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RESEARCH GUIDE

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CHOLINE

A Critical Prenatal Nutrient

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Introduction

Nutritional recommendations for pregnancy emphasize an increased need for calories, protein, calcium, iron, folic acid, and the omega-3 fatty acid docosahexaenoic acid (DHA). Public health campaigns have succeeded in creating widespread awareness of these critical prenatal nutrients and the risks associated with deficiencies. Until recently, however, the essential nutrient choline has been largely ignored in scientific, clinical, and public health discussions about prenatal nutrition.

For most of the 20th century, choline was not recognized as an essential nutrient. Researchers then discovered that adults developed nonalcoholic fatty liver disease and muscle disorders when deprived of choline. The Institute of Medicine (IOM) took notice and officially recognized choline as an essential nutrient in 1998. Even so, little attention was given to the importance of choline before, during, and after pregnancy.

Then in June 2017, delegates at the American Medical Association (AMA) Annual Meeting passed a resolution in support of including 450 mg of choline—the recommended adequate intake (AI) for pregnant women—in all prenatal vitamins. At the time of the resolution, none of the top 25 prenatal vitamins sold in the United States contained this amount.

The resolution to include evidence-based amounts of choline in all prenatal formulations has yet to be fully implemented, but it will undoubtedly have far-reaching effects. Vitamin manufacturers will need to reformulate products, clinicians will need to revise their recommendations, and millions of mothers and babies will reap the benefits. The tide in the story of prenatal choline is turning.

This research guide is written for all clinicians who counsel women of childbearing age, particularly women who are currently pregnant or who are planning to become pregnant. It is intended to bring clinicians up to date on the most current research related to the biochemistry of choline, the functions of choline in pregnancy, and the benefits of choline supplementation during the perinatal period.

Biochemistry of Choline

Choline is a water-soluble micronutrient that is derived from both endogenous and exogenous sources. Choline is generated via several pathways in the human liver, including the cytidine diphosphate (CDP)-choline pathway and the phosphatidylethanolamine N-methyltransferase (PEMT) pathway. The PEMT pathway is dependent on estrogen and therefore exceptionally active during pregnancy, when estrogen levels can reach 60 times that of non-pregnant levels.

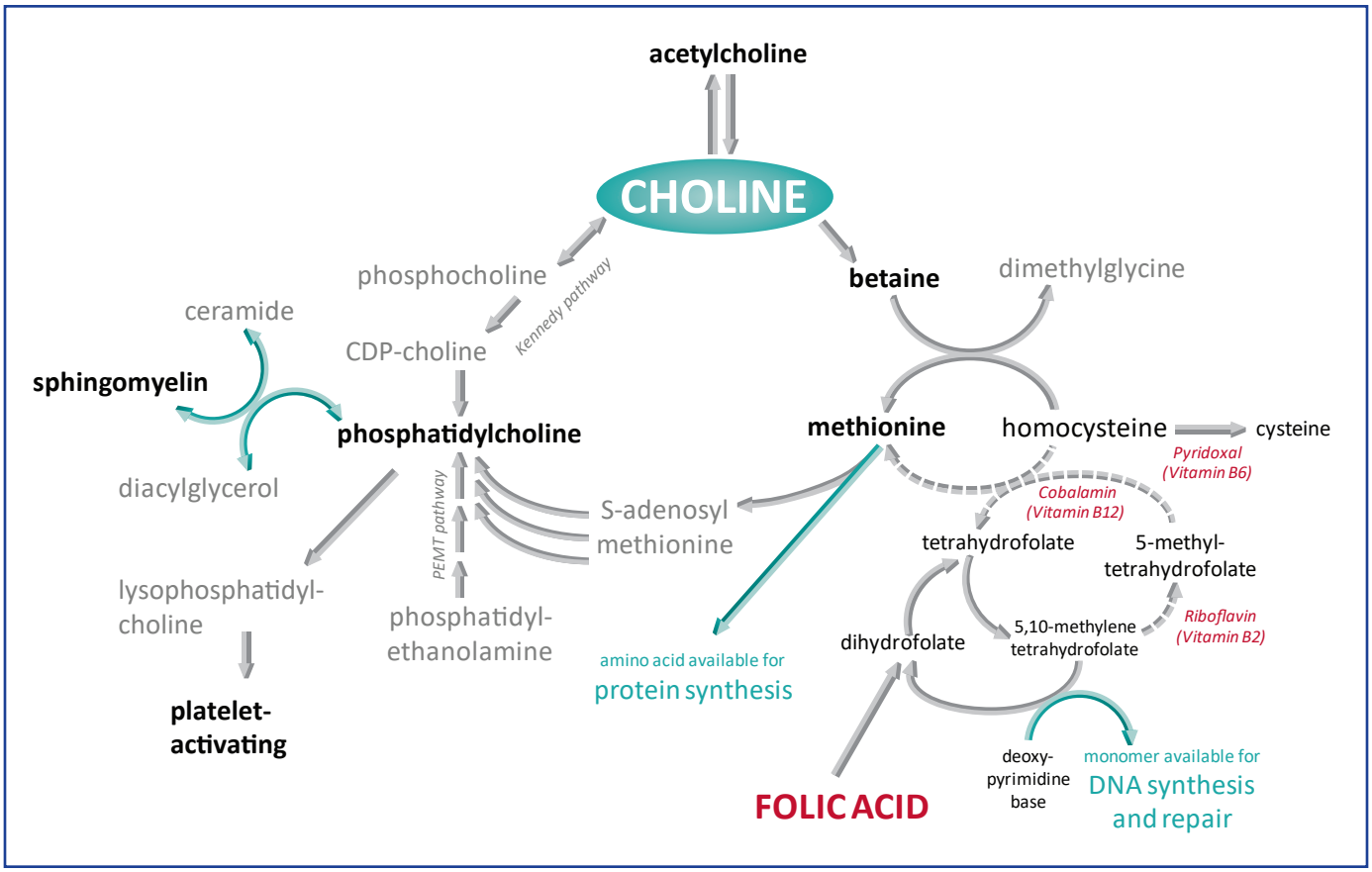
Choline is central to many biochemical processes. Choline metabolizes into 4 key biological compounds: phosphatidylcholine, sphingomyelin, acetylcholine, and betaine. These compounds play critical roles in cell division, nervous system development, and epigenetic regulation. During pregnancy, the physiologic processes that rely on choline are in full swing.

CHOLINE METABOLITES

METABOLITE	FUNCTION
Phosphatidylcholine	Phosphatidylcholine is the primary phospholipid in cell membranes. It is in high demand during periods of rapid cell division and replication.
Sphingomyelin	Sphingomyelin forms the myelin sheath around neuronal axons. It is important for neuronal communication and brain development.
Acetylcholine	Acetylcholine is a neurotransmitter that is central to cholinergic transmission in both the peripheral and central nervous systems.
Betaine	Betaine is a precursor for the methionine cycle, producing s-adenosylmethionine (SAMe), one of the body's most important methyl donors and a key player in epigenetic regulation.

A research group in the Division of Nutritional Sciences at Cornell University has published numerous papers related to the biochemistry of choline during pregnancy. The Cornell research group published a study in 2013 that compared choline metabolism in nonpregnant women to choline metabolism in women in their third trimester of pregnancy. The study found that pregnancy shifts choline metabolism away from synthesis of betaine and toward synthesis of phosphatidylcholine, via both the CDP-choline pathway and the PEMT pathway. The researchers detected an incremental increase in the transfer of PEMT-derived phosphatidylcholine

METABOLISM OF CHOLINE



across the placenta and into the fetal compartment—suggesting a specific requirement for PEMT-derived phosphatidylcholine in fetal development.

Research has also shown that choline transfers across the placenta against its concentration gradient, resulting in high concentrations in the fetal compartment. Amniotic fluid can contain up to 15 times as much choline as the maternal blood supply, and newborns are born with blood levels of choline that are 3 times that of maternal blood. Choline is indeed sequestered by the developing fetus during the prenatal period.

Choline in Pregnancy

Metabolites of choline are in high demand during pregnancy. Phosphatidylcholine aids new formation of cell membranes, sphingomyelin creates a layer of protection around developing neurons, acetylcholine communicates throughout the central and peripheral nervous systems of the developing fetus, and betaine regulates epigenetic modification via one of the body's most important methyl donors, SAMe.

The biochemistry of choline is clear, and a library of evidence from animal studies is beginning to unravel the physiologic effects of choline during pregnancy. Human studies on the role of choline during pregnancy are less abundant but intriguing nonetheless. There are both animal and human studies related to choline during pregnancy.

Animal Studies

There is clear evidence from animal research that choline is required for proper closure of the neural tube, fetal brain development, and cognition of the newborn. Studies suggest that prenatal choline supplementation improves attention and visual memory in the offspring. In animal models, choline deficiency during pregnancy impairs fetal brain development, cell division, and methylation.

Because of its epigenetic effect, several authors have suggested that prenatal levels of choline may influence the lifelong health of the offspring. Mouse studies have found that prenatal choline supplementation protected against the long-term cognitive and neuropathological

effects of seizures in adulthood. Studies in mice also suggest that prenatal choline supplementation mitigates the effects of alcohol on the offspring, offering a potential preventive measure to minimize the repercussions of fetal alcohol syndrome. Some researchers have gone so far as to suggest that prenatal choline supplementation may reduce the risk of offspring developing schizophrenia later in life.

In a mouse model of Down Syndrome, researchers found that maternal choline supplementation exerted lasting effects on cognitive function in the offspring. When pregnant mice carrying trisomic mice were supplemented with choline, the offspring demonstrated better spatial cognition, attention, and neural functioning through adulthood. No human studies have examined the effect of prenatal choline supplementation on Down Syndrome, but researchers conclude that the findings from animal studies are promising enough to warrant further investigation.

Human Studies

Human studies on the effects of choline during pregnancy are limited, but the early results are promising. Epidemiological studies suggest that choline protects against neural tube defects and may enhance childhood cognition. Clinical trials have found that choline supplementation improves stress adaptation in the developing fetus and decreases the risk of preeclampsia.

Neural tube defects, including spina bifida and anencephaly, are thought to occur when there is a deficiency of folic acid in the early stages of pregnancy. However, neural tube defects continue to occur despite widespread fortification and inclusion of folic acid in all prenatal vitamins. A prospective study of more than 180,000 pregnant women and their babies in California found that women with the lowest serum levels of choline at mid-pregnancy had more than double the risk for babies with neural tube defects—even in women consuming the recommended amount of folic acid. Mothers who had serum choline levels in the highest quartile had the lowest odds of pregnancies with neural tube defects.

Because animal studies suggest that prenatal choline may influence cognitive abilities in the offspring, researchers have begun to examine its effects in humans. A 2008 study, conducted by the National Institutes of Health (NIH), failed to confirm these findings in humans. Researchers measured concentrations of choline at various stages of pregnancy in maternal blood and in cord blood at delivery and then evaluated the child's intelligence quotient (IQ) at the age of 5. No association was found between prenatal choline concentrations within the physiologic range and child IQ at the age of 5.

In contrast to the null findings of the NIH study, researchers at the Harvard School of Public Health did detect a relationship between higher prenatal choline intake and cognitive function in children. In this prospective study, 895 mothers in Massachusetts were followed from their first trimesters of pregnancy (in 1999-2002) until their babies were 7 years old (in 2008-2011). Choline intake was estimated during the first and second trimesters, and visual memory of the children was evaluated at age 7. After adjusting for intake of related essential nutrients and other confounding factors, higher intake of choline during the second trimester was significantly associated with higher scores of visual memory at age 7.

A research group in the Division of Nutritional Sciences at Cornell University published a study in 2012 that evaluated the epigenetic effect of prenatal choline supplementation on fetal hypothalamic-pituitary-adrenal (HPA) axis reactivity. Women during their third trimester of pregnancy were supplemented with 480 mg or 930 mg of choline per day for 12 weeks. Researchers then evaluated the epigenetic effect of choline supplementation on cortisol-regulating genes and their expression in the placenta and cord blood. High-dose choline supplementation produced higher methylation of corticotropin-releasing hormone (CRH) genes in the placenta, lower CRH transcription in the placenta, and lower plasma concentrations of cortisol in the cord blood. The researchers concluded that high-dose prenatal choline supplementation modulates the expression of genes that regulate fetal HPA axis reactivity via

BENEFITS OF CHOLINE DURING PREGNANCY	
Evidence from Animal Studies	**Evidence from Human Studies**
• Promotes closure of the neural tube (Fisher 2002) • Enhances cognition of the newborn (McCann 2006) • Promotes recovery from seizures in adulthood (Wong-Goodrich 2011) • Protects against the effects of alcohol exposure in utero (Bearer 2015) • Reduces the risk of neurological and psychiatric disorders in adulthood (Freedman 2015) • Promotes fetal brain development (Mudd 2016) • Reduces cognitive effects of Downs Syndrome (Strupp 2016)	• Reduces risk of neural tube defects (Shaw 2009) • Regulates hypothalamic-pituitary-adrenal (HPA) axis reactivity in newborns (Jiang 2012) • Improves visual memory during childhood (Boeke 2013) • Reduces risk of preeclampsia (Jiang 2013)

epigenetic mechanisms. Because cortisol impairs attention and contributes to stress-related illnesses later in life, babies born to moms supplemented with choline might experience lifelong benefits of having a higher tolerance—or reduced physiologic response—to stress.

A study published in 2013 by the Cornell University research group found promising evidence that choline supplementation may reduce the risk of preeclampsia in healthy pregnant women. In this randomized clinical trial, women were assigned to take choline at a dosage of 480 mg or 930 mg per day for 12 weeks, and samples were taken from maternal blood and the placenta at delivery. Genomic testing revealed that high-dose prenatal choline supplementation significantly decreased expression of fms-like tyrosine kinase-1 (sFLT1) in the placenta and decreased sFLT1 protein concentrations in maternal blood. Given that sFLT1 is an important preeclampsia risk marker, these findings suggest that prenatal choline supplementation may improve placental angiogenesis and decrease the risk of preeclampsia.

Nutrient Synergy

Choline does not function in a vacuum but rather has the ability to compensate for or enhance the action of other essential nutrients. During the perinatal period, choline appears to synergize with folic acid and DHA to support the health of both mom and baby.

Folic Acid

Choline and folic acid have overlapping functions as well as distinct functions of their own. Both participate in one-carbon metabolism, leading to the production of the methyl donor SAMe. This means that during transient periods of folate deficiency, choline can compensate by maintaining methylation. Likewise, folate can partially compensate for transient choline deficiencies. These compensations, however, come at a cost. If choline levels are persistently insufficient, folic acid is shunted toward the methionine cycle to support one-carbon metabolism and shunted away from its critical and distinct role in DNA synthesis and repair.

A 2016 study, published by Ganz et al, found that women with genetic single-nucleotide polymorphisms (SNPs) impairing folate metabolism shunt choline toward phosphatidylcholine rather than betaine production. Betaine is an intermediary compound in the biosynthesis of SAMe, so this biological shunt would result in impaired methylation. The decreased production of betaine was observed when pregnant women consumed the recommended intake of choline (480 mg/d), but it was corrected when they consumed twice that amount (980 mg/d). The research by Ganz et al highlights the unique synergy between choline and folic acid and suggests that high-dose supplementation with choline might overcome genetic impairments in folate metabolism.

Docosahexaenoic Acid (DHA)

Choline and DHA are both involved in fetal neurodevelopment and are both components of the phospholipid bilayer of cell membranes in the brain. Phosphatidylcholine, which is derived directly from choline, is enriched with DHA in cell membranes of the central nervous system.

Animal research suggests that combined supplementation with DHA and choline enhances neurodevelopment of the fetal hippocampus better than either supplement alone. The only human study on this topic found that women supplemented with high-dose choline (930 mg/d) produced higher amounts of DHA-enriched phosphatidylcholine. This result was observed only in nonpregnant women, so further research is needed to determine the combined effect of choline and DHA supplementation during pregnancy.

Recommended Amounts

The amount of dietary or supplemental choline required for any given pregnant woman depends on her dietary habits, availability of synergistic nutrients, and genetic individuality. The AI for choline, established by the IOM in 1998, is 450 mg per day during pregnancy and 550 mg per day during lactation. Research conducted at Cornell University, however, suggests that higher amounts might be indicated to meet the dramatic increase in demand during pregnancy.

In a clinical trial published in 2012, researchers randomly assigned pregnant and nonpregnant women to take 450 mg or 930 mg of choline per day for 12 weeks. Results showed that the higher dosage of choline produced higher concentrations of choline, betaine, dimethylglycine (DMG), and sarcosine in both pregnant and nonpregnant women—without changing urinary excretion. The lack of change in urinary excretion signifies that choline supplementation at twice the recommended amount did not exceed physiologic needs. In addition, the higher dosage of choline produced a doubling in cord blood levels of DMG. These results indicate that supplementing 930 mg of choline per day during pregnancy supports one-carbon metabolism and methylation in both the mother and the fetus.

Higher intakes of choline may be particularly important for women with certain genetic variations, or SNPs. SNPs affecting choline and folate metabolic pathway enzymes have been shown to alter choline metabolism and increase the risk for nutrient deficiency.

FOOD SOURCES OF CHOLINE

Food	Serving Size	Amount of Choline per Serving
Liver	3 oz	247 mg
Salmon	3 oz	187 mg
Eggs	1 large egg	125 mg
Shiitake mushrooms	1/2 cup	58 mg
Chicken broilers or fryers	3 oz	56 mg
Grass-fed beef strip steak	3 oz	55 mg
Wheat germ	1 oz	51 mg
Milk	8 oz	38 mg
Brussels sprouts	1/2 cup	32 mg
Almonds	1 oz	15 mg

Note: Adequate intake of choline is 450 mg per day during pregnancy and 550 mg per day during lactation

Source: Wallace TC, Fulgoni VL. Usual Choline Intakes Are Associated with Egg and Protein Food Consumption in the United States. *Nutrients*. 2017;9(839).

Lactation appears to affect choline metabolism in a similar way as pregnancy: lactating women have higher plasma choline concentrations than controls but lower urinary choline excretion and decreased use of choline as a methyl donor. Just as intakes exceeding 450 mg during pregnancy appear to offer benefit, intakes exceeding 550 mg during lactation also enhance choline content of breast milk.

Dietary and Supplemental Sources

Data from participants enrolled in the National Health and Nutrition Examination Survey (NHANES) from 2009 to 2014 found that the vast majority of Americans—including pregnant women—do not consume the AI of choline per day. To be specific, only 8% of all adults and 8.5% of pregnant women in the United States consumed the recommended amount. That means that 9 out of 10 pregnant women do not consume the recommended amount of choline per day.

The NHANES data go on to show that people who eat meat, poultry, and seafood have somewhat higher choline consumption, but the only food that is significantly associated with adequate choline intake is eggs. Based on this data, researchers Wallace and Fulgoni concluded in a 2017 publication, "it is extremely difficult to achieve the AI for choline without consuming eggs or taking a dietary supplement."

Eggs provide approximately 125 mg of choline per whole egg (including the yolk), making them the primary source of choline in the American diet. A randomized controlled trial of vegetarian women of reproductive age found that consuming 6 eggs per week produced meaningful increases in plasma levels of choline metabolites. In a separate crossover trial, plasma choline levels rose in a dose-response manner with egg consumption, such that eating 3 eggs per day (compared with 0, 1, or 2) had the most beneficial effect on plasma choline levels.

Supplemental choline is available in the form of choline salts. Choline chloride is used as the standard for infant formulas. Choline bitartrate has GRAS status (generally recognized as safe) and is widely used in other supplements. Although the AMA made a 2017 resolution to include 450 mg of choline in all prenatal vitamins, it will take time to implement this recommendation. Choline is a large molecule and will dramatically increase the size or number of prenatal vitamin pills to be taken per day. Until choline is routinely included in prenatal vitamins, single-nutrient supplements may be a necessary option for women of childbearing age.

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