.01$).

Different choline-containing compounds were formed in liver from the choline-, PCho-, GPCho-, PtdCho-radiolabeled infant formula. In liver at 4 hr post treatment (*Table 1*), choline, PCho-, and GPCho-derived label were present in the greatest amounts as betaine, with PCho being the next most common metabolite formed. PtdCho derived label was principally associated with PtdCho in liver. By 24 hr in liver (*Table 2*), choline- and PCho-derived label was mainly present as betaine, with a small amount of PtdCho formed. No choline- and PCho-derived label was present as choline,

Table 1 Percent of labeled choline and choline metabolites formed in the liver of 15-day-old rat pups at 4 hr post-treatment

	Treatments			
Metabolites	^{14}C-Choline (%)	^{14}C-PCho (%)	^{14}C-GPCho (%)	^{14}C-PtdCho (%)
Betaine	48.3[a]	43.4[a]	42.5[a]	10.7[b]
Choline	2.1[a]	7.5[b]	16.5[b]	0.0[a]
PCho	22.4[a]	20.9[a]	22.5[a]	4.3[b]
GPCho	12.3[a]	13.2[a]	3.5[b]	0.0[c]
PtdCho	14.9[a]	14.9[a]	14.5[a]	84.8[b]

Rat pups 15 days of age were fed SMA infant formula containing either ^{14}C-radiolabeled choline (^{14}C-Cho), phosphocholine (^{14}C-PCho), glycerophosphocholine (^{14}C-GPCho), or phosphatidylcholine (^{14}C-PtdCho) by oro-gastric intubation. Rat livers were collected as described under "Methods and materials." Choline, betaine, phosphocholine and glycerophosphocholine containing ^{14}C-label were isolated by HPLC and quantitated using on-line radiometric detection as described under "Methods and Materials." PtdCho was isolated by thin layer chromatography, and quantitated using scintillation spectrophotometry. Data are expressed as mean percent for $n = 6$ rat pups/point.
We compared the percent of each metabolite formed from each of the different treatments administered. Within each metabolite group, percentages with different superscripts are significantly different at the $P < 0.05$ level.

GPCho, or PCho. GPCho-derived label was present as betaine, with some choline, GPCho, and PtdCho also formed. PtdCho-derived label was present mainly as PtdCho in liver with some betaine and PCho formed.

Discussion

The purpose of this study was to assess the relative bioavailability of choline and choline esters commonly found

Table 2 Percent of labeled choline and choline metabolites formed in the liver of 15-day-old rat pups at 24 hr post-treatment

	Treatments			
Metabolites Formed	^{14}C-Choline %	^{14}C-PCho %	^{14}C-GPCho %	^{14}C-PtdCho %
Betaine	85.0[a]	85.0[a]	53.7[b]	13.0[c]
Choline	0.0[a]	0.0[a]	18.3[b]	0.0[a]
PCho	0.0[a]	0.0[a]	10.7[b]	2.0[a]
GPCho	0.0[a]	0.0[a]	2.4[a]	0.0[a]
PtdCho	14.9[a]	14.9[a]	14.6[a]	84.7[b]

Rat pups 15 days of age were fed SMA infant formula containing either ^{14}C-radiolabeled choline (^{14}C-Cho), phosphocholine (^{14}C-PCho), glycerophosphocholine (^{14}C-GPCho), or phosphatidylcholine (^{14}C-PtdCho) by oro-gastric intubation. Rat livers were collected as described under "Methods and materials." Choline, betaine, phosphocholine and glycerophosphocholine containing ^{14}C-label were isolated by HPLC and quantitated using on-line radiometric detection as described under "Methods and materials." PtdCho was isolated by thin layer chromatography, and quantitated using scintillation spectrophotometry. Data are expressed as mean percent for $n = 6$ rat pups/point.
We compared the percent of each metabolite formed from each of the different treatments administered. Within each metabolite group, percentages with different superscripts are significantly different at the $P < 0.01$ level.

in milk. Studies using radioactivity are not easily accomplished in humans. The suckling pig intestine is frequently used as a model for studies on nutrient absorption because it is similar to the developing human intestine.[17] Unfortunately, little is known about choline metabolism in the pig, whereas much more is known about the metabolism of choline in the rat. Therefore, we have chosen to use a rat pup model.[18,19] We delivered radiolabel diluted with infant formula to ensure consistent treatment across all animals. If we had used rat milk, chemical composition might have varied more between treatment groups. The formula we chose contained amounts of choline and choline esters that are similar but not identical to those present in rat or human milk.[9] For example, we administered dipalmitoyl phosphatidylcholine and though palmitic acid is present in high concentration in human milk most of it is present in the 2-position of phosphatidylcholine.[20] Because 50% of ingested phosphatidylcholine is absorbed with the 1-position intact, whereas the 2-position can undergo intestinal hydrolysis for absorption but is reacylated in the intestinal mucosa, to reform intact phosphatidylcholine,[21–23] absorbed species tend to mimic the fatty acid content of the animal. Therefore fatty acid composition of phosphatidylcholine, whether saturated or unsaturated, has a limited influence on the absorption of phosphatidylcholine.

Within the stomach, label in all forms of choline remained intact. Specific activities of each form varied as expected based on formula (and stomach content) composition. The choline moiety within these compounds was absorbed and available to the tissues of the rat pup. Unesterified choline is absorbed by mediated transport and by diffusion in the rat small intestine.[24] The digestion and absorption of dietary and biliary PtdCho occurs independently from that of choline.[8] PtdCho is absorbed by enterocytes via a mechanism involving hydrolysis to form lysophosphatidylcholine, followed by reacylation within the enterocyte to reform PtdCho.[25] The mechanisms mediating GPCho and PCho absorption have not yet been described. We suggest that PCho is hydrolyzed by pancreatic alkaline and acid phosphatases and is absorbed as choline. GPCho, however, appears to be handled very differently than choline or PCho, resulting in a very different pattern of label in liver at 24 hr (*Table 2*). The lipid-soluble choline ester, PtdCho, moves more slowly from stomach and intestine into the circulation, perhaps because it is less accessible to gut transporters than the water-soluble components.

We focused on liver metabolites of choline, and only looked at choline-derived radioactivity in other tissues because liver is the major organ responsible for choline metabolism.[8] However, several other tissues (intestine and kidney) are involved in total body choline metabolism. Intestinal flora degrade some of ingested choline to form methylamines.[26–29] Methylamines are possible to detect using our chromatographic procedures, and we observed no choline-derived label in them. However, methylamines are volatile, and we cannot exclude the possibility that some methylamines were formed and lost as volatile gas before analyses. We know that choline and phosphocholine can be degraded to methylamines in the gut,[30] whereas phosphatidylcholine is not subject to such degradation.[31] The kidney also is capable of metabolizing choline to form betaine.[6,32] Under our experimental conditions choline metabolism in each of these tissues also may contribute to the relative bioavailability of choline and choline esters.

Our data suggest that there are differences in the bioavailability of the water-soluble choline compounds (choline, PCho, and GPCho) and the lipid-soluble PtdCho. Furthermore, in the liver and brain, the physical form of the derived label appears to be a significant factor for the rate of absorption and bioavailability. Once choline is absorbed, it is first accumulated by intestinal mucosa and then by liver.[33] It then enters the blood where it is carried to other tissues such as brain. Phosphatidylcholine, once absorbed, is also accumulated by liver and then a portion is secreted as plasma lipoprotein.[6] Our data indicate that, in liver, GPCho ingested in milk was metabolized differently than were choline or PCho (*Tables 1* and *2*). More GPCho-derived label was recovered as choline and PCho with less label present as betaine as compared with choline- or PCho-derived label (*Table 2*). In addition, PtdCho-derived label was metabolized very differently, with most remaining in the form of PtdCho in liver (*Tables 1* and *2*). Much of this PtdCho was probably incorporated into liver membranes. The various dietary sources of choline available in milk are, therefore, used differently by liver in the rat pup. Formulas and milks with different compositions might deliver different amounts and forms of choline to target tissues. This may have consequences for the relative balance between use of choline as a methyl donor (via betaine), acetylcholine precursor (via choline), or phospholipid precursor (via PCho and PtdCho). Whereas the data obtained from the rat pup model cannot be directly extrapolated to the human infant, our data indicate that variations in the bioavailability and utilization of choline, PCho, GPCho, and PtdCho in milk should be considered when milk substitutes are developed. Following the model of human milk should provide a safe and effective way of providing choline to the neonate.

Acknowledgments

This work was supported, in part, by funding from the National Institutes of Health (AG09525) and from Wyeth-Ayerst Laboratories. We thank Dr. Zhi-Wei Zhou for his assistance with animal feeding and tissue collection and L. Joseph Su, MPH for his statistical consultation.

References

1 Zeisel, S.H., daCosta, K.-A., Franklin, P.D., Alexander, E.A., Lamont, J.T., Sheard, N.F., and Beiser, A. (1991). Choline, an essential nutrient for human. *FASEB J.* **5,** 2093–2098

2 Zeisel, S.H. (1990). Choline deficiency. *J. Nutr. Biochem.* **1,** 332–349

3 Brown, E.S. (1964). Isolation and assay of dipalmitoyl lecithin in lung extracts. *Am. J. Physiol.* **207,** 402–406

4 Yao, Z.M. and Vance, D.E. (1988). The active synthesis of phosphatidylcholine is required for very low density lipoprotein secretion from rat hepatocytes. *J. Biol. Chem.* **263,** 2998–3004

5 Yao, Z.M. and Vance, D.E. (1989). Head group specificity in the requirement of phosphatidylcholine biosynthesis for very low density lipoprotein secretion from cultured hepatocytes. *J. Biol. Chem.* **264,** 11373–11380

6 Zeisel, S.H. and Blusztajn, J.K. (1994). Choline and human nutrition. *Ann. Rev. Nutr.* **14,** 269–296.

7 Zeisel, S.H. (1992). Choline: an important nutrient in brain development, liver function and carcinogenesis. *J. Am. Coll. Nutr.* **11,** 473–481

8 Zeisel, S.H. (1981). Dietary choline: biochemistry, physiology, and pharmacology. *Ann. Rev. Nutr.* **1,** 95–121

9 Zeisel, S.H., Char, D., and Sheard, N.F. (1986). Choline, phosphatidylcholine and sphingomyelin in human and bovine milk and infant formulas. *J. Nutr.* **116,** 50–58

10 Rohlfs, E.M., Garner, S.C., Mar, M.-H., and Zeisel, S.H. (1993). Glycerophosphocholine and phosphocholine are the major choline metabolites in rat milk. *J. Nutr.* **123,** 1762–1768.

11 Ditterman, J.C. and Wells, M.A. (1987). Determination of individual phospholipids by selective hydrolysis. *Meth. Enzymology.* **14,** 482–530

12 Sonderegger, T.B., Colbern, D., Calmes, H., Corbitt, S., and Zimmermann, E.G. (1982). Methodologic note: Intragastric intubation of ethanol to rat pups. *Neurobehav. Toxicol. Teratol.* **4,** 477–481

13 Serbus, D.C., Young, M.W., and Light, K.E. (1986). Blood ethanol concentration following intragastric intubation of neonatal rat pups. *Neurobehav. Toxicol. Teratol.* **8,** 403–406

14 Pomfret, E.A., daCosta, K., Schurman, L.L., and Zeisel, S.H. (1989). Measurement of choline and choline metabolite concentrations using high-pressure liquid chromatography and gas chromatography-mass spectrometry. *Anal. Biochem.* **180,** 85–90

15 Farris, E.J. and Griffith Jr., J.Q. (1967). *The Rat in Laboratory Investigation.* Hafner Publishing Co., New York

16 SAS. (1994). *SAS/STAT User's Guide Version 6.* SAS system, SAS Institute Inc. Cary. p. 891–996

17 Mougham, P.J., Birtles, M.J., Cranwell, P.D., Smith, W.C., and Pedraza, M. (1992). The piglet as a model animal for studying aspects of digestion and absorption in milk-fed human infants. *World Rev. Nutr. Diet.* **67,** 40–113

18 Lonnerdal, B., Yuen, M., Glazier, C., and Litov, R.E. (1993). Magnesium bioavailability from human milk, cow milk, and infant formula in suckling rat pups. *Am. J. Clin. Nutr.* **58,** 392–397

19 Lonnerdal, B., Glazier, C.E., and Lien, E.L. (1992). Selenium supplementation of infant formula: uptake and retention of various forms of selenium in suckling rats. *J. Nutr. Biochem.* **3,** 644–652

20 Jenness, R. (1979). The composition of human milk. *Seminars in Perinatology.* **3,** 225–239

21 Blomstrand, R. (1955). The intestinal absorption of phospholipids in the rat. *Acta Physiol. Scand.* **34,** 147–157

22 Whyte, M., Goodman, D.S., and Karmen, A. (1965). Fatty acid esterification and chylomicron formation during fat absorption in the rat: III. Positional relation in triglycerides and lecithin. *J. Lipid Res.* **6,** 233–240

23 Fox, J.M., Betzing, H., and Lekim, D. (1979). Pharmacokinetics of orally ingested phosphatidylcholine. In *Nutrition and the Brain.* (A. Barbeau, J.H. Growdon and R.J. Wurtman ed.), Raven Press. New York. p. 95–108

24 Sheard, N.F. and Zeisel, S.H. (1986). An in vitro study of choline uptake by intestine from neonatal and adult rats. *Pediatr. Res.* **20,** 768–772

25 Lekim, D. and Betzing, H. (1976). Intestinal absorption of polyunsaturated phosphatidylcholine in the rat. *Hoppe Seylers Z. Physiol. Chem.* **357,** 1321–1331

26 de la Huerga, J., Gyorgy, P., Waldstein, S., Katz, R., and Popper H. (1953). The effects of antimicrobial agents upon choline degradation in the intestinal tract. *J. Clin. Invest.* **32,** 1117–1120

27 Prentiss, P.G., Rosen, H., Brown, N., Horowitz, R.E., Malm, O.J., and Levenson, S.M. (1961). The metabolism of choline by the germ-free rat. *Arch. Biochem. Biophys.* **94,** 424–429

28 Flower, R.J., Pollitt, R.J., Sanford, P.A., and Smyth, D.H. (1972). Metabolism and transfer of choline in hamster small intestine. *J. Physiol.* **226,** 473–489

29 Zeisel, S.H., da Costa, K.A., and Fox, J.G. (1985). Endogenous formation of dimethylamine. *Biochem. J.* **232,** 403–408

30 Zeisel, S.H., Gettner, S. and Youssef, M. (1989). Formation of aliphatic amine precursors of N-nitrosodimethylamine after oral administration of choline and choline analogues in the rat. *Food Chem. Toxicol.* **27,** 31–34

31 Zeisel, S.H., Wishnok, J.S., and Blusztajn, J.K. (1983). Formation of methylamines from ingested choline and lecithin. *J. Pharmacol. Exper. Ther.* **225,** 320–324

32 Bagnasco, S., Balaban, R., Fales, H.M., Yang, Y.M., and Burg, M. (1986). Predominant osmotically active organic solutes in rat and rabbit renal meduallas. *J. Biol. Chem.* **261,** 5872–5877

33 Zeisel, S.H., Story, D.L., Wurtman, R.J., and Brunengraber, H. (1980). Uptake of free choline by isolated perfused rat liver. *Proc. Natl. Acad. Sci. USA.* **77,** 4417–4419