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REVIEW ARTICLE

# Effects of thiamine and benfotiamine on intracellular glucose metabolism and relevance in the prevention of diabetic complications

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**Abstract** Thiamine (vitamin B1) is an essential cofactor in most organisms and is required at several stages of anabolic and catabolic intermediary metabolism, such as intracellular glucose metabolism, and is also a modulator of neuronal and neuro-muscular transmission. Lack of thiamine or defects in its intracellular transport can cause a number of severe disorders. Thiamine acts as a coenzyme for transketolase (TK) and for the pyruvate dehydrogenase and α-ketoglutarate dehydrogenase complexes, enzymes which play a fundamental role for intracellular glucose metabolism. In particular, TK is able to shift excess fructose-6-phosphate and glycerhaldeyde-3-phosphate from glycolysis into the pentose-phosphate shunt, thus eliminating these potentially damaging metabolites from the cytosol. Diabetes might be considered a thiamine-deficient state, if not in absolute terms at least relative to the increased requirements deriving from accelerated and amplified glucose metabolism in non-insulin dependent tissues that, like the vessel wall, are prone to complications. A thiamine/TK activity deficiency has been described in diabetic patients, the correction of which by thiamine and/or its lipophilic derivative, benfotiamine, has been demonstrated in vitro to counteract the damaging effects of hyperglycaemia on vascular cells. Little is known, however, on the positive effects of thiamine/benfotiamine administration in diabetic patients, apart from the possible amelioration of neuropathic symptoms. Clinical trials on diabetic patients would be necessary to test this vitamin as a potential and inexpensive approach to the prevention and/or treatment of diabetic vascular complications.

**Keywords** Thiamine · Benfotiamine · Diabetes · Diabetic complications · High glucose

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## Introduction

Thiamine (vitamin B1) was the first vitamin of the B group to be identified in 1926 by Jansen et al. [1]. It is an essential cofactor in most organisms and has probably played a role in the earliest stages of the evolution of life [2]. Its active form, thiamine diphosphate (TDP) is required at several stages of anabolic and catabolic intermediary metabolism, such as the intracellular glucose metabolism (glycolysis, Krebs cycle, pentose-phosphate cycle) [2], and is also a modulator of neuronal and neuro-muscular transmission, probably through its activation of a ionic channel for chlorine [3].

Lack of thiamine or defects in its intracellular transport can cause a number of severe disorders. *Beriberi*, a neurological and cardiovascular disease, first described in 1630 by a Dutch physician who worked in Java and so-called because its symptoms cause walking like sheep (*beri-beri* in the local dialect), is due to a dietary deficiency of thiamine. This leads to damage of the peripheral nervous system, with pain in the limbs, weakness of the musculature and distorted skin sensation; the heart may be enlarged and cardiac output inadequate [4] and, if not adequately cured, the consequence may be death [1]. The disease is particularly common in Far Eastern countries, because of the low content of thiamine in rice, especially polished one because only the outer layer contains an appreciable amount of the vitamin, but

can occasionally be seen in severely malnourished alcoholics [4].

Absence or severe lack of thiamine in the diet can also cause the *Wernicke–Korsakoff syndrome*, or cerebral beriberi, a striking neuro-psychiatric disorder characterized by paralysis of eye movements, abnormal stance and gait, and markedly deranged mental function [4, 5].

Finally, an inborn defect in the intracellular transport of thiamine could be the cause of the TRMA syndrome (Thiamine-Responsive Megaloblastic Anemia), an autosomal recessive disorder characterized by megaloblastic anemia with ringed sieroblasts, diabetes and progressive sensorineural deafness [6].

Thiamine levels are often reduced in alcoholics, mainly due to their diet rich in carbohydrates, the impairment of thiamine absorption for the effects of chronic alcohol intake on the gut's absorptive mechanisms and the accumulation of acetaldehyde, which interferes with thiamine utilization. Thiamine deficiency in alcoholics usually is not resolved by thiamine supplementation [7]. A deficit in thiamine is often described also in patients with diabetic neuropathy [8, 9].

## Biochemistry and mechanisms of action

The chemical structure of thiamine consists in a five-membered thiazolium ring and a six-membered aminopyrimidine ring joined together by a methyl group (Fig. 1a). The active form (TDP) has a diphosphate-terminated side-chain (Fig. 1b).

Thiamine is present in free form and very low concentrations in the intestinal lumen; absorption takes place

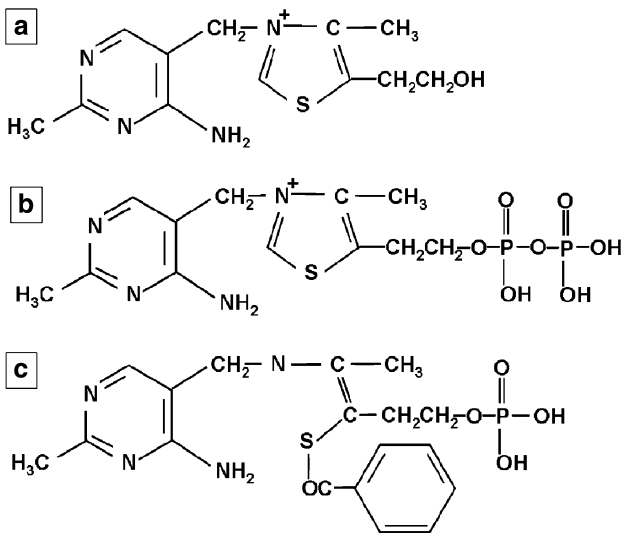


**Fig. 1** Chemical structures of thiamine (**a**), its activated form, thiamine diphosphate (*TDP*) (**b**) and the lipophilic derivative benfotiamine (**c**)

mainly in the proximal part of the small intestine, through two different mechanisms [10–12]: at concentrations lower than 1 μmol/l, thiamine is transported mainly by an active, carrier-mediated system, which involves the phosphorylation of the vitamin [13]; at higher concentrations, passive diffusion is the main mechanism. Thiamine uptake is enhanced by thiamine deficiency [14] and reduced by thyroid hormone and insulin [15], which cannot control thiamine uptake directly [16], but can modify its absorption by influencing intestinal tissue.

In the early 1960s, a group of lipid-soluble thiamine derivatives, called allithiamine because they can be found in nature in vegetables of the Allium family, was discovered [17, 18]. These derivatives have much higher absorption and bioavailability than water-soluble thiamine salts, reaching higher concentrations in blood and tissues and maintaining them longer [18, 19]. Water-soluble thiamine, as a matter of fact, is not stored in the body, but rapidly excreted in the urine and must be replenished every 5–6 h [20].

Among lipid-soluble derivatives, benfotiamine, which was developed to improve bioavailability for pharmacological administration [20, 21], seems to be the most effective [22]. It contains an open thiazole ring (Fig. 1c), which, subsequently to the passage through the mucous membranes, is closed through a reduction reaction, resolving with the production of biologically active thiamine [19], which is then converted in the active form, TDP.

TDP acts as a coenzyme for transketolase (TK) and for the pyruvate dehydrogenase and α-ketoglutarate dehydrogenase complexes, enzymes which play a fundamental role for the intracellular glucose metabolism (Fig. 2). TK, which contains a tightly bound TDP as its prosthetic group, is able to shift excess fructose-6-phosphate and glyceraldeyde-3-phosphate (G3P) from glycolysis into the pentose-phosphate shunt, thus eliminating from the cytosol the excess of these damaging metabolites (Fig. 3). G3P is one of the most effective agents of advanced-glycation end-products (AGE) formation into the cytoplasm [23] and also an end-product of the non-oxidative branch of the pentose phosphate pathway and can be produced by TK. Since the in vivo concentration of transketolase metabolites is ten-fold lower than the $K_M$ of the enzyme, the net flux and the direction of the reaction are dependent on substrate and product concentration and on $NADP^+$/NADPH ratio [24]. TK expression has been shown to be activated by high-dose thiamine and benfotiamine in the renal glomeruli of diabetic rats [25], as well as human red blood cells [26], bovine aortic endothelial cells and retinas of diabetic rats [27]. TK activity in erythrocytes is often used as a marker of a deficit in thiamine [28].

The pyruvate dehydrogenase complex is responsible for the oxidative decarboxylation of pyruvate, the final product

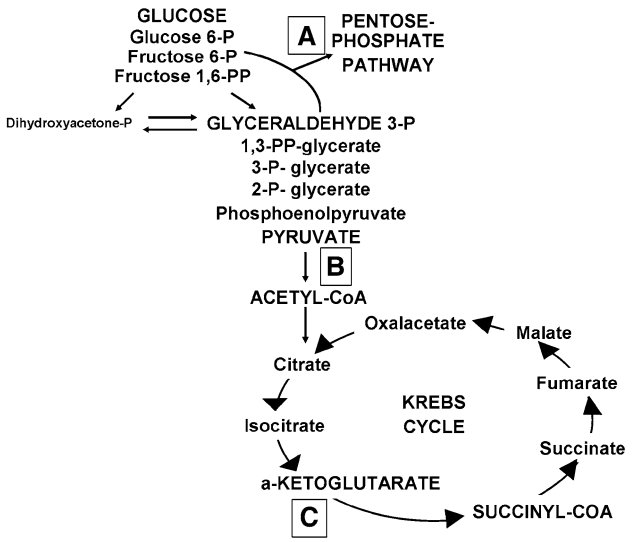


**Fig. 2** TDP acts as a coenzyme for transketolase (*A*) in the glycolytic pathway, and for the pyruvate dehydrogenase (*B*) and α-ketoglutarate dehydrogenase complexes (*C*) in the Krebs cycle, enzymes which play a fundamental role for the intracellular glucose metabolism

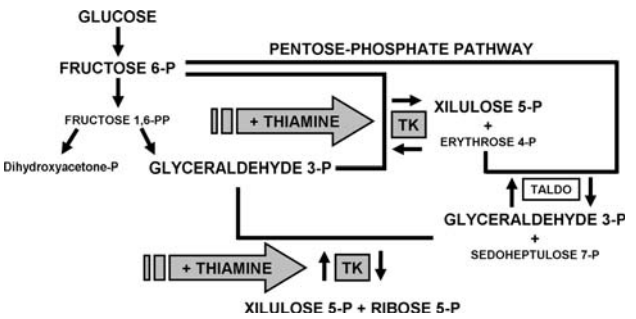


**Fig. 3** Transketolase (*TK*), which contains a tightly bound TDP as its prosthetic group, is able to shift excess fructose-6-phosphate and glycerhaldeyde-3-phosphate from glycolysis into the pentose-phosphate shunt, thus eliminating from the cytosol the excess of these damaging metabolites. *TALDO* transaldolase

of glycolysis, in the formation of acetyl-CoA, which then enters the Krebs' cycle; a deficit in thiamine is known to decrease oxidation of pyruvate, with the subsequent accumulation of pyruvate and lactate [29]; similarly, the α-ketoglutarate dehydrogenase complex is responsible for the oxidative decarboxylation of α-ketoglutarate into succinyl-CoA inside the Krebs' cycle: thiamine deficiency thwarts the correct functioning of this enzyme, thus not decreasing the metabolic flux inside the cycle [30].

## Mechanisms of glucose-induced toxicity in diabetic complications

Diabetic microangiopathy is a major cause of blindness, renal failure and nerve damage, while, in large vessels, diabetes accelerates atherosclerosis, leading to increased risk of myocardial infarction, stroke and limb amputation. The severity of microvascular complications in both type 1 and 2 diabetes has been clearly associated with the duration and degree of hyperglycaemia. Diabetic patients with a lifetime of poor glycaemic control have a high frequency of tissue complications and definitive proof of the "glucose hypothesis" was provided by two large prospective clinical trials [31, 32], which demonstrated the strong relationship linking hyperglycaemia and diabetic complications in type 1 and, respectively, type 2 diabetes.

The majority of cell types is able to down-regulate glucose transport in the presence of high ambient glucose, in order to keep intracellular glucose concentration constant. On the contrary, vascular endothelial and mesangial cells, which are among the first targets of glucose toxicity, are characteristically unable to reduce glucose transport in hyperglycaemic conditions [33, 34], leading to glucose accumulation inside the cell and overproduction of reactive oxygen species (ROS) by the mitochondrial electron transport chain: therefore high intracellular glucose levels are among the major factors responsible for diabetic tissue damage [35]. Mechanisms involved in glucose-mediated damage can be divided into two groups: one causing acute metabolic changes which are reversible when normal glycaemia is restored, the other determining cumulative impairments, that remain, or sometimes even worsen, when euglycaemia is restored [36].

Several reports in the literature show a high sensitivity of vascular cells to direct exposure to high glucose concentrations [37–39]. Early apoptosis has been demonstrated in both human umbilical vein endothelial cells and bovine retinal pericytes, following 2-day incubation in high glucose [39]. Glucose-induced apoptosis has also been demonstrated in podocytes, mesangial cells, dorsal root ganglion cells, cardiac myocytes and renal tubular epithelial cells [40–44]. Increased expression of the pro-apoptotic Bax proteins in vivo and in vitro [38] and activation of NF-κB in vitro [45] have been associated with vascular cell apoptosis.

Rapid glucose depletion was shown to enhance apoptosis in retinal pericytes by modulating the expression of the *Bcl-2* family genes [46, 47], while a ROS—mediated cellular "memory" of vascular stress after glucose normalization has been demonstrated in HUVECs and ARPE-19 retinal cells [48]. Increased expression of type IV-collagen and fibronectin long after normalization of the glucose levels have been demonstrated in endothelial cells previously grown in high glucose concentrations [49, 50]. Intermittent exposure to high glucose has been described to increase apoptosis related to oxidative stress in human umbilical vein endothelial cells, possibly through overproduction of mitochondrial superoxide [48, 51–53].

Reinstitution of good metabolic control after 6-month hyperglycaemia was shown to elevate oxidative stress and nitric oxide production in the kidney of diabetic rats [54]. These findings are consistent with the clinical observation that daily fluctuations in plasma glucose concentrations, which occur in diabetic patients, are correlated to increases in cardiovascular disease [55] and microvascular complications [56]. On the other hand, diabetic retinopathy was demonstrated to accelerate after restoration of normal blood glucose levels, in particular if improvement in glycaemic control is achieved rapidly [57–60].

Among the possible mechanisms of glucose-induced vascular damage, four hypotheses have been widely entertained and clinical trials established to study specific inhibitors: (1) increased flux through the polyol pathway; (2) increased formation of AGE; (3) PKC activation; (4) increased flux through the hexosamine pathway (Fig. 4a).

1. The key-enzyme of polyol pathway is aldose reductase (AR), which normally reduces toxic aldehydes to inactive alcohols, but, in the case of excess intracellular glucose, reduces it to sorbitol, while consuming the cofactor NADPH, with consequent hyperglycaemic pseudohypoxia [61] and increased susceptibility to intracellular oxidative stress [62, 63]. It has been demonstrated, among other effects, that treating diabetic dogs with an AR inhibitor prevents diabetes-induced damage to nerve conduction velocity [64].
2. High glucose inside the cell initially reacts with proteins, amino acids and nucleic acids via Schiff base condensation with amino groups, followed by irreversible rearrangement into an Amadori product. Further Maillard reactions slowly produce advanced glycation end products, or AGEs, which can also derive from the earlier glycation products (Schiff base and Amadori intermediates) through glycoxidation reactions or from reactive dicarbonyl fragments generated from free glucose [65]. AGEs, in turn, can modify intracellular proteins, including some involved in the regulation of gene transcription [66], while diffusing out of the cell can modify extracellular matrix [67], leading to reduced cell-to-cell adhesion and vascular dysfunction [68, 69], and circulating blood proteins, leading to activation of AGE receptors and production of inflammatory cytokines and growth factors [70–72]. Inhibition of AGE by aminoguanidine prevents structural changes in experimental diabetic retinopathy [73].
3. Intracellular high glucose increases the de novo synthesis of the lipid second messenger diacylglycerol (DAG), which in turn activates PKC synthesis [74], causing a number of negative effects inside the cell, such as decreased synthesis of endothelial nitric oxide synthase (eNOS) or increased synthesis of endothelin-1, transforming growth factor $\beta$, plasminogen activator inhibitor-1 [75] and NF-$\kappa$B [36, 76]. Inhibition of PKC prevents vascular dysfunction in diabetic retina and kidney [77, 78].
4. Excess fructose-6-phosphate derived from high availability of intracellular glucose can be transformed to glucosamine-6-phosphate and then to UDP *N*-acetylglucosamine, which acts on serine and threonine residues of transcription factors, resulting in pathological changes in gene expression [79, 80].

Brownlee et al. [76] have hypothesized that the possible common denominator ("unifying mechanism") of these apparently independent biochemical pathways is high-glucose-induced excess production of ROS by the mitochondrial electron transport chain inside the endothelium, as a result of increased flux through the Krebs' cycle. Inhibition of high glucose-induced superoxide production by superoxide dismutase (SOD) prevents diabetic nephropathy in the *db*/*db* diabetic mouse [81]. ROS, causing strand breaks in nuclear DNA, activate the poly-(ADP-ribose)-polymerase (PARP), which in turn inhibit glyceraldehyde phosphate dehydrogenase (GAPDH) activity [82], therefore pushing metabolites from glycolysis in the upstream pathways mentioned above (Fig. 4a).

## Metabolic effects of thiamine and benfotiamine in preventing diabetic complications: in vitro and in vivo studies

High glucose concentrations cause a number of pathological changes in small and large vessels, the mechanisms of which are not yet fully understood, as stated above. Potentially, thiamine can prevent cell damage induced by hyperglycaemia, both by removing excess G3P from the cytoplasm, as described above ("Biochemistry and mechanisms of action") and, through activation of α-ketoglutarate dehydrogenase, by facilitating the utilization, in the Krebs' cycle, of acetyl-CoA derived from accelerated glycolysis [83]. Addition of thiamine was shown in 1996 to normalize cell replication, lactate production and AGE formation in endothelial cells from human umbilical veins and bovine retinas, cultured in high glucose concentrations, by re-directing the glycolytic flux towards alternative pathways, whereas it had no stimulatory or inhibitory effects if added to physiological glucose concentrations [83].

In the same year, a high in vitro inhibition of antigenic late (post-Amadori) AGE formation by TDP on bovine serum albumin, ribonuclease A and human hemoglobin was demonstrated, in contrast with the "classic" AGE

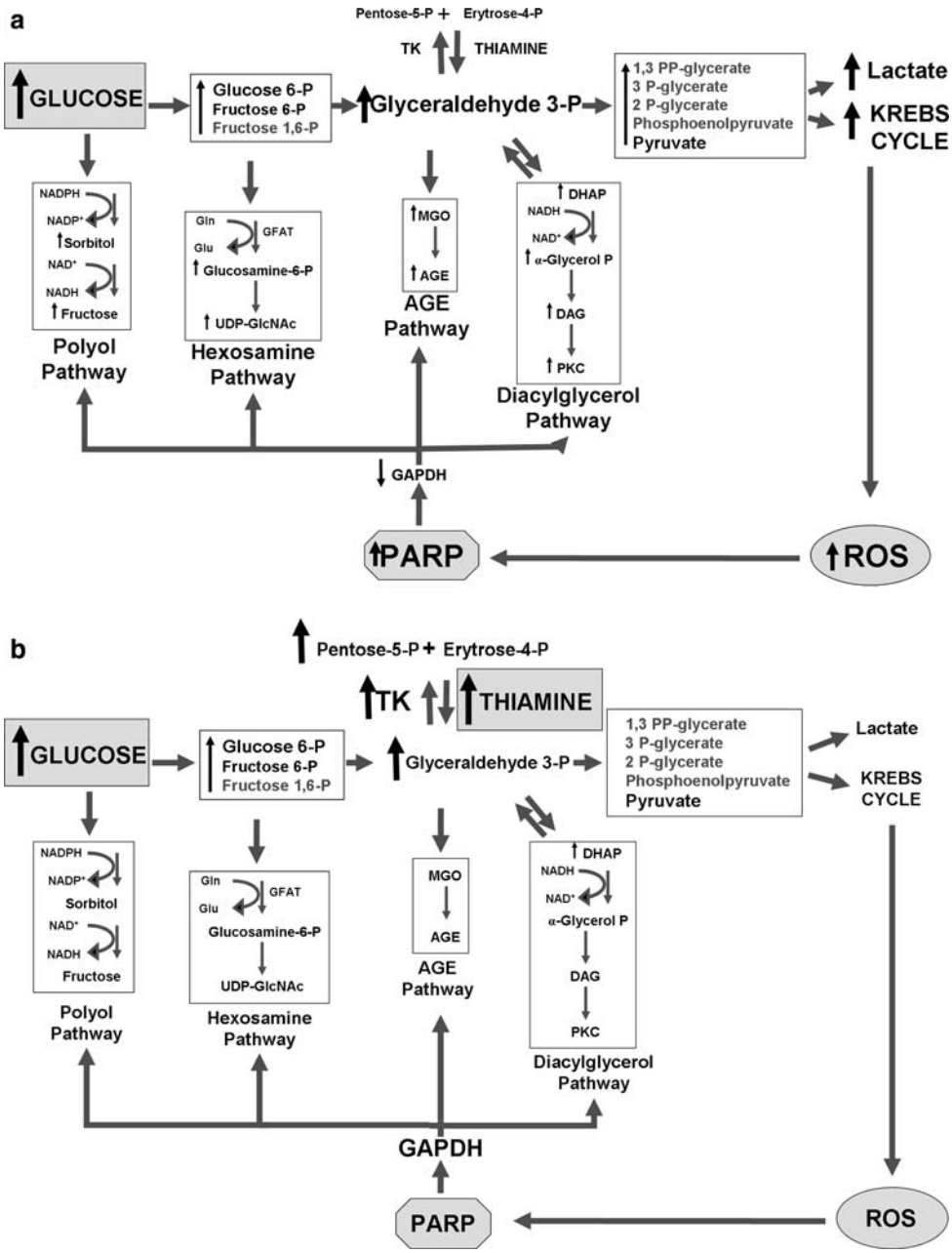


**Fig. 4** **a** Among the possible mechanisms of glucose-induced vascular damage, four hypothesis has been widely examined: (1) increased flux through the polyol pathway; (2) increased formation of AGE; (3) PKC activation; (4) increased flux through the hexosamine pathway. The common denominator of these apparently independent biochemical pathways ("unifying mechanism") seems to be high-glucose-induced excess production of ROS by the mitochondrial electron transport chain inside the endothelium, as a result of increased flux through the Krebs' cycle [36, 76]. ROS, causing strand breaks in nuclear DNA, activate the poly-(ADP-ribose)-polymerase (*PARP*), which in turn inhibit glyceraldehyde phosphate dehydrogenase (*GAPDH*) activity [82], therefore pushing metabolites from glycolysis in the upstream pathways mentioned above. **b** Thiamine/benfotiamine have been shown to normalize all the four branches in hyperglycaemic conditions in vascular cells and diabetic rats [27, 90]

inhibitor aminoguanidine, which seems to inhibit the initial phase of glycation and suggesting that the therapeutical potential of these inhibitors may be significantly enhanced by co-administration of thiamine and aminoguanidine [84].

Incubation of human red blood cells with normal and high glucose in the presence of different concentrations of thiamine, by increasing TK activity, led to a decrease in the intracellular concentrations of triosephosphates, such as G3P, and a subsequent increase of ribose-5-phosphate and sedoheptuloxe-7-phosphate, together with decreased formation of methylglyoxal, one of the most potent agent of non-enzymatic glycation [85].

Benfotiamine was subsequently reported to be similar to thiamine in correcting delayed replication and increased AGE formation in human endothelial cells [86]: an additional explanation to this, apart from the shifting of toxic intermediates of glycolysis towards alternative pathways, was that thiamine/benfotiamine exert a protective effect from high glucose damage by increasing the availability of reduced glutathione [87], which in turn depends on the recycling of oxidized glutathione through an NADPH-requiring reaction. Since the pentose phosphate shunt is an important source of NADPH and it is potentiated by thiamine [88], the latter has been suggested to act indirectly as an anti-oxidant [86, 87].

Thiamine and benfotiamine were shown to be effective in preventing two markers of apoptosis (increase of DNA fragmentation and caspase-3 activity) due to exposure to high ambient glucose in endothelial cells and pericytes, possibly by protecting against the intracytoplasmic accumulation of damaging metabolites and, consequently, AGEs, though no effects on cell cycle traversal for both high glucose and thiamine/benfotiamine were observed [39].

High doses of thiamine and benfotiamine were also shown to prevent incipient diabetic nephropathy in the streptozotocin (STZ)-induced diabetic rat model of diabetes with moderate insulin therapy, that were discovered in this study to be thiamine deficient, due to increased urinary excretion of the vitamin. Thiamine and benfotiamine were demonstrated to inhibit the accumulation of triosephosphates through their conversion to ribose-5-phosphate, increase TK expression in renal glomeruli, decrease PKC activation, oxidative stress and protein glycation, and finally inhibit the development of microalbuminuria; this was achieved without changes of either plasma glucose concentration or glycated hemoglobin [25].

Studies on the prevention of the early hallmarks of diabetic retinopathy (pericyte loss and thickening of the basement membrane) showed that thiamine is able, similarly to aminoguanidine, to normalize bovine retinal pericyte adhesion to extracellular matrix produced by human endothelial cells in high glucose concentrations, possibly through reduction of matrix protein glycation due to highly glycating glucose intermediate metabolites [68]; as a matter of fact, as structural components of the extracellular matrix, such as collagen, are the prime targets of advanced glycation processes, cross-link formation induced by AGE could cause stiffness of the basement membrane and impair tissue remodeling [89]; in a subsequent study [69], an indirect confirmation of these findings was given by the finding that pericyte viability (i.e. proliferation, cell cycle traversal and apoptosis) was not affected by culturing them on high-glucose conditioned extracellular matrices.

Hammes et al. [27] showed how benfotiamine can normalize the excess production of ROS inside the altered endothelium, both in bovine aortic endothelial cells and retinas from rats with nine months of diabetes, by inhibiting the activation of the hexosamine and the diacylglycerol-PKC pathways and AGE formation, thus leading to normalization of 3 out of the four branches of the "unifying mechanism" proposed to be at the basis of the pathogenesis of diabetic vascular complications. In another study [90], the fourth branch, the polyol pathway, was shown to be normalized by the addition of either thiamine or benfotiamine to high glucose in vascular cells, through the decrease of aldose reductase activity, sorbitol and intracellular glucose levels (Fig. 4b). Inhibition of hyperglycaemia-associated NF-$\kappa$B activation in diabetic rat retinas by benfotiamine treatment through TK activation was also demonstrated [27]. In the same work, morphological studies on rat retinas showed that benfotiamine is able to prevent the formation of acellular capillaries, thus preventing experimental diabetic retinopathy.

Thiamine was also shown to improve endothelial cell migration after endothelial injury, as well as revert decreased cell migration and increased von Willebrand factor secretion, a marker of endothelial cell damage, under hyperglycaemic conditions, thus suggesting that thiamine treatment may decrease endothelial cell dysfunction due to high glucose and improve re-endothelialization after intimal injury in diabetic and non-diabetic conditions [91].

More recently, benfotiamine was demonstrated to prevent ischemia-induced toe necrosis, improve hind limb perfusion and oxygenation and restore endothelium-dependent vasodilatation in STZ-induced diabetic mice, probably through protein kinase B (PKB)/Akt mediated potentiation of angiogenesis and inhibition of apoptosis: PKB/Akt are known to play a central role in the control of angiogenesis and endothelial cell homeostasis [92]. Moreover, benfotiamine was able to prevent the accumulation of AGEs and the induction of the pro-apoptotic caspase-3 in ischemic muscles, while normalizing Nos3 and Akt expression [92]. In the same study, benfotiamine supplementation was shown to stimulate proliferation and inhibit apoptosis of endothelial progenitor cells (EPCs) cultured in hyperglycaemic conditions, and to increase the number of circulating EPCs in diabetic mice submitted to limb ischemia.

The positive effect of benfotiamine on EPCs was confirmed by Marchetti et al. [93], who showed that hyperglycaemia impairs EPC number, uptake and binding of acLDL and lectin-1, together with EPC ability to differentiate into mature endothelial cells and be involved in de novo tube formation, when co-cultured with mature endothelial cells on matrigel. Benfotiamine administration, through the modulation of Akt/FoxO1 activity, improves the expression of endothelial cell markers in EPCs, restores eNOS levels and the ability of EPCs to take part to angiogenesis.

Stracke et al. [94] described the beneficial effects of benfotiamine, though not thiamine, on peripheral nerve function (motor nerve conduction velocity) and the formation of glycation products in nervous tissue of STZ-induced diabetic rats. Total thiamine levels in blood and nerve tissue were found to be much higher after administration of benfotiamine compared with thiamine nitrate, so that the amelioration of motor nerve conduction velocity and decrease of glycation products may be attributed to the higher tissue availability of benfotiamine. Moreover, early

administration of benfotiamine (immediately after diabetes induction) was proven to be more effective than later administration (two months after diabetes induction).

Benfotiamine was demonstrated to alleviate oxidative stress in STZ-induced diabetic mice both in cerebral cortex tissue, through a mechanism independent of AGE, tissue factor and TNF-α [9], and cardiomyocytes, in which it also improved contractile function [95]. Failure of benfotiamine in rescue AGE formation was, in these studies, probably due to the short-time exposure (14 days) to the vitamin.

High-dose thiamine therapy (70 mg/kg) normalized food intake and prevented diabetic-induced increases in plasma cholesterol and triglycerides in STZ-induced diabetic rats, thus counteracting dyslipidaemia, but did not reverse the decrease of HDL [96]. This was due to prevention of thiamine depletion and decrease of TK activity in rat liver, with a concomitant decrease in UDP-*N*-acetylglucosamine and fatty acid synthase activity. However, lower doses of thiamine (7 mg/kg) and benfotiamine at both concentrations were ineffective [96], probably because exogenous thiamine is able to increase hepatic thiamine levels more effectively than benfotiamine in the 90-min postprandial period [97]. In the same study [96], diuresis and glicosuria, but not plasma glucose concentration, of STZ diabetic rats were decreased by either thiamine and benfotiamine.

Finally, a protective effect of high-dose thiamine on detrusor contractility and the progression of diabetic cystopathy in STZ-diabetic rats was suggested [98].

## Metabolic effects of thiamine and benfotiamine in preventing diabetic complications: clinical studies

Beneficial effects of administration of thiamine and derivatives have been demonstrated in thiamine-deficient patients with chronic renal insufficiency, in whom benfotiamine administration led to higher TDP concentrations in erythrocytes accompanied with a significant improvement of the erythrocyte transketolase activity [99] and alcoholic polyneuropathy, in which vibration perception (measured at the tip of the great toe), as well as motor function, improved after benfotiamine administration; a tendency toward improvement was also evident for pain and co-ordination [100].

Little is known, however, on the possible positive effects of thiamine/benfotiamine administration in diabetic patients. A significant improvement in neuropathy symptoms (nerve conduction velocity and vibration perception threshold) and decrease in pain, together with the patients' sensation of improved clinical conditions, were signaled in diabetic subjects with polyneuropathy treated with either thiamine [8] or benfotiamine [101, 102].

It was observed that diabetic subjects tend to have lower blood thiamine concentrations than healthy controls, together with a reduced erythrocyte transketolase activity [103–105] and an increased thiamine renal clearance [106], while intestinal absorption and membrane transport of thiamine may be decreased [15]. Although the intestinal thiamine transporter is saturated by relatively low doses of thiamine, there is slow passive diffusion of thiamine at high concentrations [12].

However, since diabetic subjects usually do not manifest the typical clinical markers of thiamine deficiency, this is probably due to a specific pattern of vascular cells. Endothelial cells and pericytes are unable to regulate glucose transport, reaching high levels of intracellular glucose concentrations [33], in the presence of hyperglycaemia. Such high levels of glucose determine high production of ROS, which could oxidize thiamine, with the production of inactive compounds, such as thiochrome and oxydihydrothiochrome [107].

Benfotiamine was shown to completely prevent macro- and microvascular endothelial dysfunction and oxidative stress, as assessed by measurement of flow-mediated dilatation and hyperemia, following an AGE-rich meal in type 2 diabetic patients, possibly through reduction of endogenous AGEs and dycarbonyl (metyl-glyoxal) production, and suggesting a role for benfotiamine in atherosclerosis prevention in diabetic patients [108]. A similar effect was demonstrated for thiamine, the intravenous administration of 100 mg of which improved endothelium-dependent vasodilatation in the presence of hyperglycaemia [109]. Both studies showed that thiamine/benfotiamine effects were not due to a glucose-lowering mechanism as either compound had no effects in normoglycemic conditions.

## Conclusions

Diabetes might be considered a thiamine-deficient state, if not in absolute terms at least relative to the increased requirements deriving from accelerated and amplified glucose metabolism in non-insulin dependent tissues that, like the vessel wall, are prone to complications.

As shown in this review, thiamine and its derivatives have been widely demonstrated to have the potential to correct most of the known metabolic abnormalities induced by high glucose in isolated cells and also to prevent complications in animals with experimental diabetes.

Surprisingly enough, however, the huge amount of literature on the beneficial effects of thiamine and benfotiamine in cell and animal models has been followed so far by very few clinical trials on diabetic patients, which

would be necessary to test this vitamin as a potential and inexpensive approach to the prevention and/or treatment of diabetic vascular complications.

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