



Choline

An Essential Nutrient for Fitness



BALCHEM
ENCAPSULATES

VitaCholine[™]

The performance of physically active individuals is thought to be influenced by ergogenic nutrients. These substances may have one or more specific functions in the body. Stimulant action in the nervous system, usage as a storage form for a biochemical precursor, and agency in the turnover of metabolic by-products are prime among them.

Choline is truly a multi-tasking nutrient, important for cell structure and function, lipid metabolism, neurotransmission, cell signaling and genetic regulation. It works in cooperation with complementary B-vitamins to ensure that cellular operations run smoothly. The necessity of choline in the human diet was established fairly recently. In the past decade, new data have emerged on its special importance for individuals with impaired biosynthesis and those with increased nutrient need due to health status or extreme energy expenditure. Choline is clearly a nutrient important to athletes and others.

Water-soluble choline salts, such as choline chloride and choline bitartrate, are versatile, stable, GRAS ingredients that can easily be incorporated into a variety of food and supplement products. Label claims on nutrient content and structure-function relationships can be made, offering a point of distinction for choline-fortified food and supplement products.



Figure 1. Choline structure

Choline: Essential for athletes and others

Choline is absolutely necessary for wellness throughout the human lifespan, particularly for active individuals. It plays a key role in the optimal structure and function of the human body from the level of cells to that of whole tissues and organs. Much of choline's biological importance can be attributed to the fact that it is a biosynthetic precursor of numerous molecules that are important for diverse structural and functional purposes in the human body (1). By its phosphorylation, base exchange, oxidation, one-carbon transfer, and acetylation in the body, it makes available intermediates for many metabolic processes. It also works in a regulatory capacity, controlling gene expression and the catalytic activity of enzymes related to metabolism. Other effects are indirect, in that it affects the balance of and need for many other partially compensatory metabolites to which it is closely related, via its influence on the activity of certain regulatory genes or the catalytic proteins they express.

When free choline is needed in a tissue or organ, it is either biosynthesized, extracted from circulation (2;3), or scavenged from local endogenous supply and stored as the free base or, more commonly, as the phosphorylated moiety phosphocholine (4). In this way, it is ready and available for a variety of biological purposes. In partial fulfillment of these biological demands, choline can be made by all cells in the body via the cytidine diphosphocholine (CDP-choline) pathway (2;5) and by liver cells via the estrogen-regulated (6;7) enzyme phosphatidylethanolamine N-methyltransferase (PEMT) (8;9). However, the efficiency of choline biosynthesis in humans varies significantly with age and gender, and is modulated by hormones, individual genetics, and choline and B-vitamin status and intake behavior. Choline is regarded as an essential nutrient for humans because of its recognized biological significance, as well as the reality that its biosynthesis is known to be insufficient to meet the needs of healthy individuals (10). Choline need may be exaggerated in physically active individuals who, by engaging in sustained intensive exercise, run down their internal stores of the nutrient (11-13).

Deprivation of dietary choline is first manifest by a measurable decrease in levels of plasma choline, and a concomitant increase in plasma homocysteine. The significance of these changes is discussed later in this summary. When expenditure of choline exceeds intake and biosynthesis fails to replenish the nutrient pool, membrane phospholipids must be sacrificed and broken down to ensure choline supply to the brain (3). This results in an accumulation of fat in vacuoles of liver cells (steatosis) and consequent damage to liver cell membranes (14) and muscle cells (15). In cases of serious and sustained deprivation, cell suicide is activated in lymphocytes (16), and multiple organ dysfunction is clinically evident (15).

Choline intake prevents and reverses these symptoms of nutrient deficiency and assures that the choline pool is kept full for the multiple purposes for which it is needed. As such, a Dietary Reference Intake (DRI) was developed for choline nutrition in healthy individuals (10) and published by the Food and Nutrition Board of the Institute of Medicine (US) in 1998. Current dietary recommendations for choline are based on the daily dosage necessary to prevent these abnormalities in most individuals, *i.e.* 7 mg/kg body weight/day, which equates to 550 mg choline per day for an average adult male.

Recent population studies suggest, however, that a significant portion of the US population does not consume dietary choline at current recommended levels (17). The choline shortfall is disproportionately apparent in certain ethnic groups (18) and specific subpopulations, which is directly contrary to long held assumptions about its conditional importance and limited the intake data from early small-scale investigations (19). Many of the best food sources of choline, such as beef liver, egg yolks and wheat germ (12;13), are widely available, but not well-represented in the current American diet due to collateral fat and cholesterol content (18) or a simple lack of palatability. The carbohydrate-rich foods typical of athletes' diets are generally not good sources of choline.

Dietary supplements, choline-rich meal-replacement products, and the discretionary fortification of foods and beverages are appropriate ways to deliver necessary choline to consumers, particularly those who are physically active. Water-soluble choline salts are very easily added to virtually any processed food product, liquid, tablet or capsule, exhibiting excellent stability, high bioavailability and compatibility with other nutrients.

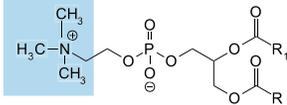
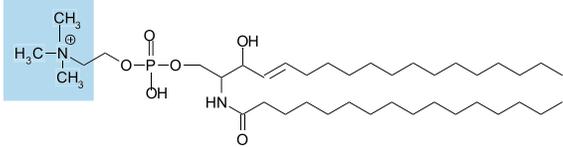
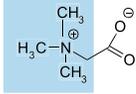
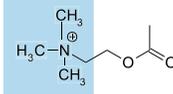
Substance	Function	Structure
Phosphatidylcholine	Structural component of the amphiphilic phospholipid membranes of all cells	
Sphingomyelin	Provides insulation along the length of a neuron.	
Betaine	Controls water transport in and out of cells. Contributes to the 'methyl pool' toward maintenance of the balance of choline, folate and B-vitamins.	
Acetylcholine	Agent of message propagation between neurons.	

Table 1. Choline as a structural precursor

Benefits of choline

Choline's functionality for athletic consumers is collectively related to its role as the precursor of neurotransmitters, membrane phospholipids, and cellular osmolytes, as well as its value in methyl-group donation (20). These factors are important in precision performance, muscle function and recovery, and training output.

Mind over muscle

With its acetylation, catalyzed by the enzyme choline acetyltransferase, free choline is converted to acetylcholine, a common but extremely important neurotransmitter in the central and peripheral nervous system. Although less free choline is converted to acetylcholine than is turned into membrane phospholipids (5), the reaction is important in that it is the only way to generate the acetylcholine molecule (3:21). In addition to its function in transmitting messages between neurons in the brain, acetylcholine serves similar purposes elsewhere in other parts of the body. Cholinergic neurons innervate individual muscular fibers at the neuromuscular junction, where they help relay messages that result in muscular reaction. Acetylcholine is also the primary neurotransmitter of the vagus nerve, which runs most of the length of the human body and connects the central nervous system with distal organs and tissues. This non-neural component of the human cholinergic system may be significant in choline's putative anti-inflammatory effect (22).

The passage of signals between neurons is facilitated by better cell-to-cell contact between the neurons and a greater availability and mobilization of neurotransmitters. It is also influenced by so-called secondary messengers and other bioactive metabolites (23). All of these factors are influenced directly by availability of choline in the course of development and maintenance of the body.

The level of choline in the brain is directly affected by the levels in plasma, which is significantly influenced by intake of the nutrient (3:24;25). When neuronal communication accelerates during periods of high activity, neurons' need for choline increases significantly. The concentration of free choline available at the terminal of a cholinergic neuron, the primary location of acetylcholine synthesis, is a limiting factor in this reaction. Choline crosses the blood-brain-barrier by facilitated diffusion, and moves from extracellular fluid into cells according to the existing concentration gradient (3:26;27). Choline intake increases the synthesis of acetylcholine and its release in muscles, the heart, and at the neuromuscular junction (28;29).

Maintenance of the nervous system during intense exercise is necessary to drive the continued neural activation of muscles and to delay the onset of so-called "central fatigue," which is associated with long periods of sustained muscular usage (30;31). Decreased choline (and consequently, acetylcholine) may be associated with delays in transmission of muscle contraction impulses (11;29). In states of dietary choline deprivation (32) and metabolic stress such as extreme exertion, membrane phospholipids (phosphatidylcholine and sphingomyelin) (3;5;33;34) may be catabolized by phospholipase enzymes in an effort to maintain levels of brain choline (35) and provide for its release into synaptic clefts in the hippocampus (36) and the neuromuscular junction. Choline intake is necessary to regulate the activity of the various enzymes that synthesize, break down and influence the release of the neurotransmitter acetylcholine (37-40), with a secondary benefit of helping in the retention of cell membrane integrity.

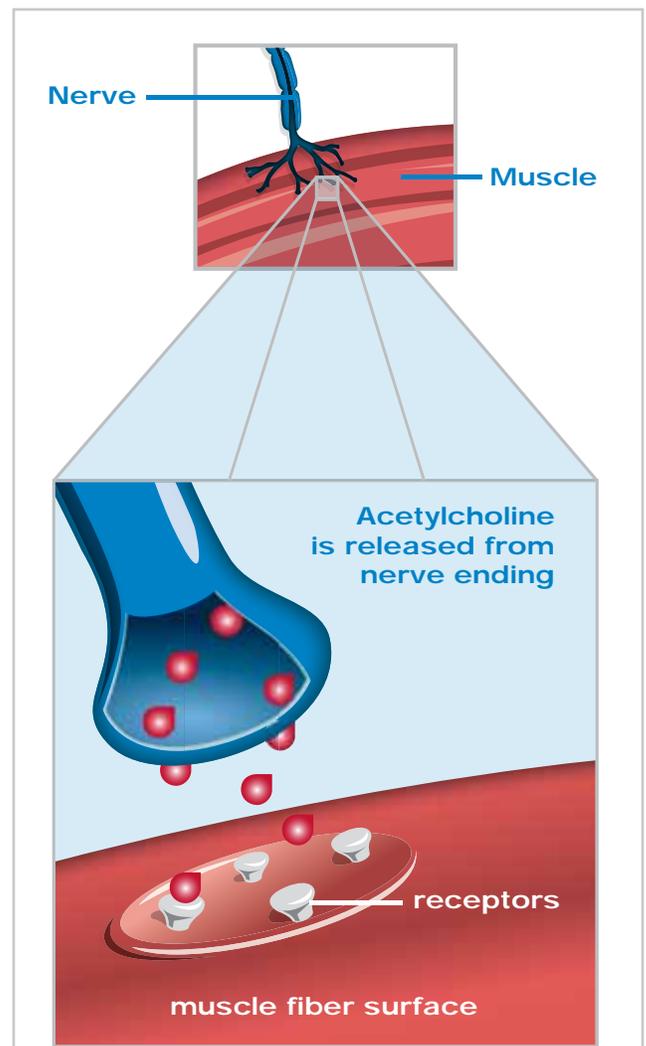


Figure 2. Choline at the neuromuscular junction

Keeping the body in balance

Choline is readily converted by the body into the important osmolyte betaine in an irreversible oxidation process. Betaine is significant in regulating the balance of influx and efflux of water in cells (41). Volume changes in cells due to hypo- and hypertonic environmental changes trigger a variety of catabolic and anabolic processes, respectively, that are challenging for cells to accommodate (42).

Once choline is oxidized in the body, it may be further catabolized in support of metabolic balance. It is important for its contribution to the so-called cellular "methyl pool." Methyl groups [CH₃-] are attachments that are transferred between biological macromolecules. They function as "epigenetic markers," which are functional tags that can activate or suppress the expression of genes and the activity of catalytic proteins (43). Methyl groups are also important in other molecular interconversions. Methylation of homocysteine, a cellular metabolite which circulates in the blood of all individuals, converts it to methionine. This essential amino acid is needed to repair and build proteins and to serve as a precursor for other substances, such as S-adenosylmethionine (SAAdoMet), an important methyl donor in its own right (44;45). SAAdoMet influences a variety of cellular processes involved in immune response (43;46). It suppresses the production of several inflammatory "first responders," activates an additional, complementary route of disposal for cellular homocysteine (cystathionine β-synthase, Vitamin B₆ cofactor), and promotes the production of the antioxidant glutathione and the necessary activity of many methyltransferase enzymes (43;46;47).

Choline insufficiency in humans, even those with adequate methionine, results in an accumulation of unmetabolized homocysteine (48). Intense, prolonged physical activity has also been shown to markedly increase plasma homocysteine in some athletes (49;50). It is theorized that increased protein or methyl group turnover may be the operative mechanism(s), and that an individual's health and nutrition status, their starting level of fitness, and the mode and intensity of exercise are significant factors in the magnitude of the effect (50). (The relationship of homocysteine with B-vitamin and creatine metabolism is explored in a subsequent section of this paper.) Unabated homocysteine creates a distinct imbalance of SAAdoMet and its immediate precursor (51), thus disrupting the methyl pool, and negatively affecting enzymes that assist in methyl group transfer.

Whether homocysteine itself is true risk factor, or merely a biomarker or by-product of biochemical dysfunction (52) remains to be firmly established. However, homocysteine has been widely linked to numerous health conditions (43) ostensibly due to its alleged cytotoxic and vascular effects (53-56). It has been implicated (57) in fibrosis of the liver (58), occlusion in the cardiovascular system (59;60) and the complex etiology of age-related cognitive decline (61). Homocysteine's mechanism of action appears to be related to reactivity of its thiol group (57;62). By reaction of the thiol group with amino acid side chains, homocysteine can affect the structure and function of important proteins and enzymes. Direct and secondary oxidative effects (43) generate the reactive oxygen species (ROS) that initiate reactions in cells and tissues and overwhelm the innate protective mechanisms of antioxidant enzymes and nitric oxide, particularly in the vascular endothelium.

Increasing choline intake has been shown to reduce plasma homocysteine in healthy and homocysteinemic subjects (53;55). An additional benefit of this functional contribution of choline is that it serves to spare the complementary nutrient folate for its other use in DNA synthesis (63). Choline's remaining two methyl groups are released in subsequent oxidation steps and diverted to other metabolic processes and biosyntheses (62).

STRUCTURE-FUNCTION CLAIM

- Choline may help reduce levels of plasma homocysteine.

Getting things moving

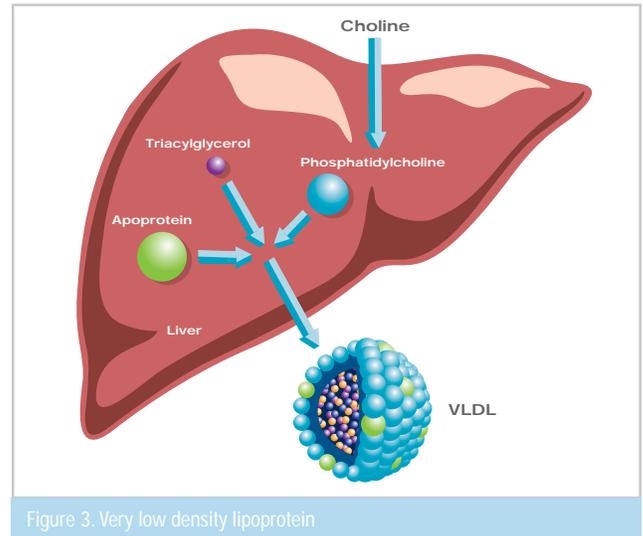
As a precursor of the phospholipid phosphatidylcholine, free choline is an important component of the structure of very low density lipoproteins (VLDL). The manufacture of VLDL occurs in the liver as a mechanism to transport non-polar lipids within the aqueous environment of circulating blood, away from the organ (64-66). VLDLs are effectively a cellular packaging system designed to transport fat to adipose tissue for storage or to muscles for immediate use (67). The choline that is used by the liver for this purpose is biosynthesized as discussed previously; the necessary remainder must be obtained from the diet (2;9).

In dietary choline deficiency, there is a decrease in triglyceride export from the liver in VLDLs, resulting in excess fat deposition (steatosis) in that organ (68). The clinical outcome of fatty liver is the result of an apparent effect on the size, shape and viability (ease of enzymatic breakdown) of the VLDL particles (69), rather than inadequate phosphatidylcholine synthesis. Liver dysfunction (70) is thought to be related to the rupture of cell membranes caused by steatosis, as well as the induction of apoptosis (controlled cell death) (71) and compensatory uncontrolled cell division in liver tissue (10;72;73). Hepatotoxicity may arise from infiltration of the liver by fatty acids, some of which are oxidized and recycled, collaterally generating reactive oxygen species (ROS) which may interact with macromolecules (e.g. DNA) or membranes (67;74). Measurements of high enzyme activity of alanine- and aspartate aminotransferase, which are released from liver cells when their membrane integrity is compromised (75), are the surrogate measure used for diagnosis.

Choline deprivation affects cellular structural integrity and lipid metabolism outside of the liver, as well. Most notably, cell membranes are catabolized and cellular suicide is activated in muscles the absence of choline (15). Triglycerides accumulate in choline deficient muscle cells as a result of increased assembly of the molecules from the pool of components (fatty acids, diacylglycerol) scavenged from broken down cell membranes (76), rather than *de novo* lipogenesis. Increasing Choline intake is a direct way to reverse these effects.

STRUCTURE-FUNCTION CLAIM

- Choline may promote healthy liver function.



Teamwork: Nutrient interrelationships and synergies

Betaine

Betaine is a primary metabolite of choline which has been extensively studied as such for its ergogenic effects in athletes. Administration of choline is known affect to levels of betaine (3;77). Choline is converted to betaine in a two-step enzymatic process in which energy equivalents (as cellular adenosine triphosphate (ATP)) are collaterally generated. Interventional studies with supplementary betaine have been conducted in humans (e.g. reference (78)); improvement in subjects' strength and power performance has been observed, though not unilaterally (79), mechanistic picture is still in development (41). It is worth noting that, because of the irreversibility of the oxidation reactions that generate it from free choline, betaine cannot contribute to the biosynthesis of the neurotransmitter acetylcholine, as choline can.

B-vitamins

Choline is commonly discussed in context of B-vitamins for their shared roles in methylation and amino acid synthesis (48;80). Changing availability of B-vitamins shifts the dynamics of reactions requiring free choline, and vice versa (81). For instance, adequacy of folate nutrition has been demonstrated to be inextricably linked to choline need (63;82-86). When folate is deficient in the diet, choline is decreased in the liver (85). Likewise, when choline is deficient in the diet, folate is found to be depleted in the liver (87). The activity of the choline-mediated pathway that turns over cellular homocysteine spares the complementary nutrient folate for its other very important use in DNA synthesis (63).

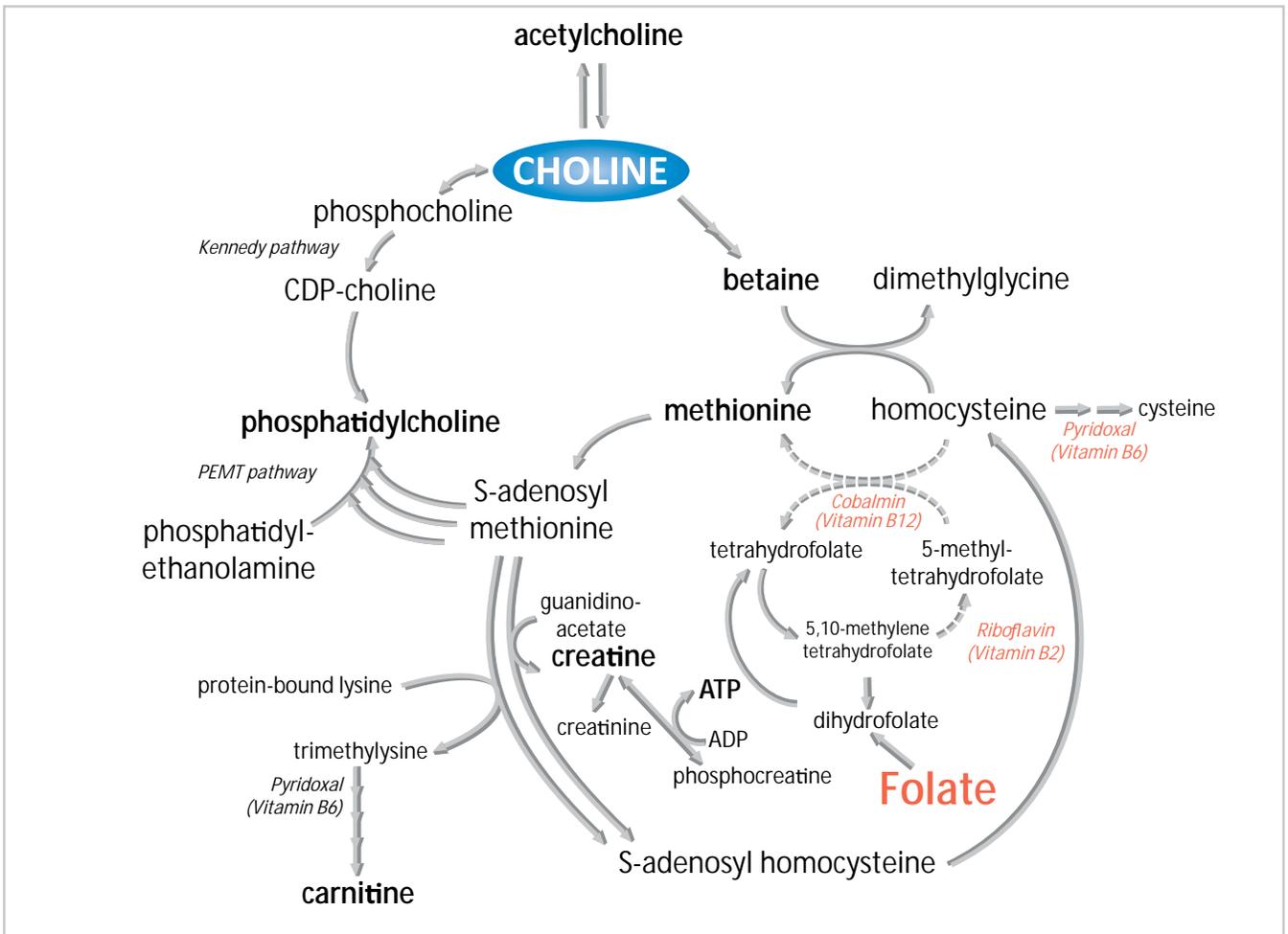


Figure 4. Choline and the methionine cycle, nutrient synergies

The dietary choline requirement for choline is increased in individuals with certain variations in genes involved in B-vitamin metabolism and one-carbon transfer, especially the gene encoding the enzyme methylene tetrahydrofolate reductase (MTHFR) (14;86). Vitamin B₂ (riboflavin) is a key component of the cofactor flavin adenine dinucleotide (FAD) for MTHFR, the enzyme which catalyzes the first steps of the biosynthesis of sulfur-containing amino acids, including that of methionine *via* homocysteine (88). Vitamin B₁₂ (cobalamin) is a cofactor for methionine synthase, the enzyme which directly catalyzes the actual conversion (89). Vitamin B₆ is involved in an enzymatic reaction that offers another route of disposal for excess homocysteine. It is also a cofactor for an enzyme in the synthesis of carnitine, which is necessary for fat transport prior to β -oxidation. If the demands of exercise require tapping into glycogen stores for energy, the need for Vitamin B₆ as a cofactor will consequently increase, making it less available for homocysteine turnover (50). It would seem reasonable that this might tax the body's choline stores, in turn, underscoring the importance of choline intake.

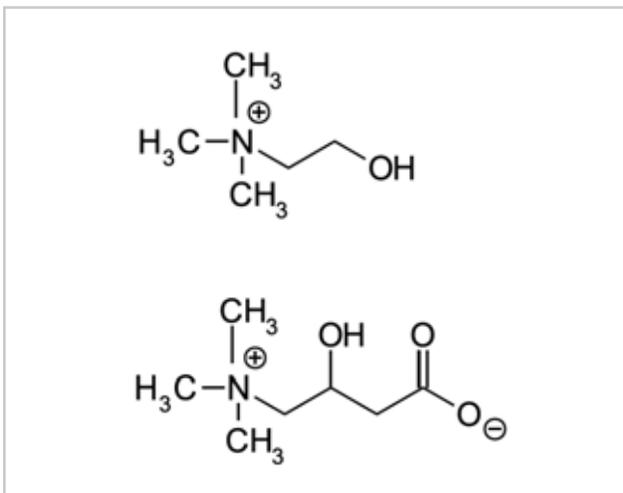


Figure 5. Similarity between choline (top) and carnitine (bottom) structures

Carnitine

Carnitine and choline are remarkably similar in their chemical structure. The biological purposes that choline and carnitine serve are mechanistically different, but are not metabolically unrelated. Carnitine is synthesized from lysine and methionine in the liver and kidney (90) in a series of reactions that require Vitamin B₆. It is necessary in the translocation of long chain fatty acids to mitochondria for β -oxidation, the first step in fat metabolism for the generation of cellular energy. Choline is important, of course, in that it makes methionine (and SAdoMet) available for carnitine's biosynthesis by its methylation of homocysteine, and it is itself necessary for hepatic fat transport.

It is not surprising that choline and carnitine status appear to be related to one another. Choline deficiency is associated with a decrease in carnitine in skeletal and cardiac muscle and in the liver (91) and diminished hepatic fatty acid oxidation (92). Supplementary choline seems to maintain serum carnitine levels by reducing carnitine's excretion; it increases carnitine's reabsorption via the kidney (93;94) and influences its redistribution (95), presumably by affecting the mechanism of molecular transport (96). Choline administration has been shown to increase carnitine in skeletal muscle, decrease body fat in animals (97) and enhance fatty acid oxidation (98). Interventional trials in humans (e.g.(99)) have not yet been undertaken with methodology and measurements adequate to allow rigorous and specific conclusions about these relationships.

Creatine

Creatine is synthesized in the liver by the methylation of guanidinoacetate by SAdoMet, in a reaction catalyzed by a methyltransferase enzyme. This process generates enormous amounts of collateral homocysteine, and much SAdoMet is expended in the conversion. Increased demand for creatine during muscular contraction will thus further increase the production of homocysteine (50), suggesting the particular importance of choline intake to keep levels of the metabolite under control.

Ergogenic nutrients work by:	Choline's contribution:
Acting as central or peripheral stimulant	Key precursor of acetylcholine
Increasing storage or availability of limiting substrate	
Reducing or neutralize metabolic by-products	Reduction of cytotoxic homocysteine
Serving as supplemental fuel source	Assists in mobilization of fat
Enhancing recovery	Supports creatine biosynthesis (important in muscle recovery) by turning over collateral homocysteine

Table 2. Potential modes of influence of choline on fitness metabolism

Conclusion

Choline's ergogenic value

Ergogenic aids generally function based on specific biological roles (100;101). Choline is notable in that it very clearly fulfills many of these purposes, based on its diverse biochemical functions in the human body. Intake of the nutrient in the diet makes it more available as a key substrate of the important acetylation reaction that generates the acetylcholine neurotransmitter. Choline also acts to convert the metabolic by-product homocysteine into the essential amino acid methionine and cellular methylating agent SAdoMet. While choline does not serve as an alternate fuel source *per se*, it does have an important purpose in controlling the deposition of fat, which is a fuel source tapped in intense exercise.

It is not surprising, then, choline and its direct metabolite betaine have been studied over the past two decades for their positive effects in physically active humans. A prevailing theme in research findings is the apparent dependence of choline's effect on human performance on the type, duration and intensity (% VO_2 max) of physical activity (20). Investigations that have been conducted to date have varied considerably in size and scope (96;98), studying groups as diverse as elite Army Rangers (102), seasoned marathon runners (11-13) and ordinary individuals.

Oral administration readily affects serum levels of choline (103) and consequently, of betaine, a metabolite which has been studied in some detail for its ergogenic value (3;77). The systemic absorption and distribution of water-soluble salts is known to be reasonably fast and, sustained over a period of 4-8 hours (25;103-105). If choline is ingested in bound (rather than free base) form, for example as cytidine diphosphocholine (CDP-choline) (3) or glycerylphosphorylcholine (α -GPC choline) (106), it is quickly hydrolyzed and metabolized as free choline is, suggesting that there is no special benefit to its delivery this way.

STRUCTURE-FUNCTION CLAIM

- Choline may reduce fatigue and increase vigor during strenuous exercise.

The metabolism of choline is dependent on an individual's choline, B-vitamin (81), folate (63;82-86), methyl pool status, and it is strongly influenced by individual genetics (107-109). In general, though, the body's processing of choline in a meal or in a supplement begins in the stomach with breakdown of the food matrix by acids and enzymes. The partially digested chyme is moved into the small intestine, where enterocytes package fats, including choline-containing phospholipids, into chylomicron lipoproteins and release them into the lymph, and subsequently to the blood (77). Choline salts are stored and circulated as in free form, most commonly phosphorylated (4). When choline reaches the upper small intestine, it is transported by a carrier (110) in a concentration-regulated mechanism, and it subsequently enters the portal circulation (77), from which the liver extracts it for use in lipoprotein construction. It is converted to betaine in the kidney, where it provides osmolytic benefits if not further metabolized itself (41).

Dietary supplements, choline-rich meal-replacement products, and many other types of foods and beverages are excellent delivery forms for choline. At typical dosage levels, water-soluble choline salts do not detract from a product's flavor profile. The nutrient is extremely stable through high temperature processing operations and remains highly bioavailable. Choline salts have broad regulatory acceptability worldwide. In certain markets, allowances also include label claims attesting to a product's choline content and aspects of the nutrient's truly unique functionality. This is an important distinction among the class of nutrients, minerals and vitamins geared toward physically active consumers.

This article is for informational purposes only and is not meant to be construed as authoritative legal or medical advice. Balchem makes no representations as to its accuracy and assumes no liability or responsibility for the content of this article.

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