Ann. Rev. Nutr. 1981. 1:95–121

DIETARY CHOLINE: BIOCHEMISTRY, PHYSIOLOGY, AND PHARMACOLOGY

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0199-9885/81/0715-0095$01.00

INTRODUCTION

The discovery that a diet supplemented with choline alters brain function has stimulated recent interest in this nutrient. At the same time, technical advances in the measurement of choline have facilitated the accumulation of information that has increased the understanding of the biochemistry and physiology of this quaternary amine. Choline is necessary for normal function of the mammalian organism. It is a precursor for the biosynthesis of phospholipids, essential components of all membranes. It is also a precursor for the biosynthesis of acetylcholine, an important neurotransmitter. Many species of animals have a dietary requirement for choline; humans deficient in choline have not yet been identified.

CHOLINE AS A NUTRIENT

Choline (trimethyl-beta-hydroxyethylammonium) is a quaternary ammonium compound that is widely distributed in plants and animals. It was first discovered by Strecker (202) when it was isolated from the bile of the pig; choline's structure was determined and was chemically synthesized in 1866 (15). Choline was recognized as a component of phospholipids, but it took more than a century before the pathway that incorporated it into lecithin was elucidated (138).

Early experiments with insulin first demonstrated choline's importance as a nutrient. Depancreatized dogs, maintained on insulin, developed fatty infiltration of the liver and eventually died. Dietary administration of raw pancreas prevented hepatic damage (7) and it was later found that choline, a component of pancreatic lecithin, was the essential dietary ingredient (26, 28, 116).

Chronic ingestion of a diet deficient in choline has major consequences, including liver damage and renal hemorrhage in some species. In the rat (9, 12, 57, 61, 105, 148), dog (77, 90), hamster (107), pig (83), and chicken (59, 66, 141), choline deficiency results in fatty infiltration of the liver, which is due to a disturbance in the ability of the hepatocyte to export triglycerides and phospholipids secondary to faulty biosynthesis of plasma lipoproteins (159). Phosphatidylcholine is an integral part of the structure of these lipoproteins and of the microsomal membranes involved in their assembly (159). Renal function is also compromised in the choline-deficient rat. Concentrating ability, free water reabsorption, sodium excretion, glomerular filtration rate, and renal plasma flow are all decreased (158), and gross renal hemorrhage occurs after short periods of choline deprivation (22, 27, 102, 171). Infertility (51), bony abnormalities (79, 130), decreased hematopoiesis (49), and hypertension (143) have all been reported as consequences

of choline-deficient diets in animals. Other nutrients can alter the pathology associated with choline deficiency. Dietary methionine can decrease choline requirements, probably by replacing choline as a methyl donor for the regeneration of S-adenosylmethionine (120, 189).

A choline deficiency syndrome has never been documented in the human. The diet of most humans contains adequate amounts of choline, as it is distributed widely throughout the plant and animal kingdom. After choline deprivation was noted to be associated with fatty liver in animals, several investigators postulated that alcoholic liver cirrhosis was due to choline deficiency. This encouraged a large number of therapeutic trials by using supplemental choline in humans with liver disease, with variable results (18, 23, 45, 169).

THE DIET AS A SOURCE FOR CHOLINE

Free choline concentration in the serum and tissues depends on the dietary intake of choline (33, 110, 222). In the adult human, serum choline concentration fluctuates within a small range when common foods are ingested (244) (Figure 1). Administration of a single large dose of choline chloride (0.05 g/kg of body weight) elevates plasma choline concentration 300–400% (119, 121). After a meal supplemented with choline has been consumed, peak plasma choline concentrations are attained within 3 h, with some elevation persisting for 8 h (119). Dietary intake of the choline-containing phospholipid, lecithin, also increases serum choline concentrations. Small, but significant, elevations of serum choline levels occur after meals that might be eaten by a liver and egg connoisseur (244) (Figure 1). Larger oral doses of lecithin (0.3 g/kg of body weight) increase serum choline concentration 400%, with significant elevations persisting for as long as 12 h (244) (Figure 1). It is this longer duration of action, and lecithin's resistance to degradation within the gut to form trimethylamine, that makes this phospholipid the treatment of choice for choline supplementation. One note of caution, a form of lecithin is currently available through "health-food" stores. Though this preparation is labeled "pure," it is actually a mixture of phosphatides that contain less than 30% phosphatidylcholine.

In the human diet, most choline is consumed in the form of lecithin, in foods such as liver, eggs, soybeans, and peanuts (Table 1) (78, 89, 155, 241). Lecithin is also added to commercially prepared foods as an emulsifying agent. For example, lecithin helps disperse water in margarine, and it increases the wetting power of cocoa powder in chocolate bars (241). A conservative estimate of total choline intake, as lecithin and free choline, is 300 mg/day, with an egg and liver connoisseur ingesting more than three times this amount (38, 241).

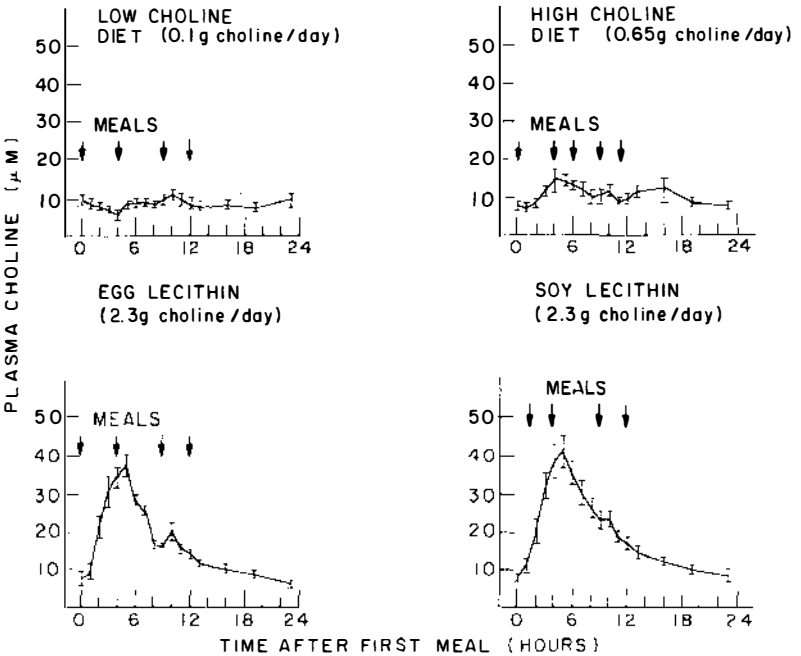

Figure 1 Plasma choline response to ingested choline and lecithin. Six adult human subjects ingested each of four diets (common foods high in choline or lecithin, common foods low in choline or lecithin, low-choline diet plus a breakfast supplement of 25 of 80% pure soy lecithin, and low-choline diet plus a breakfast supplement of 25 g of 80% pure egg lecithin; the choline content indicated for each diet was calculated by using the choline and lecithin contents of ingested foods). Plasma samples were obtained at regular intervals and were assayed for choline by using a radioenzymatic assay. Meal times are indicated by arrows. Data are expressed as mean ±SD. (From ref. 244.)

ABSORPTION OF CHOLINE

Choline is absorbed in the upper small intestine, chiefly in the jejunum (48, 87, 144, 186). In the guinea pig, this transport is unidirectional and is mediated by a saturable carrier system at choline concentrations less than 4 mM ($K_m = 110$ μM, $V_{max} = 25$ nmol/ml of tissue water X min $^{-1}$) (144). When concentrations are higher than 4 mM transfer across the jejunum is proportional to intraluminal choline concentration, which suggests a passive diffusion mechanism (144). These carriers are localized in the brush border of jejunal mucosal cells, they are not sodium or energy dependent, and they are not inhibited by betaine but are inhibited by hemicholinium-3 and deanol (144, 186, 200). In the chicken, the duodenum is net secretor of choline (secreting twice the daily dietary intake), but most of this choline, and most of ingested choline, is absorbed again in the jejunum (48).

Table 1 Choline and lecithin content of common foods[a]

Food (per 100 g)	Choline chloride (mg)	Lecithin[b] (mg)
Calf liver	650	850
Lamb chop	—	753
Beef round	—	453
Ham	—	800
Trout	—	580
Cheese	—	50–100
Egg	0.4	394
Oatmeal	131	650
Soybeans	237	1480
Wheat germ	—	2820
Polished rice	—	586
Peanuts	—	1113
Spinach	4–17	6–14
Cauliflower	78	2
Kale	89	2
Potato	40	1
Lettuce	16–20	0.2
Carrots	6–13	5–8

[a] Data from Ref. 241.
[b] Molecular weight of choline chloride is 141, the molecular weight of lecithin is approximately 800 (depending on fatty acid composition).

Once choline is absorbed it enters the portal circulation, as it is a water-soluble molecule.

Some of ingested choline is metabolized before it can be absorbed from the gut; betaine (200) and trimethylamine (11, 67–71) are the major degradation products formed. Betaine formation, responsible for destruction of as much as 50% of ingested choline, is inhibited by anoxia and physostigmine (87). The production of trimethylamine is greatest when large amounts of choline are ingested (69). Intestinal bacteria must be responsible for trimethylamine production, as its formation was markedly reduced when gut was sterilized with antibiotics (11, 69) and was absent in the germ-free rat (175) or when choline was administered intravenously, bypassing the gut bacterial flora (71).

DIGESTION AND ABSORPTION OF CHOLINE ESTERS

As noted earlier, the ultimate source of most dietary choline is the phospholipid lecithin (phosphatidylcholine). Both pancreatic secretions and intestinal mucosal cells contain enzymes capable of hydrolyzing lecithin.

Phospholipase A_2 (which cleaves the β-fatty acid moiety) is found in pancreatic juice and in the intestinal brush border (67, 68, 92, 122, 145, 168, 204). It is secreted as a zymogen, which is activated in the gut by trypsin, calcium ions, and bile salts (67, 68, 122). Within the mucosal cell phospholipase A_1 cleaves the α-fatty acid and phospholipase B cleaves both fatty acids (204). However, these enzymes cleave much less lecithin than the pancreatic phospholipase does. The net result is that most ingested lecithin is absorbed as lysolecithin (deacylated in the β-position).

Within the enterocyte lysolecithin can be converted to glycerylphosphorylcholine by further deacylation, catalyzed by lysophospholipase (phospholipase B) (80, 96, 168). Lysolecithin can also be acylated, reforming lecithin (92, 145, 205). This reaction, in which two lysolecithin molecules are converted to one molecule of glycerylphosphorylcholine and one molecule of lecithin, is catalyzed by a dismutase found in both the microsomal and soluble fractions of the mucosal cell. It is maximally active when high concentrations of lysolecithin are present, as it has a K_m for lysolecithin of 3.6 mM (205). Approximately twice as many lecithin molecules are absorbed from the gut as are reconstituted and secreted from the mucosal cell into the lymphatic circulation (10, 35, 92, 145, 188).

The lecithin as part of the chylomicron, is secreted into lymphatics and enters the systemic circulation, where it is subjected to clearance and further degradation. Phospholipase C (EC 3.1.4.3), present in brain, kidney, liver, and spleen, acts upon this lecithin, forming phosphorylcholine and diglyceride (136). In each of these organs alkaline phosphatase cleaves phosphorylcholine, liberating free choline (153). Phospholipase B activity, present in many tissues, acts upon lecithin to form glycerylphosphorylcholine, which can then be cleaved by glycerylphosphorylcholine diesterase, producing free choline (16, 17, 65, 223). Finally, the brain possesses some phospholipase D activity (which forms glycerylphosphate and free choline from lecithin). This brain enzyme has a pH optimum of 6.0, and a K_m for lecithin of 0.83 mM (184).

Glycerylphosphorylcholine is present in small quantities in the diet and, as discussed above, is formed from lecithin in several tissues. Within the gut mucosal cell, glycerylphosphorylcholine diesterase (L-3-glycerylphosphorylcholine glycerophosphohydrolase, EC 3.1.4.2) catalyzes the conversion of glycerylphosphorylcholine to glycerylphosphate and free choline (122, 168). The free choline liberated enters the portal circulation (122).

Phosphorylcholine, also present in small amounts in the diet, is rapidly cleaved within the intestinal mucosal cell by an alkaline phosphatase, liberating free choline and phosphate (153). Prostatic acid phosphatase can also cleave phosphorylcholine.

Sphingomyelin, a choline-containing phospholipid, is not degraded within the intestinal lumen (122), but is taken up by the enterocyte intact.

Within the mucosal cell it is extensively hydrolyzed, as no intact sphingomyelin reaches the lymph (164). A specific sphingomyelinase that forms phosphorylcholine and ceramide exists in liver. It has almost no activity towards lecithin (114). Phospholipase C activity, present in brain, kidney, liver, and spleen, can also act on sphingomyelin, thereby forming phosphorylcholine, which can then be cleaved by phosphatases, thereby producing free choline (93, 125).

SOURCES OF CHOLINE NOT DERIVED FROM THE DIET

The diet is not the only source of choline. Several organs possess the ability to synthesize choline molecules de novo, by the sequential methylation of phosphatidylethanolamine, forming lecithin. Of the three enzymatic pathways that catalyze lecithin biosynthesis, only this one generates new choline molecules (Figure 2). The cytidine diphosphocholine (CDP) (138) and base exchange (166, 185, 207) pathways do not cause a net synthesis of choline, but only redistribute preexisting molecules. It is only by the sequential methylation of phosphatidylethanolamine, catalyzed by the enzyme(s) phosphatidylethanolamine-N-methyltransferase (PEMT), that new choline molecules are produced (31, 42, 43, 146, 152, 160). This enzyme has its highest activity in the liver (31, 43), although it is found in kidney, testes, heart, lung, adrenal, erythrocyte, spleen, and brain (37, 117, 118). The liver utilizes the methylation pathway to meet an estimated 15% of the daily requirement for choline in the rat (about 13 μmol/g of liver per day) (31, 43, 187, 239); the rest of needed choline is derived from the diet. This pathway quantitatively synthesizes about 15–40% of the lecithin within the liver, with most of the rest derived from the CDP pathway (146, 206). In rat brain the methylation pathway synthesizes lecithin, some of which is subsequently cleaved, which produces significant quantities of free choline (36).

PEMT is a complex of two separate activities, one that adds the first methyl group, and a second that adds the final two methyl groups. S-adenosyl methionine (SAM) acts as the methyl donor for both components (117, 178). The activity of the first enzyme is limiting (43), and it is located on the inner cell membrane; the second enzyme is located on the outer cell surface, and the phospholipid substrate translocates across the membrane as added methyl groups change its charge (117). The two enzymes have different kinetic properties: The first has a pH optimum of 6.5 and has a high affinity for SAM; the second has a pH optimum of 10.0 and a low affinity for SAM (117). Choline deficiency and exposure to ethanol increase the activity of the methylation pathway in liver (84, 120, 220). Female rats have more hepatic PEMT activity than do males (31, 146).

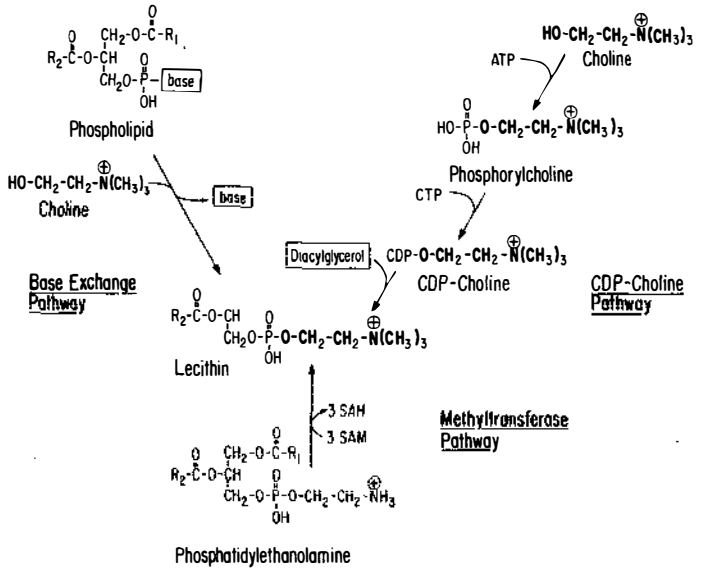

Figure 2 Pathways for the biosynthesis of lecithin. The CDP-choline pathway and the base exchange pathway form lecithin by using pre-existing choline molecules (choline indicated by darker print). The methyltransferase pathway synthesizes lecithin by methylating phosphatidylethanolamine, thereby forming the choline moiety de novo (from Ref. 37).

THE BIOCHEMISTRY OF CHOLINE

In mammalian tissue, free choline participates in four enzyme-catalyzed pathways: oxidation, phosphorylation, acetylation, and base exchange.

Oxidation

Choline is oxidized to betaine aldehyde, which is then converted to betaine by the enzyme system choline oxidase (choline dehydrogenase, EC 1.1.9.91, and betaine aldehyde dehydrogenase, EC 1.2.1.8). This enzyme activity is present in several mammalian tissues, including liver (where it is most active) (25, 108, 139, 149, 233) and kidney (24, 149). It does not seem to be present in brain, muscle, or blood of rats (24).

Choline dehydrogenase is largely a mitochondrial enzyme (72, 139, 203, 215, 235, 237), whereas betaine aldehyde dehydrogenase is cytosolic (108, 236, 242). Choline dehydrogenase has a pH optimum of 7.6–8.2 and has a K_m for choline of 0.7 mM (108, 179). In view of this low affinity for choline, it is probably not saturated with substrate in vivo. It is inhibited by its end product, betaine aldehyde [which only builds up in the presence of a betaine aldehyde dehydrogenase inhibitor, such as semicarbazide (86)] and is inhibited by amytal, antimycin, cyanide, atabrine, aureomycin, analogues of choline, 2-amino-2-methyl-1-propanol, and α,α-dimethyltriethylcholine (19, 179, 215, 225).

The maximal rate of betaine formation from choline in liver is more than 10 times faster than choline incorporation into phosphorylcholine (108, 224). Betaine, the end product of choline oxidation, cannot be reduced to form choline; it can donate one of its methyl groups to L-homocysteine, thereby producing dimethylglycine and L-methionine, in a reaction catalyzed by betaine-homocysteine methyltransferase (EC 2.1.1.5) Dimethylglycine is converted to sarcosine and then to glycine by sarcosine oxidase (EC 1.5.3.1). Thus, although the choline oxidase pathway acts to remove free choline from the body, it is able to scavenge valuable methyl groups.

Phosphorylation

Choline kinase (choline phosphotransferase, EC 2.7.1.32) catalyzes the phosphorylation of the hydroxyl group of choline by utilizing adenosine triphosphate as the phosphate donor (44). It is widely distributed in mammalian tissues, including liver (44, 138, 207), brain (109, 151), and lung (81, 85, 197, 199). It is a soluble, cytosolic enzyme, with a pH optimum of 8 to 11 and a K_m for choline of 0.033 mM, and it has a requirement for magnesium ions (44, 109). Ethanolamine is a competitive inhibitor ($K_i = 6.2$ mM) and betaine, phosphorylcholine, and adenosine diphosphate also inhibit activity (44).

Phosphorylation of choline is the first step in the CDP-pathway for the synthesis of lecithin (Figure 1) (138). In this pathway cytidine triphosphate and phosphorylcholine are combined, which generates CDP-choline and inorganic phosphate. The CDP-choline is subsequently combined with diacylglycerol (catalyzed by phosphatidylcholine glyceride transferase), which forms lecithin. In liver the rate of this reaction is 0.25 μmol/mg (wet weight) per min (207). This rate is less rapid than that measured for the choline oxidase pathway.

Acetylation

Though only a small fraction of administered choline is acetylated (55, 111, 112), this pathway has importance because of acetylcholine's role as a neurotransmitter. The reaction of acetyl coenzyme A with choline is catalyzed by choline acetyltransferase (ChAT, EC 2.3.1.6) (162). This enzyme is highly concentrated in cholinergic nerve terminals (88, 221), although it has been localized in some non-nervous tissues such as placenta (177).

Several factors influence ChAT activity in vivo: (*a*) precursor availability: (*b*) feedback inhibition of ChAT by its end product, acetylcholine; (*c*) variation in the activity of high-affinity uptake of choline into the nerve terminal; and (*d*) mass-action kinetics (i.e. increased synthesis when high concentrations of substrate or low concentrations of product are present).

ChAT from rat brain has an in vitro K_m for choline of 0.4 mM, and a K_m for acetyl-CoA of 0.018 mM. In human brain, ChAT has a K_m for

choline of 0.6 mM and for acetyl-CoA of 0.007 mM (230). In vivo, brain acetyl-CoA concentrations are 8–9 μM and brain choline concentrations are 37–100 μM, which makes it unlikely that ChAT is saturated with either substrate (183, 230). Thus, choline (and possibly acetyl-CoA) availability could determine the rate of acetylcholine synthesis.

Choline administered systemically rapidly enters the brain (58, 111, 112, 167, 176). A specific carrier mechanism, within the blood-brain barrier, that transports choline into the brain against a concentration and charge gradient has been identified. It has a K_m for choline of 0.44 mM and a V_{max} of 10 nmol/g of brain per min (167). At physiologic serum choline concentrations (which are always less than 0.1 mM) this transport mechanism is unsaturated and capable of carrying choline at a rate proportional to serum choline concentration. The systemic administration of choline chloride raises serum choline concentration, brain choline concentration, and brain acetylcholine concentration (55, 111). The acetylcholine content of peripheral cholinergic neurons and of tissues rises as well (112, 113, 218). Administration of choline as a chronic dietary supplement also increases brain choline and acetylcholine concentrations (56).

This increase in acetylcholine levels is associated with increased release of acetylcholine into the synapse. Postsynaptic activation of tyrosine hydroxylase activity, a phenomenon mediated by acetylcholine activation of a postsynaptic receptor (124, 161, 210), occurs when choline is administered (218, 219). This activation can be blocked by presynaptic denervation, or by the administration of atropine, which suggests that choline does not directly activate receptors, but must first be converted to acetylcholine in presynaptic terminals (218). Direct measurement of acetylcholine release from brain neurons is not technically possible, but in preparations of isolated phrenic nerve and in brain slices choline administration significantly increases the amount of acetylcholine released into the perfusing medium (29, 228). The above experimental observations make it likely that in vivo synthesis of acetylcholine is influenced by choline availability.

The hypothesis that acetylcholine might inhibit ChAT activity, in vivo, is based on the observed kinetics of the enzyme. Acetylcholine, when present in high enough concentrations, can compete with choline for an acceptor site on the enzyme (132, 229). Since the content of vesicular acetylcholine is inhibited in vitro by 20 mM acetylcholine, feedback control of acetylcholine is possible. The significance of this mechanism in vivo is not known. The segregation of acetylcholine into pools within the neuron may act to prevent ChAT inhibition. If feedback control plays an important role in regulating acetylcholine synthesis it becomes difficult to explain the large increases in acetylcholine concentration noted after choline administration (55, 56, 110, 111).

It has been proposed that acetylcholine synthesis is coupled to the activity of the sodium-dependent high affinity uptake of choline by the nerve terminal (20, 106, 191). This transport is influenced by the impulse activity of the neuron (13, 192), and by synaptosomal acetylcholine content (127). These observations are based upon in vitro studies performed on isolated neuronal particles (synaptosomes). In other nerve preparations evidence supports the contention that choline transport is not coupled to acetylcholine synthesis (140). It is possible that high-affinity uptake is important in the re-uptake of choline liberated from acetylcholine within the synapse, but that low-affinity mechanisms, located in the membranes of the cell body and axon, take up significant amounts of choline as well.

The reaction catalyzed by ChAT is reversible and is dependent on the concentrations of both precursors and products. The equilibrium constant of the enzyme is relatively low (e.g. 40) and therefore transmitter synthesis could be regulated by mass action (99, 174). Though these enzyme kinetics were obtained in vitro, this mechanism may help to account for the acceleration of acetylcholine synthesis in vivo by large amounts of choline.

Base Exchange Reactions that Involve Choline

This pathway has been described in several tissues, including the liver and brain (166, 173, 185). It involves the substitution of choline for serine, ethanolamine, or inositol present within endogenous phospholipids. The reaction is reversible and requires the presence of calcium ions, but it is not energy dependent. At the present time it is uncertain whether or not the "base" exchange pathway is quantitatively important in vivo, as less than 10% of intraportally injected [*methyl*-^{14}C]choline enters lecithin without passing through phosphorylcholine (185, 206).

ORGAN UPTAKE OF CHOLINE

As described earlier, many organs oxidize, phosphorylate, or acetylate choline, thereby removing it from the pool of free choline available to the tissues of the body. An alternative mechanism for decreasing available free choline would be to segregate choline into a site not readily accessible (i.e. the kidney could accumulate free choline and excrete it into the urine).

Uptake of Choline by the Kidney

Within 15 min after a dose of radioactive choline is administered systemically a significant amount of label is found within the kidney (112, 113, 150). Nephrectomized dogs show a decrease in the rate of choline disappearance from the plasma (34, 123). Some of this choline, which is taken up by the

kidney, appears in the urine unchanged (34, 112), but this probably only occurs when choline presented to the kidney exceeds a threshold concentration. In the chicken, transport of choline across the renal tubule is an active process (2–5, 180), with rate determined by choline concentration (4). Choline analogues alter renal excretion of choline: Hemicholinium-3 at low concentrations enhances transport (probably by inhibiting tubular reabsorption), whereas at high concentrations it markedly inhibits choline output (3). Quinine, cyanine 863, and tetraethylammonium also inhibit transport (4, 180), whereas betaine and carnitine have no effect (208).

Much of the choline transported into the kidney is oxidized to form betaine (4, 208). Net active excretion does not occur until sufficient choline is presented to the kidney to saturate choline oxidase. The transport maximum (1 pmol/kg per min) is reached only when choline presented to the kidney exceeds 2 pmol/kg per min, and no choline is excreted when less than 0.5 pmol/kg per min is presented (2, 4). Betaine is passively transported by the kidney and is the major metabolite of choline excreted in the urine (112). Renal free choline concentration is 70–90 nmol/g (wet weight) (112).

Mean free choline concentration in the plasma of azotemic humans is several times greater than that observed in normal controls (181), which indicates that renal clearance plays an important role in the maintenance of plasma choline concentrations in the human. Hemodialysis rapidly removes choline from plasma (181).

Uptake of Choline by the Liver

As discussed earlier, the liver has very active mechanisms for phosphorylation and oxidation of choline. All ingested choline, and free choline liberated from phospholipids, enters the hepatic circulation. The liver is a significant "sink" for choline. Hepatectomy elevates plasma choline concentration by increasing choline's half-life (34). In vivo, systemically injected choline is accumulated by liver (112, 113, 150), and in isolated perfused rat liver there is a significant net uptake of free choline (212–214, 245). A saturable mechanism [$K_a = 0.17$ mM, $V_{max} = 0.84$ μmol/g (dry weight) per min] and a nonsaturable mechanism (through which uptake is proportional to choline concentration presented to the liver) contribute to hepatic choline uptake (245). Free choline concentration within the hepatocyte is 50–100 nmol/g (wet weight) (110). The remainder of the choline accumulated by the hepatocyte is metabolized to form betaine, phosphorylcholine, and lecithin (245). The rate at which liver takes up choline is sufficient to explain the extremely rapid disappearance of choline injected systemically.

SPECIAL NOTES ON CHOLINE METABOLISM IN THE FETUS AND NEONATE

The newborn human, rat, and rabbit have extremely high plasma choline concentrations (4–7 X adult) (243). In preceding sections, sources of choline, metabolic pathways that use choline, and sinks for choline in the adult mammal have been described. Fetal and neonatal organisms differ from adults, and it is likely that some mechanisms involved in choline metabolism are different. The fetus derives all nutrition from its mother, via the placenta, the neonate ingests only milk, and the adult eats a wide variety of foods. Both the fetus and the neonate utilize a great deal of choline in growth-related membrane synthesis; the adult has finished rapid growth. Finally, several biochemical pathways, in which choline is a precursor, are crucial for survival of the neonate (i.e. lung surfactant synthesis).

Sources of Choline in Utero

During periods of rapid growth large amounts of choline are needed for membrane and myelin synthesis. The fetus, unable to eat choline-containing foods, must derive all exogenous choline from its mother, via the placenta. The human placenta is perfused with 500 ml of maternal blood per min (226). It possesses a transport mechanism for choline, with both a passive diffusion (transport proportional to maternal blood choline concentration) and an active transport component [$K_m = 0.35$ mM, $V_{max} = 75$ nmol/ml (wet weight)], which results in significant transport of choline from maternal blood into the placenta (129, 227). Choline accumulated by the placenta is rapidly converted to acetylcholine; only small amounts of phosphorylcholine and phospholipid are synthesized in comparison (226, 227). An age-related variation occurs in placental acetylcholine concentration (177), as does a similar age-related change in placental choline acetyltransferase activity, both of which peak at 16–24 weeks postconception (177). All of the acetylcholine synthesized within the placenta must be made by using exogenous choline, as this organ lacks the ability to synthesize choline de novo (177, 227). Acetylcholine within the placenta is not associated with neurons, and its function is unknown (177, 227). The placenta is incapable of taking up lecithin from maternal blood and transporting it to the fetus (30).

Postnatal Diet

In mammals maternal milk is a major component of the diet for some time after birth. The rat is weaned at 10–15 days postnatal, when it begins to try solid food (234). The human is weaned weeks to months after birth, depending on local customs. The composition of milk changes with postnatal age.

Colostrum (pre-milk, secreted for the first 5 days postnatal) is 2.9% fat, transitional milk (5–10 days postnatal) is 3.6% fat, and mature milk is 3.8% fat (128). In human milk, phospholipids represent 0.26% of the fat globule, but compose 23.4% of the fat globule membrane (a loose network of proteins, lipids, and phospholipids that amount to 7% of total lipid content), thus phospholipids in human milk account for 1–2% of lipid fat. Of this, approximately 30% is phosphatidylcholine, 30% is sphingomyelin, and the remainder is phosphatidylethanolamine (30%), phosphatidylserine (2–6%), and phosphatidylinositol (4%), with traces of lysolecithin and lysophosphatidylethanolamine (128). Most of milk lipid is triacylglycerol (98%); the major fatty acids are palmitic, stearic, oleic, and linoleic, with exact composition varying with maternal diet (128).

Most published reports on total choline in milk do not include all the available choline (choline, lecithin, and sphingomyelin) and have not been controlled for time of day, time of feeding (fore-milk versus end-milk), or time postpartum. For these reasons the figures cited below are only estimates. Human milk contains 780 nmol of lecithin per ml and 100 nmol of choline per ml (163). The average human neonate ingests approximately 600–700 ml of milk/day (126), which (with the above estimates) contains 1 mmol of lecithin and 100 μmol of free choline. Human infants are often fed commercially prepared milk substitutes, with compositions different from human milk. Many of these formulas are derived from cow's milk. This milk has a fat content of 3–5%, of which 0.2–1% is phospholipids. The major components of the phospholipid fraction are phosphatidylcholine (35%), phosphatidylethanolamine (32%), and sphingomyelin (25%). The total choline content of cow's milk varies with season of the year and with handling and is approximately 500 nmol/ml (94). Several commercially prepared formulas (Lofenalac, Albumaid-KP, Formula 3200K, MSUD-AID, and Formula 80056; all used in the treatment of inborn errors of metabolism) are much lower in lecithin content than is human milk (163). At the present time it is not possible to state what the consequences for the neonate might be if such formulas were chronically ingested.

Growth

Dilution of body stores by rapid growth occurs in the neonate, but not in the adult. Choline requirements during this period not only must meet the demands of normal turnover in tissues, but must meet the demand for incorporation into membranes of new cells. In the macaque brain, for example, 10 mg of phospholipid must be synthesized for each gram of brain weight, during a period beginning at 40 days postconception and extending through 40 days postnatal (232). Individual organs grow at different rates.

In the rat, maximal brain growth (in weight) occurs at 10–15 days after birth (52, 73, 74); in humans, maximal brain growth occurs just before term (73).

Certain aspects of cell and organ growth place special demands on choline supplies. Myelinization, the wrapping of neuron axons in a layer of phospholipid (mostly lecithin and sphingomyelin), takes place both before and after birth. In the human, myelinization begins during the second half of fetal life and is largely complete by 6 months, but it continues for 3–5 years after birth (211).

Metabolic Pathways that Require Choline and that are of Special Importance in the Neonate

The synthesis of lecithin occurs in every tissue of the neonate. In the lung, synthesis of surfactant (mostly disaturated lecithin) is crucial for the survival of the organism. Surfactant is a surface active lipid that coats the alveolus and acts to lower surface tension, thereby preventing alveolar collapse during expiration. When alveoli collapse, the infant must exert much more force to re-expand the lungs. Deficiency of surfactant is the major factor in the pathogenesis of respiratory distress syndrome in the premature human infant (47, 100, 157). The major active component of surfactant is disaturated lecithin (1, 46). In the lung, the sequential methylation pathway for the formation of lecithin is of little quantitative significance (81, 182). The CDP-choline pathway, discussed in an earlier section, is responsible for most lung lecithin synthesis. In vitro, 10–50 times more lecithin is synthesized by this pathway than by the methylation route (81). In vivo, the CDP-choline pathway is 100 times more active (85).

Choline kinase has an affinity for choline in the range of lung free choline concentrations, thus dramatic changes in kinase activity could occur if substrate availability is altered. In the offspring of rat dams fed a choline-deficient diet, phosphatidylcholine content of surfactant is reduced (69% of control) (137). No evidence is available to determine whether or not choline treatments can accelerate lung lecithin synthesis.

The metabolism of choline varies with postnatal age. Choline dehydrogenase in liver increases in activity after birth (203, 224). In liver slices (from 19 days postconception, 7 days postnatal, and adult rats), formation of phosphorylcholine and of phosphatidylcholine does not change as the rat matures (224). The distribution of choline among tissues may also change with postnatal age. More choline is transported into the brain of a 15-day-old rat than is transported into adult rat brain (167). The newborn rabbit takes up three times more choline into its brain than the adult rabbit does, yet uptake of glucose and mannitol show no developmental changes, which

demonstrates that the increase in choline transport is not due to nonspecific leakiness (41). Higher brain uptake of choline into the brain of the 1-day-old versus the adult chicken has also been reported (39).

EFFECTS OF CHOLINE ADMINISTRATION

Choline has been used to treat human diseases for more than 50 years. The major sites of its therapeutic action are the nervous system, the cardiovascular system, and the mechanisms that store and transport fats and cholesterol.

Choline and Diseases of the Nervous System

As discussed in a previous section, acetylcholine synthesis and release are augmented after the administration of pharmacologic doses of choline. Acetylcholine is an important and widespread neurotransmitter in the central nervous system. The ascending tegmental-mesencephalic-cortical neurons contain cholinergic synapses and are involved in the control of arousal, learning, motor activity, and REM-sleep (135, 154). Motor activity also depends on cholinergic synapses in the ventral-tegmental pathway, with its projections to the corpus striatum and cerebellum, and on nigrostriatal circuits, which include cholinergic striatal interneurons (98, 135, 142).

Choline was first tried as therapy for the movement disorder tardive dyskinesia because clinical evidence indicated that inadequate neurotransmission at striatal cholinergic interneurons was involved in the pathophysiology of this syndrome (62, 98, 142). Tardive dyskinesia, one consequence of long-term use of neuroleptics, is characterized by choreoathetotic movements of the face, the extremities, and occasionally the trunk (14, 60, 216). All neuroleptics currently used in the United States have potential for producing tardive dyskinesia, and prevalence estimates vary from 0.5–50% (131). The only effective therapy to date has been oral administration of choline or lecithin (which are postulated to act by bypassing inhibition of cholinergic striatal interneurons) (63, 97, 103, 104).

The septo-hippocampal cholinergic neurons are involved in memory (154). There is a great deal of evidence that, in the rat and human, activity of cholinergic neurons is vital for normal memory. Mice exhibit an age-related diminution of memory. Chronic dietary choline deficiency exacerbates this loss of memory, whereas choline-enriched diets markedly improve memory in older mice (21). Healthy humans, treated with anticholinergic drugs, develop memory deficits that resemble those seen in patients with hippocampal lesions (they have deficits in short-term memory—the

ability to store new memories) (75, 76). Choline administration alters memory: It decreases the number of trials needed to memorize lists (194, 195); and it may diminish deficits in short-term memory associated with Alzheimer's disease, a syndrome of the aged in which hippocampal cholinergic neurons are deficient (40, 53, 82, 91, 172, 190, 198). Definitive statements regarding the utility of choline supplements in the treatment of memory disorders must await carefully controlled double-blind studies. Such investigations are underway at the Massachusetts Institute of Technology and several other institutions at this time.

Physostigmine and other centrally active anticholinesterases reverse some of the symptoms of mania (50, 64). In an open study, administration of lecithin (in addition to lithium) reduced manic episodes, and when lecithin was withdrawn 75% of the patients worsened (54). One side effect reported when choline was used in the treatment of tardive dyskinesia was the worsening of existing depression (209). Some other parameters of brain function are altered by choline administration, for example electroencephalographic measurement reveals slight changes in brain electrical activity (101). It is possible that respiration could be altered as there are cholinergic synapses in the chemosensitive neurons of the ventral surface of the medulla oblongata (95).

Choline also has effects on the peripheral nervous system. Descending mesencephalic and spinal pathways contain cholinergic neurons. These neurons contribute to spinal input into sensory and motor phenomena. Peripheral cholinergic innervation extends to skeletal and smooth muscle, and to exocrine and endocrine glands. At the adrenal medulla, skeletal muscle and lacrimal and nasopharyngeal glands cholinergic neurons are the predominant, or sole modulators of activity (135). Acetylcholine is also involved in the release of vasopressin (196) and thyroid hormone (156). Choline has already been shown to augment acetylcholine release in the heart (147), neuromuscular junction (29), adrenal gland (218), superior cervical ganglion, stellate ganglion, celiac ganglion, and the ganglia of the thoracic sympathetic chain (217).

Choline and Hypertension

In both humans and animals, intravenous choline administration lowers blood pressure (8, 134, 201). This effect is prevented by pretreatment with hemicholinium-3, which suggests that choline must be taken up into the neuron to exert its hypotensive effect (193). Oral administration of choline chloride slightly lowered the blood pressure of some patients with Alzheimer's disease (40). It is possible that the hypotensive effect of choline is mediated via the vagus nerve (which is cholinergic), but choline is also a

vasodilator (134). Cardiovascular side effects of choline administration are rarely reported and are not a major clinical problem. It may be that neurons are most sensitive to changes in the availability of choline when they are firing rapidly, as would neurons in tracts that have lost significant numbers of working synapses (the remaining neurons try to make up for deficient transmission). Peripheral cholinergic neurons, firing at normal rates, might be relatively insensitive to choline administration.

Rats exposed to transient periods of choline deficiency subsequently develop irreversible hypertension (27, 143, 158). These changes may be related to alterations in renal as well as in cardiovascular function.

Changes in Blood Lipids Induced by Choline or Lecithin Treatments

Choline plays an important role in the maintenance of normal lipid transport (165b). Oral administration of choline to rats increases plasma total cholesterol, phospholipid, high density lipoprotein, and low density (β) lipoprotein (165a,b). In humans and rats, diets deficient in choline and methionine decrease cholesterol and β-lipoproteins (165b). In rabbits, choline has little effect on serum total cholesterol (86a, 140a). There have also been reports of a hypocholesterolemic effect of choline (115, 133). If choline does increase blood cholesterol concentration, it is possible that the mechanism involves mobilization of lipids from hepatic stores (165b). If choline lowers blood cholesterol, it may be acting by stimulating biliary cholesterol and lecithin excretion (181a).

Lecithin has been marketed as an over-the-counter preparation, which has been claimed to lower blood cholesterol. The effects of lecithin feeding on serum lipids have been inconsistent. In rabbits fed a high cholesterol diet, small supplements of lecithin (containing 20% phosphatidylcholine) lower serum total cholesterol and decrease the incidence of atherosclerosis (140a). In another study no such effects were noted (143a). In healthy humans oral lecithin may lower total blood cholesterol (5a, 32, 119), transiently lower serum total cholesterol for a period of 4 weeks (201a), or have no effect on total blood cholesterol (53a, 101a, 104a, 224a). In patients with type II hyperlipidemia, lecithin administration does not lower serum cholesterol (101a, 224a). One might hypothesize that any effect that lecithin has as a hypocholesterolemic agent is mediated by the enzyme lecithin cholesterol acyltransferase. This enzyme transfers a fatty acid from lecithin to cholesterol, thereby forming cholesterol ester, which is more efficiently removed from the blood (165, 170). In the human, lecithin does not stimulate esterification of plasma-free cholesterol (53a).

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