

Expanding arsenal against diabetic neuropathy through betaine: Success so far and bottlenecks

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Abstract: Diabetes mellitus is one of the main chronic metabolic syndromes that contains a number of repercussions and risk factors because hyperglycemia leads other organs to malfunction. Despite the existence of cutting-edge methods for the treatment of diabetes, the proper therapeutic medication distribution remains a serious worry in the current situation. Betaine, also known as N,N-trimethyl glycine, is an amino acid derivative with a number of advantageous health effects. This chemical is available to both humans and other animals because it is consumed and created endogenously. Additionally, some pathological conditions, such as type 2 diabetes, result in a decrease in the amount of betaine in the tissues. Betaine has been found in rodent studies to considerably lessen a number of abnormalities associated with diabetes. changes in the liver and other insulin-sensitive organs. Researchers believe that AMP-activated protein kinase is crucial to the mechanism through which betaine exerts its anti-diabetic effects. Also, betaine has been demonstrated to reduce endoplasmic reticulum stress and inflammation in rodent models of diabetes. Since betaine has shown promising therapeutic benefits in animal trials, its potential use in treating diabetes has been raised.

Keywords: diabetes; neuropathic pain; pathophysiology, betaine; metabolites

1. Introduction

Diabetes, a dangerous metabolic illness, affects around 10% of people globally. Due to its widespread occurrence and increased morbidity, this condition is an important global problem. Based on the cause, signs, and ways of treatment, there are several types of diabetes. The most common kinds of diabetes are types 1 and 2. In individuals with type 1 diabetes, the only source of endogenous insulin is the pancreatic beta-cells, which rapidly age. This is associated with serious metabolic issues and open hyperglycemia. Thus, type 1 diabetes patients need to be treated with exogenous insulin. Type 2 diabetes mellitus, which accounts for around 90% of all cases, is characterized by defective insulin synthesis and action (insulin resistance) [1]. T2DM, or type 2 diabetes mellitus, has been linked to a wide range of pathophysiological anomalies. It is well known that decreased muscle glucose uptake and elevated endogenous glucose production are two hallmarks of insulin resistance. Increased lipolysis, greater free fatty acid levels, and accumulation of intermediate lipid metabolites all contribute to increased glucose generation, decreased peripheral glucose utilization, and impaired beta-cell activity. Insulin resistance and adipose tissue inflammation are significant contributors to the onset of type 2 diabetes. It is

now commonly acknowledged that non-alcoholic fatty liver disease (NAFLD) exists in individuals with insulin resistance. Despite hyperglycemia, the capacity of the renal tubules to absorb glucose may rise, and hypothalamic insulin resistance (a condition of the central nervous system) lessens the capacity of circulating insulin to suppress glucose production. The underlying pathophysiologic abnormalities must be taken into account in order to properly treat hyperglycemia in T2DM patients. Obesity and type 2 diabetes are frequently linked. In persons with type 2 diabetes, hyperglycemia is infrequent, and diabetic consequences take time to materialize. To successfully combat insulin resistance, an initial excess of insulin is created [1,2]. However, long-term overstimulation of insulin release eventually results in the death of pancreatic beta cells. Greater physical activity and nutritional adjustments are suggested as an adjunct to pharmaceutical therapy for type 2 diabetic patients [3].

Type 2 diabetic patients who take anti-diabetic drugs report good results. However, prolonged use of these medications might have negative side effects, such as hypoglycemia and the danger of neuroglycopenia, gastrointestinal problems, and an increase in body weight. New compounds are therefore being investigated for their anti-diabetic properties to supplement current treatments and lessen the negative effects of medication [4,5].

Among various antidiabetic natural components, betaine is also a good component to control glucose levels as well as its related complications. Sometimes it is referred to as “glycine betaine” [6]. N,N,N-trimethylglycine is its chemical name, and it can be found in large concentrations in wheat bran, beets, wheat bread, and spinach. The “sodium-dependent amino-acid transport system” is primarily responsible for its rapid absorption in the digestive tract [7]. Betaine is used as a dietary supplement in both animal and human nutrition in addition to supplies obtained from food [8]. Betaine has been shown to prevent liver steatosis. Both alcohol-related fat buildup and non-alcoholic fatty liver disease are effective. Reduced lipid synthesis, enhanced lipid oxidation, and relief from inflammatory and oxidative stress all contribute to betaine’s elicited inhibition of hepatic fat buildup. Additionally, betaine may successfully shield the liver from certain poisons [9]. Betaine has also been demonstrated to benefit the intestines and gut. It strengthens intestinal epithelium, improves mucosal barrier performance, and effects tight junction proteins favorably when taken as a supplement. Betaine supplementation is associated with enhanced renal and cardiovascular health in addition to having favorable effects on the digestive system. Treatment with betaine may also diminish neurological disorders and shield against certain cancers. According to a large body of research, betaine is also helpful in treating diabetes and diseases related to diabetes [10]. The findings of the anti-diabetic potential as well as the impact on diabetic neuropathy of betaine are presented in this review.

1.1. Pathophysiology of diabetic neuropathy

After eating, the pancreas reacts by secreting insulin, which has the effect of accelerating the transport, biotransformation, and storage of glucose in skeletal muscle and adipose tissue. The liver can still provide the brain with glucose from the blood even when there is no insulin in the system. Insulin inhibits glucagon release and

decreases blood fatty acid levels, both of which have a detrimental impact on the liver's ability to produce glucose. Intracellular hypoglycemia and extracellular hyperglycemia were caused by insufficient insulin production or insulin resistance, respectively. Diabetic ketoacidosis, which is brought on by intracellular hypoglycemia, leads to the breakdown of body fat through procedures known as glycogenesis and gluconeogenesis [4,11–15]. An increase in extracellular glucose causes hyperglycemic coma and osmotic diuresis; reduced protein synthesis and gamma globulins cause cachexia, polyphagia, and delayed wound healing [16]. Since diabetes is a metabolic disorder, consequences are a constant worry for diabetics. Vascular problems might include microvascular (neuropathy, retinopathy, and nephropathy), macrovascular (coronary heart disease, peripheral vascular disease, and stroke), or mixed vascular. Compared to risks of microvascular issues, macrovascular degeneration is more significantly associated with diabetes-related mortality and morbidity in the elderly [17].

Diabetic neuropathy (DN), which is defined as “the presence of symptoms and/or evidence of peripheral nerve dysfunction in patients with diabetes following the elimination of alternative causes,” is the most common result of diabetes [18]. Data collected in 2005 show that 60–70% of diabetic people had moderate to severe DN. A defining feature of DN is progressive, length-dependent sensory loss [19]. The fingers are frequently impacted by the time symptoms reach the knees. The longest axons that innervate the foot are earliest impacted by DN. There is a link between abnormal nerve conduction studies and sensory issues [20], reduced myelinated fiber density (MFD), and decreased intraepidermal nerve fiber (IENF) densities [21]. Human sural nerve and skin biopsies [22]. There is presently no medication that may stop the loss of nerve fibers and function in DN, despite the fact that maintaining euglycemia may reduce peripheral nerve degradation [23].

1.2. Risk factors for painful diabetic neuropathy

The majority of studies addressing the risk factors for neuropathic pain in DN use cross-sectional rather than prospective research, univariate rather than multivariate analysis, and do not always specify the comparator [24]. Female sex is a risk factor for painful diabetic neuropathy [25], which is consistent with risk variables for many NP syndromes. Furthermore, a higher prevalence of pain has been consistently recorded in individuals with more severe neuropathy as characterized by clinical grading scales and sensory loss on quantitative sensory tests poor glycemic control [26], reduced renal function [25], and a high body mass index (BMI). These observations are associated with severe DN when compared to painless DN [27]. Some of these elements may be linked to neuropathy progression, while others may be linked to sensory neuron hyperexcitability and the development of pain (**Figure 1**).

A multitude of pathogenic changes in neurons, glia, and vascular cells are brought on by hyperglycemia, dyslipidemia, and altered insulin signaling. These changes can result in nerve dysfunction and, finally, neuropathy. It can advance with neurodegeneration, loss of neurotrophic signaling, endoplasmic reticulum stress, mitochondrial dysfunction, and DNA damage. It can also advance with macrophage activation. Because different cell types are more or less vulnerable to damage based

on metabolic imbalances, the role of these pathways in the onset of neuropathy varies with cell type, disease profile, and time.

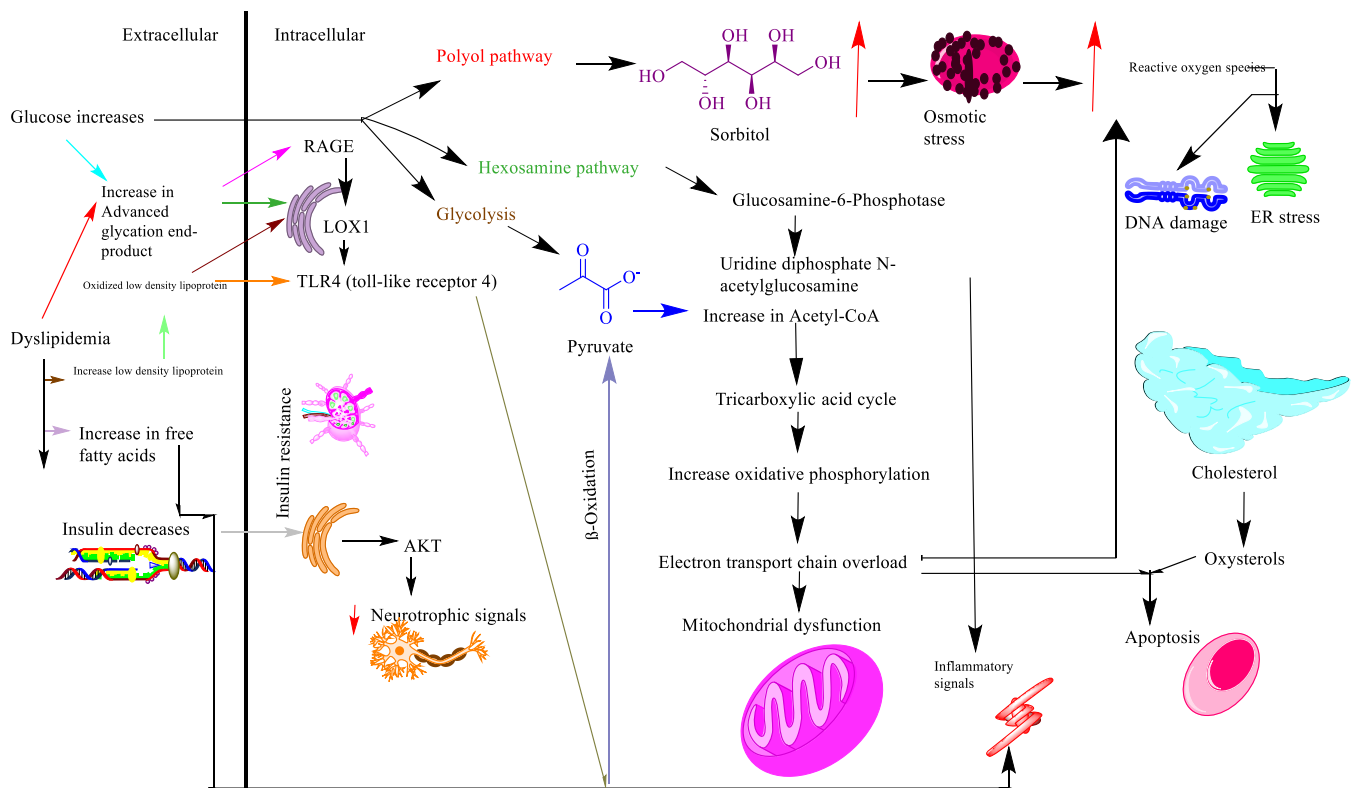
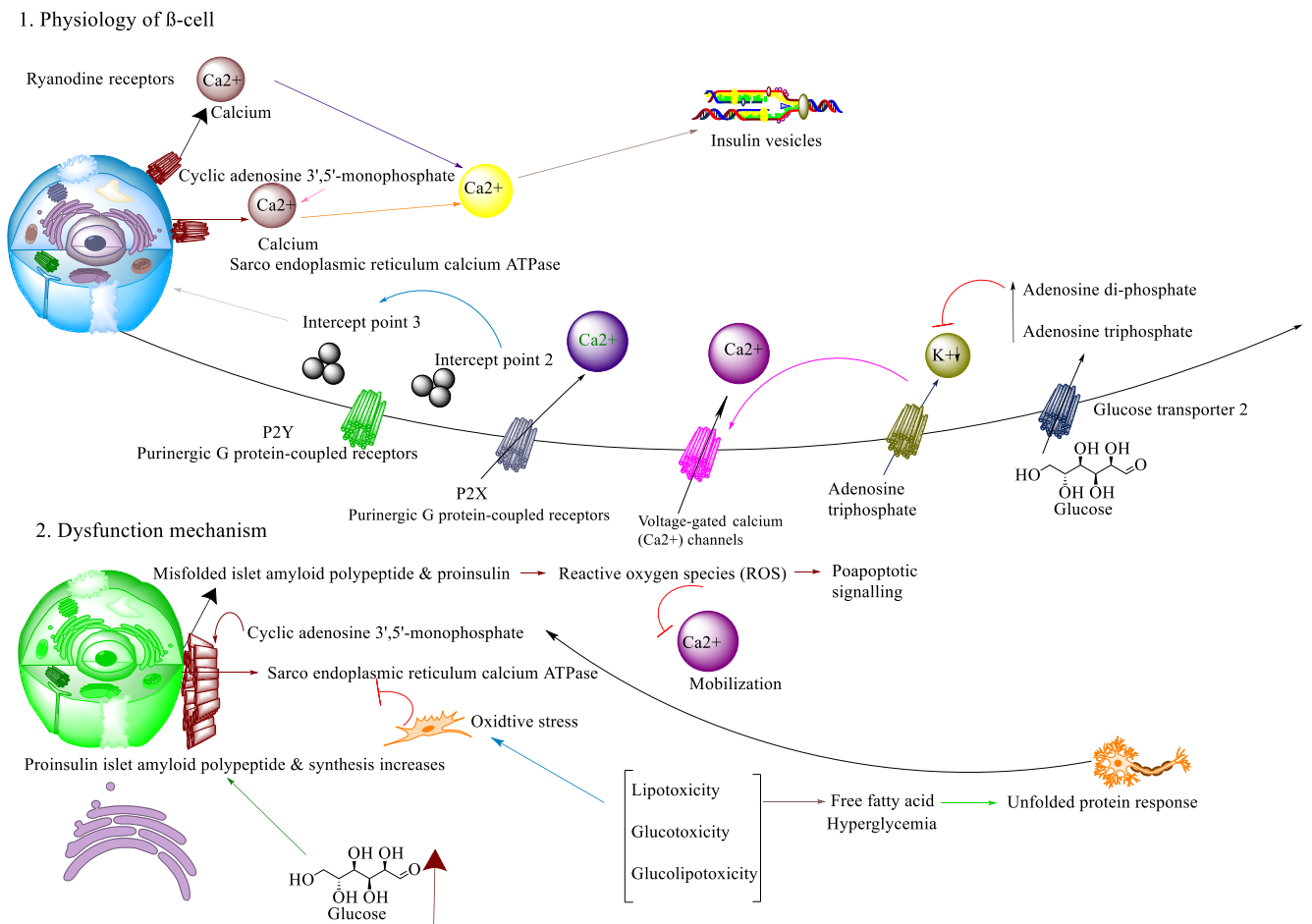


Figure 1. Diabetic neuropathy pathogenesis.

1.3. Basic mechanisms of T2DM

Maintaining cellular integrity and tightly controlling the mechanisms and pathways involved in cell physiology is necessary for ensuring optimal cell function [28]. Pre-proinsulin is converted by cells into insulin, which is then produced by the cells. The endoplasmic reticulum (ER) and several other proteins enable pre-proinsulin to mature and alter structurally to become proinsulin [29]. Proinsulin is then transferred from the ER to the Golgi apparatus (GA), where it enters developing secretory vesicles and is transformed into C-peptide and insulin [30,31]. Insulin is made and stored in granules up until a signal to release it is received. The principal trigger for the production of insulin is elevated glucose levels. It's crucial to remember that many things, such as hormones, fatty acids, and amino acids, can also cause the release of insulin [32]. The glucose transporter 2 (GLUT2), a solute carrier protein that also serves as a glucose sensor in cells, is the main pathway by which cells absorb glucose as blood glucose levels rise. When glucose enters the cell, glucose catabolism begins. This increases the intracellular ATP/ADP ratio and causes the plasma membrane's ATP-dependent potassium channels to close. As a result, the membrane depolarizes and voltage-dependent Ca^{2+} channels open, allowing Ca^{2+} to enter the cell. Insulin exocytosis results from the priming and fusing of secretory insulin-containing granules to the plasma membrane as a result of the rise in intracellular Ca^{2+} [30,32–34] (**Figure 2**).



Part 1 of the figure is in connection with part 2. In part 1 it is represented that glucose is mostly internalized via the GLUT2 transporter, and insulin release is primarily triggered by a reaction to increased glucose levels. The closing of ATP-dependent potassium channels results in membrane depolarization and the opening of voltage-dependent Ca^{2+} channels because glucose catabolism increases the ATP/ADP ratio. In the latter, Ca^{2+} influx results in insulin exocytosis. Additional Ca^{2+} channels such as P2X, P2Y, SERCA, and RYR help in Ca^{2+} mobilization and insulin secretion.

Part 2 of the diagram represented that oxidative stress is exacerbated by hyperglycemia and hyperlipidemia, which results in the production of ROS, which prevents Ca^{2+} mobilization and activates proapoptotic signals. Furthermore, ER stress is brought on by hyperglycemia and the activation of the apoptotic unfolded protein response (UPR) pathways. Persistently high glucose levels increase the creation of proinsulin and IAAP, which produces ROS. Additionally, the RY receptors (RYR) can enhance Ca^{2+} signals and, because of their advantageous positions within the cell and their capacity to mediate Ca^{2+} -induced Ca^{2+} release (CICR), they may play significant roles in stimulus-insulin secretion coupling. Involved in the stimulation of

insulin secretion, RYR increases Ca^{2+} signals when the channel is sensitized by messenger molecules produced by food metabolism or ligand-binding [35] (**Figure 2**). However, additional cell signals can also help or improve the way that β -cells release insulin. Among them, cAMP may be the most significant messenger enhancing the release of insulin. According to growing evidence, cAMP mobilizes insulin-containing secretory vesicles by reducing intracellular Ca^{2+} reservoirs, which raises intracellular Ca^{2+} concentrations [36].

Extracellular ATP is another crucial regulator of β -cell activity, and this is supported by strong data. When insulin granules are exocytosed by beta-cells in response to glucose stimulation, ATP is released. Independent of glucose, insulin exocytosis is controlled by purinergic signaling through P2Y and P2X purinergic receptors, which also promote Ca^{2+} mobilization. According to reports, P2Y purinoreceptors are connected to G-proteins [37,38] whereas P2X-type receptors are ATP-activated ligand-gated ion channels non-selective for cations [39].

For P2Y receptors, it has been suggested that insulin release may be mediated by intracellular Ca^{2+} mobilization in response to inositol-1,4,5-trisphosphate (IP3) production, which causes the release of Ca^{2+} from ER storage and enhances the exocytosis-triggering Ca^{2+} signal [40,41] (**Figure 2**).

Mechanisms leading to β -cell dysfunction

β -cell dysfunction has been traditionally associated with β -cell death [42]. However, recent evidence suggests that the dysfunction of β -cells in T2DM might be due to a more complex network of interactions between the environment and different molecular pathways implicated in cell biology [43]. Hyperglycemia and hyperlipidemia are frequently present in an excessive dietary state, similar to that found in obesity, encouraging IR and chronic inflammation. Due to genetic disparities in sensitivity, in these conditions, β -cells are vulnerable to toxic stresses such as inflammation, inflammatory stress, ER stress, metabolic/oxidative stress, and amyloid stress, which have the potential to eventually result in the loss of islet integrity [42]. FFA overexposure and hyperglycemia cause ER stress, which in turn activates the apoptotic unfolded protein response (UPR) pathways, which results in β -cell malfunction [44]. In reality, obesity-related lipotoxicity, glucotoxicity, and glucolipotoxicity cause oxidative stress and metabolic stress, both of which harm β -cells [43]. Defects in the synthesis of any insulin precursors, or insulin itself, as well as disruption of the secretion mechanism, can lead to insulin secretory dysfunction, the primary driver of β -cell failure, and a foundation of T2DM. For instance, reduced expression in the GLUT2 glucose transporter would affect the downstream signaling pathway [45], while failure in the folding of proinsulin is another finding commonly linked to deficient insulin production and diabetes [46].

2. Betaine

2.1. Source of betaine

Most of the betaine we consume comes from food, either as betaine or dietary choline. It is reasonable to suppose that dietary modifications will have an impact on the relative relevance of various sources. Betaine has a choline-saving effect, despite

the fact that both choline and betaine are crucial for human health. Dietary choline cannot entirely fulfill the needs for betaine and choline. Betaine is abundant in crops from the beet family, including spinach, beetroot, oats, and wheat. Everyone agrees that the majority of people (from a variety of demographic categories) take between 100 and 300 mg daily [47–50]. We have found that it is difficult to design a long-term diet that is satisfying and supplies more than about 800 mg per day without supplementation, despite prior suggestions that the average daily consumption of betaine may approach one gram. The diet contains a variety of different betaines, only proline betaine, which is found in citrus foods like orange juice and sprouted legumes, can alter the metabolism of the amino acid. Because proline betaine blocks glycine betaine reabsorption in the kidneys, it may appear to increase betaine excretion even in healthy persons [51]. Trigonelline, which is also present in coffee and bean sprouts, does not have the same impact.

2.2. Chemical structure and metabolism of betaine

Betaine is a necessary osmolyte and methyl group source that may be obtained from food or by oxidizing choline. Homocysteine is methylated by its metabolism into methionine, which also results in N,N-dimethylglycine (**Figure 3**) [52]. In addition to obtaining it from food, the body also produces betaine through a two-step choline oxidation process. Because endogenous metabolism cannot completely meet the demand, betaine supplementation in the diet may be necessary for maintaining or enhancing health [53]. As an osmolyte, betaine prevents osmotic stress and maintains a steady intracellular osmotic pressure. Additionally, by stabilizing the molecular structure of some proteins, it may change the characteristics of such proteins. Because it has three CH₃ groups in one molecule, betaine is an essential donor of these groups for transmethylation reactions. Betaine's function in methylation processes is crucial to the correct methylation of homocysteine into methionine. Homocysteine levels decrease, S-adenosylmethionine levels increase, and the proper levels of methionine are preserved as a result. Too much blood homocysteine and insufficient S-adenosylmethionine both raise the risk of a number of ailments, including stroke, cardiovascular disease, various neurological disorders, and others [54]. Betaine is not only involved in the methylation of nucleic acids but also in the conversion of homocysteine to methionine. Inappropriate methylation is linked to the development of cancer, genomic instability, and senescence [55].

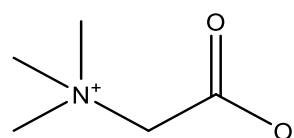


Figure 3. Chemical structure of betaine.

2.3. Metabolites of betaine

Choline is a major source of betaine, and homocysteine and methionine, which are engaged in its catabolism, are connected via the metabolism (**Figure 4**) to other metabolites that are essential to the health of humans and other mammals. People with a poor vascular risk profile frequently have low plasma betaine levels [56,57],

although plasma betaine is only weakly connected with tissue betaine [58]. Only trace amounts are passed through the urine [59,60], indicating that the usual pathway for elimination is catabolism. Despite significant variations in tissue betaine concentrations, plasma and urine betaine are under strong homeostatic control, and there is no correlation between plasma betaine levels and urinary betaine excretions. Although tissue betaine concentrations fluctuate significantly, the strong homeostatic regulation of plasma and urine betaine is mostly unaffected by osmotic variations [61] [62,63], and there is no relationship between plasma betaine concentrations and urinary betaine excretions. Despite the possibility that tissue betaine shortage and low plasma betaine concentrations are connected, plasma betaine concentrations are a limited marker. An insufficiency may result from excessive loss or insufficient choline-to-betaine metabolism [52,64]. As a result, betaine deficiency is difficult to detect. The methionine load test may be a test of betaine sufficiency [52,65].

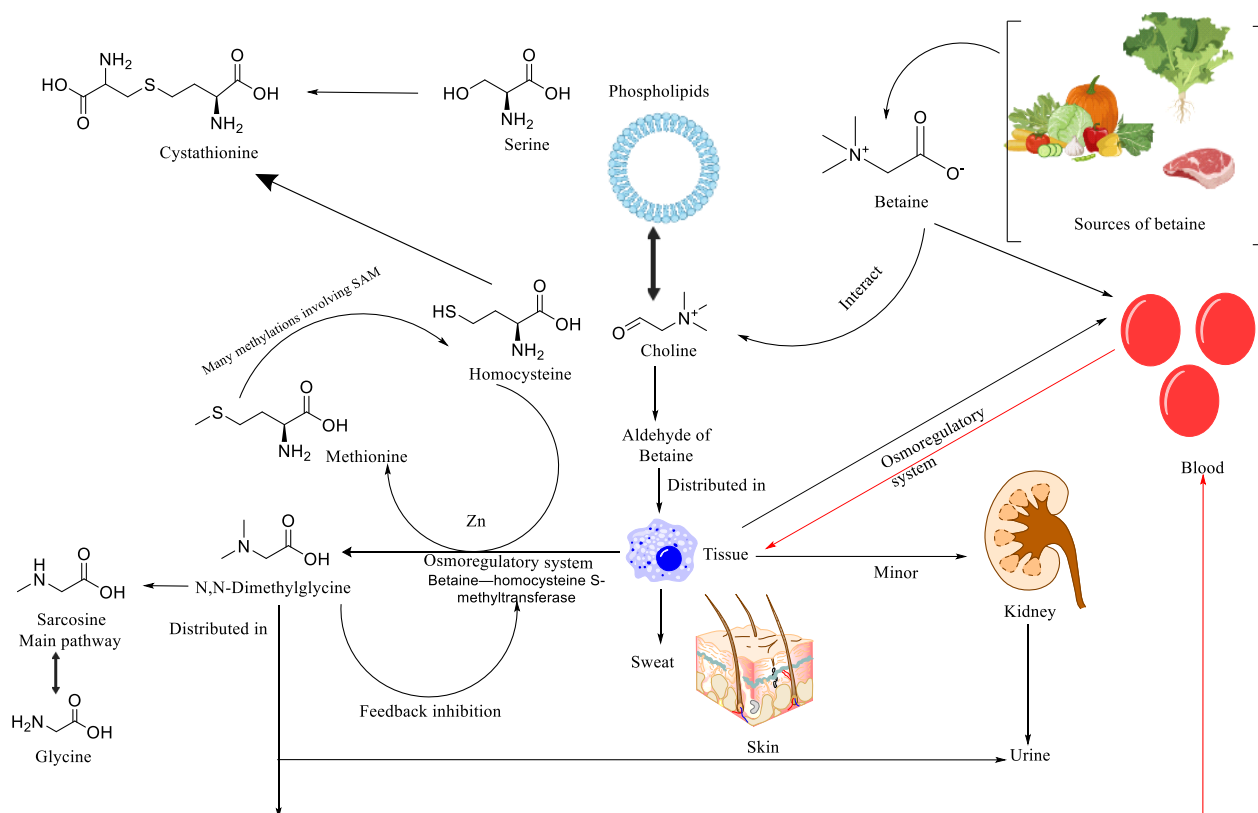


Figure 4. Betaine metabolic pathways. Betaine-homocysteine methyltransferase (BHMT). S-adenosylmethionine (SAM). Bold representation of methyl groups (and their metabolites).

Another type of shortage is a significant loss of betaine in the urine; betaine excretion is frequently excessively high or low in people with diabetes or renal failure [52,62]. Increased plasma dimethylglycine [52] implies enhanced betaine metabolism (as a reaction to homocysteine accumulation), and a lack of betaine is a major cause of elevated fasting plasma homocysteine [52,66]. Supplementing with B vitamins won't help if a betaine deficit causes elevated homocysteine levels [67–69]. Elevated homocysteine in some research populations may be a sign of betaine deficiency rather than the real issue.

2.4. Pharmacokinetics of betaine

Betaine is absorbed as an osmoprotectant from bacteria to vertebrates via the duodenum, according to many studies. In pharmacokinetic studies, betaine was found to rapidly increase plasma concentrations after oral administration in both homocystinuria patients and healthy volunteers [61,70,71]. Betaine is primarily eliminated through sweat rather than urine because it may be easily filtered by the kidneys and then reabsorbed back into the bloodstream. Based on its special transporters, betaine builds up in the body and is mostly delivered to the kidneys, liver, and brain. It appears to have a large distribution volume and spread swiftly. In a two-compartment model, the plasma concentration-time curve displayed a biexponential decline. Betaine was shown to be fairly slowly excreted by the body. Throughout the pharmacokinetic investigation, it was established that all of the routine laboratory data remained unchanged [72–74].

2.5. Pharmacological impact of betaine

2.5.1. Impact on human

Betaine has two important purposes in mammals: first, it is a significant osmolyte that is stored in most tissues to help regulate cell volume, and second, it is a CH₃ donor for the remethylation of homocysteine to methionine [75]. In the rat, where nearly all tissues have higher concentrations than the blood, the osmolyte function predicts that tissue betaine concentrations will be higher than plasma concentrations. The kidney, which may have high betaine concentrations (N100 mM) in the medulla, is where the osmo-regulated betaine transporter BGT-1 was initially discovered. However subsequent studies have shown that it is expressed in several organs [58,76–79]. Betaine is a “compensatory” or “counteracting” solute that, like the majority of osmolytes, promotes protein stability (**Table 1**) One of the most crucial roles of the renal medulla is to reduce the denaturing effects of urea, and it is particularly successful in doing so [80–83]. Most biological methylation processes need the synthesis of methionine, with S-adenosylmethionine (SAM) being the most prevalent methyl donor [84–86].

Table 1. Clinical impact of betaine in diabetic patients.

Parameters observed	Betaine responses	Diseased state of individuals
Fasting blood glucose	Reduction	People with prediabetes
Glycemic control	Increase	Obese human
Total body fat mass	Reduction	Obese human
Insulin action	Increase	Obese human
Body fat percentage	Reduction	Obese human
Betaine blood levels	Negative correlation	Patients with type 2 diabetes

2.5.2. Betaine in hyperglycemia-induced steroidogenesis

According to a recent study, mice with hyperglycemia have decreased expression of GLUT 2-4 and SGLT 1-2, which disrupts GC glucose absorption and causes energy shortage [87]. Furthermore, both primary rat granulosa cells and animals given STZ

exhibit impaired synthesis of progesterone and estradiol at high glucose levels [87]. The protective benefits of betaine in granulosa cells under high glucose circumstances could be mediated by some different mechanisms. Due to decreased absorption and entrapment in the extracellular compartment, glucose functions as an effective serum osmolyte in hyperglycemic situations [88,89].

Cells are prone to hypertonic stress and shrinkage during hyperglycemia and diabetes [90]. Cell shrinkage, by mechanical stress can induce endoplasmic reticulum stress (ERS), cytochromes *c* release and subsequent caspase-3 or caspase-12 activation and cellular apoptosis [91]. Betaine is a significant osmoprotectant that can be stored in large amounts in cells. It increases the free water content and cytoplasmic cell volume to prevent cells from contracting under hyperosmotic conditions and inhibits various proteins linked to hyperosmotic apoptosis by stabilizing proteins' fundamental structural elements [91–93]. Taken together, it is possible that betaine may act as an osmoprotectant and prevent the activation of apoptotic factors and cell cytotoxicity in GCs under hyperosmotic conditions caused by high concentration of glucose.

Betaine can also improve insulin resistance and hyperglycemia in high-calorie diet-induced diabetes in experimental animals [88,94]. Betaine dietary supplementation could improve gestational diabetes mellitus in pregnant STZ-treated mice by increasing insulin levels, alleviating insulin resistance, and restoring normal serum concentration of homocysteine as well as lipid profile characteristics. Given the previous findings, it is therefore hypothesized that betaine may affect the functions of granulosa cells in physiological and pathological conditions. Based on our knowledge, the impact of betaine on the functions of ovarian granulosa cells undergoing hyperglycemia has not been determined yet [88,94].

2.5.3. Betaine in hyperglycemia-induced vascular damage

Betaine reduces the generation of ROS, which in turn inhibits the VEGFR-2 signaling pathway and the synthesis of VEGF [95]. The STZ-induced diabetic rats' retinas showed higher levels of VEGF, HIF-1 α , and Akt expression. By preventing diabetes-induced Akt activation in the diabetic rats' retinas, betaine therapy reduced this rise in VEGF and HIF-1 α expression. The findings suggested that by inhibiting retinal neovascularization in diabetic individuals, betaine may be used to postpone the development of problems related to diabetic retinopathy. Dietary betaine supplementation has been demonstrated to enhance glucose homeostasis, decrease hepatic lipid buildup, and raise fibroblast growth factor (FGF) 21 levels in mice [96].

2.5.4. Impact of betaine on stress of oxidation

The ability of betaine to protect against oxidative stress is attributed to the fact that betaine is highly lipotropic and, when administered exogenously, it can readily pass across the membrane lipid bilayer and diffuses into intracellular compartments [97]. One theory for betaine's lipotropic characteristics is that it has an electrophilic methyl group that reduces pathological conditions brought on by oxidative and reductive stress [98]. Additionally, betaine contributes to the synthesis of methionine, which is a significant source of cellular cysteine and is used in the trans-sulfuration pathway to produce reduced glutathione, which shields cells from reactive metabolites [99].

3. Betaine impact on different organs

3.1. Impact of betaine on kidney

The degree of cell damage under heavy metal stress depends on the rate of reactive oxygen species formation and on the efficiency of detoxification and repair mechanisms. The cellular defense system against toxicity from ROS includes superoxide dismutase (SOD), catalase and glutathione peroxidase. Renal total antioxidant capacity was markedly decreased in cadmium-treated animals and this was corrected back to normal by betaine supplementation. Renal content of intracellular scavengers as SOD, GSH-Px, catalase and reduced GSH are decreased in rats exposed to cadmium. The data indicate that cadmium can induce its nephrotoxic actions via the production of ROS and interference with antioxidant defense mechanisms [100,101]. SOD is considered as the first line of defense against the deleterious effects of oxygen radicals in the cells where it scavenges ROS by catalyzing the dismutation of superoxide to H₂O₂ and O₂. The inhibitory action of cadmium on SOD may be due to competition between Cd and Zn or Cu that are required for activity of SOD [102]. Catalase is a heme protein which catalyzes the reduction of H₂O₂ to water and oxygen and thus protects the cell from oxidative damage by H₂O₂ and OH [103]. The decrease in catalase activity by cadmium may be attributed to the decreased absorption of iron, an essential trace element required for the activity of catalase.

Reduced glutathione is a sulphur-containing nucleophilic substance found in high concentration in kidney [104]. It plays a pivotal role in the protection of cells against oxidative stress and metals detoxification. The reduced GSH level was declined in the cadmium group compared to the normal group. Cadmium binds exclusively to sulfhydryl groups of GSH leading to its inactivation [105] and also exhibit an antagonistic effect with selenium and lowers its availability to GSH-Px [101,106]. Hence, betaine administration significantly corrected kidney functions.

3.2. Impact of betaine on methyl donor

Betaine possesses numerous essential functions in the organism. It is an osmolyte and thereby maintains the intracellular osmotic pressure and protects against osmotic stress. Besides, it may stabilize the structures of some proteins, affecting their properties [8]. Due to the presence of three methyl groups in one molecule, betaine is an important donor of these groups for transmethylation reactions and is highly relevant for the proper conversion of homocysteine to methionine. This leads to decreased amounts of homocysteine, increased concentrations of S-adenosylmethionine, and maintaining proper levels of methionine. Abnormalities in these leads to stroke, cardiovascular disease as well as neurodegenerative disorders [9,53–55,107]. Betaine is also involved in the methylation of nucleic acids and affect gene expression. Inappropriate methylation causes genomic instability, senescence, and cancer development [55]. Hence, the so-called “methylation diets” are recommended for health [8,54].

3.3. Impact of betaine on neurodegenerative diseases

The state of human malnutrition and high homocysteine levels have been closely

related to the progression rate of dementia and Alzheimer's disease [108], and appears to be due to GB role in up-regulation of the memory-related proteins NR1 and NR2A. Also, GB reverses tau proteins phosphorylation, deactivates protein phosphatase 2AC (PP2Ac), reduces A β accumulation, and the inflammation levels by down regulation of IL-1 β and TNF α , all of them induced by Hcy [108]. The capacity of GB to penetrate the blood-brain barrier and its transport to astrocytes by GAT2 contribute to recover the osmotic pressure and to activate the GABAergic neuronal system by interaction with GABA receptors, attenuating memory impairment and psychiatric illness such as schizophrenia [109]. The metabolism of GB increases the level of S-adenosyl-methionine (SAM) that is synthesized from L-methionine in the presence of folate and vitamin B₁₂ obtained from the homocysteine cycle. SAM is related to the reduction of hyperhomocysteinemia and depressive symptoms [110]. Moreover, GB is preferentially accumulated in hippocampal nervous tissue by a betaine/GABA transporter (BGT1) and modulates the accumulation of other key neurotransmitters or precursors to neurotransmitters [111]. Finally, the accumulation of GB is associated with a reduction of triglycerides and low-density cholesterol levels and, therefore, with the decrease in risk of cerebrovascular diseases [112].

3.4. Impact of betaine on cardiovascular disease

Betaine administration reduced Hcy levels, this could affect the coagulative and inflammatory impact and normalize plasma apoA1 levels contributing to the cardiovascular protection [113,114]. In this sense, the administration of GB has an antithrombotic effect, reduces lipid peroxidation in plasma, extends prothrombin time and partial thromboplastin time in pial arterioles and venules in mice [115]. GB concentration increased during physiological cardiac hypertrophy induced by pregnancy in Sprague-Dawley rats, and this is regulated by the enzyme BADH to protect myocytes from hyperosmotic stress. Moreover, positive correlation between mRNA, protein concentration, and enzyme activity levels with the increase in GB in late pregnancy and postpartum, this is clear evidence about the importance of GB regulation for the physiological adaptation to cardiac hypertrophy [116].

3.5. Impact of betaine on liver

Betaine exerts hepatoprotective effects under various pathological conditions [8,53,117,118]. This action is also confirmed by studies on animal models of diabetes. Similar to skeletal muscle, supplementation of betaine to mice fed an HFD increased betaine content in liver [119,120]. One of the relevant effects evoked by betaine and related to the anti-diabetic action are changes in hepatic lipid accumulation. Prevention of hepatic lipid accumulation due to betaine therapy contributes to better insulin action in liver and alleviates other diabetes-related parameters. Betaine-induced decrease in hepatic lipid accumulation results from multiple effects. One of the relevant are changes in expression of peroxisome proliferator-activated receptor alpha (PPAR α). This is a nuclear transcription factor implicated in regulating the expression of genes related to lipid metabolism. Reduced tissue expression of PPAR α is associated with diminished degradation of fatty acids, while increased expression exerts the opposite effects. Betaine was shown to up-regulate the reduced hepatic expression of PPAR α

in obese, insulin-resistant db/db mice [121], in mice [122] and rats on an HFD [123,124], and also in fructose-fed rats [125]. This is associated with decreased hepatic expression of fatty acid synthase (FAS), a key enzyme involved in the synthesis of fatty acids. Reduced FAS expression in response to betaine supplementation was confirmed in mice fed a high-sucrose diet [126] and in mice maintained on an HFD [122].

This mitochondrial enzyme is a relevant step in β -oxidation, and its activation markedly enhances the intracellular degradation of fatty acids. Betaine was revealed to increase the expression of CPT1 in liver of rats on an HFD [124]. Given that CPT1 is inhibited by malonyl-CoA, effects induced by betaine on CPT1 may be due to increased expression of pACC and the resulting diminished formation of malonyl-CoA. It was also found that betaine decreases the expression of hemoxygenase-1 (HO-1, also known as heat shock protein 32) in liver of db/db mice [127]. This enzyme is up-regulated in response to various stress conditions, such as hypoxia, inflammation, exposure to some toxins, diabetes, and others [128,129]. Thus, reduced expression of HO-1 in db/db mice receiving betaine suggests that diabetes-related stress conditions have been alleviated. Type 2 diabetes is usually accompanied by an excessive generation of glucose from various non-sugar substrates (gluconeogenesis) [6].

Studies on insulin-resistant db/db mice indicate that betaine can prevent the exaggerated expression of hepatic glucose-6-phosphatase (G6P) and phosphoenolpyruvate carboxykinase (PEPCK), two key enzymes involved in gluconeogenesis. The nuclear factor FoxO1 (forkhead box protein O1) partially mediates this effect. Up-regulation of FoxO1 in diabetes is associated with metabolic dysregulation and insulin resistance [130]. It was, however, shown that betaine supplementation to mice maintained on an HFD reduces hepatic expression of FoxO1 [131]. This might effectively limit hepatic glucose output contributing to the blood-glucose-lowering effects of betaine [132]. Apart from beneficial effects on various enzymes, betaine may also reduce the expression of genes encoding the inflammasome proteins in liver of db/db mice [127]. Enhanced expression of these proteins indicates inflammatory stress. Thus, betaine supplementation mitigates diabetes-associated inflammatory stress [129].

Betaine was shown to reduce the oxidative stress in liver of rats on an HFD [133] and db/db mice [127]. These effects may limit diabetes-related changes in liver. Anti-diabetic effects of betaine also cover alleviation of another intracellular dysfunction, i.e., endoplasmic reticulum stress, which is linked to oxidative stress and is also involved in the pathogenesis of type 2 diabetes.

Betaine was shown to reduce the expression of markers of endoplasmic reticulum stress in liver of db/db mice [127]. The hepatoprotective properties of betaine are additionally confirmed by changes in blood parameters related to liver damage. One of the relevant is bilirubin, a product of hepatic heme degradation. Increased blood bilirubin (hyperbilirubinemia) indicates hepatic injury. It was demonstrated that hyperbilirubinemia in rats maintained on an HFD is markedly alleviated by betaine supplementation [134]. Moreover, betaine treatment of mice [135] and rats [134] fed an HFD caused also normalization of blood alanine transaminase (ALT) activities. Liver damage is associated with exaggerated ALT activities in hepatic tissue and the

resulting increased release of this enzyme to blood. These favorable changes contribute to betaine-induced improvement in hepatic insulin sensitivity [118,136].

3.6. Impact of betaine on improvement of intestine functions

A large body of evidence indicates that betaine supplementation is associated with numerous beneficial effects on the organism. Therefore, the interest in betaine as a health-promoting agent in the last years is increasing. Moreover, much attention is paid to its therapeutic potential under some pathological conditions [8,9,53,55,92,107,137,138]. Betaine was shown to prevent liver steatosis (increased hepatic lipid accumulation). Its efficacy has been confirmed in the case of both non-alcoholic fatty liver disease and alcohol-related fat accumulation. Betaine-evoked prevention of hepatic lipid accumulation results from multiple effects involving decreased lipid synthesis, increased lipid oxidation, and alleviation of oxidative and inflammatory stress. Betaine may also effectively protect liver from some toxins. Besides, betaine was found to induce beneficial effects in the gut and intestine. Its supplementation enriches intestinal microbiota, improves mucosal barrier functions, positively affects the tight junction proteins, and strengthens intestinal epithelium.

3.7. Impact of betaine on inflammatory factors

Another vital issue involved in the anti-diabetic action of betaine is linked to its ability to alleviate inflammatory stress. This stress is well established to be associated mainly with obesity, insulin resistance, and type 2 diabetes. Inflammatory markers are generated in various tissues, including skeletal muscle, adipose tissue, and liver, act at the tissue level, and may also be released to the blood. The inflammatory stress impairs insulin action in the insulin-sensitive tissues, which is associated with metabolic implications. However, diabetes-related inflammation also covers many other tissues, including pancreas and brain, contributing to their progressive dysfunction. Inflammatory stress is also strongly associated with diabetic complications. Given the relevance of inflammatory stress in the onset and progression of type 2 diabetes in humans, anti-inflammatory drugs are proposed to be developed and used to support the current therapies [129,139,140]. Betaine exerts anti-inflammatory effects in various diseases [92]. In line with these data, betaine supplementation to rats with streptozotocin-induced diabetes was shown to reduce blood markers of inflammation, i.e., tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), and interleukin-6 (IL-6) [141]. Similar effects were revealed in rats with insulin resistance induced by high-fructose intake, in which betaine diminished blood levels of inflammatory cytokines (TNF- α , IL-1 β , IL-6) and interleukin-18 (IL-18) [142]. Moreover, in rats on an HFD, betaine decreased blood TNF- α levels [133]. These results indicate that betaine exerts anti-inflammatory effects and thereby alleviates diabetic states.

3.8. Impact of betaine on adipose tissues

Adipose tissue has many relevant physiological functions in the organism. Adipocytes store energy in the form of TG. Formation of TG is increased after a meal, whereas in the post-absorptive state, TG undergo decomposition into glycerol and NEFA. The lipolytic products are released from adipocytes to the blood and are used

by other kinds of cells [143]. However, excessive adipose tissue accumulation is associated with impaired glucose homeostasis, insulin resistance, and type 2 diabetes. Moreover, adiposity leads to the enhanced generation and release of proinflammatory cytokines, contributing to metabolic disorders and insulin resistance [140,144]. Adipose tissue is also an endocrine organ since it secretes multiple adipokines having significant regulatory functions. However, dysfunction in secretion or action of these adipokines is associated with numerous grave implications, including type 2 diabetes [145,146]. It was shown that betaine supplementation to mice [122,131,147,148] and rats [133] fed an HFD reduces adipose tissue accumulation and adipocyte size [149]. Multiple effects have been implicated in the mechanism underlying this action. One of them is the betaine-induced down-regulated expression of genes regulating lipid synthesis in adipose cells [148,149]. Moreover, betaine was shown to prevent hyperexpression of fat mass and obesity-associated gene (FTO) in HFD-treated mice [149]. This has beneficial implications since the results of rodent studies indicate that FTO expression is positively correlated with adiposity [150].

Another relevant effect related to the anti-diabetic action of betaine is its positive influence on endoplasmic reticulum stress. Betaine was shown to attenuate the endoplasmic reticulum stress in adipose tissue of HFD-treated mice. This is manifested by markedly diminished expression of proteins related to the endoplasmic reticulum stress, such as C/EBP homologous protein (CHOP), c-Jun NH₂-terminal protein kinase (JNK), and glucose-regulated protein 78 (GRP78) in response to betaine therapy [147]. This is an important finding since the endoplasmic reticulum stress plays a relevant role in adipose tissue dysfunction and the resulting insulin resistance. In contrast, its alleviation improves diabetes-related parameters [151]. Betaine also exerts anti-inflammatory effects in adipose tissue. Its supplementation to mice fed an HFD was shown to reduce the content of adipose tissue inflammatory markers [120,148]. Given the relevance of adipose tissue inflammation in the development of glucose intolerance and insulin resistance [139], these effects contribute to increased insulin sensitivity and better glucose tolerance.

Betaine may also alleviate adipose tissue insulin resistance, positively affecting proteins involved in insulin signaling. This effect was demonstrated in adipose tissue of mice maintained on an HFD, in which insulin resistance is associated with down-regulated expression of protein kinase B (pPKB, pAkt), extracellular-signal-regulated kinase 42 (pERK 42), and extracellular-signal-regulated kinase 44 (pERK 44). These kinases are phosphorylated/activated in response to insulin binding to its receptor and are involved in signal transduction. Betaine supplementation to insulin-resistant mice increased phosphorylation of all mentioned kinases, which may be assumed to improve insulin sensitivity [135].

Betaine is also capable of improving parameters related to cellular metabolism. Its supplementation to mice on an HFD was shown to increase mitochondria content in adipose tissue [148,149] and improve tissue oxidative capacity [152]. Increased mitochondrial biogenesis and oxidative capacity are associated with better insulin action in adipose tissue. Importantly, it was also revealed that betaine induces metabolic alterations in adipocytes that may be directly associated with reduced fat accumulation. Adipocyte lipid content results from the rate of TG formation (lipogenesis), lipid release (lipolysis), and to a less extent, intracellular lipid oxidation

[143]. It has been shown that adipocyte response to physiological and pharmacological lipolytic stimuli is markedly impaired as a result of feeding mice [149] and rats [134] an HFD.

Moreover, HFD also reduces the capability of fat cells to lipid oxidation [149]. HFD-induced lipolysis and lipid oxidation disturbances are associated with increased fat accumulation in adipose cells. However, these disturbances are effectively limited following betaine therapy. Betaine supplementation to mice and rats maintained on an HFD was shown to increase lipolysis and lipid oxidation in adipocytes derived from these animals [134,149]. This contributes to reduced lipid accumulation in fat cells, reduced adiposity, and improved insulin action. Therefore, changes induced by betaine in adipose tissue might significantly alleviate some diabetes-related disturbances.

3.9. Impact of betaine on skeletal muscles

Dysfunction of skeletal muscle metabolism plays a relevant role in developing insulin resistance and impaired glucose homeostasis. Under physiological conditions, skeletal muscle is responsible for a major part of insulin-induced glucose uptake from the blood. However, impaired insulin action is accompanied by reduced blood glucose clearance by muscle cells, which leads to hyperglycemia. This is observed in the case of exaggerated lipid supply and the resulting increased intramuscular lipid accumulation and the formation of toxic products (mainly diacylglycerides and ceramide) [153–155]. Moreover, skeletal muscle may also secrete inflammation-related cytokines, further deepening insulin resistance [156]. Associated with positive changes in metabolism and insulin sensitivity in muscle cells. Betaine substantially reduces intramyocellular lipid accumulation in mice on an HFD. This beneficial effect is predominantly due to the down-regulated expression of genes related to fatty acid synthesis and simultaneously increased expression of genes regulating fatty acid oxidation [148]. Betaine was also shown to decrease lipid accumulation and reduce the expression of fatty acid synthase (FAS) in skeletal muscle of HFD-treated rats [134]. Betaine limits intramuscular lipid accumulation via beneficial alterations in the expression of genes regulating fatty acid metabolism, thereby improving insulin sensitivity and blood glucose clearance. **Figure 5** summarizes the effects of betaine on skeletal muscle in diabetic mice and rats.

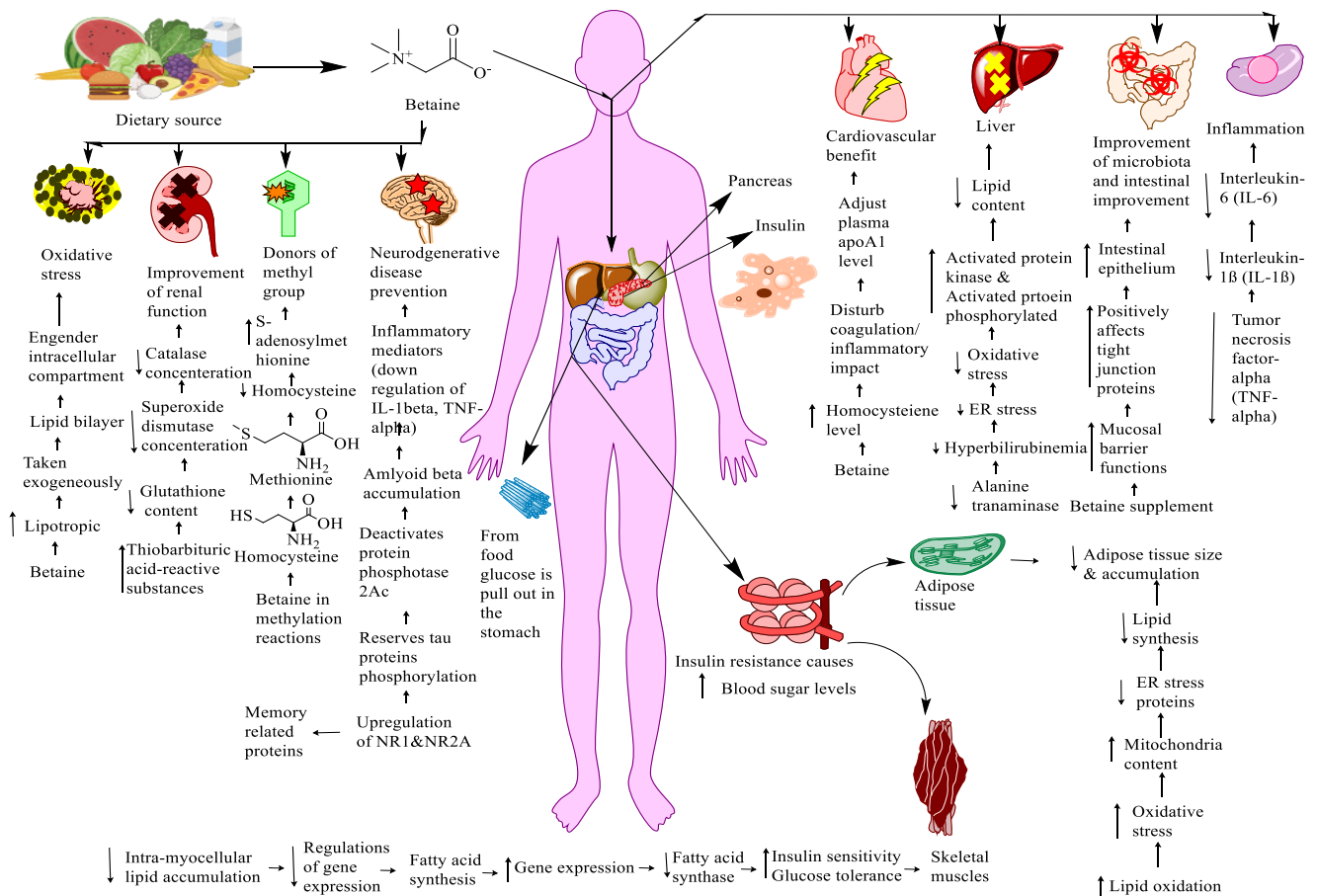


Figure 5. Impact and mechanism of betaine in various diseases.

4. Conclusion and future prospects

The function of tissue osmolytes depends on betaine, which also serves as a source of CH_3 groups and affects lipid metabolism. Some people with DM or metabolic syndrome may not have enough supply; these patients often have dyslipidemia and increased plasma homocysteine levels. Therefore, a betaine deficit may play a role in the health issues that are plaguing this growing segment of the population. A low amount of betaine supplementation may be beneficial, but long-term prospective studies are required to assess the potential advantages. Plasma homocysteine is a well-known marker of various deficits as well as a possible indicator of betaine insufficiency. It is worthwhile to conduct additional research on betaine since it has considerable therapeutic and biological properties that may be effective in the treatment of a variety of human disorders.

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Abbreviations

FFAs	Free fatty acids
Glucosamine-6-P	Glucosamine 6-phosphate
LDL	Low-density lipoprotein
LOX1	Oxidized LDL receptor 1
RAGE	AGE-specific receptor
ROS	Reactive oxygen species
TLR4	Toll-like receptor 4
UDP-GlcNAc	Uridine diphosphate N-acetyl glucosamine

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