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Role of thiamine status and genetic variability in transketolase and other pentose phosphate cycle enzymes in the progression of diabetic nephropathy

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Abstract

Background. Pentose phosphate pathway (PPP) represents a potentially 'protective' mechanism in hyperglycaemia due to shunting of glycolytic intermediates into PPP reactions. We hypothesized that thiamine status (plasma and erythrocyte levels of thiamine and its esters) together with genetic variability in key PPP enzymes—transketolase (TKT), transaldolase and TKT-like—might contribute to the progression of diabetic nephropathy (DN) and mortality of diabetics.

Methods. A total of 240 diabetic subjects with variable degree of kidney disease were included at baseline and were followed up for a median of 26 (IQR 21–50) months. Concentrations of thiamine in plasma and whole blood and TKT-catalysed reaction were determined by HPLC. Single-nucleotide polymorphisms (SNPs) (n = 14) were genotyped by means of PCR using TaqMan chemistry (Applied Biosystems, Foster City, CA, USA).

Results. Significant differences in pTh, pThDP, eryThDP and eryTKT between DN-stage groups were ascertained ($P < 0.05$) with advancing stage of DN being accompanied with increasing values of pTh, pThDP and eryTKT but not eryThDP. A highly significant negative correlation ($r = -0.41$, $P < 0.001$) was found between pThDP and eryThDP, and the tertiles of the ratio of eryThDP/pThDP were significantly associated with all-cause mortality rates ($P = 0.0072$). We also identified significant differences in the rate of DN progression between different pTDP tertile groups ($P = 0.0017$). No significant genetic effects were found.

Conclusions. The results support the role of 'functional' thiamine deficiency in the development of hyperglycaemia-related pathology. Limited intracellular availability of active TKT co-factor seems to be a dominant abnormality.

Keywords: diabetic nephropathy; pentose phosphate pathway; thiamine; thiamine deficiency; transketolase

Introduction

Diabetic nephropathy (DN) develops as a consequence of long-term metabolic and haemodynamic abnormalities associated with suboptimal compensation of diabetes mellitus. Variable degrees of fasting and/or postprandial hyperglycaemia provides substrates for several intracellular pathways (such as polyol and hexosamine pathways, dicarbonyl production and non-enzymatic glycation, etc.) that are believed to be largely responsible for the hyperglycaemia-induced cell damage [1] (see Figure 1, right part). There are, however, other metabolic pathways—such as pentose phosphate pathway (PPP)—potentially conferring protection from the hyperglycaemia-induced damage since they metabolize glycolytic intermediates (especially triose-phosphates) into harmless metabolites. PPP consists of a cascade of reactions providing (i) reducing equivalents (NADPH) for biosynthetic reactions and for regeneration of oxidized glutathione (= oxidative irreversible PPP branch with glucose-6-phosphate dehydrogenase as a rate-limiting enzyme), (ii) ribose 5-phosphates for the synthesis of nucleotides and nucleic acids and, finally, (iii) glycolytic intermediates from pentose phosphates derived from digestion of nucleic acids. The latter two functions are executed by non-oxidative (reversible) branch of PPP allowing flexible inter-conversions of 3- to 7-carbon sugars. Activity of PPP is regulated according to immediate metabolic situation and needs of organism; if not needed, products of PPP are readily converted to glycolytic intermediates and oxidized. Shunting of cumulated cytosolic glycolytic intermediates into the PPP reactions supposedly

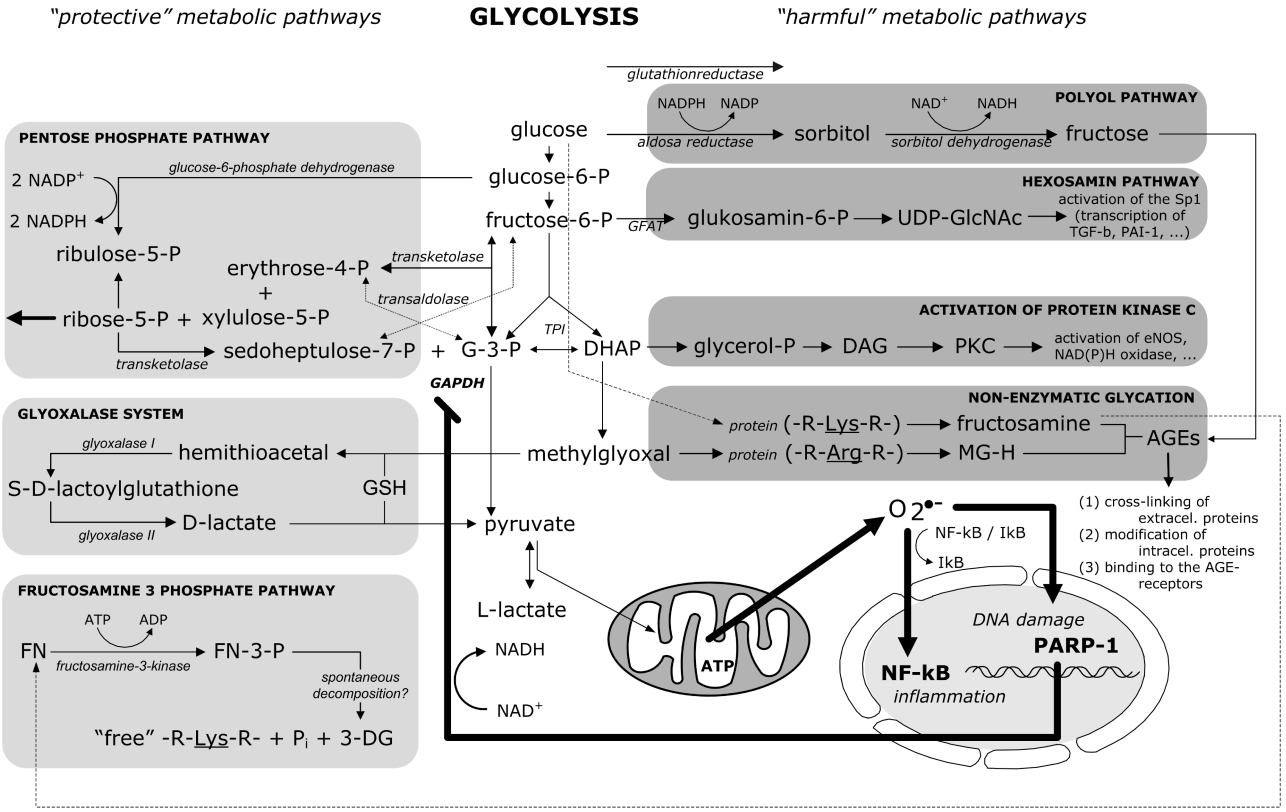

Fig. 1. Schematic overview of biochemical pathways operating during hyperglycaemia.

'disburdens' glycolysis and quantitatively limits processing of glycolytic intermediates in harmful metabolic pathways [2] (see Figure 1, left part). PPP can thus be seen as a potentially protective pathway whose metabolic flexibility is ensured by physiological enzyme kinetics and co-factor sufficiency.

Transketolase (TKT) (EC 2.2.1.1) and transaldolase (TALDO) (EC 2.2.1.2) are key enzymes of non-oxidative branch of PPP determining its efficiency. TKT requires thiamine diphosphate (active vitamin B_1) as a co-factor. Processing large amounts of glucose automatically increases demand for dietary thiamine (the condition is called high calorie malnutrition), and thus, thiamine deficiency could have an inhibitory effect on PPP and increase the hyperglycaemia toxicity. Interestingly, another TKT-like (TKTL1) enzyme has been identified encoding full-length plus truncated proteins with TKT enzyme activities [3]. It was suggested that, apart from enzymatically active TKT/TKT homodimers, other heterodimers of TKT/TKTL1 can be formed with variable enzyme activities [4].

Initial data supporting the role of thiamine metabolism dysfunction in diabetes were provided by Thornalley *et al.* [5] showing high prevalence of thiamine deficiency in type 1 (T1DM) and type 2 diabetes mellitus (T2DM) subjects compared with non-diabetic subjects. In the pilot study of T2DM with microalbuminuria, they were able to show significant regression of urinary albumin excretion after a 3-month treatment with high-dose thiamine [6]. Since kidney failure has a crucial impact on the metabolism and vast array of low-molecular-weight substances, this can also include thiamine parameters. However, nothing is known so far about thiamine metabolism and eventual thiamine deficiency in diabetic subjects with manifest diabetic kidney disease, i.e. broad range from microalbuminuria to proteinuria and variable degree of chronic kidney disease (CKD). Furthermore, there are no data available about the possible contribution of genetic variability in key PPP enzymes to impaired thiamine metabolism or susceptibility to diabetic complications such as DN. Therefore, specific aims of the study were: (i) quantification of plasma and whole blood levels of thiamine and its esters (Th, ThMP and ThDP) in diabetics with variable kidney function, (ii) determination of erythrocyte TKT activity and thiamine effect, and (iii) association between genetic variants in candidate loci involved in the non-oxidative branch of PPP (*TKT*, *TALDO1* and *TKTL1*) and selected parameters of thiamine metabolism and DN progression using a follow-up study design.

Materials and methods

Subjects

The study comprised a total of 240 diabetic patients (126 men/114 women) with diabetes duration at least 10 years and the following inception DN staging: normoalbuminuria ($n = 74$), persistent microalbuminuria [$n = 31$, urinary albumin excretion (UAE) 30–300 mg/24 h], persistent proteinuria ($n = 96$, UAE >300 mg/24 h) and end-stage renal disease (ESRD) with renal replacement therapy ($n = 39$, GFR <15 mL/min/1.73 m^2 and/or permanent renal replacement therapy) followed up in specialized nephrology units of Brno university hospitals in 2006–07. The diagnosis of DN was based on periodical measurements of UAE and GFR (by creatinine clearance from 24-h urine collection) and presence of ophthalmologically verified diabetic retinopathy. Of the total 240 diabetics, 69 subjects had T1DM, 4 subjects had latent autoimmune diabetes in adults (LADA) and 167 subjects had T2DM. Mean duration of diabetes

Table 1. Baseline clinical characteristics of diabetic subjects in the categories defined according to the DN stage

Parameter	Normoalbuminuria (n = 74)	Microalbuminuria (n = 31)	Proteinuria (n = 96)	ESRD (n = 39)	P
Age (years)	50.5 (37.0–64.0)	68.0 (53.0–74.0)	66.5 (61.0–74.0)	68.0 (62.0–77.0)	<0.01
DM duration (years)	17.0 (15.0–24.0)	9.0 (6.0–18.0)	18.0 (12.0–23.0)	18.0 (15.0–28.0)	<0.01
BMI	26.2 (23.4–29.3)	30.1 (25.6–34.7)	30.0 (26.2–33.2)	28.3 (24.4–30.8)	<0.01
FPG (mmol/L)	7.6 (6.2–9.8)	7.8 (6.8–8.3)	9.1 (7.2–11.5)	8.0 (6.2–10.3)	NS
HbA1c (%)	7.0 (5.6–8.1)	5.7 (5.1–6.8)	7.2 (5.6–9.4)	5.7 (4.8–6.6)	<0.01
Total cholesterol (mmol/L)	4.8 (4.3–5.2)	4.6 (4.1–5.4)	4.9 (4.1–6.0)	4.4 (3.5–5.3)	NS
TAG (mmol/L)	1.25 (0.82–1.90)	2.00 (1.28–2.78)	2.07 (1.43–3.15)	2.11 (1.70–2.73)	<0.01
S-urea (mmol/L)	5.6 (4.9–6.6)	8.3 (5.4–13.5)	12.0 (7.9–17.5)	23.8 (20.8–27.3)	<0.01
S-creatinine (μmol/L)	93.0 (82.5–108.0)	115.0 (89.0–160.0)	145.0 (118.0–212.0)	550.0 (479.0–640.0)	<0.01
UAE (mg/24 h)	8.0 (6.00–12.00)	70.0 (20.0–200.0)	81.0 (36.0–351.0)		<0.01
proteinuria (mg/24 h)	93.0 (72.0–110.0)	125.0 (60.0–180.0)	1363.0 (583.0–3339.0)		<0.01
GFR (mL/s)	1.70 (1.38–1.96)	1.07 (0.71–1.79)	0.78 (0.50–1.30)		<0.01

Data are expressed as median **(**IQR**)**. Comparisons were made by Kruskal–Wallis ANOVA.

was 18.2 ± 9.5 years, and mean age was 60.5 ± 16.1 years. Clinical characteristics of the study participants are shown in Table 1. Subjects did not receive any type of thiamine supplementation. The study was performed in accordance with the principles of the Declaration of Helsinki and approved by the Ethical Committee of Medical Faculty, Masaryk University Brno. Informed consents were obtained from all patients prior to their inclusion in the study.

Follow-up

The follow-up data were available for 109 subjects (followed up until June 2009). The following end points were considered: (a) renal (i.e. progression of DN by stage or reaching the ESRD requiring regular haemodialysis or transplantation), (b) cardiovascular mortality (fatal myocardial infarction, stroke, heart failure and sudden death), and (c) all-cause mortality. For (a), non-ESRD cases at baseline only were considered since ESRD (n = 39) could not progress any further. Furthermore, progression of CKD over the follow-up period was assessed as (i) an average decline of measured GFR (ΔGFR = difference of GFR at baseline and at the end of follow-up expressed per month) and (ii) rise of serum creatinine (analogically ΔS-Cr per month).

Assessment of thiamine status

Plasma and whole blood were stored at −80°C from the time of sampling until the measurements. After thawing, samples were centrifuged at 10 000 × g for 10 min at 4°C. Supernatants were deproteinized by perchloric acid at 0°C in the dark [7]. Immediately after centrifugation, Th and its phosphate esters were derivatized to thiochrome derivatives by freshly prepared alkaline solution of potassium ferricyanide as previously described [8]. Before each injection onto a chromatographic column, a pH of derivatized sample was adjusted to 6.8 ± 0.2 by diluted phosphoric acid. Modification of previously described ion-paired chromatographic method [7] using an HPLC system consisting of the Shimadzu LC-10 series (Shimadzu, Kyoto, Japan) was used for the separation of thiochromes. Separations were accomplished in gradient mode at 30°C at a flow rate 1 mL/min using a reversed-phase column Luna C18(2) (150 × 4.6 mm ID, 5 μm) protected with SecurityGuard C18 (8 × 3.0 mm ID, 5 μm) (Phenomenex, Torrance, CA, USA). Initial mobile phase (pH 7.0) consisted of KH_2PO_4 (0.2 mol/L) with TBAH (0.3 mmol/L) and methanol (80:20, v/v). The elution was performed using a linear gradient 0–10% methanol in the period of 2–4 min followed by an isocratic step with 10% methanol for 6 min. Injection volume was 20 μL. The analytes were detected at an excitation wavelength of 365 nm and emission wavelength of 435 nm. The within-run imprecision of the method was assessed by analysing 10 aliquots of the same sample (internal quality control) and was 2.4% for Th (27.6 nmol/L), 5.7% for ThMP (4.2 nmol/L) and 3.0% for ThDP (18.3 nmol/L). The between-run variation assessed by analysing the same sample on 15 consecutive days was 6.1% for Th (5.0 nmol/L), 5.4% for ThMP (1.3 nmol/L) and 4.2% for ThDP (50.0 nmol/L). Plasma (p) and whole blood (wb) values of thiamine and its esters were expressed in nanomole per litre. Haemoglobin (Hb, gram per litre) was determined using the Drabkin method (Drabkin's reagent; Sigma, St Louis, MO, USA) according to manufacturer's recommendation. Erythrocyte active TKT co-factor ThDP was calculated as a difference between wb and p ThDP, and the levels were recalculated to Hb concentration and expressed in picomole per gram Hb.

TKT activity was determined by kinetic NADH-dependent method utilizing triosephosphate isomerase and glycerol-3-phosphate dehydrogenase [9]. A reaction mixture contained ribose 5-phosphate (14 mM), NADH (0.2 mM), Tris–HCl (82 mM, pH 7.8), triosephosphate isomerase (40.1 U/mL), glycerol-3-phosphate dehydrogenase (3.7 U/mL) and ThDP (0.38 mM). TKT activity was assessed at 37°C from the rate of NADH decrease between 10 and 120 min of the reaction. The results of TKT activity were expressed in Units per gram Hb. The within-run imprecision of the assay obtained by analysing 18 aliquots of the same haemolysate with TKT activity of 1.36 U/g Hb was 5.1%. The between-run imprecision of the same sample on nine consecutive days was 7.5%. The so-called 'thiamine effect', i.e. the relative increase of TKT activity by *in vitro* addition of ThDP, was calculated, and values >15% were used as markers of eventual clinically significant thiamine deficiency.

Single-nucleotide polymorphism selection and genotyping methods

Tagging single-nucleotide polymorphisms (SNPs) in the *TKT* (MIM 606781), *TALDO1* (MIM 602063) and *TKTL1* (MIM 300044) genes (total of n = 14 SNPs genotyped in all 240 subjects) were selected using the algorithm implemented in SNPbrowser/HapMap based on the following criteria: (i) minor allele frequency ≥10% in Caucasian population, (ii) pairwise r^2 ≥0.8 with any of the unselected SNPs and (iii) availability of genotyping assay. For the panel of selected 14 SNP markers, see online supplementary Table 1. Genotyping of all variants was performed using the TaqMan® SNP Genotyping Assays in ABI Prism® 7000 Sequence Detection System with genotype discrimination performed using SDS 2.3 software (all from Applied Biosystems, Foster City, CA). The overall genotyping success rate was 98.2%. Repeated genotyping of 5% of samples, performed as a quality control, yielded identical results. *TKT* haplotypes were constructed *in silico* based on genotype data using the Bayesian-based algorithm (PHASE) [10].

Statistical analysis

Comparisons between groups were performed by non-parametric tests (Mann–Whitney or Kruskal–Wallis ANOVA), and correlations were assessed by Spearman correlation coefficients. For each SNP, Hardy–Weinberg equilibrium (HWE) and differences in genotype frequencies were tested by chi-square test, and differences in allele frequencies by Fisher exact test. For SNPs (rs766420 and rs5945412) in the *TKTL1* gene on chromosome X, allele frequencies were compared in men and women separately and in the whole samples using Fisher exact test. The Bonferonni correction was applied to adjust for multiple comparisons where appropriate (P_{corr} = 0.5/number of tests). Estimation of haplotype frequencies was performed by the Bayesian-based algorithm (PHASE) [10]. Experiment-wise significance of differences in estimated

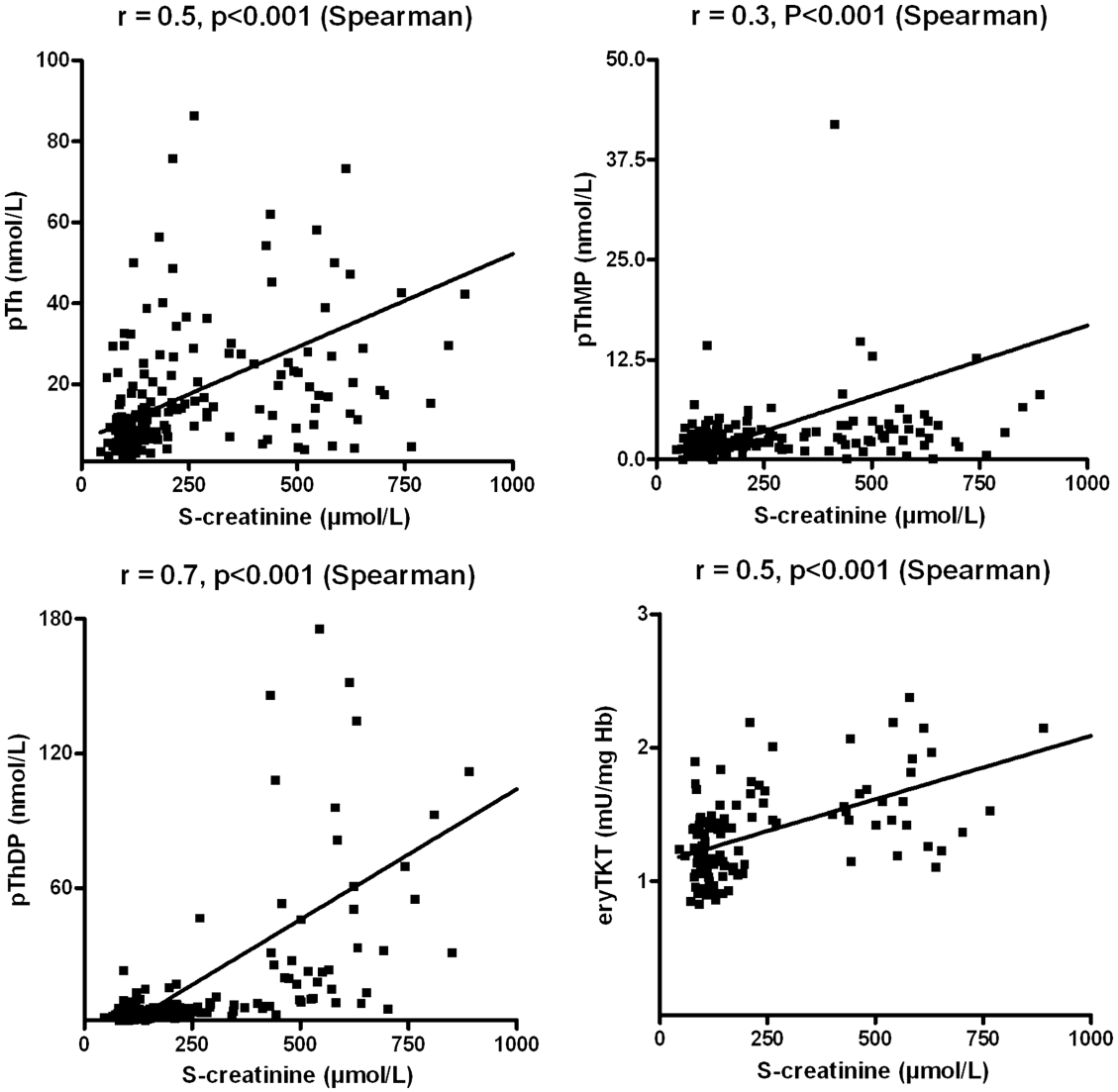

Fig. 2. Correlations between serum creatinine and thiamine parameters.

haplotype frequencies between groups was assessed empirically via permutation testing (5000 permutations). Time-to-event analysis using Kaplan–Meier curves and linear regression were used to assess predictive value of thiamine parameters for given end points and CKD parameters.

Results

Thiamine status parameters in diabetics—baseline cross-sectional data

First of all, since age, diabetes duration and serum creatinine (S-Cr) differed significantly between the groups (reflecting progressive character of the kidney disease), we analysed their potential correlation with thiamine parameters. No significant correlations were ascertained between thiamine parameters and diabetes duration (and neither with fasting plasma glucose and HbA1c) (all $P > 0.05$). We found positive correlations between age and pTh and between S-Cr and nearly all thiamine parameters (pTh, pThMP, pThDP and eryTKT activity; all $P < 0.05$) except for eryThDP. For correlations between S-Cr and thiamine parameters, see Figure 2.

Almost all parameters (pTh, pThMP, pThDP and eryTKT but not eryThDP) significantly differed between the four groups defined by the stage of DN (all $P \leq 0.001$) (see Figure 3 for selected trends). While pTh and pThDP together with eryTKT exhibited gradual increase through DN stages, active TKT co-factor eryThDP did not follow such pattern (in ESRD group eryThDP levels even dropped). When comparing all subjects with kidney disease (i.e. non-normoalbuminuria) pooled into a single DN group ($n = 166$) with normoalbuminuria subjects (non-DN group), significantly higher levels of pTh, pThDP and eryTKT activity (but not eryThDP) in DN group were detected ($P < 0.001$, $P = 0.004$ and $P < 0.001$, respectively) (see Table 2).

As the study sample contains subjects with both T1DM and T2DM, in order to exclude the effect of diabetes type, all the above-mentioned analyses were performed for T2DM group only (larger group—smaller sample size

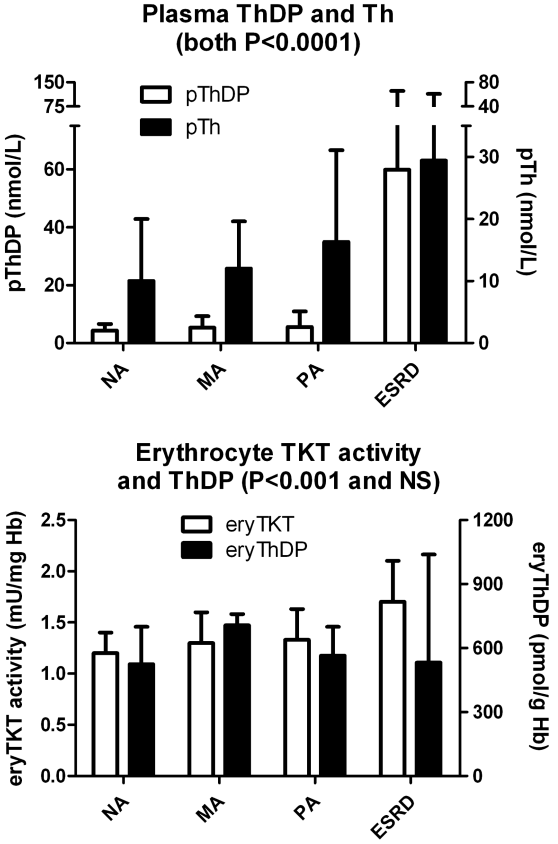

Fig. 3. Thiamine status parameters between the four groups defined by the stage of DN (data are expressed as mean ± SD).

effect). The results were the same as for pooled T1DM and T2DM subjects.

Genetic association and genotype vs. phenotype analysis

Genotype distributions of all *TKT* and *TALDO1* variants (plus *TKTL1* in women) in both DN and non-DN groups fulfilled the HWE criteria. Having applied the Bonferonni correction for multiple comparisons, we did not find any significant difference in neither allele nor genotype frequencies between diabetics with and without DN ($P_{corr} > 0.0036$). Genotype and allele frequencies of all polymorphisms studied are shown in online supplementary Table 2. Haplotype distribution of *TKT* variants did not differ significantly between DN and non-DN groups ($P > 0.05$, 5000 permutations).

Relationships between genotypes of all 10 SNPs in the *TKT* locus vs. eryTKT activity (i.e. genotype–phenotype relationships) were analysed; however, after correction for multiple comparisons, none of the differences remained significant ($P_{corr} > 0.005$).

Thiamine status parameters vs. clinical end points—follow-up data

Median (IQR) follow-up of the diabetic subjects was 26 (21–50) months. Cumulative incidence of DN progression was 11.5% (non-ESRD at inception only), for cardiovascular mortality 6.4%, and for all-cause mortality 16.4%. For thiamine parameters exhibiting significant differences between groups (i.e. pTh, pThDP, eryThDP and eryTKT activity), tertiles were used to construct subgroups of subjects. Using Kaplan–Meier time-to-event analysis, we identified significant differences in renal end point between different pThDP tertile groups ($P = 0.0017$) and in all-cause mortality between different pThDP, eryThDP and eryTKT tertile groups ($P = 0.00045$, $P = 0.00047$ and $P = 0.030$, respectively), in all cases with a clear biological gradient (see Figure 4). Interestingly, survival rates for tertiles of eryThDP and eryTKT activities exhibit a completely reverse pattern. While the worst survival rate was observed for the highest tertile of pThDP and eryTKT, for eryThDP, this was true for the lowest tertile. There seems to be an obvious discrepancy between the TKT activity and the availability of its co-factor, since testing for correlation revealed no correlation between eryThDP and eryTKT ($P > 0.05$). We tested whether eryThDP levels simply reflect total (wb) and plasma ThDP and Th levels; however, pThDP and eryThDP exhibited highly significant negative correlation ($r = -0.41$, $P < 0.001$), while pTh and eryThDP did not correlate at all ($P > 0.05$). Therefore, we calculated the ratio of eryThDP/pThDP and eryThDP/pTh and, analogically, constructed tertiles of these ratios. There was a clear and significant biological gradient ($P = 0.0072$), the lowest tertile of eryThDP/pThDP ratio being associated with the lowest survival rate (see Figure 5). A similar pattern could be observed for eryThDP/pTh ratio, but the differences did not reach statistical significance.

When a similar test (with tertiles of eryThDP/pThDP and eryThDP/pTh) was performed for DN progression end point, no significant differences were ascertained, although

Table 2. Thiamine status parameters in diabetics

Parameter	Non-DN ($n = 74$)	DN ($n = 166$)	P
pTh (nmol/L)	6.70 (5.10–10.50)	12.70 (7.60–22.50)	<0.001
pThMP (nmol/L)	2.20 (1.40–2.50)	2.30 (1.50–3.50)	NS
pThDP (nmol/L)	4.00 (2.70–5.40)	5.30 (3.40–10.70)	0.004
eryThDP (pmol/g Hb)	512.8 (431.0–624.8)	550.3 (414.5–660.5)	NS
eryTKT activity (U/g Hb)	1.110 (0.970–1.350)	1.425 (1.150–1.600)	<0.001
Thiamine effect ≥15% (%)	6.9	6.1	NS

Data are shown as medians and interquartile ranges (IQR). Comparisons of continuous variables were performed by Mann–Whitney tests, and for thiamine effect, Fisher exact test was used.

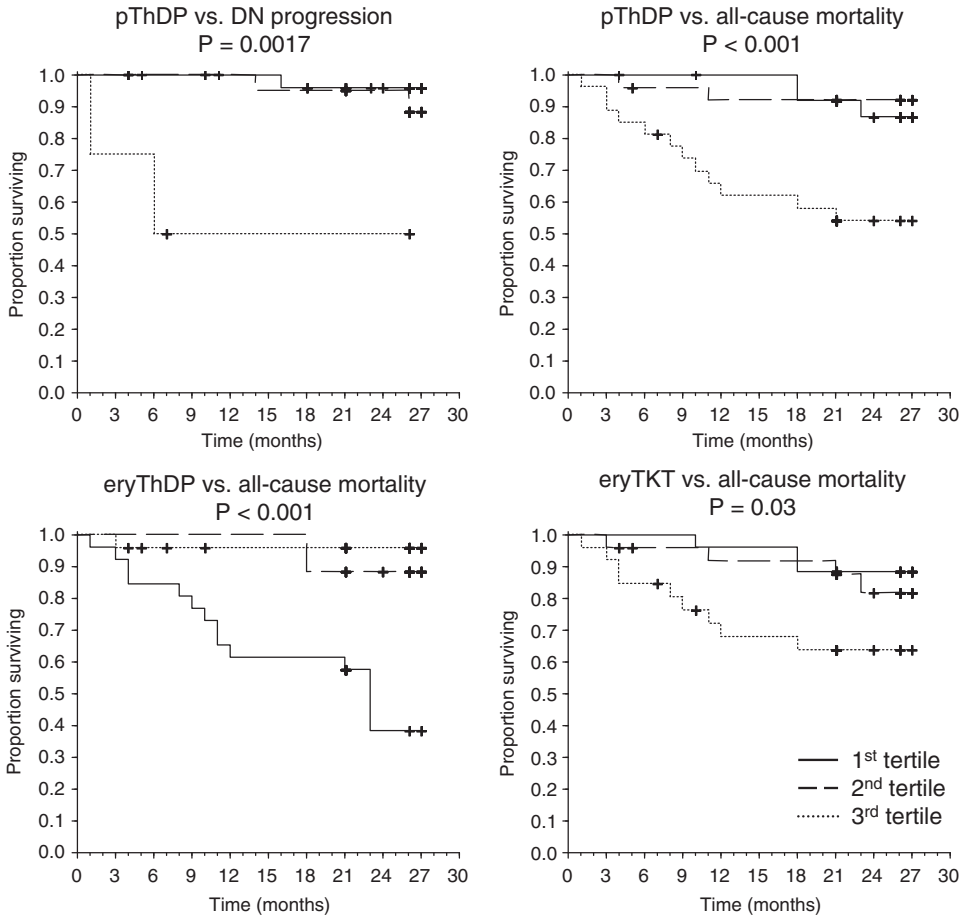

Fig. 4. Kaplan–Meier curves for renal end point and all-cause mortality by pThDP, eryThDP and eryTKT tertile groups.

the same trend was observed (the lowest tertile associated with shortest time-to-event interval); however, the rather low incidence of DN progression over the follow-up period (only 11.5% of subjects reached this end point) was unfortunately too low to allow for valid testing. Similarly, no correlation was assessed for ΔGFR and ΔS-Cr and eryThDP/pThDP or eryThDP/pTh ratios ($P > 0.05$).

Discussion

Thiamine and its derivatives play a fundamental role in energy metabolism, especially in glucose metabolism (co-factor of pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase) and in PPP (co-factor of TKT). Experimental findings, too, support the role of thiamine metab-

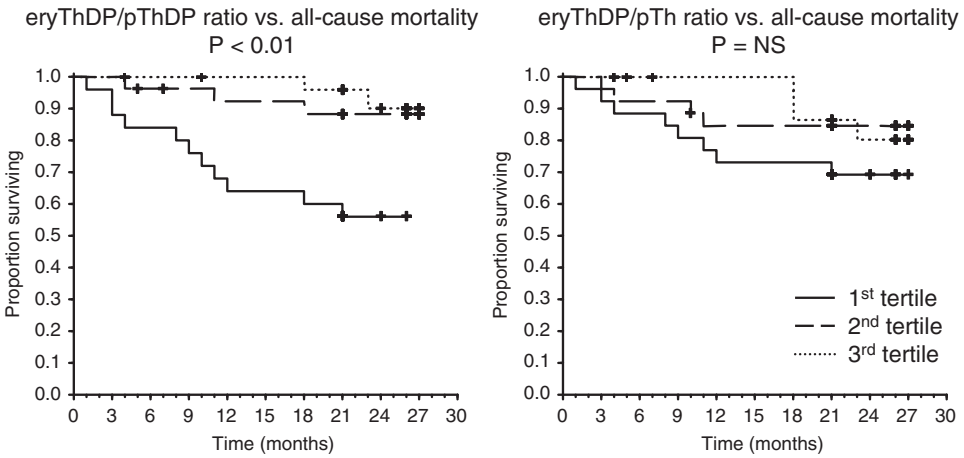

Fig. 5. Kaplan–Meier curves for all-cause mortality by eryThDP/pThDP and eryThDP/pTh ratio tertile groups.

olism in disorders of glucose metabolism, namely diabetes. Unlike natural hydrophilic co-factor thiamine, lipid-soluble TKT activator benfothiamine easily crosses plasma membranes, and its cellular concentration could thus be effectively increased. There are several reports from *in vitro* as well as *in vivo* experiments showing that TKT activation by benfothiamine effectively inhibits all four biochemical pathways responsible for hyperglycaemia-induced damage [11,12] and suppresses the development of DN [13] and retinopathy [12] in experimentally induced diabetes in animals (without primary change of diabetes compensation). A pilot human study revealed a moderate therapeutic effect of benfothiamine on diabetic polyneuropathy [14] and normalization of markers of reactive oxygen species-induced damage [15].

In the present study, we assessed thiamine status in diabetic subjects with variable stage of diabetic kidney disease with aims to dissect basic relationships between disease parameters and thiamine handling. The major findings can be summarized as follows: firstly, (1) plasma levels of Th and its derivatives pThMP and pThDP (but not intracellular eryThDP) rise in parallel to advancement of DN severity and rise of S-Cr (i.e. decline of renal function). This is most likely a result of decreased renal clearance of thiamines (molecular mass being $\leq$0.5 kDa) and not of increased intake, because patients with diabetic kidney disease in proteinuric and ESRD range are, if anything, recommended to restrict their protein intake (often coming from the same source as thiamine). Therefore, conventional parameters for assessment of thiamine status based on plasma levels are uninformative in patients with impaired kidney function.

Secondly, (2) the seemingly protective rise of plasma thiamines is not accompanied by concomitant rise of ThDP levels in erythrocytes; however, eryThDP levels do not correspond with eryTKT activity which does increase. It might be speculated that although diabetics with more advanced complications still do increase activity of TKT and thus PPP efficiency, it might not be enough to prevent development of cellular damage mediated by glycolytic intermediates during hyperglycaemia. A further increase might be prevented just by the deficiency of eryThDP. Thornalley *et al*. previously demonstrated significantly increased expression of thiamine transporters in erythrocyte plasma membranes of T1DM and T2DM subjects compared with healthy volunteers [5]; however, there is nothing known about thiamine transporter regulation in uraemia. Again, it might be speculated that increased plasma levels of thiamines in CKD paradoxically down-regulated thiamine transporters and do not allow for further increase of PPP activity. Another possible explanation can be impaired activity of thiamine pyrophospokinase during hyperglycaemia resulting in insufficient production of active TKT co-factor. Whether deficient transport into cells, impaired activation of thiamine or both, experimental data are lacking to support these speculations presently and to allow any practical recommendation regarding thiamine supplementation in diabetics with CKD.

Thirdly, (3) thiamine status parameters were related to all-cause mortality in our cohort of patients. All-cause mortality exhibited significant association with decreased levels of active TKT co-factor eryThDP whose intracellular deficiency seems not to be a consequence of decreased plasma levels of its precursor. Both findings (2) and (3) therefore indicate that major abnormalities in thiamine metabolism most likely take place on the level of transport between extra- and intracellular compartment and within the cells. Future studies are warranted to elucidate these mechanisms.

Finally, (4) we did not discover any major genetic effect in the PPP enzyme loci (*TKT*, *TALDO1* and *TKTL1*) on either a DN susceptibility or enzyme phenotype. This still does not rule out the role of genetic variability completely (small size effects could well be operating); however, large-scale case–control study and ideally sufficient sample of healthy subjects to dissect genotype vs. phenotype interactions would be required for this purpose.

Several possible weaknesses of the current study have to be mentioned. First of all, duration of the follow-up study was unfortunately suboptimal for the robust assessment of the relationship between kidney disease progression and thiamine parameters. A longer follow-up might bring more light to the issue of predictive value of thiamine parameters for diabetic kidney disease progression. Another possible weakness applies to methodology. Measurement of thiamine metabolites—although carefully optimized and validated by use of internal quality controls—was performed without an internal standard suitable for pre-column derivatization with potassium ferricyanide. However, this is a drawback of nearly all clinical studies measuring thiamines by HPLC published, since no standardized internal standard for use in clinical settings is agreed.

In conclusion, results of the recent study comprising large sample of diabetic subjects with diabetic kidney disease characterized for parameters of thiamine metabolism support the role of 'functional' thiamine deficiency in the development of hyperglycaemia-related pathology. Limited intracellular availability of active TKT co-factor seems to be a dominant abnormality. Future studies are needed to determine the mechanisms responsible for limited intracellular availability of active thiamine co-factor.

Supplementary data

Supplementary data is available online at http://ndt.oxfordjournals.org.

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Conflict of interest statement. None declared.

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Risk factors for the development of albuminuria and renal impairment in type 2 diabetes—the Swedish National Diabetes Register (NDR)

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Abstract

Background. The aim of this study was to identify clinical risk factors associated with the development of albuminuria and renal impairment in patients with type 2 diabetes (T2D). In addition, we evaluated if different equations to estimate renal function had an impact on interpretation of data. This was done in a nationwide population-based study using data from the Swedish National Diabetes Register.

Methods. Three thousand and six hundred sixty-seven patients with T2D aged 30–74 years with no signs of renal dysfunction at baseline (no albuminuria and eGFR >60 mL/min/1.73 m^2 according to MDRD) were followed up for 5 years (2002–2007). Renal outcomes, development of albuminuria and/or renal impairment [eGFR <60 mL/min/1.73 m^2 by MDRD or eCrCl >60 mL/min by Cockgroft–Gault (C–G)] were assessed at follow-up. Univariate regression analyses and stepwise regression models were used to identify significant clinical risk factors for renal outcomes.

Results. Twenty percent of patients developed albuminuria, and 11% renal impairment; thus, ~6–7% of all patients developed non-albuminuric renal impairment. Development of albuminuria or renal impairment was independently associated with high age (all $P < 0.001$), high systolic BP (all $P < 0.02$) and elevated triglycerides (all $P < 0.02$). Additional independent risk factors for albuminuria were high BMI ($P < 0.01$), high HbA1c ($P < 0.001$), smok-