

Review Article and Clinical Experience:

EMERGING MULTIPLE PROPERTIES OF HIGH DOSE THIAMINE AND B6-B12 VITAMINS Therapeutic Possibilities for Diabetic Vascular Complications

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ABSTRACT

High prevalence of low plasma thiamine level in diabetes (T1DM and T2DM) linked to a marker of vascular disease. The deficiency of thiamine in diabetes increases the susceptibility of vascular cells to the injurious effects of hyperglycemia (“PAHA” and “PAHAM”), and hence increased the risk of developing microvascular complications. Thiamine supplementation to high dose as adjunct therapy may prevent the development of vascular complications in clinical diabetes, also may result in the reversal of diabetic dyslipidemia. One of the most important function of B6 Vit is to suppress the activation and translocation of NFκB. The combination of B12 Vit and folate show the protective effects on the developments of myocardial infarction and stroke. In conclusion, the FDC drug Neurobion® show several therapeutic possibilities with multiple clinical benefits for patients with diabetes mellitus. Thiamine repletion with thiamine or benfotiamine may prevent or delay the development of diabetic microvascular complications in vivo.

Keywords: thiamine, B6-B12 vitamins, diabetic vascular complications

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Abbreviations: ACC = Acetyl-CoA Carboxyl; A.OX = Antioxidant; CRP = C-reactive protein; DHAP = Dihydroxyacetonephosphate; DRI = diet reference intake; FAS = Fatty Acid Synthase; FDC = fixed dose combination; G3P = Glycerol-3-Phosphate; GA3P = Glyceraldehyde-3-Phosphate; GFAT = Glutamin: Fructose-6-Phosphate Amidotransferase; GFR = glomerular filtration rate; GPDH = Glycerophosphate Dehydrogenase; IGT = impaired glucose tolerance; Oxphos = Oxidative Phosphorilation; “PAHA” = PKC, Age, Hexosamine pathway, activated Aldose reductase; “PAHAM” = PKC, Age, Hexosamine pathway, activated Aldose reductase, Mitochondrial dysfunction; PARP = poly (ADP-ribose) polymerase; PLP = pyridoxal-5-phosphate; R-5-P = Ribose-5 Phosphate; R-PPP = Reductive Pentosephosphate Pathway; SGLT = Sodium-GLucose co-Transporter; SMC = smooth muscle cell; TK = transketolase; T1DM = Type 1 Diabetes Mellitus; T2DM = Type 2 Diabetes Mellitus; UDP-GlcNAC = Uridine Diphosphate-N-Acetylglucosamine.

INTRODUCTION

Significant advances in understanding of the link of hyperglycemia to microvascular complications have been made. The multiple biochemical dysfunction linked to complications development is initiated by increased cytosolic glucose as triosephosphates concentration (in endothelial and mesangial cells) which causes exceeds of triosephosphates leading to diabetic complications (Figure-1). As seen in Figure-1, oxidative stress may exist through 4 possible mechanisms called: “PAHA” (PKC, Age, Hexosamine pathway, activated Aldose Reductase pathways). This oxidative stress may

cause β cell dysfunction and insulin resistance. Excellent glycemic control and powerful antioxidant may reduce the oxidative stress, and hence improved β cell function and improved insulin sensitivity may pursue. The short description of each component of “PAHA” can be followed on the following pages. “PAHA” is an abbreviation of Indonesian language which means “THIGH”.

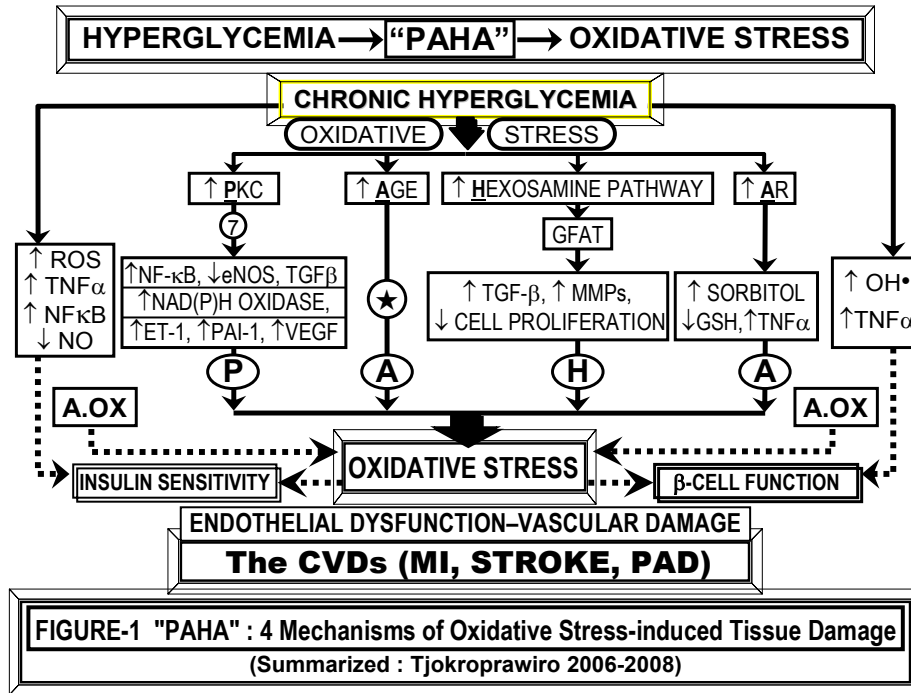
PKC Pathway (P)

Body proteins become irreversibly modified by sugars in a process known as the Maillard reaction, leading to tissue “browning”. Diacylglycerol (DAG) and PKC are critical intracellular signaling molecules that can regulate many vascular functions, including permeability, vasodilator release, endothelial activation, and growth factor signaling. Activation of PKC may also be involved in the induction of growth factors expression (VEGF, TGF- β) and signaling molecules (VEGF, ET-1). In addition, PKC activation can also impact other signaling pathways such as those using MAP kinase or nuclear transcription factor.

In the glomeruli of rats with diabetes, the α , β , δ , ϵ , and ζ are isoforms of PKC which all have been shown to be activated. Ruboxistaurin mesylate, a PKC inhibitor, has high affinity for the β 1, and β 2 isoforms, and has been

shown to block many vascular abnormalities in endothelial cells and contractile cells from the retina, arteries, and renal glomeruli. In animal with diabetes, ruboxistaurin mesylate has been shown to prevent or reserve many early hemodynamic changes observed in diabetic retinopathy, nephropathy, and even neuropathy.

Chronic oral treatment with this PKC-β isoform inhibitor in genetically diabetic mice prevented mesangial expansion and glomerular dysfunction. Ruboxistaurin mesylate is now being in clinical trials for diabetic retinopathy and neuropathy.



AGE Pathway (A)

AGEs can alter cellular function by binding to receptors, such as the receptor for AGEs (RAGEs), a transmembrane receptor. This binding may produce a cascade of cellular signaling events, such as activation of MAP kinase or PKC, which can lead to cellular dysfunction. Other receptors, such as the macrophage scavenger receptors, p60, p90, and galectin-3 have been reported to bind AGEs. Clinical trial using amiguanidine, an inhibitor of AGE formation, have been shown to be inconclusive due to the presence of its toxicity. However, the use of soluble RAGE-inhibitor to block binding to RAGE in animal models of diabetes has been reported to prevent several effects of hyperglycemia.

Hexosamine Pathway (H)

As seen in Figure-1, via the activated GFAT due to high glucose blood level, TGF-β may increase which in turn induces the accumulation of matrix protein components of the mesangium and inhibits cell proliferation (increased MMPs and decreased cell proliferation). The

accumulation of mesangial matrix is a marker to diabetic glomerulosclerosis, and a progressive decline in the surface area available for glomerular filtration and diabetic nephropathy may pursue.

Aldose Reductase or Polyol Pathway (A)

Aldose reductase uses nicotinamide adenine dinucleotide phosphate (NADPH) to reduce glucose to sorbitol which is then oxidized level of sorbitol is believed to contribute to the development of cataract. The activated aldose-reductase pathway may activate TNF? production, in which the latter will result in SMC proliferation. The decline in cellular NADPH caused by increases in aldose reductase flux may decrease the generation of nitric oxide (NO) in endothelial cell. The decreased NO may increase the expression of ICAM and VCAM, which induces platelet aggregation, suppresses vasodilator, induces SMC proliferation, and alter the cellular redox balance. Aldose reductase inhibitors have been shown to prevent some of the pathologic changes in rodent models of diabetic retinopathy, nephropathy, and neuropathy.

THE LATEST EVIDENCES ABOUT VITAMINS B1, B6, AND B12

The three components of B-Vitamins (B1, B6, and B12) have been studied, and they are reported to show potential roles in diabetic complications especially microvascular (peripheral neuropathy, nephropathy, retinopathy). More detailed information about the B-Vitamins (esp. B1 - Vitamin) will be briefly described, but focused on B1-Vitamin. The emerging multiple benefits of B1 -Vitamin repletion, even mild B1 - Vitamin deficiency in diabetes should be treated. High dose B1 - Vitamin supplementation should be prescribed as adjunct nutritional therapy to prevent dyslipidemia and the development of diabetic vascular complications. The excess of triose phosphates due to high cytosolic glucose concentrations in chronic hyperglycemia is one of potential cause for biochemical dysfunctions to the development of diabetic complications. The accumulation such substances can be minimized by disposal through ribose-5 phosphate (R-5-P) via the reductive pentosephosphate pathway (R-PPP). Importantly, the R-PPP is impaired in clinical diabetes with mild B1 – vitamin deficiency. The expression and the activity of the B1 – vitamin – dependent enzyme, transketolase (TK) – the peacemaking enzyme of the R-PPP is consequently lowered. For practical point of view, the author has summarized four pathways called “PAHA Pathways” (PKC, AGE, Hexosamine, Aldose Reductase or Poliol Pathway) which are responsible for the occurrence of diabetic vascular complications.

The potential roles of B1 – Vitamin (B1-Vit) in diabetic complications can be grouped into four : (1) Beneficial effects of B1 -Vit. on pancreatic β -cell function and glucose tolerance; (2) Beneficial effects of high dose B1 – Vit therapy in the biochemical dysfunctions and the prevention of microvascular complications; (3) Positive effects of high dose B1-Vit treatment in biochemical dysfunctions and reversal of diabetic dyslipidemia; (4) Other physiological effects of high dose therapy with B1-Vit: decreased glucosuria and diuresis (it may be linked to changes in sodium-glucose co-transporter = SGLT and vasopressin-stimulated increase in GLUT-4 activity).

Low plasma B1 -Vit (thiamine) level is prevalent in patients with type-1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), associated with increased thiamine clearance. Plasma thiamine concentration was decreased 76% in T1DM and 75% in T2DM. The low plasma thiamine concentration (due to increased thiamine clearance = Cl thiamine) concentration in diabetes was linked inversely to plasma sVCAM-1.

Increased Cl thiamine is probably due to decreased re-uptake of thiamine in renal proximal tubules. Thiamine clearance was dysfunctional in diabetic patients with normal GFR. One study in 2007 suggested the renal mishandling of thiamine in diabetic patients is an early marker of renal dysfunction and linked to the locus of renal thiamine re-uptake, particularly relates to proximal tubule dysfunction.

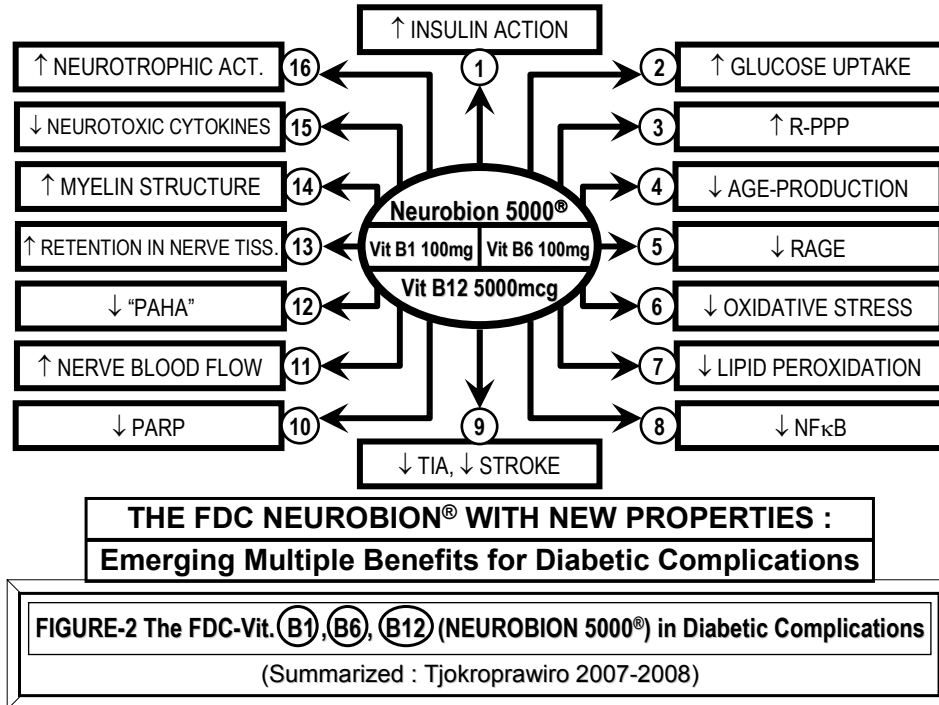
Vitamin B6 (pyridoxine) supplementation has been found beneficial in preventing diabetic neuropathy and retinopathy, and the glycosylation of proteins. It has been demonstrated in 2001 that B6 -Vit can inhibit oxygen radical production, which in turn prevents the lipid peroxidation, protein glycosylation, and (Na⁺-K⁺)-ATPhase activity reduction induced by the hyperglycemia. Vitamin B6 has been supposed to have anti inflammatory effects. It has been demonstrated that the anti-inflammatory effects of B6–Vit is mediated by suppression of NFkB activation. Vitamin B6 inhibited LPS-induced nuclear translocation of the NFkB, the proinflammatory transcription factor, with reduction of the extent of LPS-induced I κ B β degradation in RAW cells. Low levels of pyridoxal-5-phosphate (PLP), the active metabolite of B6 –Vit, are associated with high C-reactive protein (CRP) concentrations. The coronary artery disease (CAD) risk as a result of low PLP was additive when considered in combination with elevated hs-CRP concentrations or with an increased ratio of LDL to HDL.

Several prospective and intervention studies have demonstrated that increase levels of folate and B12 – Vitamin (B12-Vit) lower plasma homocystein levels, an established risk factor for myocardial infarction and stroke. Recently, the result of one study in 2007 concluded that low B12 –Vit, particularly in combination with low folate, may constitute a risk-increasing factor for the development of transient ischemic attack (TIA) and stroke. For the possible protective effects of B-Vitamins, certain thresholds of folate, B12 –Vit, and PLP seen to be crucial.

Vitamin B6 shows anti-inflammatory effects by suppressing NFkB activation. Low plasma PLP levels are inversely related to major markers of inflammation and independently associated with increased CAD risk. Low vitamin B12 plasma levels, particularly with low folate levels, increased the risk of cerebral ischemia. This effect may be mediated at least partly the elevations of homocysteine levels Taken together, vitamins B1, B6, and B12 (as built in Neurobion-5.000® tablet, a Fixed Dose Combination (FDC) of 100mg B1, 100mg B6 and 5.000 mcg B12-Vitamins), show potential therapeutic roles in the development of

diabetic complications. The emerging multiple benefits of such B-Vitamins in one tablet of Neurobion® can be prescribed as the promising adjunctive drug for the treatment or prevention of diabetic patients with vascular complications. The latest evidences about

vitamin B1, B6, B12 are compiled by the author as seen in Figure-2.



THE ROLES OF THIAMINE (VITAMINE- B1) IN DIABETIC COMPLICATIONS

Although the B-Vitamins (B1, B6, B12) are involved in the development of diabetic complications, the following description will be focused on the thiamine or B1-Vitamin (B1-Vit) and its potential roles. Thiamine is an essential micronutrient with a dietary reference intake (DRI) for annual healthy adult human subjects of 1.1mg daily for female and 1.3mg daily for males (Standing Committee on the Scientific Evaluation of Dietary Reference Intake 1998, Expert Group on Vitamin and Minerals FSA UK, 2003). As previously mentioned, there are 4 (four) groups of thiamine roles.

Beneficial Effects of Thiamine on Pancreatic β-Cell Function and Glucose Tolerance

Since mild thiamine deficiency may be prevalent in diabetes (particularly with nephropathy) the effects of thiamine deficiency on pancreatic β-cell function and peripheral insulin sensitivity is also of interest. In thiamine-deficient rats, there was increased fasting glucose and decreased fasting insulin concentration and there was also impaired insulin secretion with impaired

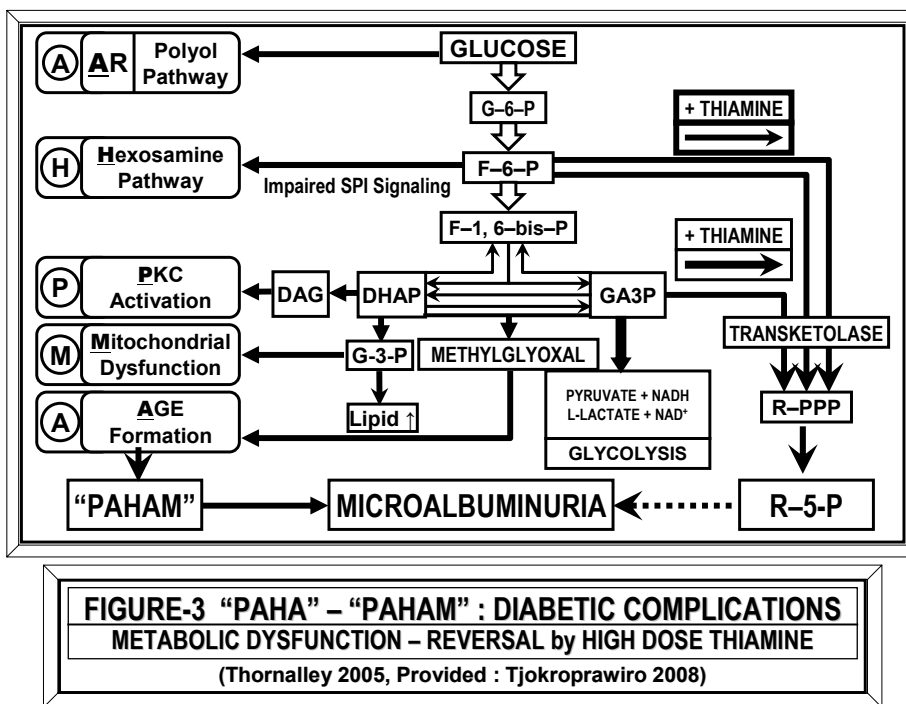
glucose tolerance (IGT) and increased plasma glucagons level in an oral glucose tolerance test (Rathanaswami et al 1988, 1989). Thornalley (2001, 2005, 2007) concluded that thiamine deficiency may impair pancreatic β-cell function and may contribute to IGT in the human population. Dietary fiber intake was inversely associated with fasting glucose, and fiber intake correlated strongly with thiamine intake. HORN study (1998) concluded that part of the association between fiber intake and glucose tolerance was attributable to dietary thiamine intake. Thornalley (2005) suggested that thiamine deficiency impairs pancreatic β-cell function and it may contribute to IGT in the human population. Further epidemiological analysis and intervention trials with thiamine or benfotiamine (thiamine prodrug) may establish a basis for prediction of T2DM by highly dose thiamine derivative therapy.

Beneficial Effect of High Dose Thiamine Therapy

There are 2 (two) beneficial effects of high dose thiamine therapy: effect in the biochemical dysfunctions and effect on the prevention of microvascular complications. High dose therapy with thiamine and the

thiamine derivative benfotiamine are new proposed as a novel therapy to counter biochemical dysfunctions

leading to the development of diabetic microvascular complication.



For practical point of view, the biochemical dysfunctions in the development of diabetic vascular complication can be easier understood by following Figure-3 which is consistent with "PAHA" as illustrated in Figure-1. Cytosolic hyperglycemia leads to the accumulation of triosephosphates (GA3P, and G3P) as seen in Figure-3. These substances are closely related to glycolytic intermediates which then initiate multiple pathways of biochemical dysfunctions (Soriano et al 2001, Guzik et al 2002, Lassegue et al 2003), that are activation of polyol pathway – activated Aldose reductase enzyme (A), increased formation of DAG-Activation of PKCβ (P), activation of Hexosamine pathways (H), increased formation of methylglytolal and related formation of AGEs (A), increased flux through the glycerophosphate shuttle and Mitochondrial dysfunction with increased oxygen radical production (M), activation of vascular NADPH oxidase (by PKC), activation and uncoupling of eNOS (by O₂-production), activation of PARP (due to DNA damage in response to oxidative stress and probably glycation process) and activation of vascular AGE receptor (RAGE)

Thiamine improves endothelium-dependent vasodilation in the presence of hyperglycemia in patients with diabetes mellitus (Arora et al 2006). The mechanisms is not due to a glucose-lowering effects, hence

administration of thiamine might improve endothelial function and therefore delay and slow the development and progression of atherosclerosis, especially in patients with IGT and T2DM who are prone to develop acceleration atherosclerosis. Thornalley et al (2001) have examined on human RBCs the possibility that activation of the R-PPP by high dose thiamine and benfotiamine might divert triosephosphates and F-6-P to R-5-P and relieve biochemical dysfunctions as occur in hyperglycemia (Figure-3).

Under hyperglycemic conditions in vitro, there was (Du et al 2003): increased PKC activity, activation of PARP, increased intracellular concentrations of the Hexosamine pathway, increased intracellular AGEs, activation of the oxidant sensitive transcription factors NF-kB, increased v.Willebrand factor secretion, delayed replication, and increased apoptosis.

All these responses of hyperglycemia might be prevented by both high dose thiamine and benfotiamine (Hammes et al 2003, Mabley et al 2004). It therefore seemed that high dose thiamine and benfotiamine may prevent or delay the development of microvascular complications in experimental diabetes in vivo (Thornalley, 2005). Both thiamine and benfotiamine prevented the development of incipient nephropathy, as judged by the prevention of microalbuminuria.

Transketolase (TK) expression and activity was decreased in renal glomeruli of diabetic rats. High dose thiamine and benfotiamine therapy normalized TK expression and activity and thereby increased the conversion of triosephosphates to R-5-P as seen in Figure-3 (Thornalley 2005). This was associated with decreased activity of membrane and cytosolic PKC, decreased protein glycation and oxidative stress. Surprisingly, these achievements were obtained without changes in hyperglycemic status and glycaemic control (Babci-Jadidi et al 2003). Hammes et al 2003 reported that high dose therapy with benfotiamine (80mg/kg BW/day) normalized the number of retinal acellular capillaries in STZ diabetic rats and hence prevented the development of retinopathy. High dose therapy with thiamine (70mg/kg/day) and benfotiamine (100mg/kg BW/day) prevented the development of neuropathy in STZ diabetic Wistar rats; AGE accumulation was also decreased (Stracke et al 2001). Conclusions – These studies show that thiamine repletion with thiamine or benfotiamine may prevent or delay the development of diabetic microvascular complications in vivo.

Positive Effects of High Dose Thiamine Treatment in Biochemical Dysfunction and Reversal of Diabetic Dyslipidemia

As seen in Figure-4, the metabolic mechanisms for the suppression of hepatic lipogenesis in diabetes by high dose thiamine is illustrated by Thornalley (2005). High dose thiamine may decrease the expression of lipogenic enzymes (GPDH, ACC, FAS) by inhibiting the activation of Hexosamine pathway in liver and adipocytes. If flux through the hepatic Hexosamine pathway is important in lipogenesis in diabetes, high dose treatment with thiamine and benfotiamine is expected to counter this effect. High dose treatment with thiamine (70mg/kg BW/day) prevented diabetes induced increase in plasma cholesterol, triglycerides in diabetic rats, but did not reverse the diabetes – induced decrease of HDL (Avena et al 2000). High dose thiamine decreased TK activity in the liver of diabetic rats. There was a concomitant decreased in hepatic UDP-NAC and FAS activities (Figure-4).

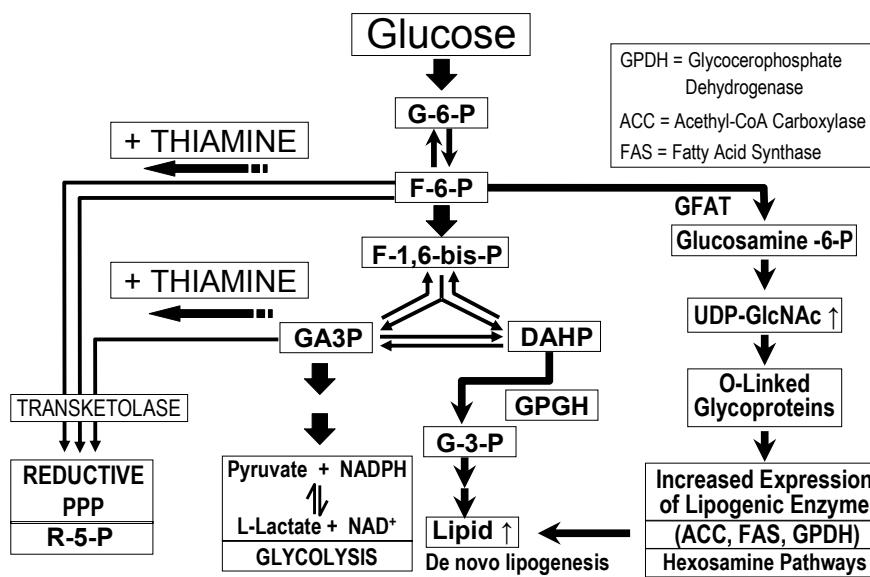


FIGURE-4 Metabolic Mechanisms: The Roles of Thiamine in DM
(Thornalley 2005)

Thiamine has also been shown to decrease aortic SMC proliferation induced by hyperglycemia and hyperinsulinemia. High dose thiamine but not benfotiamine therapy reversed diabetic dyslipidemia. Such a different response is attributed to more effective delivery of thiamine to the liver in the post prandial period by high dose thiamine than high dose benfotiamine.

Other Physiological Effects of High Dose Therapy with Thiamine

Both high dose thiamine and benfotiamine decreased glucosuria and diuresis of STZ diabetic rats in dose dependent manner (Babaei-Jadidi et al 2004). Since plasma glucose concentration was not changed, this

suggest that both thiamine and benfotiamine increased the renal reuptake of glucose. Glucose reabsorption in the kidney occurs mainly via sodium – glucose co-transporter (SGLT). Thiamine and benfotiamine – induced decrease in glucosuria may be linked to changes in SGLT and vasopressin – stimulated increased in GLUT-4 activity. Decreased diuresis may be linked to reversal of diabetes – induced activation of PKC by high dose thiamine ad benfotiamine (Babaei-Jadidi et al 2003) and consequent reversal of inhibition of water re-uptake by aquaporins in renal collecting duct cells.

THE SUMMARIZED ROLES OF B6-VITAMIN IN DIABETIC COMPLICATIONS

The 3 (three) biological compounds of B6-Vitamin are pyridoxine, pyridoxal, pyridoxamine. The multiple actions of B6-Vitamin in diabetes is due to its protective effects on O₂- production which leads to prevent: AGE formation, protein glycosylation, lipid peroxidation and Na/K-ATPase activity reduction. Vitamin-B6 has been

supposed to have anti-inflammatory effects which is mediated suppression of NFκB activation

THE SUMMARIZED ROLES OF B12-VITAMIN IN DIABETIC COMPLICATIONS

Vitamin- B12 may improve the nerve regeneration and restores nerve function: to improve neurotropic activity, to increase retention in nerve tissue, to decrease neurotoxic cytokines, and to improve myelin structure. The low B12-Vitamin, particularly in combination with low folate, may constitute a risk-increasing factors for the development of TIA and stroke

NEUROBION-5000® : THE FDC OF B1, B6, B12-VITAMINS

Neurobion-5000® is a high Fixed Dose Combination of 100mg B1, 100mg B6, 5000mcg B12-Vitamins. Its potential benefits of B1, B6, and B12 –Vitamins are illustrated in the following Table-1.

TABLE-1 Vitamins-B1, B6, B12 (Neurobion 5000®) with Multiple Benefits (Summarized : Tjokprawiro 2007-2008)	
Vit. B1 and Vit. B6	Vit. B12 (Cyanocobalamin)
① ↑ Insulin Action	① ↑ Retention in Nerve Tissue
② ↑ Glucose Uptake	② ↑ Myelin Structure
③ ↑ Pentose Bypass	③ ↓ Neurotoxic Cytokines
④ ↓ AGE-Production	④ ↑ Neurotrophic Activity
⑤ ↓ Oxidative Stress	
⑥ ↓ Lipid Peroxidation	
⑦ ↑ Nerve Blood Flow	
⑧ ↓ DAG, ↓ AGE	
⑨ ↓ PARP	
⑩ ↓ Triosephosphates	
POTENTIAL ROLES OF NEUROBION® IN DIABETIC COMPLICATIONS	

Neurobion ampule of 3ml contains: 100mg B1, 200mg B6, 5000mcg B12 vitamins. This preparation can be injected intravenously by using Formula 1 – 1 – 1 which means: 1 ampule (3 ml) Neurobion®, in 100ml normal

saline, infused in 1 hour. The Table-2 illustrated below consists of many drugs for the treatment of diabetic neuropathy. This recommendation (ADA-2008) is made based on the pathogenetic mechanisms.

Table 2. Treatment of Diabetic Neuropathy based on the Pathogenetic Mechanism (A Statement by ADA-2008)

Abnormality	Compound	Aim of Treatment	Status of RCTs
1. Polyol Pathway ↑	Aldose reductase inhibitors Sorbitinil Tolrestat Ponalrestat Zopolrestat Zenarestat Lidorestat Fidarestat AS-3201 Epalrestat	Nerve sorbitol ↓	Withdrawn (AE) Withdrawn (AE) Ineffective Withdrawn (marginal effects) Withdrawn (AE) Withdrawn (AE) Effective in RCTs, trials ongoing Effective in RCTs, trials ongoing Marketed in Japan
2. Myo-inositol ↓	Myo-inositol	Nerve myo-inositol ↑	Equivocal
3. Oxidative Stress ↑	α-Lipoic acid	Oxygen free radicals ↓	Effective in RCTs, trials ongoing
4. Nerve Hypoxia ↑	Vasodilators ACE inhibitors Prostaglandin analogs phVEGF165 gene transfer	NBF ↑ Angiogenesis ↑	Effective in one RCT Effective in one RCT RCTs ongoing
5. Protein Kinase C ↑	Protein kinase C-β inhibitor (ruboxistaurin)	NBF ↑	RCTs ongoing
6. C-peptide ↓	C-peptide	Nerve myo-inositol ↑	Studies ongoing
7. Neurotrophism ↓	Nerve growth factor (NGF) BDNF	Nerve regeneration, growth ↑ Nerve regeneration, growth ↑	Ineffective Ineffective
8. LCFA metabolism ↓	Acetyl-L-carnitine	LCFA accumulation ↓	Ineffective
9. GLA synthesis ↓	g-Linoleic acid (GLA)	EFA metabolism ↑	Withdrawn
10. NEG ↑	Aminoguanidine	AGE accumulation ↓	Withdrawn
AE = Adverse Event; AGE = Advanced Glycation End product; BDNF = Brain-Derived Neurotrophic; EFA = Essential Fatty Acid; LCFA = Long-Chain Fatty Acid; NBF = Nerve Blood Flow; NEG = Nonenzymatic Glycation; RCT = Randomized Clinical Trial			

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