Novel insight on the impact of choline-deficiency in sepsis

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Abstract: Choline, an essential dietary component, is a selective α7 nicotinic acetylcholine (ACh) receptor agonist. It plays an important role in various metabolic and non-metabolic pathways including inflammatory pathways. Systemic inflammatory response syndrome activated by bacterial endotoxins is a critical clinical condition associated with high rates of morbidity and mortality and related multiple organ dysfunction; immunological imbalance is associated with deteriorated outcome of septic patients. Choline-deficiency can be seen in different physiological and pathological states, and can be linked to the pathway of a septic condition. Assessment of the nutritional status has a significant impact on improving treatment output, healing process and prognosis of septic patients. The aim of this review is to explore the significant impact or the protective and supportive role of choline on selective pathological and/or inflammatory pathways in experimental or clinical models of sepsis.

Keywords: Choline; choline deficiency; endotoxaemia; inflammatory markers; sepsis

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Introduction

Choline is a necessary dietary component that maintains normal function and integrity of the body cells (1-3). It is a biological molecule presented in tissues as phosphatidylcholine (PtdCho) or sphingomyelin (SM). Choline is a major donor for methyl groups essential, among others, for DNA methylation and repair, signaling pathways, lipid and cholesterol transport, and metabolism. The important choline metabolites include acetylcholine (ACh), platelet-activating factor, lysophosphatidylcholine (LysoPC), phosphocholine (Pho), glycerophosphocholine (GPCho), plasmalogens, and betaine (2).

Choline has been officially approved by the US Food and Nutrition Board as an essential nutrient for the maintenance of health and the human adequate dietary intake of choline has been addressed (4). Choline is available in the diet either in a free form or in a bound form (esters) which represent most of the body stores of each, such as Pho, GPCho, SM or PtdCho (2). Dietary choline is absorbed by the small intestine and its uptake is mediated by specific choline transporters in the intestine, where eventually it is converted into PtdCho (also known as lecithin) (5); PtdCho is the main phospholipid (>50%) in the mammalian cell membrane (3). The various forms of choline have different bioavailability; the ester form of choline enters via lymph and bypasses the liver (6), while the free form of choline enters the portal circulation and is mostly uptaken by the liver (7). Choline plays a major role in the production of the essential amino acid methionine from homocysteine via the choline derivative betaine (8); it has an important metabolic role in different organs and tissues including brain, liver, kidneys, placenta and mammary glands. Choline can be synthesized endogenously by the liver via de novo mechanism as well. Although the de novo mechanism catalyzed by the liver enzyme phosphatidylethanolamine-
N-methyltransferase (PEMT) to produce new moiety of choline in the form of PtdCho compensates for the lack of dietary choline, the endogenous biosynthesis of choline is considered insufficient to meet all human requirements for choline (9).

The recommended adequate intake for choline is 425 mg/day for women, 450 mg/day for pregnant women, 550 mg/day for men and lactating women as well. Nevertheless, several factors like genetic makeup, age, gender, menopausal status, ethnic and racial backgrounds of an individual affect choline metabolism and subsequent choline requirements (4,10). Therefore, the risk of incidence of choline-deficiency depends upon different factors i.e. choline requirements in premenopausal women are decreased since the endogenous biosynthesis (de novo) of choline in the liver is estrogen-sensitive (11). In women, a single nucleotide polymorphism that has been identified in the PEMT gene is responsible for estrogen-induction of de novo biosynthesis of choline (11,12), thus it seems that choline-deficiency could occur as a genetically promoted disorder.

Choline-deficiency

The role of choline is crucial and its deficiency is considered an unhealthy state that leads to body organ dysfunction both in humans and animals (13). Insufficient dietary intake can be observed in about two weeks (14-16). Although choline is ubiquitous in different food items—a fact that makes choline-deficiency rare—choline-deficiency can be seen in physiological (e.g., intensive exercise, pregnancy and lactation) and pathological states (e.g., alcoholism and malnutrition) (17,18). Dietary lecithin (phosphatidylcholine) is considered the common source of choline; most of the choline mass is stored in phospholipid-bound form. Plasma free-choline depletion has been associated with the development of hepatic disorder (19). A very susceptible group to choline deficiency are patients on parenteral nutrition (PN); these patients have limited absorption capabilities (20), a fact that further deteriorates liver structure and function (19,21). It is noteworthy that in addition to hepatic metabolism, some of the dietary choline is degraded by intestinal flora before absorption (7,22). Despite the fact that PN contains the choline precursor “Methionine”, the plasma-free choline concentration has been found significantly lower compared to normal levels in patients on PN in both adults and older children and it has been associated with increased liver enzymes (20,23-25).

There are many studies that have uncovered several pathological conditions under the impact of choline-deficiency such as steatohepatitis, cirrhosis, hepatic cell degeneration and hepatocellular carcinoma (17,26-28), disruption of normal glucose metabolism and induction of insulin resistance (18,29), impairment in cardiac function with structural changes (30), renal tubular and cortical necrosis (31,32), ocular haemorrhagic lesions and disruption of brain development and cognitive function (33,34). In addition, choline-deficiency induces metabolic derangement leading to organ damage through the triggering of different pathways, including tumor necrosis factor alpha (TNF-α), hyperhomocysteinemia and lipid peroxidation (20). Ossani et al. (32) and Repetto et al. (35) reported that dietary choline-deficiency induces oxidative damage in the liver, kidney, heart and brain with an increment in lipid peroxidation (in rats there is an increase in plasma levels of oxidative agents and a decrease in plasma levels of antioxidants). Repetto et al. (35) found a 33% decrease in the total reactive antioxidant potential (TRAP) plasma level due to a decrease in the intracellular glutathione (GSH) and a decrease in tissue lipid soluble α-tocopherol, and a 5-fold increase in thiobarbituric acid-reactive substances (TBARS) plasma level due to stimulation of lipid peroxidation. These changes usually precede the appearance of irreversible histopathological damage of the organs. In vitro studies revealed that hepatocytes that have grown in choline-deficient media produced increased reactive oxygen species (ROS) from mitochondria in comparison to the ones grown in choline-rich media (36-41). In rats, dietary choline-deficiency caused an accumulation of lipid peroxides which led to further DNA damage in hepatocytes (42) and lymphocytes (43).

Choline-deficiency has been shown to decrease the activities of lysozyme and acid phosphatase, contents of complement 3 and immunoglobins (IgM), and to downregulate the mRNA levels of antimicrobial peptides, liver-expressed antimicrobial peptides (LEAP-2A, LEAP-2B), defensin-3 and hepcidin in the intestinal segments of juvenile Jian carp; furthermore choline-deficiency impaires the intestinal antimicrobial defense of juvenile Jian carp (44). Notably, that choline acts also as an endogenous cholinergic agonist (45); since experiments in rats have shown that cholinergic agonists induce an increase in the secretion of lysozyme and defensin in the intestine (46), the impaired intestinal antimicrobial defense caused by choline-deficiency might be partially related to the decreased cholinergic agonist levels (44). In addition, a study by Wu
et al. (44) found that choline-deficiency in fish suppressed intestinal antimicrobial defense by decreasing antimicrobial component levels and induced intestinal inflammation via upregulation of pro-inflammatory cytokines’ expression and downregulation of anti-inflammatory cytokines’ expression. Conversely, Wu et al. (47) have shown that diet rich in choline enhances serum lysozyme activity and complement 3 content, decreases pro-inflammatory cytokines interleukin 1 beta (IL-1β) and TNF-α mRNA levels while increases anti-inflammatory cytokines IL-10 mRNA levels in the main immune organs of juvenile Jian carp. The same researchers revealed that choline-deficiency upregulated the mRNA levels of nuclear factor kappa of activated B cell (NF-κB) and increased signal transducer and activator of transcription proteins (STAT) signaling pathways whereas downregulated mRNA levels of cellular protein inhibitor of kappa B (IκB) in the intestine of fish (44). In mammals, NF-κB is one of the critical signaling molecules for regulating transcription of cytokines (48) and its overactivation aggravates inflammatory reactions in rats (49). In fish, the upregulated mRNA level of proinflammatory cytokine TNF-α and the downregulated mRNA level of anti-inflammatory cytokines IL-10 and transforming growth factor-beta 2 (TGF-β2) could be related to the changes of signaling molecules NF-κB and IκB in the intestine caused by the choline-deficiency (44). Furthermore, choline-deficiency upregulated the mRNA level of Toll-like receptor 4 and Myeloid differentiation primary response 88 (MyD88) in the intestine of the fish (44,50).

Choline-deficiency promotes cellular apoptosis due to defective DNA repair (51-53). Previous reports have shown that consumption of a choline-deficient diet leads to reversible hepatocellular modifications characterized by hepatosteatosis, liver and muscle damage and increased lymphocytes apoptosis (53-56); in addition, da Costa et al. (54); James et al. (57) and Shin et al. (58) reported hepatocyte and myocyte death when cultured in a choline-deficient media that may justify the elevation of serum liver enzymes and creatine phosphokinase in human blood when humans are subjected to choline-deficiency.

The above mentioned facts and findings motivated the scientists and researchers to continue working on the disruption of crucial physiological processes as well as on the deterioration of many pathological conditions under the impact of choline-deficiency.

This review focuses on (I) the role of choline as an essential substance in the control and modulation of different immunological and inflammatory pathways in multiple models of sepsis (animal and human); (II) the consequences of choline-deficiency on the immunological and inflammatory response, and ultimately; (III) the importance of the nutritional status in septic patients.

**Choline and sepsis**

Sepsis is a life-threatening condition that arises when the body’s immune response is provoked against an infection. In modern medicine, sepsis remains as a critical clinical condition associated with high rates of morbidity and mortality (59). A major challenge in intensive care medicine is the treatment of a serious infection related to multiple organ dysfunction, generally termed as sepsis, severe sepsis or septic shock (60), which is considered as a critical and costly condition in the intensive care units worldwide. Gram-negative bacteria are the most common cause of septic shock. The toxic effects of gram-negative bacteria are due to a non-secreted, heat-stable endotoxin called lipopolysaccharide (LPS) (61,62).

A series of inflammatory reactions called systemic inflammatory response syndrome are triggered by LPS. TNF-α and ILs secreted by LPS-activated cells into the systemic circulation cause a stimulation of the hepatic cells to release acute phase proteins such as C-reactive protein (CRP) for immunological regulation (45,63); an immunological imbalance is associated with a deteriorated outcome of septic patients (64). Matrix metalloproteinases (MMPs) released into the circulation from damaged vascular endothelium might also have a role in the pathophysiology of sepsis (65). MMPs production is up-regulated by pro-inflammatory cytokines (TNF-α, IL-1 and IL-17), as well as by acute phase proteins (serum amyloid A), with counter-regulatory inhibition by IL-4 and IL-13 (66,67). Some MMPs regulate cytokine and other inflammatory molecular responses after the initiation of sepsis by activating protease-activated receptor-1 (67-69); the increased MMP/TIMP ratio (TIMP is tissue inhibitor metalloproteinases) seems to be more related to a tissue response to LPS linked injury rather, than to their involvement in the acute phase reaction (67).

Findings of Kocaturk et al. in 2016 (70) showed that choline treatment suppressed the increased MMPs and TIMPs serum concentrations in experimentally-induced sepsis in male and female mongrel dogs (0.2 mg/kg intravenous LPS-*Escherichia coli*); the increase was related to the acute phase response and organ damage, while choline prevented the reduced of Igs (IgM, IgG) concentration
induced by endotoxinaemia (70).

Many researchers have explored the significant correlation between choline and immunity in human and animals (44,45,47,71-73). Nolan and Vilayat in 1968 (74) reported that the hepatic injury and mortality due to endotoxinaemic shock induced by intraperitoneal injection of LPS-*Escherichia coli*, was significantly increased in adult female Holtzman rats fed on a choline-deficient diet; on the contrary, Rivera et al. (75) showed that a choline-rich diet protects the liver and improves survival rates in endotoxinaemic shock induced by intravenous injection of LPS-*Escherichia coli* in female Sprague-Dawley rats. With regards to sepsis and especially conditions that simulate sepsis i.e., a stressful surgery, it has been noticed that serum-free choline decreased during and after elective abdominal surgery, total abdominal hysterectomy, vaginal or cesarean childbirth, brain tumor resection and traumatic brain injury (76,77). Laboratory studies have shown that serum-free and phospholipid-bound choline concentrations decline in response to surgical and traumatic injuries in humans (76,78,79) and in dogs (77). Moreover, a stressful condition can induce a variety of metabolic and neuroendocrine changes (80-83) including the increase of cortisone, prolactin, adrenocorticotropic hormone and β-endorphine (80,83-85). In 2002, Ilcol et al. (78) found that serum choline levels were inversely correlated with the levels of stress hormones. Furthermore, under the insult of choline-deficiency, the endogenous biosynthesis (de novo) of phosphatidylcholine is promoted to compensate the demands in choline leading to increased homocysteine (86); the latter is involved in the regulation of cytokines and inflammation (87,88). In humans increased dietary choline leads to decrease in the homocysteine levels (89).

Furthermore, choline treatment (I) improved the hematological and serum biochemical findings of endotoxin-induced sepsis [0.02 or 1 mg/kg intravenous LPS-*Escherichia coli* in a saline solution] in adult mongrel dogs via activation of α7 nicotinic acetylcholine receptor (aαnAChR) (90,91); (II) attenuated the endotoxin-induced decrease in serum activity of butyrylcholinesterase and paraoxonase 1, and to a lesser extent the increases of CRP, haptoglobin and ceruloplasmin during experimentally induced sepsis in adult male and female mongrel dogs (92); (III) attenuated the increase of serum acute phase proteins (93) which are involved in the cholinergic anti-inflammatory pathway (90,91) and (IV) attenuated and even in some cases suppressed the increased MMPs and TIMPs but did not affect MMP-2 in response to a single dose of LPS-induced endotoxinaemia (70). The latter effect could be ascribed to the contribution of choline in maintaining endothelial integrity, membrane phospholipids’ structural integrity (94), and down-regulation of TNF-α expression (91). TNF-α has been reported to up- or down-regulate the expression of MMP-9, MMP-13, and MMP-14 (95) while nicotine treatment was reported to decreases expression of MMP-14 (96).

Administration of choline inhibited the harmful effects evoked by endotoxin on the vascular bed damage and leakage and protected immunoglobulin responses to LPS in lymphocytes (70,90,97) and other immune and non-immune cytokine producing cells (90). Furthermore, intracerebroventricular administration of the choline metabolite LysoPC reversed the hypotension and protected against lethality induced by endotoxin (98). Thus, it has also been suggested that choline-containing phospholipids, like lysoPC (98) and phospholipids have therapeutic effects (98,99) and improve survival in experimental models of sepsis induced by cecal ligation and puncture or intraperitoneal injection of *Escherichia coli* in mice (98) as well as in Yorkshire pigs (99).

Ilcol et al. (90) showed that experimental endotoxin-induced sepsis in dogs altered circulating choline status in a dose and time dependent manner; choline levels were in relation to serum cortisol and markers for tissue injury and/or organ dysfunction (90). Endotoxinaemia is accompanied by liver and kidney dysfunction; liver and/or kidneys excision in dogs slows clearance of free choline from circulation (100) while renal failure results in elevated levels of serum-free choline in human (101-104). In adult mongrel dogs injected with sublethal dose of endotoxin (1 mg/kg), serum levels of aspartate aminotransferase, alanine transaminase, alkaline phosphatase, gamma-glutamyltransferase, lactate dehydrogenase, creatine kinase, creatine kinase-MB, urea, creatinine, and uric acid decreased by choline administration (90) indicating that choline protects, at least in part, liver, renal, skeletal, and cardiac muscle injuries. Choline’s ability to attenuate endotoxin-induced elevations of biochemical markers for tissue injury and/or organ dysfunction was much higher at a high-dose (1 mg/kg) rather than a low dose (0.02 mg/kg) in endotoxin-treated dogs; according to Ilcol et al. (105) the mechanism of choline protection against experimentally induced endotoxin, could be attributed to increased availability of free choline and the consequent increase of central (1,106) and/or peripheral cholinergic neurotransmission (105); the increased cholinergic neurotransmission in the peripheral
The parasympathetic system could lead to an activation of vagal anti-inflammatory systems (105,107,108) and subsequent inhibition of endotoxin-induced toxic mediators from endotoxin-sensitive cells (Figure 1).

Furthermore, choline attenuates the elevation in serum TNF-α in response to 1 mg/kg dose of endotoxin, while a choline-rich diet decreases serum TNF-α by inhibiting its release from the Kupffer cells (75). Increased availability of free choline could increase membrane phospholipid synthesis (1,106,115) and/or decrease membrane breakdown (116) and diminish the vulnerability of tissues to elevated toxic mediators during endotoxinaemia. In dogs, circulating choline status is altered during experimental endotoxinaemia (72). In asthmatic patients, choline treatment decreases TNF-α, IL-4 and IL-5 release from mononuclear cells (73); in addition to that, ACh, a major choline metabolite, inhibits the production of TNF-α and IL-1 from human macrophage (117) and lymphocytes (118) and mouse microglia (119) via α7nAChR (Figure 1).

In non-terrestrial animals, like juvenile Jian carp...
(Cyprinus carpio var. Jian), it has been shown that when dietary choline was increased up to a certain level, the inflammation induced against *Aeromonas hydrophilia* challenge (intrapertoneal injection with *Aeromonas hydrophilia* as a semilethal dose of endotoxin) was attenuated via decrease of TNF-α, IL-1β, and TGF-β2 mRNA relative expression in the immune organs (47). In line with TNF-α expression in liver (120), TGF-β1 expression in rat hippocampus decreased after choline supplementation (121). Furthermore, mammalian target of rapamycin (mTOR) signaling pathways have been found to be involved in the function of the immune system (122), i.e., in mice, inhibition of mTOR reduced the release of TNF-α, IL-6 and IL-10 from activated macrophages (123). In addition, mTOR activates interferon regulatory factor-5 and -7 which are considered the principal transcription factors for pro-inflammatory cytokine genes in activated HEK293T cell-line (123). In female mice macrophage, phosphatidic acid, the hydrolysis product of PtdCho, enhanced the production of TNF-α, IL-1β, IL-6, nitric oxide and prostaglandin E2 by regulating the activity of mTOR-p70S6K1 (124). Wu et al. (125) found that dietary choline regulated the relative gene expressions of mTOR and the eukaryotic initiation factor 4E-binding protein-2 (4E-BP2) in muscle, hepatopancreas and intestine of juvenile Jian carp; thus, choline may also affect cytokines’ release through modulation of mTOR pathway (47).

Wu et al. (47,125) showed that dietary choline could enhance fish disease resistance and improve the survival of fish when subjected to *Aeromonas hydrophilia* challenge suggesting that dietary choline regulates the inflammation and enhances non-specific and specific immunity through serum activities of lysozyme and lysosome acid phosphate, hemagglutination titer, content of the complement 3 and 4, and leucocytes phagocytic activity of fish after challenge. In addition, according to Parrish et al. (45) endogenous choline may act on the α7nAChR and play an important role in regulating innate immune responses to maintain homeostasis. On the contrary, choline-deficiency decreased the survival in cobia fish (126) and hydro tilapia (127) and resulted in severe destruction of the mid-gut gland epithelial cells of juvenile shrimp (128).

In mice, both T and B lymphocytes express multiple muscarinic and nicotinic acetylcholine receptors (mAChRs and nAChRs, respectively); ACh can bind to mAChRs and nAChRs on T and B cells leading to modulation of their function (129); in a rat cultured spleen cell, ACh enhanced the Con A-induced T-cell proliferation (130). ACh also promoted anti-inflammatory response by mediating vagus nerve-based cholinergic anti-inflammatory response (48). Despite the fact that choline acts as a selective α7nAChR agonist (45), it failed to inhibit the systemic level of TNF-α in knockout mice during endotoxinaemia (45), indicating that choline may partially mediate cytokines’ expression via α7nAChR signaling. Therefore, the immunoregulatory effect of choline on the immune system could ascribe its effect on the cholinergic system (Figure 1).

NF-κB is a crucial protein complex for DNA transcription and cell survival, it regulates gene expression of the inflammatory cytokines in macrophages, monocytes and endothelial cells (48). Choline markedly decreased TNF-α level associated with suppressed activation of NF-κB in endotoxin-stimulated RAW-264.7 mouse macrophage-like cell (45). Moreover, NF-κB is an important transcriptional activator that regulates RNAs transcription (48). In fact, choline-deficiency impairs global DNA methylation (131) (Figure 2).

Furthermore, in juvenile Jian carp fish, choline-deficiency was associated with a decrease in red blood cells (RBC) and white blood cells (WBC) while RBC count increased by increasing the dietary choline levels to a certain level (47). This fact indicates that choline contributes to innate immunity (45) since low RBC and WBC levels decrease the immunity and increase the susceptibility to diseases (47,132).

Taking into consideration that the development and the growth of tissues and organs depend on cell proliferation (133) and the latter depends on the structural integrity of cells (134), it seems that choline is a major contributor in these vital processes through its essential role to maintain the structural integrity of the cell biological membranes (135) and DNA biosynthesis and repair (136).

Kortstee in 1970 (137) reported that several aerobic microorganisms can decompose choline and grow with choline as the sole C- and N-source *in vitro*; meanwhile, the gut microflora can metabolize choline to trimethylamine (138), suggesting that choline may play an important role in controlling intestinal microflora. On the other hand, dietary choline administration induced a significant increase in intestinal lactobacillus microflora count, while intestinal *Escherichia coli* and *Aeromonas hydrophila* counts were the lowest (47).

**Conclusions**

This review draws a picture of the role of choline in the manipulation and modification of the different pathological
responses under the impact of septic insult that has been triggered in different models. The protective and supportive role of choline in a war against sepsis and inflammatory diseases has been predicted through assessment of different findings in different studies. However, the understanding of the molecular pathophysiology of sepsis and of the role of choline in it, is far from complete.

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Footnote

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