THE BENEFITS OF THIAMINE (BENFOTIAMINE) USE IN DIABETES MELLITUS

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Abstract

Overweight, insulin resistance (IR), impaired glucose tolerance (IGT), diabetes mellitus (DM) and cardiovascular disease (CVD) share the oxidative stress. Oxidative stress can contribute to the development and progression of diabetic complications; therefore the antioxidant treatment can be an effective strategy in preventing diabetic complications.

Thiamine deficiency is more and more considered to be one of the risk factors for developing DM, both in patients with type 1 and type 2 DM. At the same time, even a modest thiamine deficiency is involved in the apparition and progression of irreversible chronic complications of DM. High thiamine (benfothiamine) doses can prevent or delay the apparition of both DM and its chronic micro- and macrovascular complications. Benfotiamine prevents retinopathy, nephropathy and experimental diabetic neuropathy, being implicated mainly in reducing oxidative stress. Clinical trials on diabetic patients are necessary to test the potential of these vitamins in preventing and/or treating diabetic vascular complications.

***Key words**: thiamine, benfotiamine, oxidative stress, diabetes mellitus.*

Thiamine deficiency is more and more considered to be one of the risk factors for developing DM (Diabetes Mellitus); low thiamine levels have been found both in patients with type 1 and type 2 DM. At the same time, even a modest thiamine deficiency is involved in the apparition and progression of irreversible chronic complications of DM, and high thiamine doses can prevent or delay the apparition of both DM and its chronic micro- and macrovascular complications. Oxidative stress caused by hyperglycemia can also contribute to the development and progression of diabetic complications; therefore the antioxidant treatment can be an effective strategy in preventing diabetic complications. Benfotiamine prevents retinopathy, nephropathy and experimental diabetic neuropathy through complex mechanisms, being implicated mainly in reducing oxidative stress.

Oxidative Stress mechanisms in DM

Oxidative Stress represents the excessive forming of highly reactive molecules such as ROS (reactive oxygen species) and RNS (reactive nitrogen species) (1-3). ROS include

the free radicals: $^{\bullet}O_2^-$(superoxide), $^{\bullet}OH$ (hydroxyl), $^{\bullet}RO_2$ (peroxyl), $^{\bullet}HRO_2^-$ (hydroperoxyl) and the non-radicals: H_2O_2 (hydrogen peroxide), HClO (hydrochlorous acid) (1,2,4). RNS include the free radicals: $^{\bullet}NO$ (nitric oxide), $^{\bullet}NO_2^-$ (nitrogen dioxide) and the non-radicals: $ONOO^-$ (peroxynitrite), HNO_2 (nitrous oxide), RONOO (alkyl peroxynitrates) (1,2,4). Under normal circumstances, glucose is metabolized through glycolyse. Intracellular glucose increase causes:

1. Glucose flux increase to sorbitol on polyol pathway;
2. Increase of F-6-P (fructose-6-phosphate) on hexosamine pathway;
3. Activation of DAG (diacylglycerol) − PKC (protein kinase C) pathway;
4. Formation of AGEs (advanced glycosylation end-products), through the non enzymatic reaction of glucose and dicarbonyl compounds with basic amino acids (lysine and arginine) **(5-9,10,11,12,13,14).**

1. **Sorbitol pathway:** in most cells, glucose excess can be metabolized to sorbitol and fructose by AR (aldose reductase) and SDH (sorbitol dehydrogenase), increasing NADPH oxidation (the reduced form of $NADP^+$) to $NADP^+$ (nicotinamide adenine dinucleotide phosphate) and reducing NAD^+ (nicotinamide adenine dinucleotide) to NADH, the reduced form of NAD^+ (figure 1) (5,15,16,12,13).

Sorbitol pathway causes ED (endothelial dysfunction) through three mechanisms:

– increased sorbitol accumulation intensifies osmotic stress (5);

- increase of cytosolic ratio $NADH/NAD^+$, as a result of redox imbalance similar to the one from tissular hypoxia, called *hyperglycemic pseudohypoxia* (17);

- the accumulation of triosephosphates stimulates methylglyoxal (MG) formation, increasing thus the oxidative stress (5).

2. **Hexosamine pathway:** F-6-P is converted into glucosamino-6-phosphate by GFAT (glutamine-fructose-6-phosphate amidotransferase) enzyme (figure 1) (5,18).

3. **DAG/PKC pathway:** PKC activation is caused by the synthesis *de novo* of DAG, through GP (glycerol-3-phosphate) acylation to PA (phosphatidic acid). In cells with reduced AR activity, such as endothelial cells, DAG is synthesized *de novo* from glycolyse intermediates, DHAP (dihydroxyacetone phosphate) and GA-3-P (glyceraldehyde-3-phosphate) (figure 1) (19). PKC activation causes at the cellular level:

– modifications of vascular permeability *directly* or *indirectly* – by inducing of VEGF (vascular endothelial growth factor) in VSMC (vascular smooth muscle cells) (20);

– modifications of the blood flow by reducing eNOS (endothelial nitric oxide synthase) activity and/or increasing ET-1 (endothelin-1) synthesis (21);

– thickening of basement membrane through TGF-β (transforming growth factor-β) which mediates the synthesis increment of type IV collagen and fibronectin (5);

– fibrinolysis modifications through elevated plasmatic expression of PAI-1 (plasminogen activator inhibitor-1) (5);

– oxidative stress increase through NADPH oxidase activation (5).

The link between oxidative stress and PKC activation is indicated by the fact that E vitamin (antioxidant) inhibits PKC activity (22). In diabetic animals, oral administration

of a PKC inhibitor ameliorate the increase of glomerular filtration rate and the accelerated glomerular mesangial expansion, partially correcting UAE (urinary albumin excretion) (5,23).

4. **AGE pathway – non enzymatic glycosylation:** reactive dicarbonyl compounds (MG, glyoxal and 3-deoxyglucosone), obtained through the degradation of glycosylation intermediates, can contribute to the formation of AGEs *in vivo* (figure 1) (5,24,12,13). The so called *carbonyl stress* is involved in accelerated vascular dysfunction, both in DM and in uremia. MG is probably the most important AGE formed in endothelial cells (5,25,26).

Hyperglycemia can increase ROS by activating NAPDH oxidase, by inactivating the antioxidant enzymes such as SOD (superoxide dismutase) and catalase, or eNOS (5,11).

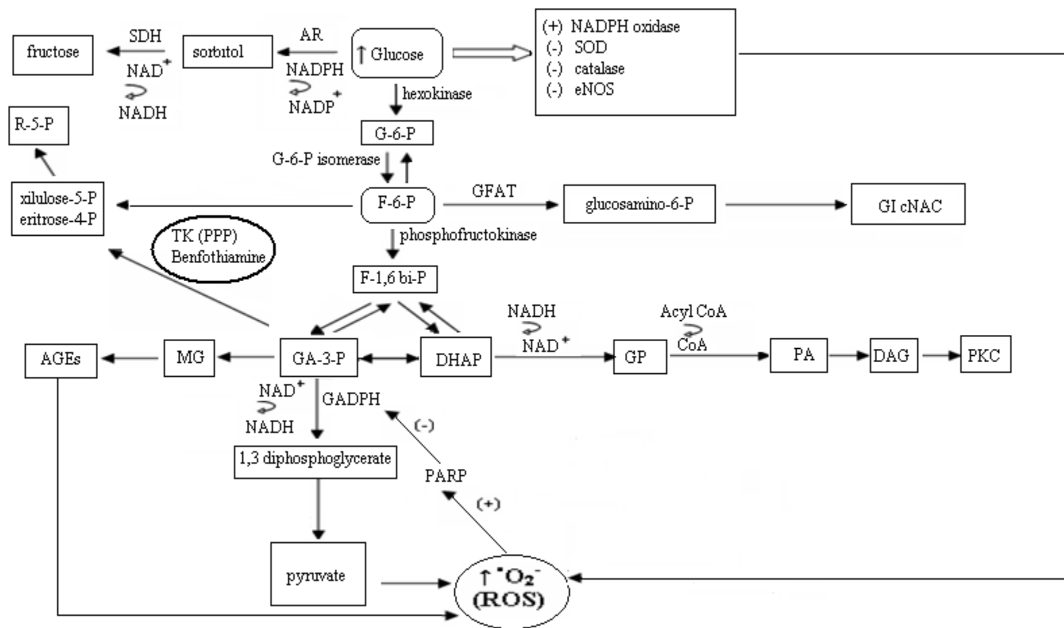

Figure 1. Oxidative stress mechanisms in DM (modified after C.G. Schalkwijk, R.B. Jadidi).

AGEs advanced glycosylation end-products; $NADP^+$ nicotinamide adenine dinucleotide phosphate; NADPH reduced form of $NADP^+$; SOD superoxide dismutase; eNOS endothelial nitric oxide synthase; AR aldose reductase; SDH sorbitol dehydrogenase; G-6-P glucose-6-phosphate; GFAT glutamine-fructose-6-phosphate amidotransferase; F-6-P fructose-6-phosphate; GlcNAC N-acetylglucosamine; F-1,6 bi-P fructose 1,6 biphosphate; NAD^+ nicotinamide adenine dinucleotide; NADH reduced form of NAD^+; DHAP dihydroxyacetone phosphate; GA-3-P glyceraldehyde-3-phosphate; GAPDH glyceraldehyde-3-phosphate dehydrogenase; TK transketolase; PPP pentose phosphate pathway; PARP Poly ADP-ribose polymerase; ROS reactive oxygen species; $^{\bullet}O_2^-$ superoxide; Acyl CoA - Acyl Coenzyme A; GP glycerol-3-phosphate; PA phosphatidic acid; DAG diacylglycerol; PKC protein kinase C; MG methylglyoxal; R-5-P ribose-5-phosphate.

Overweight, IR (insulin resistance), impaired glucose tolerance (IGT), DM and CVD (cardiovascular disease) share the oxidative stress (27,28).

Hyperglycemia causes the forming of $^{\bullet}O_2^-$ in excess at the mitochondrial level, fact which determines the activation of the other

pathways involved in endothelial dysfunction pathogenesis in DM (5).

$^{\bullet}O_2^-$, $^{\bullet}NO$ and $ONOO^-$ play an important role in cardiovascular complications in DM (1).

$^{\bullet}O_2^-$ is generated through oxygen reduction in different ways: NAD(P)H oxidase, xantine oxidase, cyclo-oxygenase, eNOS, mitochondrial transport chain in normal oxidative phosphorylation (figure 2) (1,27-30).

Under normal conditions, $^{\bullet}O_2^-$ is rapidly eliminated through antioxidant defense mechanisms: $^{\bullet}O_2^-$ is transformed into H_2O_2 by Mn-SOD (mangan superoxide dismutase) at mitochondrial level and by Cu-SOD (copper superoxide dismutase) at cytosolic level (1,27); H_2O_2 can be transformed into H_2O and O_2 by GSH-Px (glutathione peroxidase) at mitochondrial level or catalase at lysosomal level; at the same time, H_2O_2 can be converted into $^{\bullet}OH$, in the presence of Fe (iron) or Cu (figure 2) (5).

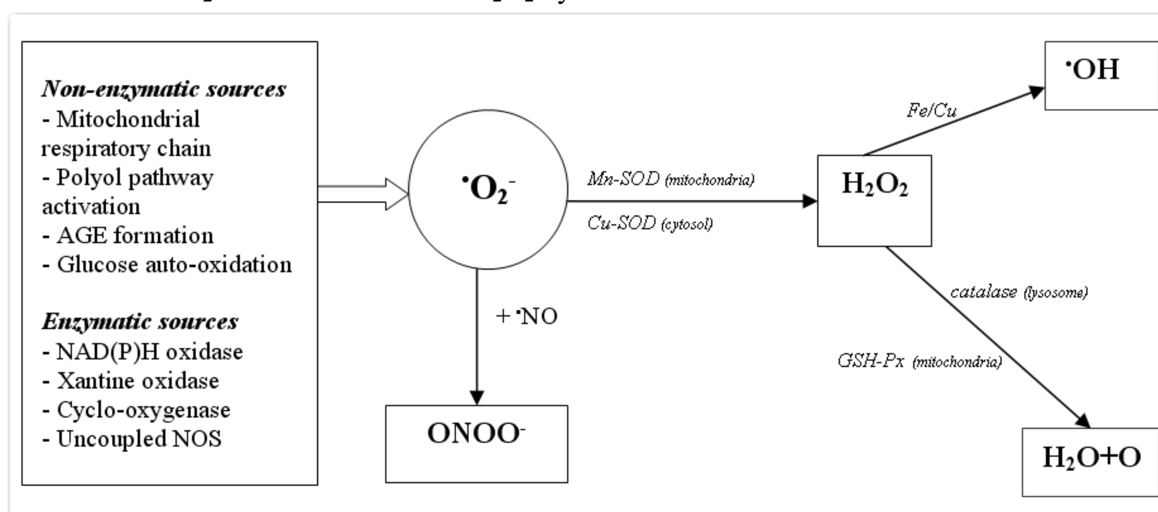

Figure 2. Reactive species in DM (modified after J. S. Johansen).

AGEs advanced glycosylation end-products; NADPH reduced form of $NADP^+$; $NADP^+$ nicotinamide adenine dinucleotide phosphate; NOS nitric oxide synthase; $^{\bullet}O_2^-$ superoxide; $^{\bullet}NO$ nitric oxide; $ONOO^-$ peroxynitrite; Mn-SOD mangan superoxide dismutase; Cu-SOD copper superoxide dismutase; H_2O_2 hydrogen peroxide; GSH-Px glutathione peroxidase; $^{\bullet}OH$ hydroxyl; H_2O water; O_2 oxygen, Fe iron; Cu copper.

$^{\bullet}O_2^-$ can activate AGE pathway, polyol pathway, hexosamine and PKC pathways, involved in the development of micro- and macrovascular complications in DM. $^{\bullet}O_2^-$ and H_2O_2 activate stress signaling mechanisms, such as NF-kB (Nuclear Factor-kB), p38-MAPK (p38 Mitogen-Activated Protein Kinase) and STAT-JAK (Signal Transducer and Activator of Transcription- Janus kinase), causing the migration and proliferation of VSMC (1).

$^{\bullet}NO$ has anti-platelet, antiproliferative, anti-inflammatory and vasodilatation effect (1,30). $^{\bullet}NO$ is produced normally from L-arginine by eNOS in the blood vessels (1,2)**, having an endothelium-dependent vasorelaxation effect** (activates guanil-atciclase in VSMC), antiproliferative effect, inhibits leucocitary and platelet adhesion (31) in vascular endothelium (1,2), regulates the expression of cytokines VCAM-1(vascular cell adhesion molecule-1) and MCP-1

(monocyte chemotactic protein-1) (31), effect due partially to the inhibition of the transcription factor NF-kB (1,32). The protective or negative effect of •NO is determined by the presence of $^{\bullet}O_2^-$; •NO reacts with $^{\bullet}O_2^-$, forming citotoxic **ONOO$^-$**, which favors lipid peroxidation (2), alters the functions of biologic membranes (1,3,28-31), contributing to ED pathogenesis. $ONOO^-$ causes DNA modification, which activates the nuclear enzyme PARP (Poly ADP-ribose polymerase) (28), reduces •NO bioavailability, inhibiting •NO antiproliferative effect (3). $ONOO^-$ oxidizes BH_4 (tetrahydrobiopterin) - an important cofactor for NOS - resulting uncoupled eNOS and producing $^{\bullet}O_2^-$ instead of •NO (3).

The sources of oxidative stress in DM are: non-enzymatic, enzymatic and mitochondrial sources (1).

Non-enzymatic sources: hyperglycemia can directly increase ROS formation. Glucose auto-oxidation can generate •OH radicals (2,12); it also, glucose reacts with proteins, forming Amadori products and then AGEs. In hyperglycemia there is an elevated glucose metabolism on polyol (sorbitol) pathway, followed by an increased $^{\bullet}O_2^-$ production (figure 2) (1).

Enzymatic sources: NOS, NAD(P)H oxidase and xantine-oxidase (figure 2) (1,34-36). In the absence of L-arginine or of one of the five cofactors: FAD (flavin adenine dinucleotide), FMN (flavin mononucleotide), hem, BH_4 and Ca^{2+}-calmoduline, NOS can produce $^{\bullet}O_2^-$ instead of •NO (3,34,35,36). Guzik et al indicated an elevated $^{\bullet}O_2^-$ production in DM, which is predominantly mediated by NAD(P)H oxidase, whose activity is significantly higher in the vascular tissues of diabetic patients (32). PKC is stimulated in DM through polyol pathway, Ang II (angiotensin II) and NAD(P)H oxidase activation (34,35,38-44).

Mitochondrial respiratory chain: $^{\bullet}O_2^-$ generation at mitochondrial level is the main factor of oxidative stress developing in DM (1,45,46,47). The excessive production of pyruvate through glycolyse generates $^{\bullet}O_2^-$ at the level of the complex II in the respiratory chain; this represents the initial ”snow ball” which turns oxidative stress into an „avalanche”, by stimulating the production of ROS and RNS on the cytokines pathway, mediated by NF-kB, PKC and NAD(P)H oxidase (1).

There are different opinions regarding ROS primary source in muscle and fatty tissues: free fatty acids (FFA) or glucose (48).

Substrate increase for citric acid cycle (Krebs) causes the formation of Acetyl- CoA (Acetyl coenzyme A) and NADH at the mitochondrial level (48,49). Acetyl-CoA, derived either from glucose through pyruvate, or from FFA beta-oxidation, combines with oxalacetate, resulting citrate, which enters the Krebs cycle and is transformed into isocitrate. Isocitrate dehydrogenase, NAD^+-dependent, generates NADH. When NADH in excess can not be reduced through oxidative phosphorylation, one electron is transferred to oxygen, forming $^{\bullet}O_2^-$ (48,50). Excessive NADH formation can be prevented through the inhibition of FFA oxidation (48,51). Elevated intracellular FFA reduce GLUT4 (glucose transporter 4) translocation through the plasmatic membrane, causing IR at muscle and adipocyte level (49,52-55), associated with ED (56) and with the decrease of antioxidant defense (57).

ED mechanisms in DM

ED represents an important factor in the pathogenesis of diabetic micro- and macroangiopathy (1).

ED appears in DM through three mechanisms:

- the **direct** mechanism (1,47). Glucose transport in vascular and endothelial smooth muscle cells is accomplished through facilitated diffusion, insulin-independent, being auto regulated in smooth muscle cells, but not in endothelial cells, where elevated plasmatic glucose concentration determines an increase of intracellular accumulation of glucose and its metabolites, generating *in vitro* an increased production of extra-cellular matrix components (fibronectin and collagen) and of pro-coagulant proteins: vWF (von Willebrand factor) and tissular factor, reducing proliferation, migration and fibrinolytic potential (1,58-63).

- the **indirect** mechanism (synthesis of growth factors, cytokines and vasoactive agents in other cells) (1,64).

- **metabolic syndrome components** (65).

ED estimation is realized indirectly through the measurement of endothelium-dependent vasodilatation, plasmatic levels of endothelial derivates: ET-1, sCD146 (cluster of differentiation 146, also known as MCAM-melanoma cell adhesion molecule), sThrombomodulin, t-PA (tissue-type plasminogen activator), PAI-1, sE-selectin, sVCAM-1, sICAM-1 (soluble Inter-cellular adhesion molecule 1), vWF, cellular fibronectin, type IV collagen and microalbuminuria (1).

The role of ED and oxidative stress in diabetic complications pathogenesis

Insulin can action on the insulin receptors of endothelial cells, producing both NO and ET-1 (1). Insulin stimulates NO production in endothelial cells by activating intracellular enzyme PI3K (phosphoinositol-3-kinase) and Akt (protein kinase B) (5,66), which phosphorylates and activates eNOS; ET-1 production is MAPK-dependent (5,67). β cells are sensitive to ROS, lacking antioxidant enzymes: catalase, GSH-Px and SOD (48,68).

Oxidative stress damages mitochondria and reduces significantly insulin secretion (48,50,69,70). The first phase of glucose-dependent insulin secretion can be suppressed by H_2O_2, chemical substitute for ROS, which reduces GAPDH (glyceraldehyde-3-phosphate dehydrogenase) activity (48,71). Excessive production of free radicals at mitochondrial level influences the first phase of glucose-induced insulin secretion (71,72), important pathogenic mechanism of postprandial hyperglycemia (73), a cause of ED (48). The reduction of insulin secretion associates with ROS increase induced by FFA, both *in vitro* and *in vivo* (48). FFA increase oxidative stress generation in humans and causes ED, effects which are diminished by antioxidants (48,74). Postprandial hyperglycemia associates with oxidative stress and ED (48,75). Hyperglycemia and FFA determine $^{\bullet}O_2^-$ production in excess at mitochondrial level and NO through NOS, while PKC and NF-kB are activated and stimulate NADPH, with excessive generation of $^{\bullet}O_2^-$. $^{\bullet}O_2^-$ and NO excess favors $ONOO^-$ formation, a important oxidant which modifies DNA; modified DNA is the obligatory stimulus for PARP enzyme activation, which reduces GAPDH activity, with GLUT 4 reduction in adipocyte and muscles, IR, diminution of β-cellular secretion and ED (48). Elevated seric AGEs correlate with the level of endothelium-dependent vasodilatation in patients with type 2 DM. AGEs stimulate ET-1 production by endothelial cells through NF-kB activation

and associate with ED (76,77). AGEs present significant proinflammatory and pro-oxidant effects, playing an important role in the development of diabetic complications (77,78).

ROS cause **ED** *directly* (through lipid membrane peroxidation, NF-kB activation and interference with NO availability) and *indirectly* (through growth factors and cytokines); there is evidence for the major role of TGF-β, IGF-1 (insulin-like growth factor-1), EGF (epidermal growth factor) in diabetic nephropathy (5,79), and of VEGF in severe diabetic retinopathy (5,80).

TNF-α (tumor necrosis factor-α) is an inflammatory cytokine produced by neutrophiles, macrophages and adipocytes, which stimulates IL-6 (interleukin), CRP (C-reactive protein), causing ED and IR; it also contributes to diabetic nephropathy pathogenesis (5).

TGF-β1 activates PKC, Amadori products and AGEs, Ang II, and the cytokines of endothelial cells; an increased glomerular expression of TGF-β1 has been observed in patients with diabetic nephropathy (5,81), TGF-β1 being an important mediator of diabetic nephropathy progression (5,82-84) through glomerular and tubular modifications (progressive thickening of glomerular basement membrane, mesangial matrix expansion, reduction of glomerular filtration and protein excretion increase) (5,81,83). TGF-β1 has anti-inflammatory effect at the level of vascular cells, reducing VCAM-1 expression and MCP-1 (5,84).

In diabetic retinopathy, micro-vessels damage at retinal level and vascular permeability increase are significantly stimulated by **VEGF** interaction with the endothelium (5,85). Elevated VEGF has been observed at vitreous level in patients with proliferative diabetic retinopathy, compared to those without this disease; VEGF antagonists reduce retinopathy in animals (5). Decreased Ang II production associates with reduced VEGF concentrations in the vitreous fluid of patients with proliferative diabetic retinopathy (5,86).

NF-kB regulates the genes of VCAM-1, E-selectin, ICAM-1, IL-1, IL-6 and IL-8, tissular factor, PAI-1 and NOS, being implicated also in vascular damage in DM. On the other hand, NF-kB activates the antioxidant enzyme SOD.

Elevated **CRP** levels have been detected both in type 1 DM and type 2 DM, being a precursor of the CVD (5,87). The proinflammatory-proatherogenic role of CRP in endothelial cells: decreases NO and prostacyclin, increases ET-1, adhesion molecules, MCP-1, IL-8 and PAI-1. In monocytes/macrophages, CRP determines tissular factor secretion, ROS increase and the release of proinflammatory cytokines, monocytary adhesion, increases oxidized LDL (low-density lipoprotein) uptake. CRP stimulates the activity of NF-kB, MAPK and the expression of type 1 receptor of Ang II, causing ROS increase and VSMC proliferation (5).

Adiponectinemia is reduced in patients with obesity, type 2 DM and coronary diseases (5). Adiponectin inhibits *in vitro* adhesion molecules expression, reduces monocytes adhesion to endothelial cells, stimulates NO production (5,88) and inhibits TNF-α in macrophages (5). In humans, hypoadiponectinemia associates with vasoreactivity damage (5,89,90),

inflammation, ED and vascular complications (5).

Monica Negrean et al evaluated ***the acute effects of dietary AGE upon the vascular function*** in patients with type 2 DM. FMD (flow-mediated dilatation), indicator of **the macrovascular function**, decreased after HAGE diet (high-AGE content) (initially at 2 h, 4 h, and 6 h); the maximum reduction was registered at 4h; at the same time, there has been observed a deterioration of **the microvascular function**, an increase of AGE seric concentration, ED markers (E-selectin, ICAM-1, VCAM-1) and of oxidative stress (TBARS- thiobarbituric acid reacting substance), proving the important influence of food preparing method upon postprandial vascular dysfunction in patients with type 2 DM (77). **E-selectin** grew 4 h after HAGE (77,78); **ICAM-1** grew 2 h after HAGE and decreased 2 h after LAGE (low-AGE content), with significant differences between 2h and 4h (77); **VCAM-1** increased after HAGE and decreased after LAGE, with significant differences between 2h and 4h (77,78); seric **TBARS** (measures lipid peroxidation and oxidative stress) increased significantly 2 h after HAGE and insignificantly 2 h after LAGE (77,78); seric **MG** increased 4 h **after** HAGE, correlated with FMD modification (77,78). **Seric CML** (carboxymethyllysine) increases insignificantly 4 h after HAGE and decreases 6 h after LAGE (77). Urinary AGEs increase only after HAGE (78).

In patients with type 2 DM, the HAGE diet carried out over a period of six weeks significantly increases AGEs, markers of inflammation (PCR, TNFα) and ED (VCAM-1), glycosylation and LDL oxidation, stimulating NF-kB, LDL vascular toxicity and the development of micro- and macrovascular complications (77).

Elevated oxidative stress levels in hyperglycemia are also indicated by the increased levels of lipid hydro-peroxides (5) and of the urinary excretion of 8-iso-PGF2α (8-iso-prostaglandin F2α) (5).

In patients with type 2 DM with CGMS (continuous glucose monitoring system), Monnier et al observed a correlation of increased free radicals production (elimination over 24 h of 8-iso-PGF2α) with glucose fluctuations range (and not with glycaemia average over 24 h, a jeun glycaemia or HbA1c) (7,91). 8-iso-PGF2α concentration was four times higher in patients with maximum glycaemic variability, compared to those with minimum glycaemic variability. The exposure of thin subjects without DM to the same glycaemic excursions noted by Monnier et al doubled nytrotyrosine concentration in the circulation (7,91). Hyperglycemic spikes reduce NO availability (7,57). Both hypertriglyceridemia and postprandial hyperglycemia induce ED through oxidative stress (73,92); there is an independent but cumulative effect of hypertriglyceridemia and postprandial hyperglycemia upon endothelial function, having oxidative stress as common mediator (7,92). Hyperlipidemia generates oxidative stress at mitochondrial level through the same mechanisms as hyperglycemia (7,93).

The close relationship between microalbuminuria and ED in DM can represent an explanation for the fact that microalbuminuria is a marker of atherotrombosis risk (1). ED can cause (micro)albuminuria *directly* (through glomerular pressure increase and glomerular

basement membrane thickening) and *indirectly* (through the modification of glomerular mesangium and of epithelial cells function, paracrine) (1). In both type 1 and type 2 DM, micro- and macroalbuminuria associate with ED markers (1).

In type 2 DM , there is a close relation between ED, inflammation and UAE (94). ED is involved in the development of diabetic retinopathy, nephropathy and atherosclerosis, in both types of DM (94), being more severe in diabetic women (95).

In patients with type 2 DM, UAE correlates significantly and independently with the plasmatic level of vWF and sE-selectin (94). The levels of vWF and sVCAM-1 are elevated and associate with an increased risk of cardiovascular mortality and microalbuminuria progression (94), and vWF associates also with the apparition of retinopathy and diabetic neuropathy (5, 96). AR excessive activation in endothelial cells of retina in humans can be a mechanism for diabetic retinopathy (5,97).

Diabetes mellitus - thiamine-deficient status

The complications of DM can be prevented by the redistribution of triosephosphates excess also towards PPP (pentose phosphate pathway), which is damaged in clinical and experimental DM because of the moderate thiamine deficit (98); DM can hence be considered **thiamine-deficient status** (99,100). The expression and activity of thiamine dependent enzyme, *TK (transketolase),* is also diminished (99). 18%, respectively 76% of the patients with DM from two studies showed a thiamine plasmatic concentration lower than the normal minimum concentration (99). Patients with type 1 and 2 DM present similar diminutions of plasmatic thiamine (101). A small thiamine deficit is frequent in patients with DM (100,102,103), who show an elevated thiamine Cl (clearance) (103).

Jadidi et al suggest that a limit thiamine deficit can increase nephropathy development risk (6). Plasmatic thiamine is reduced in patients with type 2 DM and microalbuminuria; plasmatic thiamine concentration and thiamine urinary excretion correlated negatively with sVCAM-1 (103).

The increased activity of αETK (α erythrocyte transketolase) shows a subnormal thiamine status:

– a level of αETK < 1.16 shows a low risk of thiamine deficit,

– a level of αETK between 1.16-1.25 shows a moderate risk of thiamine deficit,

– a level of αETK > 1.26 shows an elevated risk of thiamine deficit (9).

Patients with DM show a level of αETK significantly higher than healthy subjects; αETK level was significantly reduced after seven days of treatment with benfotiamine (320 mg/day) (9).

Thiamine recommended dietary intake (RDI), estimated using NUTTAB 95, FSANZ data base (Food Standards Australia New Zealand), was 1.1 mg/day in women and 1.2 mg/day in men) (104,105). Thiamine dietary intake was classified: < RDI, > RDI and thiamine supplements > 4 mg/day (104). Thiamine deficit is diagnosed by determining plasmatic thiamine, erythrocyte TK, or the percentage of pyrophosphate thiamine (104). A thiamine urinary excretion > 0.20 μmol/24 h

shows a sufficient dietary intake for the adult with DM (101).

Lonsdale et al observed a thiamine deficit in individuals whose diets had a high content of simple CH (carbohydrates), especially highly processed food items (sulfites destroy thiamine) (104,106). Individuals whose diet has a high content of refined CH should take thiamine supplements (104). Lonsdale recommends 150 mg of thiamine in doses spread out over the course of the day for individuals with thiamine deficit symptoms and for those with elevated thiamine need (104,106).

Thornalley et al evaluated thiamine status (plasmatic, erythrocytary and urinary level of thiamine) in patients with type 1 and type 2 DM, with and without microalbuminuria, and healthy subjects, as well as the relationship with ED markers (103). Plasmatic thiamine was with 76% lower in patients with type 1 DM and with 75% lower in patients with type 2 DM, compared to healthy subjects (103). Thiamine Cl was 24 times higher in type 1 DM and 16 times higher in type 2 DM, compared to healthy subjects (103). Plasmatic thiamine concentration correlates negatively with thiamine Cl and with $FE_{thiamine}$ (fractional excretion of thiamine) (103). ETK activity correlates negatively with UAE (103). Experimental DM associates with a significant diminution of plasmatic thiamine, with the diminution of TK activity and of TK protein level in renal glomeruls and with thiamine Cl increase (103,107). Clinical DM is associated with a significant plasmatic thiamine deficit, with increase of thiamine Cl, $FE_{thiamine}$ and plasmatic sVCAM-1. Plasmatic thiamine diminution in clinical DM is not caused by dietary thiamine deficit, but probably by thiamine Cl increased, due to reduced thiamine reabsorption in proximal renal tubules (103). The genetic expression which codifies thiamine transporters, THTR-1 (thiamine transporter-1) and THTR-2 (thiamine transporter-2), and also RFC-1(reduced folate carrier-1), TMP transporter (thiamine monophosphate), is regulated through the pathway *SP1 (specificity protein 1) promoter elements* (103); in hyperglycemia, SP1 signalizing is affected in tubular epithelium through *O-glycosylation* increased, on hexosamine pathway (108). Thus, in experimental (6) and clinical DM, thiamine uptake by tubular epithelium is affected by the diminution of THTR-1 and THTR-2 genetic expression (103).

P. J. Thornalley et al showed that thiamine plasmatic deficit in DM is hidden at erythrocytary level by the increase of THTR-1 and RFC-1 transporter proteins level, by the increase of genetic expression which codifies THTR-1 and RFC-1 in erythrocytary precursors, reticulocytes and erythroblasts (103). RFC-1 genetic expression is damaged in diabetic retina (103).

In patients with DM, at glomerular level, TK activity and level are 60% lower (101,103); a similar thiamine uptake affectation can occur also in retina and peripheral nerves (103, 109).

In gestational diabetes, 50% of women have a low TK level (104), compared to 25-30% of pregnant women without diabetes (104).

R.B. Jadidi et al recommend to patients with DM to avoid even a small thiamine deficit and to use increased dosages of thiamine in order to prevent clinical diabetic nephropathy (6).

The benefits of thiamine and benfotiamine in DM

Thornalley PJ et al showed that high-dose thiamine prevents microvascular complications development in experimental DM, without improving metabolic control (98,104); at the same time, it corrects dyslipidemia in experimental DM, normalizing cholesterol and triglycerides (98).

β-cellular dysfunction and IGT occurred in thiamine deficit and the possible connection between IGT and dietary thiamine shows a possible role of thiamine treatment in preventing type 2 DM (98). It is recommended to avoid even a moderate thiamine deficit in DM, and high doses of thiamine supplements can be considered as adjuvant nutritional therapy in preventing dyslipidemia and vascular complications in clinical DM (98).

Thiamine deficit in DM can increase vascular cells fragility, increasing hence the risk for developing microvascular complications (103); it has been demonstrated *in vitro* that the correction of thiamine deficit and of TK diminished activity can reduce vascular cells damage (99), decreasing thus microvascular complications risk in DM (103).

Benfotiamine (S-Benzoylthiamine monophosphate), a coenzyme for TK, pyruvate dehydrogenase, α ketoglutarate dehydrogenase, is a lipid-soluble thiamine derivative; orally administrated, it causes a much better absorption than water-soluble thiamine, reaching a maximum plasmatic level which is five times higher than that of water-soluble thiamine, and a maximum bioavailability which is 3.6 times higher than this one (110).

Benfotiamine, activating *PPP* and *TK (transketolase)* enzyme (6,81), inhibits three major pathways implicated in ED pathogenesis (hexosamine pathway, AGE and PKC), as well as NF-KB activation, associated to hyperglycemia; TK transforms GA-3-P and F-6-P into xilulose-5-P and eritrose-4-P, and then into R-5-P (ribose-5-phosphate) (figure 1). The ability of benfotiamine to inhibit the three pathways simultaneously can be clinically useful in preventing the development and progression of diabetic complications (6,9,99,111,112,**113)**

Brownlee M. demonstrated the positive effect of benfotiamine upon glucose intracellular metabolism, by increasing TK activity and activating physiologic glycolyse (114), reducing AGE formation; the author supports the use of benfotiamine in preventing the chronic complications of DM.

Benfotiamine prevents retinopathy, nephropathy and experimental diabetic neuropathy (9,98,111,115,116).

In vascular cells cultures, benfotiamine – through TK activation – reduces the genetic expression and the activity of AR, as well as sorbitol level. Physiologic activity of αETK increases only after benfotiamine (110).

TK activation by benfotiamine reduces glycolyse intermediates products formation and *endothelial activation*, preventing retinopathy and experimental diabetic nephropathy (6,111). *Endothelial activation* is characterized by an elevated expression of adhesion molecules and chemoatractants (MCP-1 and IL-8), being essential in ED (117).

X. Du et al observed the effect upon some ROS markers in patients with type 1 DM to whom *benfotiamine 300 mg twice/day* was

administered in combination with *ALA (α lipoic acid) 600 mg twice/day* over a period of 28 days. The authors showed:

- the diminution of angiopoietine-2 level (marker of MG increase in endothelial cells) after 14 days of treatment (113)

- the reduction by 40% of hexosamine pathway activity - GlcNAc (N-acetylglucosamine) measurement in circulating monocytes after 14 days of treatment; incomplete normalization of hexosamine pathway in monocytes, in contrast with its complete normalization in rat retina, shows different benfotiamine accumulation in different cellular types or a slowed turnover of intracellular monocytary proteins (113).

- 6-keto-PGF1α (6-keto-prostaglandinF1α) activity increase (endothelial enzyme with anti-atherogenic effect) after 28 days of treatment (type 1 DM associates with the reduction by 70% of the activity of prostacyclin - synthase 6-keto-PGF1α) (113).

DM is characterized by significant postprandial ED, caused by hyperglycemia, hypertriglyceridemia, AGEs and dicarbonyl compounds. Benfotiamine blocks *in vitro* MG formation induced by hyperglycemia and ED (112).

Thiamine and benfotiamine effects in diabetic nephropathy

High doses of thiamine and benfotiamine represent a potential new strategy in preventing clinical diabetic nephropathy (99).

PKC_{β} activation in renal glomeruli determines diabetic nephropathy, by stimulating the expression of VEGF, TGF-ß, and type IV collagen. High doses of thiamine and benfotiamine reduce PKC expression, preventing hence incipient nephropathy (6,98,118).

High doses of thiamine and benfotiamine stimulate TK expression in renal glomeruli, increase triosephosphates conversion (GA-3-P and a F-6-P) into pentose phosphates (R-5-P) and inhibit microalbuminuria, PKC, protein glycosylation and oxidative stress, preventing hence the development of diabetic nephropathy (6)**; it remains** to be seen if thiamine and benfotiamine also prevent the progression of incipient nephropathy towards clinical nephropathy (99).

In diabetic rats, high doses of thiamine and benfotiamine prevent microalbuminuria and proteinuria (99). High-dose thiamine prevents MG accumulation, by inhibiting triosephosphates accumulation.

RAGE (AGE receptors) activation in renal glomeruli is implicated in mesangial expansion and glomerular sclerosis in incipient nephropathy (99). High-dose thiamine blocks AGE accumulation, preventing incipient nephropathy (99) and microalbuminuria in experimental diabetes (101). High-dose thiamine prevents diabetic nephropathy in experimental diabetes, with no improvement of glycaemic control (101).

High-dose thiamine (3×100 mg/day) reduces UAE (marker of incipient diabetic nephropathy) in patients with type 2 DM with microalbuminuria after 1–3 months of therapy, ameliorating incipient diabetic nephropathy; after 3 months of therapy, the regression of microalbuminuria to normal albuminuria occurs in 35% of the patients (101).

Thiamine and benfotiamine effects in diabetic neuropathy

Thiamine and benfotiamine have benefic effects in diabetic neuropathy (13,99,112,

115,118,119-122,123). In patients with diabetic polyneuropathy, a daily dose of 400mg benfotiamine over a period of three weeks has significantly ameliorated Katzenwadel neuropathy score; the best results were registered for the symptom „pain" (119).

BENDIP (Benfotiamine in Diabetic Polyneuropathy), a randomized, placebo-controlled, double-blind study showed in patients with diabetic polyneuropathy a significant TSS (Total Symptom Score) improvement after 600 mg of benfotiamine per day over a period of 6 weeks; the best results were registered for „pain"; the study also showed a significant NSS (Neuropathy Symptom Score) reduction after 6 weeks of therapy with 600 mg of per day (115,122).

Benfotiamine, administrated to patients with diabetic polyneuropathy in a dose of 320 mg over a period of 2 weeks, and afterwards in a maintenance dose of 120 mg over a period of 10 weeks, significantly increases nerve conduction velocity after **12 weeks and reduces** the vibration perception threshold **by** 30% compared to the therapy starting point (124).

Benfotiamine effects upon postprandial adiponectinemia

HAGE diet induces *acute ED* and *adipocyte dysfunction*: 2 h after HAGE, a significant diminution of **adiponectinemia** and leptinemia has been registered compared to the initial values (78). **Ştirban A.** et al observed that HAGE food items transitory decrease postprandial adiponectinemia (at only 2 h) in patients with poorly controlled type 2 DM, an effect which is prevented through a benfotiamine pretreatment and the modification of food cooking method (116).

There is a significant correlation between the modifications of TBARS and adiponectinemia 2 h after HAGE; a three days benfotiamine therapy reduces a jeun TBARS and postprandial MG, and restores at the same time adiponectinemia (116). Benfotiamine prevents the postprandial increase (after HAGE) of AGEs, oxidative stress and ED, reducing postprandial adipocyte stress and maintaining adiponectinemia level (116). Benfotiamine pretreatment significantly reduces postprandial hyperglycemia, in spite of the similar postprandial insulin level, suggesting the improvement of postprandial insulin sensitivity after benfotiamine (116).

Benfotiamine effects upon FMD (marker of NO bioavailability) and reactive hyperemia

In patients with type 2 DM, the three days pretreatment with 1050 mg of benfotiamine per day prevents FMD diminution at 2 h, 4 h and 6 h after HAGE, FMD being completely reestablished at 6 h; it prevents at the same time reactive hyperemia damage and causes its complete recover at 4 h (112). HAGE causes a postprandial vasodilatation significantly lower than the one after HAGE + benfotiamine only at 2 h; postprandial vasodilatation after HAGE can be explained by the elevated meal-induced insulin secretion, with the increase of NO production and vasodilatation at macrovascular level, partially counterattacked by $^{\bullet}O_2^-$ production (112).

Benfotiamine can prevent HAGE-induced micro- and macrovascular dysfunction in type 2 DM, by reducing endogenous and dicarbonyl AGE production and by decreasing

oxidative stress (112). There is an inverse correlation between CML increase and microvascular function damage and between MG increase and macrovascular function (FMD) damage, CML having a more important role in regulating microcirculation, and MG in regulating macrocirculation (112). Benfotiamine prevents postprandial increase of MG (maximum at 4 h) and CML in humans, having an important role in preventing atherosclerosis in diabetic patients (112). Benfotiamine, preventing **oxidative stress** (decreases TBARS at 2 h), can improve IR (112). Three days benfotiamine pretreatment cancels the increase of ED seric markers (E-selectin, ICAM-1, VCAM-1), occurred postprandial after HAGE (112). Benfotiamine has direct and indirect **anti-inflammatory effects**, preventing CRP increase, which occurred at 6 h after HAGE (112). Benfotiamine does not improve each and every parameter of the a jeun status (FMD, reactive hyperemia, E-selectin, VCAM-1, ICAM-1, CRP, fibrinogen); this fact is explained by the short duration of the treatment (112).

Clinical trials on diabetic patients are necessary to test the potential of these vitamins in preventing and/or treating diabetic vascular complications.

Although the life-style change remains the best preventing method, numerous antioxidant substances are being discovered (48,120,121), which can become important means in stopping DM and its complications.

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