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Thiamine and the Cellular Energy Cycles: A Novel Perspective on Type 2 Diabetes Treatment

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ABSTRACT

None of the currently available therapeutic interventions for type 2 diabetes mellitus address the intracellular metabolism of glucose through the main energy pathways of the cell. Thiamine (vitamin B1) is a water-soluble vitamin and essential normal dietary component. When modified in the body to the pyrophosphate derivative, it acts as a coenzyme for pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase & transketolase which are required for the utilization and consumption of glycolytic and hexose monophosphate pathway intermediates & form an integral part of intracellular and glucose metabolism. Thiamine deficiency decreases the activities of these enzymes, leading to imbalances in the metabolic pathways. The effects of these imbalances are more pronounced in diabetes mellitus where renal dysfunction produces mild thiamine deficiency.This indepth review presents a novel perspective on, the cellular energy cycles, thiamine dependant enzymes,pharmacotherapeutics of type 2 diabetes especially thiamine and their impact on type 2 diabetes treatment. Thiamine, with its well established safety record, easy accessibility and affordability could be an invaluable adjunct for our type 2 diabetic population and help to improve the quality of their lives by giving them some respite from the complications of type 2 diabetes and perhaps reduce the need of more expensive oral hypoglycaemic agents required by them.

INTRODUCTION

Cellular survival is dependant upon the energy pathways ingrained within them. Their comprehension is imperative in understanding the role of their component enzymes in type 2 diabetes treatment and the inextricable linkage of a few of them to thiamine.

GLYCOLYSIS OR THE EMBDEN MEYERHOFF PATHWAY

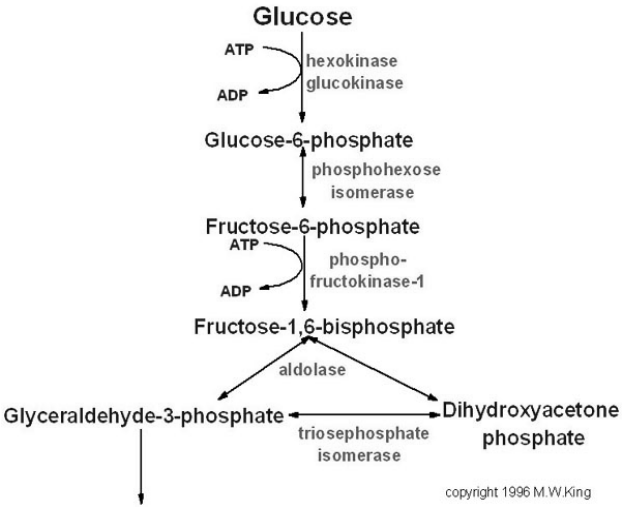
This is an ancient metabolic, cytosolic pathway that converts glucose into pyruvate under anaerobic conditions (i.e. it doesn't require much oxygen) and further into lactate or ethanol. The free energy released from this forms high energy compounds ATP and NADH.Under aerobic conditions CO2 and substantially more ATP is produced [1]. The pathway of glycolysis comprises of 2 clear divisions (Fig1). The first is a chemical priming phase in which, glucose is converted to fructose 1, 6-bisphosphate. In the second phase fructose1, 6 biphosphate is degraded to pyruvate, with a utilization of adenosine triphosphate in the priming phase and its production along with NADH in the energy yielding phase which fuels mitochondrial ATP synthesis via oxidative phosphorylation.

CITRIC ACID CYCLE

After glycolysis,where glucose is broken down into pyruvate only releasing fractional amounts of ATP, further aerobic processing of glucose is conducted through the Kreb Cycle, synonymous with tricarboxylic acid or citric acid cycle(Fig. 2). Intracellularly the mitochondria serve as site of citric acid cycle and oxidative phosphorylation activities.

Steps of the Krebs or Tricarboxylic (Citric Acid) Cycle

1. The pyruvate dehydrogenase complex converts pyruvate to acetyl coA by combining acetylation of coenenzyme A which now enters the citric acid cycle. The steps in the Krebs cycle are as follows
2. The previous Krebs cycle product oxaloacetate combines with acetyl coenzyme A to form citrate by the action of citrate synthase.



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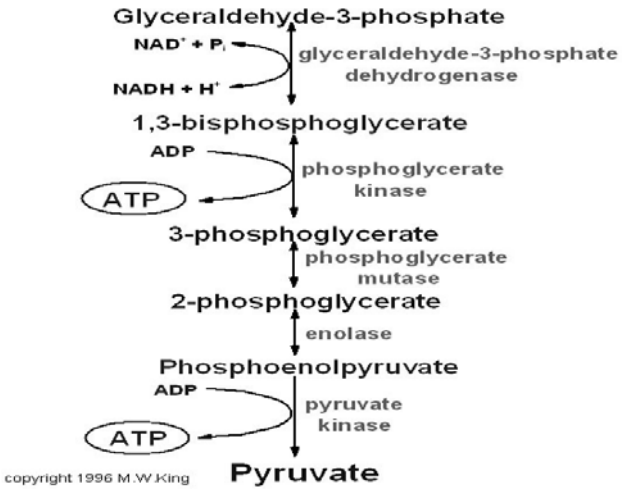

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Fig. 1 A) Phase1 (Priming Phase) of Embden Meyerhoff Pathway
1B Phase 2 (Energy Yielding Phase) of Embden Meyerhoff Pathway
Fig 1 A & B: A Schematic Pathway of glycolysis from glucose to pyruvate.and its connection to the reductive pentose pathway and citric acid cycle. Adapted from Michael W. King, Ph.D / IU School of Medicine / miking at iupui.edu / © 1996–2011.

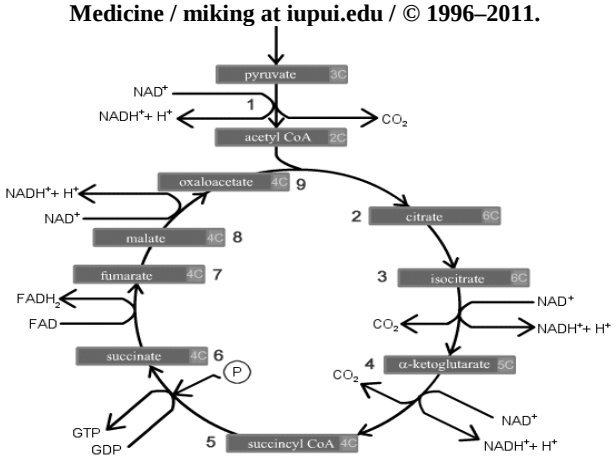


Fig. 2: Krebs cycle (www. library.thinkquest.org)

3. Aconitase acts upon citrate which is converted into its isomer isocitrate.
4. Isocitrate dehydrogenase oxidizes isocitrate to form α-ketoglutarate releasing and NADH2.
5. Alphaketoglutarate dehydrogenase oxidizes α-ketoglutarate to succinyl CoA also releasing carbondioxide and NADH2.
6. Succinate thiokinase causes succinyl Co A to release coenzyme A
7. Succinate dehydrogenase oxidizes succinate to fumarate, also converting FAD to $FADH_2$.
8. Fumarase hydrolyzes fumarate to malate.
9. And lastly malate dehydogenase oxidizes malate to oxaloacetate, simultaneously reducing NAD^+ to $NADH_2^+$.

The overall chemical reaction of the tricarboxylic acid cycle is:

$$\text{Acetyl-CoA} + 3NAD^+ + FAD + GDP + P_i + 2H_2O \longrightarrow 2CO_2 + 3NADH + FADH_2 + GTP + 2H^+ + HSCoA \ ^2$$

THE MITOCHONDRIAL CATALYTIC REPERTOIRE

The pyruvate dehydrogenase complex:

Both prokaryotic and eukaryotic species carry among others, conglomeration of proteins into a mega, specifically arranged multienzyme structural

complex termed (a "metabolon").

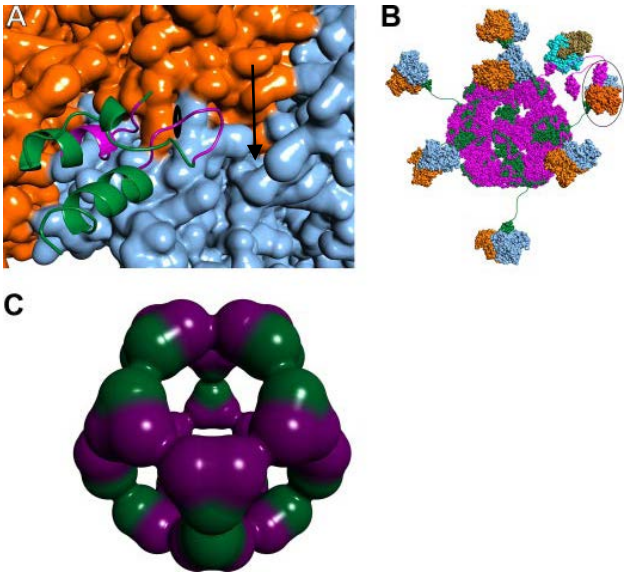


Fig. 3. Protein-protein Interactions in the Native human PDC. Adapted from Brautigham (2006)

A: Close-up view of E3BD (ribbons representation) bound to E3 (surface) (Brautigham 2006). One monomer of E3 is colored orange, and the other is blue. The approximate position of the dyad axis of the E3 dimer is shown by the black symbol and arrow. Most of E3BD is colored green, but those residues with atoms that would clash with a second bound E3BD are shown in purple.

B: Schematic model of the native human PDC. The dodecahedral 60-meric core of the human PDC is modeled using the structure of the catalytic domain of B. tearothermophilus E2 (Izard 1999). The E2p polypeptides are colored magenta, with E3BP polypeptides colored green. The E3 dimers are shown in blueand orange, with a single E3BD bound per dimer of E3 (Brautigham 2006), as indicated by the data. In this model, it is possible for 20 E3 dimers to bind; only 7 are shown for clarity. A single E1p heterotetramer docked to the E1pBD of E2p is represented, subunits shown in tan and cyan. The structure of the human versions of E1p bound to E1pBD is unknown; shown here is the structure from B. stearothermophilus (Frank 2004). The circled E3 has an LBD of E. coli E2p docked to the active site. E2p and E3BD are therefore noncovalently cross-linked via their mutual interaction with E3.

C: Possible arrangement of E2p and E3BP components in a 40/20 core.Shown is a dodecahedral arrangement of 20 heterotrimers composed of 2 E2p proteins (purple) and one E3BP (green)(Brautigham 2008).

Of these enzyme complexes of the metabolon, the pyruvate dehydrogenase complex is highly evolutionarily conserved mitochondrial α-ketoacid dehydrogenase complex, along with the branched-chain α-ketoacid dehydrogenase complex (BCKDC), and the α-ketoglutarate dehydrogenase complex (KGDC)[4,5].

Nomenclature of the pyruvate dehydrogenase complex and its subunits

The complex has 3 main components with multiple subunits and multiple names.(Fig 3)

The heterotetramer **PDEI p PYRUVATE DEHYDROGENASE (EC1.2.4.1)** comprises of 2 alpha and 2 beta subunits[6]. Its alpha 1 subunit is designated as PDE1A-2,(pyruvate dehydrogenase (lipoamide) alpha2). Its gene PDHA2 is located on chromosome 4 having length of 1383 bp/460 aa[7,8] Whereas the alpha 2 subunit is designated asPDH E1-A type1 (*i.e.*) synonym PHE1A. Its gene PDHA1 is located on chromosome X which has length of 15922 bps[9] and a mol.wt of 160KDa . The Pyruvate dehydrogenase E1 component subunit beta, or pyruvate dehydrogenase (lipoamide) beta mitochondrial, synonym, PDE1-B, has gene located on chromosome 3 having length of 6198 bp[10-12]. The key function of the complex E1alpha subunit containing the active site is to be the rate limiting enzyme, unidirectionally funneling intermediate metabolites from glucose breakdown to either the oxidative metabolic pathways or fatty acid and cholesterol synthesis[13].

PDE2p contains **dihydrolipoyl transacetylase enzyme activity (EC2.3.1.12)** encoded by DLAT Dihydrolipoamide acetyl tranferase gene, present on human chromosome 11 band q23.1. It has mol wt 200 KDa[14]. Interestingly, this long arm region of chromosome 11 often presents with translocations in cellular genetic abnormalities[15].

PDE3/GCSL/LAD/PHE3 (EC 1.8.1.4) component contains the dihydrolipoyl dehydrogenase activity. E3 activity is encoded by the DLD located on chromosome 7 and length 28799[16]. It has a mol.wt of 110 KDa. This protein has four different sites: the flavin adenine dinucleotide binding site, the nicotinamide adenine dinucleotide binding site, the centre site and the interface site. The protein forms a homodimer with the FAD and NAD binding regions on one unit and

the interface domain of the other unit forming the active centre[17].

Structural Association of the 3 Units:

The human pyruvate dehydrogenase multi enzyme complex (PDC) is a nuclear encoded mitochondrial matrix 9.5 megadalton catalytic organization of copies of three catalytic components i.e. heterodimeric pyruvate dehydrogenase (E1p 30copies) (thiamine diphosphate (ThDPdependant), homodimeric dihydrolipoyl transacetylase (E2p12 copies) and dihydrolipoamide dehydrogenase dimer (E3) (FAD containing) residing in the inner mitochondrial membrane[4](Fig. 3) . The (E1p) and E3subunits surround a 60-meric dodecahedral core of 40 copies of E2p and 20 copies of a monomeric non catalytic component, E3-binding protein (E3BP), which specifically tethers E3 dimers to the pyruvate dehydrogenase complex[18]. Each E2p subunit contains two consecutive lipoic acid-bearing domains (LBDs), termed as L1 and L2, one subunit binding domain (SBDp) which binds E1p and the inner-core/catalytic domain containing the E2 p active site responsible for the self assembly of the core which connects with the other independent domains by unstructured linkers[3](Fig.3). Similarly, each E3BP subunit consists of a single LBD (referred to as L3), the E3-binding domain (E3BD) and the noncatalytic inner core domain. It is presumed that the lipoyl bearing domains LBDs (L1, L2, and L3) and 60 subunits of the transacetylase seem to form a free circulation of lipoyl groups among which the acetyl groups are freely exchanged[18] and shuttle between the active sites of the three catalytic components of the PDC during the oxidative decarboxylation cycle[19]. Unspecified copies of each PDC regulatory enzyme pyruvate dehydrogenase kinases and pyruvate dehydrogenase phosphatases are also strung non-covalently to the core by the LBD2[5,20]. These regulate PDC activity by a reversible phosphorylation/dephosphorylation mechanism that involves covalent modification of E1p subunit[21]. The PDC complex catalyzing through its E1p, E2p and E3 components linked by substrate channeling carries out the oxidative decarboxylation of pyruvate to yield acetyl-CoA and reducing equivalents (NADH). It serves as the gate keeper enzyme that strategically links glycolysis, Krebs cycle and lipogenic pathways[4,5]. In the nervous system it is involved in the production of acetyl choline and for myelin synthesis[5]. The complex also requires 5 different coenzymes: CoA, NAD+, FAD+, lipoic acid and thiamine pyrophosphate (TPP). While thiamine pyrophosphate, lipoic acid and FAD+ are tightly bound to enzymes of the complex and the other two (CoA and NAD+) are employed as carriers of the products of PDH complex activity.

Active Site of PDE1

These are 2 in number, each binding cofactors thiamine pyrophosphate TPP and magnesium ion. The alpha subunit binds magnesium ion and pyrophosphate fragment whereas beta subunit binds the pyrimidine fragment of TPP, forming together a catalytic site at the interface of the subunits[6]. There is active communications between the cofactors in the enzyme complex. Only one of the two thiamine molecules is in the chemically activated state while the inactive one ionizes at much lower magnitude in a model of ping pong kinetics .The active site synchronization over a distance of 20 Angstroms via proton wire through an acidic tunnel in the protein , keeps the active sites in an alternating activation state[22]

Regulation of the PDH Complex

Alterations in the nonphosphorylated active/ phosphorylated inactive state of the PDE1 component which also catalyzes the rate limiting step in the overall PDC reaction is the prime pathway in which the rate of glucose oxidation is maintained *in vivo* in mammals[21]. This process is deputed to two dedicated enzymes pyruvate dehydrogenase phosphatases (PDP) and pyruvate dehydrogenase kinases (PDK) which have 4 isoforms 1-4 and are tethered to the PDC by LBD of the E2 p subunit[18]. Phosphorylation by PDK inactivates E1 resulting in the PDH-b (the inactive form) and thus, decreased PDC activity. Whereas dephosphorylation by PDP has the opposite effect and results in PDH-a, the non-phosphorylated, active form of PDH[5,22-25]. PDK activity is

stimulated by the overall products of the reaction, *i.e.* by NADH and acetyl-Coa, Mg and ATP, causing negative allosteric effects on PDH-a, (active) form and are inhibited by Ca and pyruvate[22]

Phosphorylation of the heterotetrameric (α2 β2) E1p component is essential for the inactivation of the human PDC which occurs at 3 serine residues of the alpha subunit. Two of these sites are located in the conserved phosphorylation loop A[6] which forms one wall of the active site channel and helps to anchor ThDP to its active site.Site 3 is in the phosphorylation loop B which provides coordination to magnesium chelated by the THDP potassium. Phosphorylation of any of the 3 sites inactivates E1p and drastically reduces the affinity for pyruvate[24]. Disordered loops of E1p arise from phosphorylation and result in downregulation of the PDC activity. Binding of the cofactor ThDP induces ordering of both the loops which then can mediate decarboxylation and reductive acetylation of the pyruvate. Phosphorylation of PDC is crucial in regulating carbohydrate and lipid metabolism[14,25]. Starvation and diabetes increase phosphorylation that inactivates PDC, leading to impaired glucose oxidation[26,27]. On the other hand prevention of PDC phosphorylation by specific PDK inhibitor, dichloracetate increases reactive oxygen species levels in the mitochondria leading to cellular apoptosis and the inhibition of tumour growth[28,29]. Therefore the regulation of PDC flux by reversible phosphorylation is a potential target for obesity and cancer[30,31].Finally the expression of PDK2and PDK4 is down regulated by insulin in the long term[32,33]. In the animal model, downregulation of skeletal muscle pyruvate dehydrogenase in the rat model before and after the onset of diabetes mellitus has been observed[34].

Dephosphorylation/activation of the PDC is ascribed to two Mg and Ca dependant genetically and biochemically distinct isoforms of pyruvate dehydrogenase phosphatase PDP heterodimeric (PDP1&PDP2), which are important regulators of PDC activity. PDP1 has both a catalytic (PDPc) subunit bound to the inner mitochondrial membrane eand a regulatory (PDPr) subunit[35]. Both PDP1 components are targeted by insulin which enhances PDPc activity and lessens PDPr negative control resulting in enhanced overall PDP1 efficiency.These effects are at the core of insulin signaling of PDH[36]. PDP2, recently discovered in rat tissues consists of a catalytic subunit insensitive to Ca, 10 fold less sensitive to Mg than PDP c is also considered a target in insulin signaling[37,38]. In humans too, down regulation of PDP in obese subjects is a malfunction that signals insulin resistance[39].

Diseases Produced by Defective PDC

As the PDC has prime significance in intermediary metabolism, mutations in the genes encoding for PDCsubunits produce severe clinical phenotypes[40]. Congenital defects in E1p in the X linked gene lead to lactic acidemias, encephalopathies, neuronal dysfunction in infancy[40]. Mutations in the E2, E3BP cause primary biliary cirrhosis leading to liver failure[41,42], autoimmune hepatitis[43] and neurodegenerative conditions such as Alzheimer's disease. Combined enzyme deficiencies of α-ketoacid dehydrogenase complexes pyruvate dehydrogenase complex, BCKDC and ketoglutarate dehydrogenase complexes have been observed due to genetic changes in human E3[44] resulting in lactic acidemias and maple syrup urine disease[45-47]. Other anamolies of the PDC include autoantibodies leading to paediatric biliary cirrhosis[47]. Additionally, the aberrant down-regulation of pyruvate dehydrogenase complex activity by reversible phosphorylation has been shown to be contributory to hyperglycemic states observed in type-2 diabetes[25], increasing the chances of pyruvate dehydrogenase complex as a therapeutic target for a 150 million people affliction *i.e.* diabetes). Failure of functioning of the pyruvate dehydrogenase complex and specially of its E1p subunit due to lack of thiamine vitamin B1 would therefore inevitably lead to poor handling of glucose and its substrates and could manifest as deleterious effects in type 2 diabetics.

The Alphaketoglutarate Dehydrogenase Complex

The human 2 ketoglutarate dehydrogenase complex while extensively studied has not yet been reconstructed in vitro and reliance on other mammal models persists[5,48](Fig 4**).**

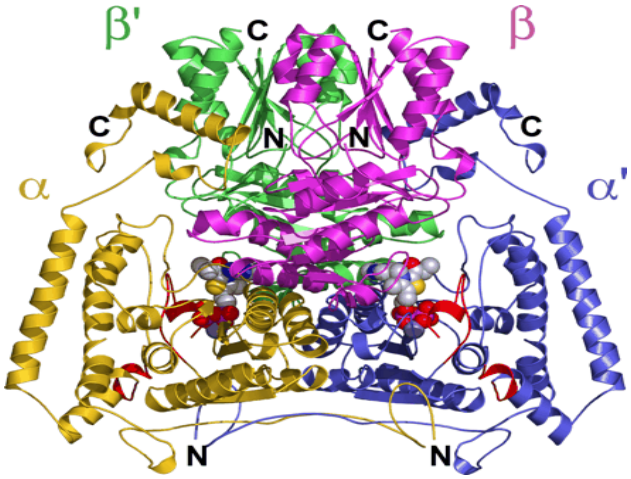


Fig 4 Representative Model for Human 2 Ketoglutarate Dehydrogenase Complex:
Identical conformation in the two active sites of the human E1b heterotetramer.of the Human Branched alpha ketoacid Dehydrogenase Complex .Ribbon representation of S320Pα human E1b in the crystal form containing an entire heterotetramer in the asymmetric unit. The N and C termini are indicated. All figures of molecular structures were created with the program PyMol (DeLano Scientific, San Carlos, CA). Jun Li. The Journal of Biological Chemistry, 2007;282, 11904-913.

Structure of Alphaketoglutarate Dehydrogenase Complex

This 4 to 10 mega Dalton supramolecular complex is organized around a polyhedral form of a cubic core of 24/60 lipoate bearing dihydrolipoyl succinyltransferase E2 subunits (8 trimers) arranged with octahedral (432) symmetry[5] associated with non covalently attached multiple copies of dihydrolipoamide E1k and dihydrolipoamide E3K individually held via its E1/E3 binding domains which serve as scaffolds for the E2 core. There is also biochemical evidence of E3 binding to the aminoacid terminal region of E1 terminal allowing for separation of a stable E1-E3 submolecular complex from the E2 core[49].Also attached are regulatory kinase and phosphatase units[50]. Further lipoyl bearing domains LBDs of the E2 core are attached serving as swing arms impart substrate chanelling by sequentially visiting the different active sites in each of the three E1, E2 and E3 catalytic components [51] to transfer acyl groups to the active site of E2 leading to oxidative decarboxylation of the alpha ketoacids[51]

Nomenclature and Function of the Alphaketoglutarate Dehydrogenase Subunits:

The complex has 3 main enzymatic components with multiple subunits & copies and varied names: oxoglutarate dehydrogenase (lipoamide); EC: 1.2.4.2 (E1k), dihydrolipoamide S-succinyltransferase; EC:2.3.1.61 (E2k) and dihydrolipoamide dehydrogenase; EC:1.8.1.4 (E3k)[52].

1. **Alpha ketoglutarate dehydrogenase/2 oxoglutarate dehydrogenase E1k heterotetramer** (2 alpha and 2 betachains) (53) component has 6 copies (lipoamide) polypeptide enzyme having mol wt 115.94 kDa (from nucleotide sequence) and sequence length 34160 aminoacids.It is encoded by the OGDHgene localized on chromosome 10 , 54290aa & 7 at p13-p14[54] containing 22 exons spanning 102483 bpairs[55,56]. It contains a thiamine diphosphate cofactor and catalyzes thiamine diphosphate dependant decarboxylation of 2 oxoglutarate and subsequent reductive acylation of the oxidized lipoyl moiety LBD (lip-LBD-S2) which is covalently bound to the E2 component dihydrolipoamide succinyl transferase[5]. Thiamine diphosphate is tightly but not covalently bound to the 2-oxoglutarate dehydrogenase component[57] ThDP remains an essential cofactor and alphaketoglutarate dehydrogenase complex in the form of homo dimers alpha2, homo tetramers alpha 4 or heterotetramers alpha 2 beta 2 contain ThDP binding pockets that constitute two or four active sites for this enzyme which operate independently without an obligatory alternating mechanism in the E1b component[58] and overall activity is abolished at 50% phosphorylation (1 of 2 sites) within each active channel similar to PDC[59].
2. **Dihydrolipoamide S- succinyltransferase E2k** core has 24 /60 copies containing lipoyl active site as well as active sites for E1 and E3 subunits based on similar mammalian

PDC structural studies and molecular wt of 64.5 KDa[5]. It is encoded in gene DLST located on chromosome 14 q24.2-q24.3 with a length of 21815 base pairs [60]. This inner core plays an essential role in mediating the E1 catalyzed decarboxylation of 2 oxoglutarate and reductive acylation of the lipoyl moiety and E3 catalyzed reoxidation of the dihydrolipoyl moiety.

3. Located in the mitochondrial lumen, **Dihydrolipoamide dehydrogenase E3k** or E3component a flavoprotein (dimer) has 12 copies, a sequence length of 28796 aminoacids and is 54.15kDa in weight. It is encoded in the DLD gene localized to 7q31-q32 [61], its function is to catalyze the transfer of electrons from dihydrolipoamide to NAD+ and bears close structural and functional approximation to the PDE3 component of pyruvate dehydrogenase and its full complex contains 6 dimers [5].

Function

The alphaketoglutarate dehydrogenase complex EC 1.2.4.2 also termed as oxoglutarate dehydrogenase complex, acts on alphaketo-glutarate/2 oxoglutarate a key intermediate in the krebs cycle converting to succinyl co A, produces NADH and CO2 in an irreversible reaction[62] KGDHC catalyzes a vital step in the Krebs cycle, which is also a step in the metabolism of the potentially excitotoxic neurotransmitter glutamate. It allows amino acids to enter the citric acid cycle and produce energy; this is a reversible reaction in which glucose which enters the cycle can leave it to make amino acids thus linking amino acid pathways to the citric acid cycle. It also participates in lysine degredation and tryptophan metabolism. Alpha-KGDH is vital for maintaining NADH supply to the respiratory chain and is limited only when alpha-KGDH is also inhibited by ROS. In addition being a key target, it is also able to generate ROS during its catalytic function which is regulated by the NADH/NAD+ ratio[63]. Its cofactors are TPP bound to E1, lipoic acid covalently bound to lysine on E2 which accepts the hydroxyethyl carbanion from TPP as an acetyl group, coenzyme A which is substrate for E2 and accepts the acetyl group from it, FAD bound to the E3 subunit reduced by lipoamide and NAD which is substrate for E3 and reduced by FADH2 [64].

Regulation:

Basic short term regulation of KGDHC is through adenosine diphosphate ADP, P (i) and Ca2+; these positive effectors increase manifold the affinity of ketoglutarate dehydrogenase complex to alpha-ketoglutarate. While KGDHC inhibitors are NADH, adenosine triphosphate, succinyl-CoA, and thioredoxin protects KGDHC from self-inactivation during catalysis [65]. Alpha-KGDH is also sensitive to oxidative stress and a number of metabolites modify the activity of KGDHC, including inactivation by 4-hydroxynonenal. In the human brain, comparison of KGDHC activity to other enzymes of energy metabolism like aconitase, phospho-fructokinase and the electron transport complexes shows it to be lower than all of them. Therefore impairment of KGDHC function is likely to disturb brain energy metabolism and result in brain disease[66].

Diseases Produced by a Dysfunctional Alpha-ketoglutarate Dehydrogenase Complex

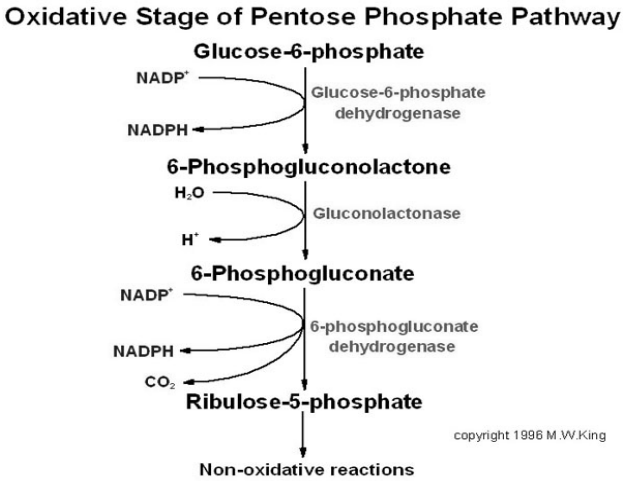
The alpha-ketoglutarate dehydrogenase complex (KGDHC) is an important mitochondrial enzyme and the reduction in 2-oxoglutarate dehydrogenase complex activity can be linked to several aspects of brain dysfunction such as elevation of GABA shunt and glycolysis.Such changes may maintain normal energy metabolism but enhance the vulnerability of the cells to changes such as occur with oxidants[67] and neuropathology in a number of neurodegenerative diseases[66]. Deficiencies of alpha ketoglutarate dehydrogenase complex C are likely to impair brain energy metabolism and therefore brain function, and lead to manifestations of brain disease associated with modifications in mRNA of E2k and E3 sub units in sub thalamic brain regions[67]. In Wernickes encephalopathy there is AKGDH and thiamine deficiency associated with increased oxidative stress markers, lipid peroxidation resulting in neuronal cell death in pons, thalamus and cerebellum[68,69]. In general, the clinical manifestations of KGDHC deficiency relate to the severity of the deficiency. A range of disorders have been recognized: varying

from psychomotor retardation in childhood, to intermittent neuropsychiatric disease with ataxia and other motor disabilities, such as Friedreich's and other spinocerebellar ataxias[70], as well as neural diseases where mental deficits are also visible such as Parkinson's disease, and Alzheimer's disease (AD)[70]. KGDHC is not evenly distributed in human brain, and the neurons that appear more vulnerable to such damage lie in human temporal cortex. Variations in KGDHC that are not damaging during reproductive life become so with aging perhaps by predisposing this mitochondrial metabolon to oxidative damage[66]. In Parkinsons Disease which has been deeply investigated, KGDHC Activity is reduced, coupled to elevated levels of monoamine oxidase B[71] and cytosolic accumulation of cytochrome c which inturn activates other pathways, including cell death cascades and enzyme inhibition which alters Ca2+ homeostasis[72] The KGDHC enzyme is further a target for ubiquitination-dependent degradation in mitochondria by binding of Siah2, the RING finger ubiquitin-protein isopeptide ligase 2, encoded by gene siah2[73]. Diabetes mellitus, thiamine dependent megaloblastic anaemia and sesorineural deafness associated with deficient alpha ketoglutarate dehydrogenase activity have also been reported[74].

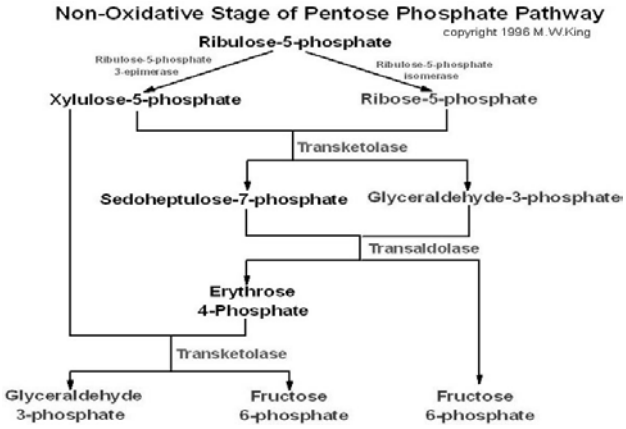
The Pentose Phosphate Pathway (also called Phosphogluconate Pathway or Hexose Monophosphate Shunt):

There exist 2 wings ,oxidative and reductive of the pentose phosphate pathway(Fig 5). The oxidation steps, utilizing glucose-6-phosphate (G6P) as the substrate, occur at the beginning of the pathway and generate 2 moles of NADPH. The reactions catalyzed by glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase are essential for the conversion of hexoses to pentoses[75].

The non-oxidative reactions of the pentose phosphate pathway are mainly functioning to produce ribose 5 phosphate, and equally significantly to convert dietary 5 carbon sugars into both 6 (fructose-6-phosphate) and 3 (glyceraldehyde-3-phosphate) carbon sugars which can then be utilized by the pathways of glycolysis[76].



A) Digrammatic Representation of the Oxidative Stage of Hexose Monophosphate Shunt



B) Reductive or Non Oxidative Stage of the Hexose Monophosphate Shunt

Fig. 5: (A): Digrammatic Representation of the Oxidative Stage of Hexose Monophosphate Shunt and (B) Reductive Stage of the Hexose Monophosphate Shunt

Transaldolase (involved in redesigning of the carbon backbone of the PPP substrates) and transketolase are the primary enzymes involved in the non-oxidative steps of the pentose phosphate pathway:

Importantly transketolase the TPP dependant enzyme functions at two sites to transfer 2 carbon groups from substrates of the PPP, thus also reorganizing the carbon atoms that enter this pathway and generating the 3 carbon sugar glyceraldehyde-3-phosphate which can be shunted

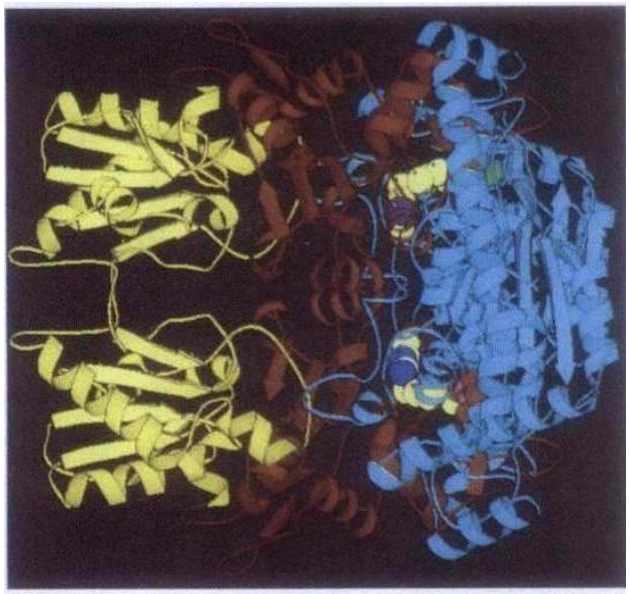
to glycolysis and oxidized to pyruvate.[64]

Functions of the Pentose Phosphate Pathway in Normal and Diseased Conditions:

The Pentose phosphate pathway is primarily energy forming, and non mitochondrial with only a cytoplasmic enzymatic presence entrusted to utilizing 6 carbon sugars, and producing in turn 5 carbon sugars for the synthesis of neucleotides, nucleic acids and reducing equivalents in the form of NADPH. The pentose phosphate pathway is a metabolic redox estimator and regulates transcription during the anti-oxidant response, as a shift from primary carbon metabolism, is fastest in oxidative stress[77]. NADPH cofactor serves as reducing equivalent in the endoplasmic reticulum lumen for fatty acid and steroid biosynthesis in hepatic and, adipose tissue, adrenal cortex[78]. High levels of PPP enzymes are in neutrophils and macrophages as they utilize NADPH to produce ROS to destroy engulfed microbes in a process termed as respiratory burst[79]. G6PD deficiency effects red blood cell viability dependent on PPP generated NADPH , a glutathione reducer, the absence of which results in hemolysis seen with certain drugs and diseases like malaria which cause oxidative stresss[80]. Cancer cells are known to access successfully the glucose flux in the pentose phosphate pathway supporting NADPH and reactive oxygen species production and glutathione reduction[81] responding to both incremental and decremental reactive oxygen species[82]. Electron leakage from the mitochondrial electron transport remains essential (through the action of ribonucleotide reductase) in generating deoxyribonucleiotides from nucleotides as well producing ROS in collusion with oncogenes[83] and molecular oxygen[84] promoting genetic damage in normal cells and therapy resistance in cancerous cells[85].Malignant cells also use reduced glutathione[81] or NADPH to combat oxidative stress and to support the oxidation of fatty acids in detached cells [86].

Transketolase Enzyme (TKT) (EC 2.2.1.1):

Transketolase is the premier cytosolic enzyme of the reductive pentose phosphate pathway. Its 3 genes TKT, Transketolase like TKTLI and Transketolase like TKTL2 encode for proteins with transketolase activity.All of them participate in the reductive pentose pathway reactions catalyzing transfer of a 2 carbon fragment from a ketose donor to an aldose (acceptor substrate)[87].



Adapted from Kochetov2005, Lindquist 1992

Fig. 6: Schematic View of Transketolase Dimer Showing its Different Components. The 3 components are colour differentiated: N terminal domain, light blue, middle domain, light brown & C terminal domain yellow.The bound cofactor ThDP is shown as a CPK model and Ca++ion in green

Nomenclature

Transketolase: synonymous with TKT1 &TK is composed of and encoded by the TKTgene located on chromosome 3 (30390 bp)[89-91].

Transketolase like protein 1: named as TKT2, TKR, TK 2, Transketolase 2, Transketolase-related protein has molwt of 60-70 KDaltons depending on splice variation encoded by the TKTL1 gene located on chromosomeX Length: 25052 bp[92, 93].

Transketolase like protein 2 termed TK is composed of 913 aminoacids encoded by gene TKTL2 located on chromosome 4 having length of 2742 bp[94].

TKT Structure:

Transketolase (TK) is a homodimer [95] (Fig 6) and the least structurally complicated member of thiamine diphosphate (ThDP)-dependent enzymes PDHC & OGDHC [96]. Each monomer consists of three distinct regions the N terminal or PP binding region, the middle or pyrimidine binding region and C terminal region[87]. The first 2 regions are associated with coenzyme binding while the role of the third remains unknown [85, 97].

Thiamine Binding Site:

TKT has two active centres with one THDP molecule attached to a binding motif [98, 99] and a bivalent cation (Ca affinity more than Mg[100]) tightly bound at each centre by noncovalent interactions[101]. Thiamine binding site is located within a deep furrow which allows only the C2 atom of the thiazolium ring to be exposed to the donor substrate [101]. A highly conserved starter sequence glycine-aspartate-glycine GDG and concluding sequence asparagine- asparagine (NN) represent this site between residues 154 and 185[101]. Further the interactions of the non-covalently bound coenzyme ThDP-magnesium with the protein component are at five critical sites containing arginines (Arg 101, Arg 318, Arg 395, Arg 401 and Arg 474and Asp155)[101] contribute to dimer formation, stability or catalytic activity[102,96]. The dimerization process involves initial binding of magnesium to the aspartate in the starter sequence which inturn interacts with the pyrophosphate molecule of the thiamine diphosphate through hydrogen bonding[101], followed by one transketolase monomer engaging the pyrophosphate moiety and the other with the thiazolium and pyrimidine rings of ThDP [88,97]. The importance of this interaction is reflected in the noticeable refractoriness in Wernickes encephalopathy to thiamine treatment alone in hyomagnesemic alcoholics[103]

Mechanism of Transketolase Reaction:

This enzyme has a 2 stage catalytic cycle central to which is the TPP molecule, initiated by the deprotonation in its thiazolium ring due to interaction with Glu 418 of apotransketolase.

Role of Transketolase in Disease and Therapy:

Transketolase enzyme genetic variants and depreciated enzyme activities were noted in neurodegenerative diseases like Wernickes Korsakoff syndrome and Alzheimers disease[104]. Upregulation of the TKT L1 gene has been found in a number of malignant disorders resulting in enhanced total transketolase activity and cellular proliferation in human colon cancer[105], thyroid[106], cervical[107], ovarian cancer [108], nephroblastoma and adenocarcinoma. Its increased expression is found to be a potential diagnostic biomarker for breast cancer[109] and prognostic biomarker for nasopharyngeal[110] and laryngeal squamous cell carcinoma[111]. The reason may lie in the role of tranketolase in the reductive pentose pathway which remains a source a carbons such as in ribose required for neucleotide synthesis, NADPH and reduced glutathione in addition to aromatic acids and fatty acids required for cellular growth in general and explosive growth in particular. Transketolase has begun to emerge as a target in the cellular immune response in multiple sclerosis [112]. Human transketolase can be used in structure-based drug design as target for inhibition in the treatment of cancer[113] and in the search for new transketolase inhibitors as non permanently charged thiamine analogs ,which are substrates for the thiamine activator thiamine pyrophosphokinase. These pyrophosphate analogs antagonize the ability of transketolase in vitro[113].

In diabetes mellitus type 2 experimental model, the role of transketolase in the reductive pentose pathway and its activation by administration of lipid soluble thiamine derivative benfotiamine is well documented and undeniable[114] and further clinical research is ongoing.

Pharmacotherapeutics of Type 2 Diabetes:

Treatment is done using 4 categories of oral antidiabetic drugs.

1. Insulin secretagogues: Sulfonylureas, meglitinides, D-phenylalanine derivatives
2. Those reducing insulin resistance:
   i) Biguanides
   ii) Thiazolidinediones (glitazones)
4. Those decreasing carbohydrate absorption from the gut: Alpha Glucosidase inhibitors.

Insulin Secretagogues:

i) **Sulfonylureas:**

These act by stimulating insulin release from pancreatic B cells. Sulfonylureas may also act by decreasing hepatic insulin clearance[115]. They increase insulin concentration often failing to improve first phase insulin release in response to a glycemic challenge. There is secondary failure and tachyphylaxis to sulfonylurea therapy following prolonged use. Their adverse effects are hypoglycaemia, GIT disturbances, cholestatic jaundice, agranulocytosis, aplastic and hemolytic anemia, generalized hypersensitivity and dermatological reactions[116]. There is also a debate on associated cardiovascular mortality – due to blockage of KATP channels of the hearts and vascular tissues [117].

Second generation sulfonylurea glimepiride is useful as single therapy in previously drug naïve patients and also in combination with non-secretagogue medication[118]. Glimepiride may be linked to lower incidence of hypoglycaemia[119] and may improve insulin sensitivity [120]. It also has an insulin sparing action[121].

ii) **Meglitinides:**

Like the sulfonylureas, meglitinides also stimulate insulin secretion. Repaglinide has a very rapid onset of action and therefore it is indicated for use in controlling post prandial glucose excursion. There is no sulfur in its structure therefore it can be used in Type 2 diabetic allergic to sulfonylureas[116].

iii) **D-phenylalanine derivatives:**

Netaglinide is the latest insulin secretagogue to become available. It selectively enhances early insulin release providing excellent meal time glucose control while reducing total insulin exposure[122]. It has a half life of 1.5 hrs and short action of 4 hrs therefore; it does not require dosage adjustment in geriatric patients and also those with mild hepatic disease or nephropathy.

iv) **Biguanides:**

These agents don't cause hypoglycemia and are thus called euglycemic agents. Current proposed mechanisms of biguanides include glycolysis simulation in tissues, reducing glucose absorption from GIT with increased glucose to lactate conversion, reduced hepatic and renal gluconeogenesis, in the GI tract and reduction of plasma glucagon levels[123]. Most frequent toxicity are gastrointestinal (anorexia, nausea, vomiting, abdominal discomfort and diarrhea). It is contra indicated in patients with hepatic disease or in conditions predisposing to tissue anoxia because of risk of lactic acidosis[124]. The most commonly used drug from this group is metformin which is available by itself as glucophage. In combination with glyburide (sulfonylurea) and an extended release form (glucophage XR). The metformin/glyburide combination shows better glycemic control and ultimately results in lesser chronic complications because both prongs of Type 2 diabetes i.e relative insulin deficiency and insulin resistance are handled simultaneously. With the glucophage XR better compliance is achieved due to simplification of the drug regimen and therefore enhanced ability to treat a chronic disease like Type 2 diabetes mellitus.

v) **Thiazolidinediones (glitazones):**

They are also considered to be euglycemic and are effective in 70% users. Three drugs have been used clinically from this group (Troglitazone, Rosiglitazone and Pioglitazone). Troglitazone a severely hepatotoxic and its removal from public use is well known. These are selective agonists for nuclear peroxisome proliferator – activated receptor – gamma (PPAR GAMMA) whose activation enhances insulin responsive genes that regulate carbohydrate and protein metabolism[125]. The PPAR GAMMA receptors are found in muscles, fat and liver. Thiazolidinediones also affect vascular endothelium, immune system, ovaries and tumor cells. As they affect genes,

these should not be used during pregnancy, any significant disease and heart failure [124].

vi) **Alpha Glucosidase Inhibitors:**
Competitive inhibitors of intestinal alpha glucosidases namely acarbose and miglitol decrease the post meal digestion and assimilation of simple and complex carbohydrates such as starch and disaccharides[126]. These are effective also in prediabetic individuals and successfully restored β cells function. Therefore, diabetes prevention may be a further indication for their usage[127]. Prominent adverse effects include flatulence, diarrhea and abdominal pain. Some time hypoglycemia may occur with concurrent sulfonylurea treatment. There use cautioned in patients with inflammatory bowel and hepatic disease.

NEW DRUGS FOR TYPE 2 DIABETES:

Currently available

The Incretin hormones released by the gut, gastric inhibitory peptide (GIP) and Glucagon like peptide1 (GLP-1) (liraglutide) stimulate insulin secretion upon nutrient entry into the gut, suppression of glucagon release,slow gastric emptying and decrease food intake[128, 129]. Therefore, they have an antidiabetogenic potential. Incretin mimetics e.g. Exenatide LAR from exendin 4 is currently in use and most resistant to DPP4 degredation. GIP has also been shown enhancing β cell proliferation and inhibiting apoptosis in islet cell lines[130,131]. Additionally functional GIP receptors have been identified on adipocytes and shown to stimulate glucose transport, accelerating fatty acid synthesis and stimulating lipoprotein lipase activity in animal models[131,133,134]. Several novel GIP analogues have been developed which act as stronger GIP agonists, showing resistance to degradation by Dipeptidyl Peptidase-4 (DIPP-4)[135] and demonstrating increased insulinotropic and blood glucose lowering activity[135]. Dipeptidyl peptidase Inhibitors (vildagliptin & sitagliptin) suppress breakdown of Glucagon like peptide1 (GLP-1) show great potential and are undergoing clinical testing. Antihyperglycemic synthetic analogs of amylin a hormone which are produced by the pancreas to lower blood sugar levels are available in injectable form and require close monitoring. Dapagliflozin a renal glucose reabsorption inhibitor reduces glycemic reabsorption independent of insulin, promises to be a new drug for type 2 diabetes treatment[136]. Testosterone replacement therapy in diabetic hypogonadal men decreases insulin resistance[137] probably by protective effect on pancreatic beta cells through its action on inflammatory cytokines[138].

Experimental new drugs

A vanadium and allixin based drug[139] and macrophage migration inhibitory factor MIF blocking inhibitory synthetic oral drug reducing blood sugar levels was tried in the mouse model and found to be effective in both the type 1&2 diabetc model[140]. Lisofylline, a fat metabolism inhibitor which prevents buildup of ceramide a by product of fat metabolism in mouse skeletal muscle decreased the insulin resistance and thus appears to be a novel new approach for type 2 diabetes[141] . It also has the ability to protect insulin producing cells by inhibiting cytokines produced by immune cells leading to apoptosis and cellular dysfunction and is thus effective in type1 diabetes[142]. LXR agonists have shown potential and require further testing in human and model systems[143]. Growth factors and protein kinase C inhibitors may act as innovative therapies for diabetic retinopathy[144].

Surgical interventions

Recently a type of gastric bypass surgery has been successful in normalizing blood sugar in a small number of normal to moderately obese type 2 diabetics [145, 146]. This surgery may possibly reduce death rate by 40% from all causes in morbidly obese people [147].

Micronutrient Approaches to Treatment of Diabetic Complications

People with diabetes have reduced antioxidant capacity which lays the basis for usage of antioxidant vitamins such as β carotene or vitamin C or E. A reduced level of ascorbic acid (Vitamin C) leaves the body more at mercy to the

detrimental effects of aldose reductase, an enzyme responsible for many diabetic complications, such as cataracts and peripheral neuropathy [148]. Quercetin is another powerful aldose reductase inhibitor. It has been shown to inhibit aldose reductase by upto 50% [149]. Vitamin E is a free radical scavenger. It may play a preventive role in diabetic retinopathy by decreasing DAG levels, normalizing protein kinase C activation, normalizing blood flow in retinal and renal microvasculature and restoring NO mediated endothelium dependent relaxation[150, 151]. Renal and retinal vascular flows and responses were normalized in individuals who had diabetes of less than 10 years duration with high dose oral vitamin E therapy given for short periods while unchanged glycaemic control was observed[152].

Magnesium and chromium deficiency have been associated with poor diabetic control, insulin resistance, macro vascular disease and hypertension [153] and decreased glucose tolerance respectively[154]. Reduction of neuronal damage in diabetics by inhibiting glutamate dehydrogenase via vitamin B6 therapy has also been observed[155].N-Reduced glutathione precursor NAcetyl Cysteine is a gene expression and cellular metabolism modulating antioxidant and its role in prevention of β cell oxidatory damage by acting as NFKB (a genetic regulator) inhibitor and subsequent deintensification of inflammatory responses is well documented[156]. Trace element vanadyl sulfate that behaves like insulin normalized hyperglycemic levels in diabetic animals and decreased the insulin need by upto 75% [157]. In human with Type 2 diabetes, low doses of vanadyl sulfate enhanced insulin responsive glucose uptake, glycogen production and decreased endogenous glucose formation. This resulted in reduced lipid oxidation and plasma free fatty acids levels[158]. Alpha lipoic Acid has powerful antioxidant activity, insulinomimetic action and provides protection from insulin resistance linked diabetic stress while improving glucose utilization [159]. Hyperglycemia reduction in diabetic rats was observed along with improvement in GSH levels with selenium therapy[160].Calcium AEP has benefited both type 1 and type 2 diabetics as it is alpha cell membrane integrity factor required for cellular membrane function. The hormone dehydroepiandrosterone (DHEA) undergoes a decrease in levels with aging that many researchers have linked to impair glucose metabolism. It was found to be as effective in reducing body fats and maintaining insulin responsiveness as exercise[161]. Thiamine is also now showing potential as therapy for type 2 diabetes.

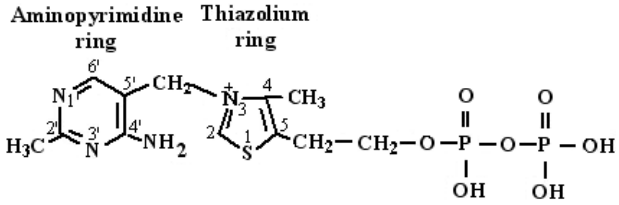


Fig. 7: Structure of thiamine diphosphate molecule.

Thiamine (termed aneurin or antineuritic vitamin initially) was the premier discovery of the B vitamins and thus ranked vitamin B1 (Fig. 7). It has relative temperature, acid stability and water solubility containing a pyrimidine ring and a thiazole nucleus linked with a methylene bridge. Thiamine is an essential micronutrient with a dietary reference intake (DRI) for normal healthy subjects of 1.1 mg/day for females and 1.3 mg/day for males[162]. Found in range of foodstuffs such as cereal grains. Its rich sources are brown rice, bran, oat meal, flax, poultry, egg yolks, beef, pork, liver, nuts, fruits and vegetables such as oranges, asparagus, kale, cauliflower, potatoes[163].UK law demands compulsary fortification of flour with thiamin of not less than 0.24mg/100g flour to replace losses during milling. In Pakistan no compulsory fortification is done and the general public consumes milled white flour which is easily available and probably thiamine deficient. Thiamine is naturally found in 4 forms in varying degrees of phosphorylation in TMP thiamine monophosphate, TPP thiamine pyrophosphate or diphosphate and TTP = thiamine triphosphate. It is commercially available as salt in its mononitrate HCl (also natural byproduct) and relatively inaccessible semi lipid soluble form S-acyl derivative benfotiamine and truly lipid soluble thiamine disulphide derivatives sulbutiamine and fursultiamine. Out of these, Thiamine HCl is the water soluble, easily accessible and commonly used vitamin supplement available with the trade name Benerva.

Pharmacokinetics:

Thiamine Absorption in Normal Conditions:

Thiamine is released from its administered form by phosphatase and pyrophosphatase in the proximal part of the small intestine, following which absorption occurs mainly from this site with some from the stomach and the colon; thiamine absorbed in the colon may originate from intestinal microflora. Its absorption is hindered by alcohol consumption and folic acid deficiency[164].An organic cation requires high affinity organic anion transporters THTR1[165], THR2 and reduced folate transporter RFC-1 of both folic acid thiamine monophosphate (TMP) intracellularly[166, 168] across cell membranes at normal physiological concentrations. At high expression levels RFC1 transports TPP out of the cells[167]. At higher concentrations thiamine crosses cell membranes in its open unionized form of the thiazolium ring even by passive diffusion.THTR2 is placed on the luminal surface of the gastrointestinal epithelial cells and THTR1 is on the basolateral surface mainly but not exclusively[169]. THTRI is expressed widely in human tisseues with particular high expression in skeletal muscles, placenta, heart liver and kidney[168,170].Mutations in the SLC19A2 (D93H, S143F and G172D) cause malfunctioning of the thiamine transporter THTR1, thiamine deficiency and thiamine responsive megaloblastic anaemia (TRMA)[171,172]. THTR2 is widely expressed most abundantly in placenta, kidney and liver[173]. Also highly expressed RFC-1, is in human tissues including mitochondrial membranes[168, 174]. It has affinities for TMP and TPP of 26µM and 32µM respectively [167,168]. Cellular efflux is the probable reason for the presence of thiamine in plasma and cerebrospinal fluid[175-177].Thiamine in the glomerular filtrate is reabsorbed by the renal brush border membrane high affinity transporters where influx was increased by an outward directed H+ gradient[178] RFC-1 is expressed on the apical and basolateral surface of the proximal tubular epithelial cells[179]; it may mediate the reuptake of TMP and provide a solution to the normal absence of TMP in the urine. Proton antiport membrane transport may operate in both intestinal and renal proximal tubular thiamine uptake[180]. In the plasma thiamine is bound to plasma proteins primarily albumin. A hormonally regulated rat serum thiamine binding protein TBP is believed to be important for tissue distribution[163].

Assessment of Thiamine Status:

Erythrocytes contain approximately 90% of total thiamine in the blood and therefore conventionally their transketolase levels have generally been considered to be the measure of thiamine status in the body[181] .Thiamine deficiency is assessed conventionally by measuring the percentage below complete saturation of the thiamine dependant enzyme transketolase (TK) in RBCs-"thiamine effect". The normal value of the thiamine effect in human subjects is in the range 0-15%, mild deficiency is 15-25% and severe thiamine deficiency >25%[182]. Latest research has however questioned its reliability as thiamine transporters THTR1 and RFC1 in erythrocytes are upregulated in thiamine deficieny and RBC TK levels are not decreased in tandem[183]. Furthermore it doesn't account for changes in TK expression in RBC and other precursor cells. The expression of TK is decreased in thiamine deficiency[184]. Currently assessment of mononuclear TK activity and plasma thiamine concentration determination using HPLC flourimetric determination with respect to normal healthy controls gives greater insight into thiamine status[185-187]. More recently in capillary enzyme reaction and capillary electrophoresis methods are emerging as potential alternative monitoring and determining techniques for thiamine in samples[188].

Thiamine Metabolism within the Cells:

Once transported into the cells by THTR1 and THTR2 thiamine is converted to TPP by thiamine pyrophosphokinase (TPPK). Human TPPK has high expression in the testes, small intestine and kidney with moderate expression in brain, liver, placenta and spleen[167]. When TMP enters cells by RFC-1 it is hydrolyzed to thiamine by phosphatases[168]. Thiamine deficiency decreased the activity of TPPK[189] and was implicated in decreased hepatic levels of TPP with normal levels of thiamine in STZ diabetic rats[190].Within mitochondria TPP is slowly hydrolyzed to TMP which may leave the mitochondria via the same transporter. High concentrations of thiamine

monophosphate inhibit thiamine pyrophsphokinase activity noncompetitively[191] and inhibit the entry of TPP into the mitochondria competitively[189]. A small amount of TPP is further phosphorylated to thiamine triphosphate (TTP) by thiamine pyrophosphate kinase and hydrolyzed to TPP by TPPphosphatase [192,254]. TPP is hydrolyzed to TMP and to thiamine by phophatases[168]. Plasma half life is relatively short (2days)[193] but its tissue half life is approximately 9-18 days[194]. Thiamine is stored largely in skeletal muscle and the highly perfused organs such as heart, brain, liver and kidneys[163]. Subcellularly only 10% of total TPP is available for binding to transketolase most of it is associated with the mitochondria[185].Thiamine and its acid metabolites are are excreted primarily in the urine [195].

PHARMACODYNAMICS OF THIAMINE

Mechanism of Action

Thiamine diphosphate binds to a evolutionarily highly conserved domain located in a deep cleft in the active sites of the thiamine dependant enzymes resulting in the activity of these enzymes [196.]

Cruciality of Thiamine Diphosphate in TPP Dependant Enzyme Functions and the Structural Implications of This Molecule in Their Activity

The physiological function of thiamine is mainly fulfilled by TPP (TDP). Structurally the basis of thiamine action and activation of all ThDP-dependent enzymes lies in thiamine catalysis and deprotonation of the thiazolium ring and contribution of the aminopyrimidine side chain in this effect[197- 198] while the pyrimidine ring with its dual proton donor and acceptor capability functioning as a proton transfer system.

On the basis of these chemical alterations TPP functions as coenzyme for mitochondrial enzymes pyruvate dehydrogenase (PDH[199] and α ketoglutarate dehydrogenase[200] of the citric acid cycle. TPP is also a cofactor for the cytosolic enzyme TK of the reductive pentose pathway [201] and of the branched chain α ketoacid dehydrogenase. However TTP is a cofactor for neuronal phosphorylation with unusual phosphorylation of histidine residues[202]and may have a role in neurotransmitter signaling[203, 204] as well.

Symtoms of Severe Thiamine Deficiency:

Thiamine derivatives and thiamine dependant enzymes are universally present in all cells of the body thus a thiamine deficiency would seem to affect all organ systems especially the heart and the nervous system due to their high oxidative metabolism as witnessed in its severest form as beriberi (dry, wet or infantile)[195].Dry beriberi manifests itself as peripheral neuropathy, leading to bilateral dysfunction of sensory and motor nerves resulting in impaired reflexes and causing calf muscle tenderness[205].Wet beri beri symptoms include mental confusion, peripheral neuropathy, muscular atrophy, swelling, rapid pulse, cardiomegaly and heart failure[164]. Infantile beriberi occurs in breast fed infants of thiamine deficient mothers and presents with symptoms ranging from cardiac, aphonic or pseudomeningetic form of the disorder.Infants with cardiac beri beri frequently exhibit a loud piercing cry, vomiting and tachycardia[195]. A diet rich in thiaminase found in raw shellfish, raw fresh water fish may result in vitamin b1 deficiency[206].Wernickes Korsakoff syndrome a vitamin B1 deficiency alcoholism related disorder[207], gastrointestinal diseases, HIV AIDS, chronic diseases lead to thiamine deficiency due to malnutrition[205].

Thiamine Overdose

Symptoms occur rarely include tachycardia, warmth, flushing, irritability, sweating, nausea, restlessness and allergic reactions.

Drug Interactions

Pharmacokinetic interactions at the level of drug metabolism include microsomal enzyme induction by prolonged anticonvulsant pheytoin resulting in decreased plasma levels of thiamin in patients with seizure disorders such as epilepsy[208]. 5-Antimetabolite fluorouracil, a cancer chemotherapeutic agent inhibits the phosphorylation of thiamin to thiamin pyrophosphate (TPP)[209]. While borderline thiamine consumption and diuretic (furosemide) or marked alcohol abuse may enhance thiamine deficiency risk through enhanced diuresis

resulting in decreased reabsorption[210,195].

Safety Evidence

The water soluble thiamine HCl form is safe in humans in oral doses less than or equal to several hundred milligrams via oral route[211]. A UK EVM found that a small clinical trial in Alzheimers patients[212]revealed no adverse effects of thiamineHCl at daily oral intakes of 6000 to 8000mg for five to six months[211]. A randomized double blind placebo controlled trial was conducted in India for therapy of primary dysmenorrhea, a daily oral dose of 100mg thiamine was given to 556 females for 60-90 days and no adverse effects were noted[213].In extremely rare cases of allergic sensitivity were noted solely in patients using thiamine by the parenteral route and were probably due to the injection vehicle and it not been reported to be carcinogenic or mutagenic. No known genetic microsomal variations increase susceptibility to thiamine toxicity [214].

THIAMINE AND DIABETES

Thiamine Transport in Diabetes

Experimental evidence suggests that thiamine transport maybe abnormal in diabetes.In experimental diabetes, these was diminished intestinal absorption of thiamine and TMP[215]. Mild deficiency of thiamine in diabetes may induce increased expression of THR1 as found in frank thiamine deficiency [216]. Experimental diabetes has been found associated with decreased expression of RFC1[217]. Mild thiamine deficiency in diabetes may therefore lead to induced expression of tissue THTRI and THTR2 transporters to improve tissue acquisition of the available thiamine and decrease expression of RFC-1transporter activity to retain tissue TPP.

Thiamine Depletion Impact on Glycemic Control, Thiamine Dependant Ezymes Retina, Nephron, β Cells of Pancreas and Peripheral Nerves in Experinental Diabetes

Streptozocin **i**nduced diabetic rats with supportive insulin therapy to regulate hyperglycemia, 54% decreased of plasma thiamine concentration was reported in contrast to normal controls [190]. This was induced in the diabetic state despite high dietary intake (9 fold) in excess of DRI for rats . The primary cause was marked increased renal clearance of thiamine which was increased by 8 fold[218]. In streptozotocin-induced diabetic rats, there was decreased transketolase expression and activity in renal glomeruli, liver, skeletal muscle and RBCs after 12 weeks of diabetes was found with associated progressive increase in the renal clearance of thiamine and increased albuminuria with duration of diabetes, suggesting that abnormal renal handling of thiamine may occur early in the process of impairment of renal function in diabetes [190,218].Since mild thiamine deficiency may be prevalent in diabetes, particularly with nephropathy, its impact on β cell function is of interest. Isolated pancreatic islets of thiamine deficient rats had diminished baseline, glucose and sulphonylurea (tolbutamide) induced insulin secretion of insulin [219]. There was also impaired insulin secretion with impaired glucose tolerance (IGT) and increased plasma glucagon concentration in an oral glucose tolerance test[219].

In experimental diabetes, similar low plasma thiamine concentration was associated with low TK activity and expression in renal glomeruli [218].Reduced activity of PDH was also noticed due to thiamine depletion[220]. Similar impairment of thiamine-related metabolism may occur in the diabetic retina and peripheral nerves[221,217] pre-disposing these tissues to the adverse effects of hyperglycaemia.

Effect of Thiamine Therapy in Diabetes: On Glycemic Control in Experimental and Animal Model:

Lack of improvement glycemic control in STZ diabetic rats by high dose thiamine or benfotiamine therapy was noticed [218, 222, 114, 223]. Thiamine therapy was found to decrease hyperglycemia in cirrhosis[224], insulin resistance of muscle and inadequate insulin secretion by β cells [225]. In thiamine responsive megaloblastic anaemia too hyperglycemia is linked to impaired insulin secretion due to mutated high affinity thiamine transporter[226]. Therapeutic intervention by thiamine in both cases is likely to involve improved β cell metabolism and insulin secretion. This effect was

not noticed in permanent insulin deficiency of the STZ diabetic rat model where most of the pancreatic β cells are damaged or destroyed and resultantly no improvement in glycemic control is observed. It is not yet known if thiamine or benfotiamine improve glycemic control in type 2 diabetic animal model.

Mild Thiamine Deficiency in Diabetics and Improved Post Therapy Thiamine Status in Clinical Studies:

Mild thiamine deficiency has been observed in diabetics in different international studies.There is paucity of data on thiamine and thiamine dependant enzyme status in clinical diabetes mellitus. In Japan a study of 46 diabetic patients (7 type 1, 39 type 2) with moderate glycemic control (glycated hemoglobinA1c 9%) found lower diabetic RBC TK activity in 79% of patients and a concomitant decrease in thiamine level in 76% of diabetics. Oral thiamine supplementation 3-80mg/day increased thiamine levels (20 patients) and TK activity (15 patients)[227]. A larger study of 100 type 2 diabetic patients (glycated HbAic 9.2%) in Israel, TK activity was lower than the minimum normal range in 18% of diabetics[228]. A smaller Italian study of 10 type 1 diabetic children with normal renal function found plasma thiamine concentration to be decreased by 34% with respect to normal healthy controls and was normalized in a placebo controlled intervention with lipophilic thiamine derivative benzoxymethyl thiamine (50mg/day) [229].

In the Hoorn Study, a study of glucose tolerance in 2196 human subjects, (50-75 years old) without diabetes dietary fibre intake was inversely associated with fasting glucose and fibre intake correlated strongly with thiamine intake. Thiamine intake had a strong association with 2 hour postprandial glucose concentration unlinked to fibre intake and fasting glucose. Leading to the conclusion that part of the connection between fibre intake and glucose tolerance was associated with dietary thiamine intake[230].

These studies suggest that thiamine deficiency impair β cell function and thiamine deficiency may have a contribution in IGT in the human population. Further epidemiological analyses and intervention trials with thiamine or benfotiamine may establish a basis for prevention of type 2 diabetes by high dose thiamine derivative therapy.

Intervention of High Dose Thiamine Therapy in Biochemical Dysfunction in Diabetes and the Prevention of Microvascular Dysfunction, Neuropathy, Dyslipidemia Complications:

Microvascular disease (nephropathy, retinopathy and neuropathy) a common debilitating manifestation of chronic diabetes mellitus, have no effective therapy. Hyperglycaemia in diabetic subjects is an essential element for development of both microvascular and macrovascular complications risk factor DCCT 2003[231]. High doses of thiamine and its derivative S-benzoylthiamine monophosphate (Benfotiamine) are proposed as a new therapy to counteract biochemical dysfunction leading to the development of microvascular complications[114].High dose thiamine and Benfotiamine may counter the development of microvascular complications by activation of the reductive pentosephosphate pathway[223].

In streptozotocin induced rats (STZ) ,diabetic rats were given high doses of thiamine orally and it prevented the development of incipient nephropathy as observed by prevention of micoalbuminuria. The mechanism appeared to be, normalizing transketolase expression and activity in the non oxidative pentose pathway causing increased conversion of triosephosphates and fructose 6 phosphate to ribose-5-phosphate[190]. This was also observed for human RBCs in vitro[232]. Both thiamine and benfotiamine prevented decreased replication, increased apoptosis and AGE accumulation induced by hyperglycemia in human umbilical endothelial cells in vitro[233]. In retinopathy observed in streptozotocin diabetic wistar rats high dose thiamine therapy (80mg/day) normalized the number of retinal acellular capillaries which were found to be 3 fold increased initially, proving a role for benfotiamine in retinopathy prevention[114].

High dose thiamine therapy (70mg/day) and benfotiamine 100mg/day prevented the development of neuropathy in STZ wistar rats as judged by improved nerve conduction velocity and AGE accumulation was decreased[234]. In a double blind placebo controlled clinical trial of 24 diabetic

patients with benfotiamine (80mg/day-pyridoxine (180mg/day)-cyanocobolamin (0.5 mg/day) for 2 weeks and half of this for another 10 weeks there was sufficient increase in nerve conduction velocity [234]. In a 6 week open trial with 36 patients receiving upto 4 times higher doses than already mentioned there was significant improvement in pain, vibration and current perception on the peroneal nerve[235]. These studies show that thiamine repletion with thiamine and benfotiamine may prevent the development of diabetic microvascular complications and neuropathy in vivo. Higher risk of coronary heart disease in diabetes mellitus2-3 fold increase in men and 3-5 fold increase in women relative to the non diabetic population is also linked with hyperglycaemia, hyperinsulinaemia, high blood pressure, low grade inflammation, elevated levels of triglycerides, cholesterol and plasminogen activator inhibitor-1[236] and hyperhomocysteinuria[237]. Dyslipidemia is a critical component in diabetic coronary heart disease, which is associated with increased levels of VLDL particles which initiate the development of small dense LDL and HDL particles through hexosamine pathway that pose the primary atherosclerotic risk [238]. Preceding enhanced hepatic lipoprotein secretion was a change from lipid oxidation to lipogenesis and increased lipoprotein synthesis appeared to be the key for dyslipedemia progression. In the liver of transgenic mice overexpression of the glutamine: fructose-6-phosphate amido transferase (GFAT), the rate limiting enzyme in the hexosamine pathway was associated with hyperlipidemia [239]. Interestingly by activation of the hexosamine pathway the glucose-mediated induction of lipogenic enzymes, glycerophosphate dehydrogenase (GPDH), fatty acid synthase (FAS) and acetyl-CoA carboxylase, was stimulated in liver and adipocytes [240](Fig 8). Therefore it appears that the lipogenesis in diabetes is strongly associated with the flux through the hepatic hexosamine pathway. High dose thiamine treatment prevented diabetic dyslipidemia in experimental diabetes which was associated with reversal of diabetes-induced signaling via the hexosamine pathway of increased expression of lipogenic enzymes [241]. It has been shown recently that high dose therapy with thiamine counters this effect in experimental diabetes by induction of the expression of transketolase (TK) and saturation of TK with thiamine pyrophosphate (TPP) cofactor; this increases the activity of TK and activates the reductive pentosephosphate pathway (Fig 8). In turn, this diverts metabolic flux away from the hexosamine pathway, decreased lipogenesis and correct diabetic dyslipidaemia as shown below[241](Fig 8). High dose therapy with thiamine therapy (70mg/day) thwarted diabetes induced increase in plasma cholesterol and triglycerides in diabetic rats but could not reverse the diabetes induced depletion of HDL in streptozotocin diabetic rats on maintenance insulin therapy[241]. This was achieved by prevention of thiamine depletion and the resultant decrimental TKactivity in the liver of diabetic rats.There was also a concomitant decrease in fatty acid synthase activity and UDP acetyl glucosamine. Normalization of food intake was also observed with 70 mg thiamine therapy of diabetic rats. Therefore probably suppression of food intake with high dose thiamine therapy prevented diabetic dyslipidemia in experimental diabetes.

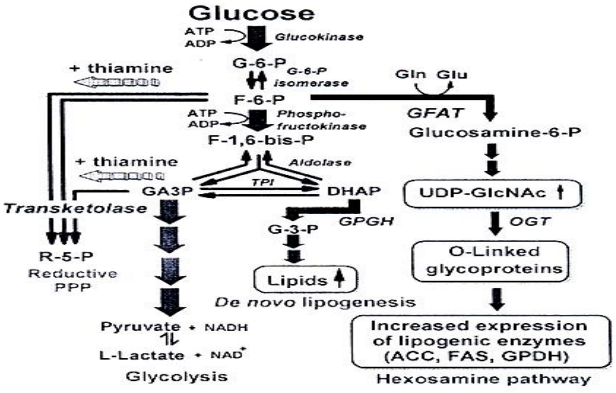


Fig. 8: Metabolic Mechanism for Supression of Hepatic Lipogenesis in Diabetes by Thiamine.Adapted from PJ Thornalley 2006

Therapeutic intervention with thiamine and benfotiamine[190] and subsequent reversal of inhibition of water reuptake by aquaporins in renal collecting tubules may also play a role [242, 243].High dose thiamine therapy also normalized food consumption in STZ rats which maybe linked to the effects of thiamine metabolites TPP and thiamine triphosphate on dopamine signaling in the brain related to sensory specific satiety[244 ,245].

However similar data for the other two pyruvate dehydrogenase and alphaketoglutarate dehydrogenase with regard to high dose thiamine is rarer.A clinical trial in diabetic type 2 individuals to study the effect of high dose thiamine therapy is still awaited.

High dose thiamine might also decrease incipient nephropathy since it also did this in experimental diabetes linked to reversal of multiple mechanisms of biochemical dysfunction: activation of protein kinase C and polyol pathways, oxidative stress and increased protein glycation[233]. Suppression of both dyslipidaemia and microalbuminuria in experimental diabetes by high dose thiamine therapy occurred by mechanisms other than those utilized by current clinical therapy. It is expected, therefore, that in type 2 diabetic patients, beneficial effects of thiamine may be achieved in addition to those produced by conventional therapy. This is of great importance as none of the currently available oral hypoglycemic medications aim at the mechanisms causing microalbuminuria and dyslipidemia in diabetics. Correction of dyslipidaemia and decreased microalbuminuria would be a significant advance, since both are risk factors for cardiovascular disease the major cause of mortality of diabetic patients[246] [247]. Drugs such as cerivastatin decreased total and LDL cholesterol, triglycerides, microalbuminuria and increased HDL cholesterol in type 2 diabetic patients. However, normal levels of these metabolite values were not achieved[248]. Statins also exhibit adverse effects (deranged LFTs and muscle damage) in some individuals [123].

Comparison of B1 Therapy to B-Complex Therapy:

Interestingly pharmacologically combined therapy of vit B1, B6 and B12 did not augur well in diabetics having diabetic nephropathy and substantial adverse outcomes associated with high dose vitamin B6, B9 and B12 co-supplementation in patients with advanced diabetic nephropathy was brought to light[249] Recently concluded Diabetic Intervention with Vitamins to Improve Nephropathy (DIVINE) study produced an unexpected accelerated decline in renal function (16.5ml/min versus 10.7ml/min per1.73m2: p=0.02). There was also an increase in number of vascular disease events, defined as a composite of myocardial infarction, stroke, revascularization and all cause mortality (risk of outcomes: 23.5% versus 14.4% (P=0.04). Another Heart Outcomes Prevention Evaluation (HOPE 2), 5552 patient trial found no effect of high dose B6, B9 and B12 supplementation on death from cardiovascular disease, whereas risk of stroke was decreased and the risk of unstable angina increased[250].Another study on Homocystinemia in End Stage Renal Disease (HOST) study found no proof that combination of high dose B6, B9 and B12 supplements reduced risk or improved survival in cardiovascular disease related events[251]. Thus in entirety disappointing results of the DIVINE study also raise caution levels for future human trials on high dose vit B6, B9 and B12 therapy in patients with diabetic nephropathy. The reasons could have been multipronged ranging from toxic accumulation of folate and B12 in patients of diabetic nephropathy with low GFR[249] or competitive inhibition of TMP and TPP transport at the level of RFC1 transporter by high dose folate[252-254] at key sites such as the kidney and vascular cells thus adversely affecting sharing of thiamine between tissues rich in thiamine and those deficient in it[183].

SUMMARY

Thus final summarization of these studies indicates that high dose thiamine repletion may decrease the risk of micro and macrovascular disease and counter incipient nephropathy in diabetes. The effect of thiamine occurred independent of control of hyperglycaemia, blood pressure and statin/fibrate therapy, suggesting that high dose thiamine therapy may produce improvements in the prevention of dyslipidaemia and diabetic nephropathy in addition to those produced by current therapy for control of hyperglycaemia, blood pressure, cholesterol and lipids. Since dyslipidemia and microalbuminuria are reversible in type 2 diabetic patients[253, 248], it is possible that high dose thiamine therapy might improve renal function and metabolic control through reduction in biochemical dysfunction and improvement in thiamine dependant enzyme

activities in diabetic patients with existing dyslipidaemia and microalbuminuria . However, it appears that there may be noticeable variations in these parameters on the basis of geographical, racial pharmacogenetic and factors. So the need of the hour was an indepth study as a double blind placebo controlled clinical trial to study the effect of high dose thiamine therapy on biochemical profile and activities of thiamine dependant enzymes in type 2 diabetic patients in our multiracial population in Pakistan.

THERAPEUTIC IMPLICATIONS

Based on the data above, the first ever randomized, double blinded, placebo controlled clinical intervention trial registered with the World Health Organization involving high dose B1 therapy was conducted by Dr.Saadia ShahzadAlam of the Pharmacology Deptt (Co-Principal Investigator 1) of Federal Postgraduate Medical Institute Lahore for a period of 5 months to study the effect of high dose thiamine therapy on biochemical profile and activities of thiamine dependant enzymes on type 2 diabetics in the Pakistani population[255] . This trial was also pioneering internationally on the subject of diabetic nephropathy and the effect of thiamine supplementation on it[255]. 40 type 2 micoalbuminuric diabetic patients at the Diabetes Clinic of Shaikh Zayed Hospital Lahore were administered 300mg/day (100mg tablets Administration of 300mg B1 TDS) / placebo for 3 months followed by a 2 month washout period [255].

The results of this trial were quite interesting and have been published internationally[255-257], plasma thiamine levels of both thiamine and placebo groups were significantly depleted as compared to normal controls. There were significant baseline derangements of incipient diabetic nephropathy (microalbuminuria), glycemic control parameters FBS and glycated hemoglobin, lipid profile including total cholesterol, HDL, LDL, triglycerides and VLDL in type 2 microalbuminuric diabetics as compared to healthy individuals. Following 3 months 300 mg/day thiamine administration there was significant improvement of urinary albumin excretion, and preservation of glomerular filtration rate suggested that these occurred due to thiamine replenishment and decreased glycated hemoglobin and LDL cholesterol levels were observed in the washout period as a delayed effect[255-258]. Additionally following thiamine therapy significant reduction in plasma levels of sVCAM-1, noticeable and an inverse linkage between thiamine therapy and vWF was apparent in this group as compared to placebo, suggested noticeable benefit with reduction in the risk factors of type 2 diabetes[255-258]. Significant changes in other serum and urinary biomarkers profile were also observed in type 2 diabetics following thiamine therapy in a simultaneously carried out proteomic study[259-262, 265]. Three thiamine dependant enzymes PDE3, PDE1β, AKGDHE1 and Transketolase were determined to be dysfunctional at baseline in type 2 microalbuminuric diabetic patients in comparison to normal healthy controls, and improved in both activity and gene expression with high dose thiamine therapy [263, 264] While importantly no hepatic or renal adverse effects were encountered prior, during therapy or as a residual effect, post washout thus fortifying the previously established human safety track record of thiamine. [255 -258]

We hope that these findings would contribute to knowledge regarding the role of thiamine therapy at 300mg/day dosage on biochemical profile and molecular aspects of those vital thiamine dependant enzymes and help in providing improved, safe and more effective treatment for type 2 diabetic patients with incipient nephropathy, dyslipidemia with expected decrease risk of heart disease and kidney failure.

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