Betaine as a Functional Ingredient

Subjects: Food Science & Technology

Contributor: Dejan Dobrijević , Kristian Pastor , Nataša Nastić , Fatih Özogul , Jelena Krulj , Bojana Kokić , Elena Bartkiene , João Miguel Rocha , Jovana Kojić

Betaine is a non-essential amino acid with proven functional properties and underutilized potential. The most common dietary sources of betaine are beets, spinach, and whole grains. Whole grains—such as quinoa, wheat and oat brans, brown rice, barley, etc.—are generally considered rich sources of betaine. This valuable compound has gained popularity as an ingredient in novel and functional foods due to the demonstrated health benefits that it may provide.

betaine metabolic pathways disease prevention food sources

1. Introduction

Betaine (trimethylglycine) is a natural product. It is a glycine derivatized by three extra methyl groups. Its chemical structural formula in 2D and 3D forms is represented in **Figure 1**. It is a stable and harmless natural constituent that exists in plants, animals, and microorganisms. It was reported that betaine is naturally present in beets, spinach, wheat bran, wheat germ, and aquatic invertebrates [1][2]. Betaine is endogenously produced via choline metabolism or exogenously ingested through dietary intake [3]. Either ingested as a dietary supplement or from food, betaine has a similar bioavailability, being broken down to dimethylglycine and lastly to sarcosine in the mitochondria of kidney and liver cells [4].

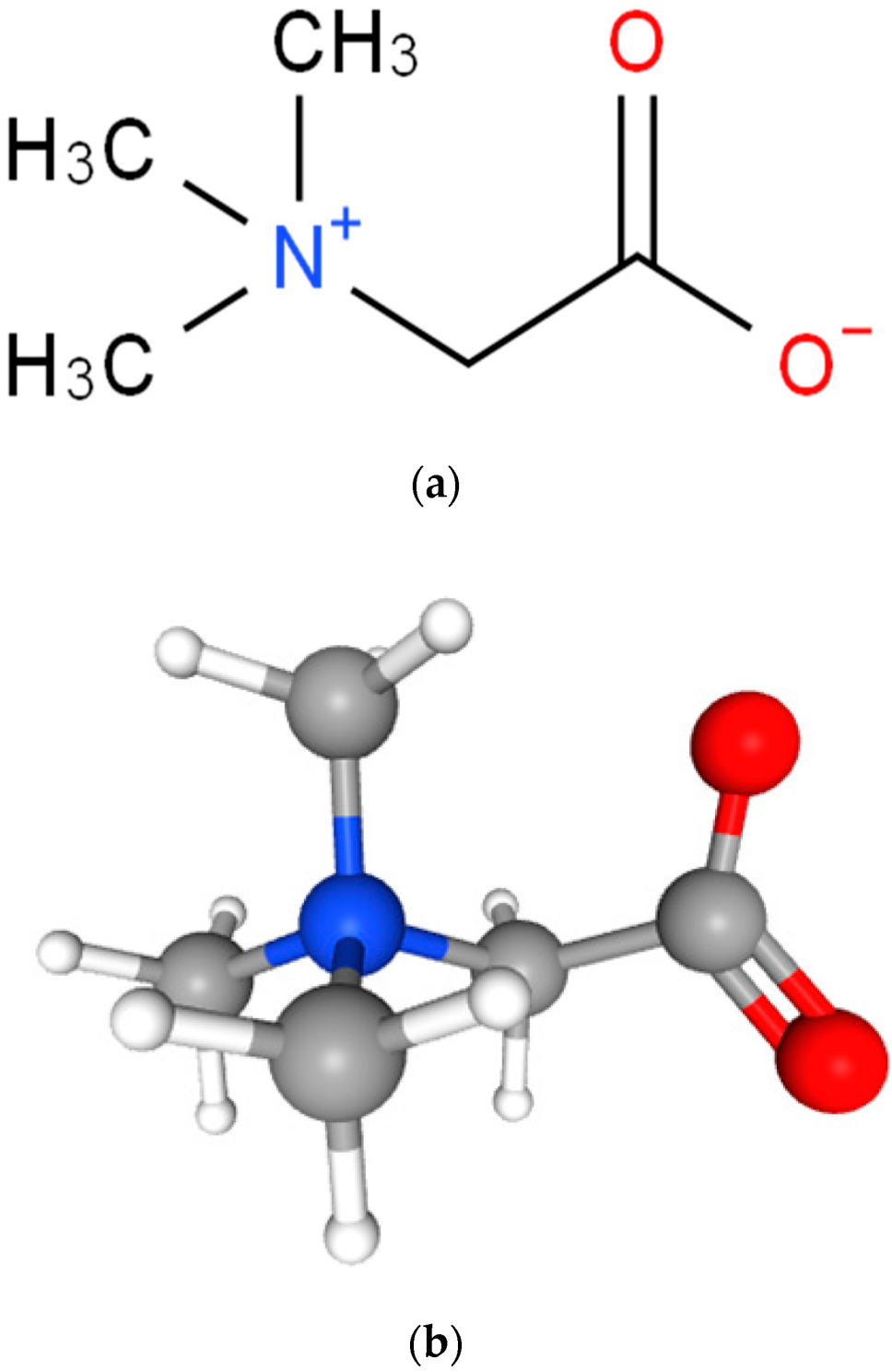

Figure 1. Structural formulae of betaine: (**a**) 2D-structure; and (**b**) 3D-structure.

In the United States of America (USA), betaine is generally recognized as a safe ingredient, that is, a GRAS (Generally Recognized as Safe) ingredient, while in Europe it has approval for use in food from the European Commission (EC) [5], which allows the safe use of betaine in food in an amount of at least 500 mg per food serving. The above-mentioned health approval is associated with betaine's contribution to the methionine cycle. Commercial betaine is available in three different forms: natural anhydrous betaine; synthetic anhydrous betaine;

and betaine hydrochloride [5]. For secondary industries, betaine can be produced by chemical synthesis or by relatively expensive isolation from sugarbeets or byproducts of beet processing. Natural betaine has superior functional properties compared to its synthetic analogue, and its use is preferred by the pharmaceutical, cosmetic, and healthcare industries [6][7][8][9].

One of the main roles of betaine is to help regulate homocysteine levels in the blood [10]. Homocysteine is an amino acid that, when present in high levels, has been linked to a higher risk of heart disease, strokes, and other health problems. Betaine helps to convert homocysteine into other beneficial substances, thus helping to maintain healthy levels of this amino acid in the blood [11][12]. However, it was reported that betaine might have a harmful impact on blood lipids. Zawieja et al. [13] performed a meta-analysis on the impacts of betaine supplementation at a daily amount of at least 4 g on blood lipid status in adults. No significant impact was found for triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, or plasma total cholesterol. Supplementation with 4 g/d of betaine for 6 weeks could relatively increase plasma total cholesterol, which is important for cardiovascular health. Ashtary-Larky et al. [14] also carried out a meta-analysis on the impacts of betaine supplementation on cardiovascular illness indicators. They reported that supplementation with betaine at doses of 4 g/d might cause harmful effects on the lipid profiles of people with health disorders. On the other hand, supplementation with less than 4 g/d of betaine generated beneficial reductions in homocysteine levels. These findings indicate that a desirable quantity of betaine supplementation is below 4 g/d.

Betaine has also been shown to have potential benefits for exercise performance and muscle strength. Some studies have suggested that betaine supplementation may improve endurance, reduce fatigue, and enhance muscle power and strength [15]. In addition, betaine has been investigated for its potential role in supporting liver function and protecting against liver damage, as well as for its anti-inflammatory and antioxidant properties [11][16]. Overall, betaine is a compound with a range of potential health benefits, although further research is required to fully understand its mechanisms of action and effectiveness in various contexts.

Furthermore, betaine has been employed as a feed additive in pig and poultry rations since it is regarded as a cheap alternative feed supplement for improving nutrient utilization [17]. Betaine is generated by choline oxidation or delivered through diet in the animal organism. Over the past years, many studies have evaluated the impacts of betaine on various animal species. Betaine plays a role in the transmethylation reaction for the synthesis of some active constituents, such as carnitine, creatine, etc., as well as in the higher usage of nutrients and the digestibility and bioavailability of methionine. Enhancing immune status and decreasing heat or oxidative stress are also considered significant functions of betaine [18]. Most of the published research has been focused on the growth-stimulating, higher milk-yielding, carcass-modifying, immune-boosting, and stress-dropping properties of betaine in various species [19][20][21].

2. Metabolism and Physiology

In mammals, betaine plays three important metabolic and physiological roles: (i) it is an organic osmolyte, which helps to maintain normal cell volume under osmotic stress and can accumulate to molar concentrations; (ii) it

provides protection against protein denaturation, thus being called a ‘chemical chaperone’; and (iii) besides methylfolate, it is the only molecule that provides methyl groups for homocysteine remethylation [22][23][24][25].

Besides mammals, cellular uptake of betaine occurs in various other organisms, such as bacteria and invertebrates. Belonging to the chemical class of amino acids, betaine is mainly transported with the aid of γ-aminobutyric acid [26]. Studies performed on animals have shown rapid absorption of betaine after a meal in the small intestine via the duodenum [27]. Studies on humans have shown rapid absorption and distribution of betaine within 1–2 h after ingestion. The constant concentration of betaine in human serum ranges from 20 to 70 µmol/L. According to Schwahn et al. [25], rapid absorption and distribution of betaine were found both in healthy subjects and patients with homocystinuria, with a maximum concentration of 0.94 mmol/L after 0.90 h and an elimination half-life of 14.38 h. Distribution and elimination kinetics in homocystinuric patients appear to be accelerated. Even at high doses of 100 mg per kg of human weight, betaine is mostly used up through metabolic pathways and not excretion. However, betaine can be present in the urine of individuals with kidney disorders and diabetes. Subacute studies in rats have shown that betaine is not toxic when added at 0–5% of the total diet. However, due to large intakes, the ratio of red blood cells could be slightly disturbed. Many authors suggest that the maximum daily intake of betaine is 9–15 g, with 20 g being the maximum [25][28][29].

The fundamental role of betaine in microorganisms and plant cells is cell protection from possible inactivation, which may occur due to osmotic stress [30]. Exposure of plants to hostile environmental conditions, such as drought, high salinity, and unfavorable temperatures, leads to increased betaine synthesis in cell mitochondria [31]. Betaine can fulfill its role as an osmoregulator only when it is not catabolized. Modulation of water content and cell volume is of crucial importance for living organisms. Cells have the ability to adapt to varying degrees of osmotic pressure by accumulating inorganic ions of low molecular weight (such as sodium, potassium, and chlorine ions) and organic osmoregulators (such as methylamines, amino acids, and sugar alcohols). The role of inorganic ions in osmoregulation is limited because their higher concentrations could affect protein structure and thus enzyme functions [32][33]. Betaine’s osmolytic action is the result of a dipolar structure and a good solubility in water, whereby, along with the other organic osmolytes, it demonstrates a lower degree of interaction with enzyme functions and metabolic processes in cells [34]. Studies have shown that betaine has a very low potential to bind to the protein surface, thereby allowing cells to control the water surface tension without affecting lipase stabilization [35]. A cytosolic methyltransferase enzyme, betaine-homocysteine S-methyltransferase, which uses betaine as the methyl donor for the remethylation of homocysteine to form methionine and dimethylglycine, is likely to help keep the cellular osmotic equilibrium by maintaining the regular betaine concentration. Its inhibition reduces betaine degradation [25][36].

Betaine plays its primary role as an osmolyte in the kidney tissue, thus protecting the mammalian kidney medulla cells from osmotic stress and enabling the control of the concentration gradient and the accumulation of metabolic waste products in the urine [25][31][37]. It can accumulate in the kidneys of humans, protecting the cells from the high concentration of electrolytes and urea. Furthermore, it can regulate the water balance and movement through the epithelial tissue [38]. Thus, betaine increases water retention in cells by replacing inorganic salts and protecting intracellular enzymes from osmotic pressure or temperature-induced inactivation [36][39]. For example, when

spinach is grown on soil with high salinity, betaine accumulates in the chloroplast and prevents water from leaking out of the cells due to the increased osmotic pressure. Mitochondria in salmon liver cells adsorb betaine when exposed to increased osmotic pressure, thus allowing the body to use less energy to maintain the required amount of water in cells. For this reason, betaine is being added as an osmoregulator to ponds to protect fish when moving through water of different salinity degrees [31].

As already mentioned above, betaine plays its second major role in methionine metabolism by converting and detoxifying homocysteine to methionine in the human liver and kidneys, acting as a methyl group donor [40][41]. Even a slightly elevated level of homocysteine in the blood could be considered a biomarker of increased risk of cardiovascular, cerebral, and peripheral vascular diseases, neurodegenerative disorders, and cognitive decline [42] [43]. Moreover, higher homocysteine concentrations have been associated with low concentrations of B vitamins, such as folate and vitamins B-12 and B-6, which are involved with a one-carbon metabolism, pointing to its disturbance [44]. Research has proven that betaine intake can contribute to the lowering of circulating homocysteine levels in patients with homocystinuria and chronic renal failure, but even in healthy subjects [45][46]. The association between dietary intakes of betaine and the concentration of homocysteine has been assessed by Chiuve et al. [47]. A cross-sectional analysis of 1477 women proved that total betaine intake, together with its precursor choline, was inversely associated with plasma homocysteine levels. Furthermore, the authors concluded that the remethylation of homocysteine may be more dependent on the betaine pathway when other methyl sources are low, which might be a result of either inadequate folate intake or heavier alcohol consumption [47]. According to the study of Lee et al. [44], performed on food-frequency questionnaires on 1325 male and 1407 female participants in the USA, a higher choline-plus-betaine intake managed to decrease the concentration of post-methionine-load homocysteine. Betaine and choline intakes were, therefore, associated with both fasting and post-methionine-load total homocysteine concentrations, especially in participants with low folate and vitamin B-12 status [44]. Since betaine is found in high amounts in wheat aleurone, research by Price et al. [37] investigated the impact of a diet rich in whole-grain foods on 79 healthy participants over four weeks. The results showed a significant increase in plasma betaine concentrations, as well as dimethylglycine and methionine as products of betaine-mediated homocysteine remethylation, and a significant decrease in plasma homocysteine and LDL cholesterol levels. No significant effects on plasma choline or B vitamins (folate, riboflavin, and vitamin B-6) were thereby observed [37].

Betaine could be produced in the human body by a series of enzymatic reactions that mainly occur in the mitochondrial cells of the liver and kidneys. These transmethylation reactions imply the transfer of methyl groups via the methionine cycle in vital biological processes, as shown in **Figure 2** [48].

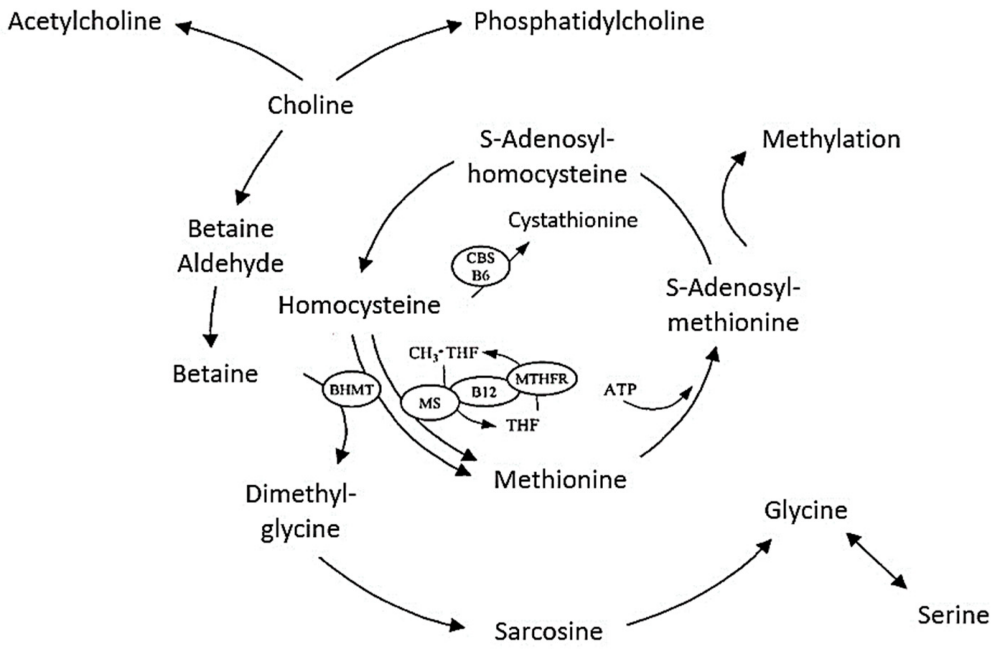

Figure 2. Methionine metabolic pathway.

The transfer of a methyl group from betaine is conducted via the enzyme betaine-homocysteine methyltransferase (BHMT), whereby betaine is converted to dimethylglycine. In this metabolic pathway, methionine is formed from homocysteine. Alternatively, methionine could be created from 5-methyltetrahydrofolate (CH_3-THF), which uses the enzyme methionine synthetase (MS) to transfer a methyl group via vitamin B-12 (cobalamin). The resulting compound—methylcobalamin, then donates a methyl group to homocysteine, thus forming methionine. The enzyme methylenetetrahydrofolate reductase (MTHFR) is involved in the release of the methyl group from CH_3-THF (5-methyltetrahydrofolate). Transmethylation metabolic pathways closely link choline, betaine, methionine, CH_3-THF, and vitamins B-6 and B-12 in order to convert homocysteine to methionine (**Figure 2**). This cycle also creates S-adenosyl methionine (SAM), which is considered the main methyl group donor in the human body. Moreover, SAM is involved with many other metabolic processes, such as the synthesis of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), carnitine, creatine, neurotransmitters, phospholipids, and the process of tissue regeneration, to name a few [25][40][49].

Dietary betaine, as well as L-carnitine and choline, which can be found in red meat, dairy products, chicken, eggs, and fish, have the ability to be broken down to trimethylamine (TMA) in the gut, which is absorbed and converted to trimethylamine N-oxide (TMAO) in the liver via the enzyme flavin-containing monooxygenase-3 (FMO3). The metabolite TMAO gained interest as being present in the highest concentrations in the tissues of the Greenland shark, which holds the distinction of being the longest-living vertebrate in the world [50]. This compound has been associated with several chronic disorders in humans, although the mechanisms of action are still not well understood [51]. A multiethnic cohort study by Fu et al. [51] aimed to investigate associations of TMAO and its precursors (betaine, choline, and carnitine) with inflammatory and cardiometabolic risk biomarkers in 1653 participants, ranging in age from 60 to 77 years old. Higher concentrations of TMAO and carnitine and lower concentrations of betaine were shown to be related to greater insulin resistance. In general, plasma TMAO

concentrations were associated with a number of trimethylamine-producing bacterial taxa and, along with their precursors, may contribute to inflammatory and cardiometabolic risk pathways [51].

3. Health-Promoting Properties

3.1. Redox Potential

The mechanism of betaine's antioxidant activity is still not entirely clear. However, it could probably act dually: directly, as a "scavenger" of reactive oxygen species (ROS), and indirectly, by improving the activity of enzymatic antioxidants, e.g., superoxide dismutase (SOD) [52].

The ability of betaine to scavenge ROS has not been determined so far by classical chemical assays, such as the ferric-reducing antioxidant power (FRAP) test. On the other hand, betaine's antioxidant activity has been determined in animal and plant models, suggesting that the interaction of betaine with the organism could be essential for its redox activity [53].

The indirect antioxidant activity of betaine is reflected in its ability to regulate sulfur-containing amino acid (SCAA) metabolism. SCAAs play a pivotal role in the synthesis of several intracellular antioxidants, such as glutathione. Betaine can increase intracellular levels of SCAA by increasing the level of methionine [54].

3.2. Liver Diseases

3.2.1. Nonalcoholic Fatty Liver Disease (NAFLD)

NAFLD is a condition of fat accumulation in the liver without the presence of excessive alcohol consumption or other specific causes of hepatic steatosis. Although NAFLD was previously considered a relatively benign condition, numerous studies have identified the potential for progression to cirrhosis and hepatocellular carcinoma. Betaine, as a lipotrope, can reduce the accumulation of fat in the liver by increasing the oxidation of free fatty acids and reducing lipogenesis, and it additionally shows an anti-inflammatory effect [55]. Vesković et al. [56] presented a mouse NAFLD model induced by a methionine- and choline-deficient diet. In the study, the betaine-treated group showed alleviation of inflammation and steatosis, a decrease in enlarged mitochondria, and an increase in the number of autophagosomes. Additionally, betaine supplementation is important for prevention. It is known that a high-fat diet leads to an increase in the concentration of S-adenosylmethionine. Deminice et al. [57] demonstrated that betaine supplementation in rats leads to a fourfold decrease in S-adenosylmethionine and, thus, prevents the occurrence of fatty liver and liver damage. Despite numerous preclinical studies that claim a positive effect of betaine on the course of liver diseases, there is not enough data from large randomized studies to support its safe application for the clinical treatment of NAFLD [58].

3.2.2. Alcoholic Liver Disease (ALD)

ALD refers to liver damage caused by chronic, excessive alcohol consumption. Alcohol cannot be deposited in the body and is therefore oxidized in the liver, which is the primary place of alcohol metabolism. During the breakdown of ethanol (alcohol in alcoholic beverages), highly toxic chemical compounds such as acetaldehyde are created, which trigger inflammation and, ultimately, the destruction of hepatocytes. Betaine supplementation can prevent alcohol-induced depletion of glutathione and cysteine in hepatocytes and, thereby, improve the antioxidant protection of these cells [59]. Li et al. [60] demonstrated that a diet enriched with betaine can reduce blood alcohol levels in rats continuously fed with alcohol. In a study conducted by Rajdl et al. [61], 117 male volunteers drank 375 mL of white wine per day for a month. In the group that consumed betaine along with wine, it was shown that this amino acid can reduce the adverse effects of moderate alcohol consumption by reducing homocysteine levels. Additionally, a study by Shen et al. [62] suggests that aberrant DNA methylation is associated with the pathogenesis of ALD, i.e., prolonged alcohol consumption may lead to DNA hypomethylation. Therefore, betaine, as a methyl donor, could have significant preventive properties in ALD.

3.2.3. Other Liver Diseases

Betaine could also have beneficial effects on other hepatic diseases, such as drug-induced liver injury and hepatitis B and C infections [63]. Zhai et al. [64] reported that betaine has significant hepatoprotective effects in a rat model where liver injury was induced by carbon tetrachloride (CCl_4). The results showed that betaine pretreatment significantly reduced levels of hepatic transaminases as well as hepatic levels of malondialdehyde. Additionally, levels of glutathione peroxidase and SOD were significantly increased. In a study conducted by Nezgoda et al. [65], 41 children with chronic hepatitis B infection in remission of acute lymphoblastic leukemia were treated with a betaine–arginine complex. Namely, the authors reported a significant reduction in pain syndromes, hepatomegaly, and the activity of hepatic aminotransferases. Another hepatotropic virus, the hepatitis C virus (HCV), is an important cause of chronic liver diseases worldwide. Interferons type I and II signaling is crucial in activating anti-viral genes, which can be suppressed by HCV. Betaine might have an important role in modulating HCV-induced inhibition of interferon signaling. Moreover, the addition of betaine to the standard anti-HCV therapy could overcome resistance to pegylated interferon α [11].

3.3. Chronic Kidney Disease (CKD)

CKD is characterized by progressive damage and a reduction in the total number of nephrons, leading to a loss of kidney function. It is caused by structural and/or functional abnormalities in the kidneys and manifested by the presence of pathohistological abnormalities and/or elevated plasma biomarkers of tissue damage, with or without a decrease in the glomerular filtration rate. Low betaine plasma levels are associated with increased kidney damage, oxidative stress, and inflammation. Therefore, betaine plasma level might be a useful biomarker for identifying CKD stages [66]. A randomized case-control study by Ephraim and Jewell [67] included 24 cats with CKD. In a group that was fed food supplemented with 0.5% betaine for 10 weeks, body composition improved, while plasma biomarkers indicated better kidney health. In another study by Ephraim and Jewell [68], a total of 28 dogs with CDK were examined. Consumption of test foods (low soluble fiber plus betaine (0.5% betaine, 0.39% oat beta-glucan, and 0.27% short-chain fructooligosaccharides) or high soluble fiber plus betaine (0.5% betaine, 0.59% oat beta-glucan,

and 0.41% short-chain fructooligosaccharides)) led to a decrease in several uremic toxins. Furthermore, Sharma et al. [69] demonstrated that orally administrated betaine in rats with sodium arsenite-induced nephrotoxicity could have significant nephroprotective properties. As observed in preclinical studies, betaine has shown beneficial effects on CKD. Therefore, betaine-enriched food formulas might have the potential to be used as an additional treatment for patients with CKD [70].

3.4. Cardiovascular Diseases

It is well known that elevated levels of homocysteine in the blood plasma represent a risk factor for the development of cardiovascular diseases. Homocysteine is a thiol amino acid that is formed by demethylation of the essential amino acid methionine. Its adverse effects include oxidation of LDL cholesterol, increased production of collagen, reduced bioavailability of nitric oxide, and prothrombotic features. Betaine, as a methyl donor, can remethylate homocysteine, i.e., convert it to methionine [71]. According to Ashtary-Larky et al. [14], betaine supplementation at a dose of 4 g/day might have homocysteine-lowering effects. Doses of ≥4 g/day are not recommended since they can have a lipid-augmenting effect. Additionally, elevated plasma levels of homocysteine can alter the levels of apolipoprotein A1, which can cause abnormal maturation of high-density lipoprotein (HDL) particles. Keeping in mind that betaine can reduce homocysteinemia, this could normalize plasma apolipoprotein A1 levels, contributing to cardiovascular protection [72].

3.5. Carcinogenesis

Carcinogenesis (oncogenesis) is the process by which healthy cells are transformed into malignant cells. Currently, the associations between dietary intake of betaine and cancer risk remain obscure, mainly due to a lack of strong evidence as only a few studies are available. Van Puyvelde et al. [73] reported that betaine supplementation does not affect breast cancer risk. In a study by Lu et al. [74], no significant associations between betaine intake and lower colorectal cancer risk were observed. Additionally, in a study by Guertin et al. [75], the association between serum betaine level and colorectal cancer risk was not significant. On the contrary, Seyyedsalehi et al. [76] claim that an increased betaine intake might decrease the risk of colorectal cancer. Regarding prostate cancer, Kar et al. [77] claim that betaine has the ability to cause apoptosis and inhibit cell growth in the DU-145 human prostate cancer cell line. Han et al. [78] conducted a prospective cohort study of 6,528 men who were followed up over the years. In those with a confirmed diagnosis of prostate cancer, lethal risk was inversely associated with betaine intake. Data about betaine usage in oncology is scarce and varies to a significant extent. To overcome the lack of precision in risk estimates, more comprehensive studies are needed.

3.6. Neuroprotective Properties

Neuroprotection refers to different strategies and mechanisms to protect neuronal elements against damage due to acute injury or neurodegenerative disorders, such as Alzheimer's disease. Betaine was investigated in terms of Alzheimer's disease. In their study on HMC3 cells, Meng et al. [79] identified betaine as an autophagy inducer. The result was the clearance of amyloid-beta via the PI3K/AKT pathway. The same signaling pathway was investigated by Huang et al. [80]. In this study, betaine was able to ameliorate cognitive deficits in rats by regulating the

PI3K/AKT pathway. Furthermore, it has been reported that betaine is depleted in the brains of patients with multiple sclerosis. Knowing that methionine metabolism is dysregulated in this disease, betaine, as a methyl donor, could have beneficial effects. In a study by Singhal et al. [81], it was reported that betaine can activate neuroprotective transcriptional programmers in the mouse model of multiple sclerosis. Neuroprotective agents also aim to limit inflammation and reduce oxidative damage, which is especially important in reperfusion injuries and cerebral ischemia. Li et al. [82] reported a favorable effect of betaine pretreatment in a rat model of brain infarct. Biochemical analyses revealed a decrease in pro-inflammatory cytokine production and a reduction in oxidative stress damage. Additionally, the volume of the brain infarct was reduced.

3.7. Body Composition and Sport Performance

Several studies on pigs and chickens in the late 1990s showed that the use of betaine could increase meat yield. Such an effect motivated researchers to look into the effects of betaine on human body composition [15]. In a double-blind, randomized, placebo-controlled trial by Cholewa et al. [83], 23 young women without prior training experience were randomly assigned to a betaine or placebo group. In a betaine group, individuals were supplemented with 2.5 g of betaine per day. After an eight-week period of structured resistance training, significant changes in lean mass and muscle thickness, as well as sports performances, were found. Nobari et al. [84] conducted another double-blind, randomized, placebo-controlled trial, which included 29 male soccer players who were randomly assigned to a betaine or placebo group. In a betaine group, individuals were supplemented with 2 g of betaine per day. After a fourteen-week period, biochemical analyses revealed increased testosterone levels in supplemented individuals, while there were no significant changes in lean body mass or body fat. According to a meta-analysis by Ashtary-Larky et al. [85], betaine supplementation does not improve body composition. No significant changes in body composition indices, such as body mass, body mass index, body fat percentage, fat mass, or fat-free mass, were noted. There are a lot of discrepant results in this field, indicating that future studies are required.

References

1. Day, C.R.; Kempson, S.A. Betaine chemistry, roles, and potential use in liver disease. Biochim. Biophys. Acta 2016, 1860, 1098–1106.
2. Zhao, G.; He, F.; Wu, C.; Li, P.; Li, N.; Deng, J.; Zhu, G.; Ren, W.; Peng, Y. Betaine in Inflammation: Mechanistic Aspects and Applications. Front. Immunol. 2018, 9, 1070.
3. Willingham, B.D.; Ragland, T.J.; Ormsbee, M.J. Betaine Supplementation May Improve Heat Tolerance: Potential Mechanisms in Humans. Nutrients 2020, 12, 2939.
4. Cholewa, J.M.; Guimarães-Ferreira, L.; Zanchi, N.E. Effects of betaine on performance and body composition: A review of recent findings and potential mechanisms. Amino Acids 2014, 46, 1785–1793.

5. European Commission. Commission Regulation (EU) No. 432/2012 of 16 May 2012 establishing a list of permitted health claims made on food, other than those referring to the reduction of disease risk and to children's development and health. Off. J. Eur. Union 2012, 1, L136. Available online: http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex:32012R0432 (accessed on 17 April 2023).
6. Altinisik, S.; Zeidan, H.; Yilmaz, M.D.; Marti, M.E. Reactive Extraction of Betaine from Sugarbeet Processing Byproducts. ACS Omega 2023, 8, 11029–11038.
7. Pastor, K.; Pezo, L.; Vujić, D.; Jovanović, D.; Ačanski, M. Discriminating cereal and pseudocereal species using a binary system of GC–MS data—A pattern recognition approach. J. Serb. Chem. Soc. 2018, 83, 317–329.
8. Psodorov, Đ.; Ačanski, M.; Psodorov, D.; Vujić, Đ.; Pastor, K. Determination of the content of wheat and buckwheat flour in bread using GC-MS system and multivariate analysis. J. Food Nutr. Res. 2015, 54, 179–183.
9. Pastor, K.; Ačanski, M.; Vujić, Đ. Chapter 3: A Review of Adulteration Versus Authentication of Flour. In Flour and Breads and Their Fortification in Health and Disease Prevention, 2nd ed.; Preedy, V.R., Watson, R.R., Eds.; Academic Press: Cambridge, MA, USA; Elsevier Inc.: Amsterdam, The Netherlands, 2019; pp. 21–36.
10. McRae, M.P. Betaine supplementation decreases plasma homocysteine in healthy adult participants: A meta-analysis. J. Chiropr. Med. 2013, 12, 20–25.
11. Arumugam, M.K.; Paal, M.C.; Donohue, T.M., Jr.; Ganesan, M.; Osna, N.A.; Kharbanda, K.K. Beneficial effects of betaine: A comprehensive review. Biology 2021, 10, 456.
12. Truitt, C.; Hoff, W.D.; Deole, R. Health functionalities of betaine in patients with homocystinuria. Front. Nutr. 2021, 8, 627.
13. Zawieja, E.E.; Zawieja, B.; Chmurzynska, A. Betaine supplementation moderately increases total cholesterol levels: A systematic review and meta-analysis. J. Diet. Suppl. 2021, 18, 105–117.
14. Ashtary-Larky, D.; Bagheri, R.; Ghanavati, M.; Asbaghi, O.; Tinsley, G.M.; Mombaini, D.; Wong, A. Effects of betaine supplementation on cardiovascular markers: A systematic review and Meta-analysis. Crit. Rev. Food Sci. Nutr. 2022, 62, 6516–6533.
15. Cholewa, J.M.; Newmire, D.E.; Rossi, F.E.; Guimarães-Ferreira, L.; Zanchi, N.E. Chapter 60: An overview of betaine supplementation, sports performance, and body composition. In Nutrition and Enhanced Sports Performance, 2nd ed.; Bagchi, D., Nair, S., Sen, C.K., Eds.; Academic Press: Cambridge, MA, USA, 2019; pp. 691–706.
16. Heidari, R.; Niknahad, H.; Sadeghi, A.; Mohammadi, H.; Ghanbarinejad, V.; Ommati, M.M.; Jamshidzadeh, A. Betaine treatment protects liver through regulating mitochondrial function and

counteracting oxidative stress in acute and chronic animal models of hepatic injury. Biomed. Pharmacother. 2018, 103, 75–86.

17. Mishra, A.; Verma, A.K.; Das, A.; Singh, P.; Bisht, P.; Wankar, A.K. Exploring the potentials of betaine supplementation in poultry and pig: A review. IJAN 2021, 38, 1–14.
18. Uyanga, V.A.; Oke, E.O.; Amevor, F.K.; Zhao, J.; Wang, X.; Jiao, H.; Lin, H. Functional roles of taurine, L-theanine, L-citrulline, and betaine during heat stress in poultry. J. Anim. Sci. Biotechnol. 2022, 13, 23.
19. Shah, A.M.; Ma, J.; Wang, Z.; Zou, H.; Hu, R.; Peng, Q. Betaine supplementation improves the production performance, rumen fermentation, and antioxidant profile of dairy cows in heat stress. Animals 2020, 10, 634.
20. Dunshea, F.R.; Oluboyede, K.; DiGiacomo, K.; Leury, B.J.; Cottrell, J.J. Betaine improves milk yield in grazing dairy cows supplemented with concentrates at high temperatures. Animals 2019, 9, 57.
21. Lakhani, P.; Kumar, P.; Alhussien, M.N.; Lakhani, N.; Grewal, S.; Vats, A. Effect of betaine supplementation on growth performance, nutrient intake and expression of IGF-1 in Karan Fries heifers during thermal stress. Theriogenology 2020, 142, 433–440.
22. Zeisel, S.H.; Blusztajn, J.K. Choline and human nutrition. Annu. Rev. Nutr. 1994, 14, 269–296.
23. Burg, M. Molecular basis of osmotic regulation. Am. J. Physiol. 1995, 268, 983–996.
24. Caldas, T.; Demont-Caulet, N.; Ghazi, A.; Richarme, G. Thermoprotection by glycine betaine and choline. Microbiology 1999, 145, 2543–2548.
25. Schwahn, B.C.; Hafner, D.; Hohlfeld, T.; Balkenhol, N.; Laryea, M.D.; Wendel, U. Pharmacokinetics of oral betaine in healthy subjects and patients with homocystinuria. Br. J. Clin. Pharmacol. 2003, 55, 6–13.
26. Wettstein, M.; Weik, C.; Holneicher, C.; Haussinger, D. Betaine as an osmolyte in rat liver: Metabolism and cell-to-cell interactions. Hepatology 1998, 27, 787–793.
27. Kettunen, H.; Peuranen, S.; Tiihonen, K.; Saarinen, M. Intestinal uptake of betaine in vitro and the distribution of methyl groups from betaine, choline, and methionine in the body of broiler chicks. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 2001, 128, 269–278.
28. Lever, M.; Sizeland, P.C.; Bason, L.M.; Hayman, C.M.; Chambers, S.T. Glycine betaine and proline betaine in human blood and urine. Bioch. Biophys. Acta 1994, 1200, 259–264.
29. Frontiera, M.S.; Stabler, S.P.; Kolhouse, J.F.; Allen, R.H. Regulation of methionine metabolism: Effects of nitrous oxide and excess dietary methionine. J. Nutr. Biochem. 1994, 5, 28–38.
30. Virtanen, E. Piecing together the betaine puzzle. Feed Mix Mag. 1995, 3, 12–17.

31. Burg, M.; Ferraris, J.; Dmitrieva, N. Cellular response to hyperosmotic stresses. Physiol. Rev. 2007, 87, 1441–1474.
32. Burg, M.B. Molecular basis for osmoregulation of organic osmolytes in renal medullary cells. J. Exp. Zool. 1994, 268, 171–175.
33. Petronini, P.G.; De Angelis, E.M.; Borghetti, P.; Borghetti, A.F.; Wheeler, K.P. Modulation by betaine of cellular responses to osmotic stress. Biochem. J. 1992, 282, 69–73.
34. Yancey, P.H.; Clark, M.E.; Hand, S.C.; Bowlus, R.D.; Somero, G.N. Living with stress: Evolution of osmolyte systems. Science 1982, 217, 1214–1222.
35. Söderlun, T.; Zhu, K.; Jutila, A.; Kinnunen, P.K.J. Effects of betaine on the structural dymanics of Thermomyces (Humicola) lanuginosa lipase. Coll. Surf. B Biointerfaces 2002, 26, 75–83.
36. Pícha, J.; Vaněk, V.; Buděšínský, M.; Mládková, J.; Garrow, T.A.; Jiráček, J. The development of a new class of inhibitors for betaine-homocysteine S-methyltransferase. Eur. J. Med. Chem. 2013, 65, 256–275.
37. Price, R.K.; Keaveney, E.M.; Hamill, L.L.; Wallace, J.M.; Ward, M.; Ueland, P.M.; McNulty, H.; Strain, J.J.; Parker, M.J.; Welch, R.W. Consumption of wheat aleurone-rich foods increases fasting plasma betaine and modestly decreases fasting homocysteine and LDL-cholesterol in adults. J. Nutr. 2010, 140, 2153–2157.
38. Kettunen, H.; Peuranen, S.; Tiihonen, K. Betaine aids in the osmoregulation of duodenal epithelium of broiler chicks, and affects the movement of water across the small intestinal epithelium in vitro. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 2001, 129, 595–603.
39. Courtenay, E.S.; Capp, M.W.; Anderson, C.F.; Record, M.T., Jr. Vapor pressure osmometry studies of osmolyte-protein interactions: Implications for the action of osmoprotectants in vivo and for the interpretation of “osmotic stress” experiments in vitro. Biochemistry 2000, 39, 4455–4471.
40. Craig, S.A. Betaine in human nutrition. Am. J. Clin. Nutr. 2004, 80, 539–549.
41. Spaggiari, M.; Calani, L.; Folloni, S.; Ranieri, R.; Dall’Asta, C.; Galaverna, G. The impact of processing on the phenolic acids, free betaine and choline in Triticum spp. L. whole grains milling by-products. Food Chem. 2020, 311, 125940.
42. Bates, C.; Mansoor, M.; Pentieva, K.; Hamer, M.; Mishra, G. Biochemical risk indices, including plasma homocysteine, that prospectively predict mortality in older British people: The national diet and nutrition survey of people aged 65 years and over. Brit. J. Nutr. 2010, 104, 893–899.
43. Smith, A.; Smith, S.; De Jager, C.; Whitbread, P.; Johnston, C.; Agacinski, G. Homocysteine-lowering by B vitamins slows the rate of accelerated brain atrophy in mild cognitive impairment: A randomized controlled trial. PLoS ONE 2010, 5, e12244.

44. Lee, J.E.; Jacques, P.F.; Dougherty, L.; Selhub, J.; Giovannucci, E.; Zeisel, S.H.; Cho, E. Are dietary choline and betaine intakes determinants of total homocysteine concentration? Am. J. Clin. Nutr. 2010, 91, 1303–1310.

45. Wilken, D.E.; Wilken, B.; Dudman, N.P.; Tyrell, P.A. Homocystenuria-the effects of betaine in the treatment of patients not responsive to pyridoxine. N. Engl. J. Med. 1983, 309, 448–453.

46. Brouwer, I.A.; Verhoef, P.; Urgert, R. Betaine supplementation and plasma homocysteine in healthy volunteers. Arch. Intern. Med. 2000, 160, 2546–2547.

47. Chiuve, S.E.; Giovannucci, E.L.; Hankinson, S.E.; Zeisel, S.H.; Dougherty, L.W.; Willett, W.C.; Rimm, E.B. The association between betaine and choline intakes and the plasma concentrations of homocysteine in women. Am. J. Clin. Nutr. 2007, 86, 1073–1081.

48. Barak, A.J.; Beckenhauer, H.C.; Tuma, D.J. Betaine, ethanol, and the liver: A review. Alcohol 1996, 13, 395–398.

49. Parkhitko, A.A.; Jouandin, P.; Mohr, S.E.; Perrimon, N. Methionine metabolism and methyltransferases in the regulation of aging and lifespan extension across species. Aging Cell 2019, 18, e13034.

50. McFadden, J.W.; Myers, W.A. Trimethylamine N-Oxide in Humans and Dairy Cows: Should We Be Concerned? In Proceedings of the Virtual Cornell Nutrition Conference, Ithaca, NY, USA, 19–22 October 2020.

51. Fu, B.C.; Hullar, M.A.J.; Randolph, T.W.; Franke, A.A.; Monroe, K.R.; Cheng, I.; Wilkens, L.R.; Shepherd, J.A.; Madeleine, M.M.; Le Marchand, L.; et al. Associations of plasma trimethylamine N-oxide, choline, carnitine, and betaine with inflammatory and cardiometabolic risk biomarkers and the fecal microbiome in the Multiethnic Cohort Adiposity Phenotype Study. Am. J. Clin. Nutr. 2020, 111, 1226–1234.

52. Zhang, M.; Zhang, H.; Li, H.; Lai, F.; Li, X.; Tang, Y.; Min, T.; Wu, H. Antioxidant Mechanism of Betaine without Free Radical Scavenging Ability. J. Agric. Food Chem. 2016, 64, 7921–7930.

53. Alirezaei, M.; Khoshdel, Z.; Dezfoulian, O.; Rashidipour, M.; Taghadosi, V. Beneficial antioxidant properties of betaine against oxidative stress mediated by levodopa/benserazide in the brain of rats. J. Physiol. Sci. 2015, 65, 243–252.

54. Hassanpour, S.; Rezaei, H.; Razavi, S.M. Anti-nociceptive and antioxidant activity of betaine on formalin- and writhing tests induced pain in mice. Behav. Brain Res. 2020, 390, 112699.

55. Chen, W.; Xu, M.; Xu, M.; Wang, Y.; Zou, Q.; Xie, S.; Wang, L. Effects of betaine on non-alcoholic liver disease. Nutr. Res. Rev. 2022, 35, 28–38.

56. Vesković, M.; Labudović-Borović, M.; Mladenović, D.; Jadžić, J.; Jorgačević, B.; Vukićević, D.; Vučević, D.; Radosavljević, T. Effect of Betaine Supplementation on Liver Tissue and

Ultrastructural Changes in Methionine–Choline-Deficient Diet-Induced NAFLD. Microsc. Microanal. 2020, 26, 997–1006.

57. Deminice, R.; da Silva, R.P.; Lamarre, S.G.; Kelly, K.B.; Jacobs, R.L.; Brosnan, M.E.; Brosnan, J.T. Betaine supplementation prevents fatty liver induced by a high-fat diet: Effects on one-carbon metabolism. Amino Acids 2015, 47, 839–846.
58. Mukherjee, S. Role of betaine in liver disease-worth revisiting or has the die been cast? World J. Gastroenterol. 2020, 26, 5745–5758.
59. Rehman, A.; Mehta, K.J. Betaine in ameliorating alcohol-induced hepatic steatosis. Eur. J. Nutr. 2022, 61, 1167–1176.
60. Li, J.; Li, X.M.; Caudill, M.; Malysheva, O.; Bardag-Gorce, F.; Oliva, J.; French, B.; Gorce, E.; Morgan, K.; Kathirvel, E.; et al. Betaine feeding prevents the blood alcohol cycle in rats fed alcohol continuously for 1 month using the rat intragastric tube feeding model. Exp. Mol. Pathol. 2011, 91, 540–547.
61. Rajdl, D.; Racek, J.; Trefil, L.; Stehlik, P.; Dobra, J.; Babuska, V. Effect of Folic Acid, Betaine, Vitamin B6, and Vitamin B12 on Homocysteine and Dimethylglycine Levels in Middle-Aged Men Drinking White Wine. Nutrients 2016, 8, 34.
62. Shen, H.; French, B.A.; Tillman, B.C.; Li, J.; French, S.W. Increased DNA methylation in the livers of patients with alcoholic hepatitis. Exp. Mol. Pathol. 2015, 99, 326–329.
63. Wang, C.; Ma, C.; Gong, L.; Dai, S.; Li, Y. Preventive and therapeutic role of betaine in liver disease: A review on molecular mechanisms. Eur. J. Pharmacol. 2021, 912, 174604.
64. Zhai, Y.; Tang, H.; Zhang, Q.; Peng, Y.; Zhao, L.; Zhang, B.; Yang, Y.; Ma, J.; Zhu, J.; Zhang, D. The Protective Effect of Lycium barbarum Betaine and Effervescent Tablet against Carbon Tetrachloride-Induced Acute Liver Injury in Rats. Nat. Prod. Commun. 2023, 18, 1934578X231161419.
65. Nezgoda, I.; Moroz, L.; Singh, S.; Singh, O. Modern approaches in management of children with chronic hepatitis b in remission of acute lymphoblastic leukemia. Georgian Med. News 2020, 308, 71–79.
66. Alvarenga, L.; Ferreira, M.S.; Kemp, J.A.; Mafra, D. The Role of Betaine in Patients with Chronic Kidney Disease: A Narrative Review. Curr. Nutr. Rep. 2022, 11, 395–406.
67. Ephraim, E.; Jewell, D.E. Betaine and Soluble Fiber Improve Body Composition and Plasma Metabolites in Cats with Chronic Kidney Disease. Front. Biosci. 2023, 15, 8.
68. Ephraim, E.; Jewell, D.E. Effect of Added Dietary Betaine and Soluble Fiber on Metabolites and Fecal Microbiome in Dogs with Early Renal Disease. Metabolites 2020, 10, 370.

69. Sharma, S.; Kaur, T.; Sharma, A.K.; Singh, B.; Pathak, D.; Yadav, H.N.; Singh, A.P. Betaine attenuates sodium arsenite-induced renal dysfunction in rats. Drug Chem. Toxicol. 2022, 45, 2488–2495.

70. Moreira, L.S.G.; Fanton, S.; Cardozo, L.; Borges, N.A.; Combet, E.; Shiels, P.G.; Stenvinkel, P.; Mafra, D. Pink pressure: Beetroot (Beta vulgaris rubra) as a possible novel medical therapy for chronic kidney disease. Nutr. Rev. 2022, 80, 1041–1061.

71. Luo, M.; Wang, T.; Huang, P.; Zhang, S.; Song, X.; Sun, M.; Liu, Y.; Wei, J.; Shu, J.; Zhong, T.; et al. Association of Maternal Betaine-Homocysteine Methyltransferase (BHMT) and BHMT2 Genes Polymorphisms with Congenital Heart Disease in Offspring. Reprod. Sci. 2023, 30, 309–325.

72. Rosas-Rodríguez, J.A.; Valenzuela-Soto, E.M. The glycine betaine role in neurodegenerative, cardiovascular, hepatic, and renal diseases: Insights into disease and dysfunction networks. Life Sci. 2021, 285, 119943.

73. Van Puyvelde, H.; Dimou, N.; Katsikari, A.; Indave Ruiz, B.I.; Godderis, L.; Huybrechts, I.; De Bacquer, D. The association between dietary intakes of methionine, choline and betaine and breast cancer risk: A systematic review and meta-analysis. Cancer Epidemiol. 2023, 83, 102322.

74. Lu, M.S.; Fang, Y.J.; Pan, Z.Z.; Zhong, X.; Zheng, M.C.; Chen, Y.M.; Zhang, C.X. Choline and betaine intake and colorectal cancer risk in Chinese population: A case-control study. PLoS ONE 2015, 10, e0118661.

75. Guertin, K.A.; Li, X.S.; Graubard, B.I.; Albanes, D.; Weinstein, S.J.; Goedert, J.J.; Wang, Z.; Hazen, S.L.; Sinha, R. Serum Trimethylamine N-Oxide, Carnitine, Choline, and Betaine in Relation to Colorectal Cancer Risk in the Alpha Tocopherol, Beta Carotene Cancer Prevention Study. Cancer Epidemiol. Biomark. Prev. 2017, 26, 945–952.

76. Seyyedsalehi, M.S.; Rossi, M.; Hadji, M.; Rashidian, H.; Marzban, M.; Parpinel, M.; Fiori, F.; Naghibzadeh-Tahami, A.; Hannun, Y.A.; Luberto, C.; et al. Dietary Choline and Betaine Intake and Risk of Colorectal Cancer in an Iranian Population. Cancers 2023, 15, 2557.

77. Kar, F.; Hacioglu, C.; Kacar, S.; Sahinturk, V.; Kanbak, G. Betaine suppresses cell proliferation by increasing oxidative stress–mediated apoptosis and inflammation in DU-145 human prostate cancer cell line. Cell Stress Chaperones 2019, 24, 871–881.

78. Han, P.; Bidulescu, A.; Barber, J.R.; Zeisel, S.H.; Joshu, C.E.; Prizment, A.E.; Vitolins, M.Z.; Platz, E.A. Dietary choline and betaine intakes and risk of total and lethal prostate cancer in the Atherosclerosis Risk in Communities (ARIC) Study. Cancer Causes Control 2019, 30, 343–354.

79. Meng, Q.; Chen, Y.; Chen, Y.; Wang, L.; Zhao, L. Betaine Induced Autophagy to Protect Neurons Against Amyloid Beta in Alzheimer's Disease. Res. Square 2021.

80. Huang, B.; Hu, X.; Hu, J.; Chen, Z.; Zhao, H. Betaine Alleviates Cognitive Deficits in Diabetic Rats via PI3K/Akt Signaling Pathway Regulation. Dement. Geriatr. Cogn. Disord. 2020, 49, 270–278.

81. Singhal, N.K.; Sternbach, S.; Fleming, S.; Alkhayer, K.; Shelestak, J.; Popescu, D.; Weaver, A.; Clements, R.; Wasek, B.; Bottiglieri, T.; et al. Betaine restores epigenetic control and supports neuronal mitochondria in the cuprizone mouse model of multiple sclerosis. Epigenetics 2020, 15, 871–886.
82. Li, Q.; Qu, M.; Wang, N.; Wang, L.; Fan, G.; Yang, C. Betaine protects rats against ischemia/reperfusion injury-induced brain damage. J. Neurophysiol. 2022, 127, 444–451.
83. Cholewa, J.M.; Hudson, A.; Cicholski, T.; Cervenka, A.; Barreno, K.; Broom, K.; Barch, M.; Craig, S.A.S. The effects of chronic betaine supplementation on body composition and performance in collegiate females: A double-blind, randomized, placebo controlled trial. J. Int. Soc. Sport. Nutr. 2018, 15, 37.
84. Nobari, H.; Kargarfard, M.; Minasian, V.; Cholewa, J.M.; Pérez-Gómez, J. The effects of 14-week betaine supplementation on endocrine markers, body composition and anthropometrics in professional youth soccer players: A double blind, randomized, placebo-controlled trial. J. Int. Soc. Sport. Nutr. 2021, 18, 20.
85. Ashtary-Larky, D.; Bagheri, R.; Tinsley, G.M.; Asbaghi, O.; Salehpour, S.; Kashkooli, S.; Kooti, W.; Wong, A. Betaine supplementation fails to improve body composition: A systematic review and meta-analysis. Br. J. Nutr. 2022, 128, 975–988.