WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 2, 1-13. Research Article ISSN 2277– 7105

BENFOTIAMINE AND HEART RATE VARIABILITY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS AND CARDIAC AUTONOMIC NEUROPATHY

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Article Received on
22 Nov. 2018,
Revised on 12 Dec. 2018,
Accepted on 01 Jan. 2019

DOI: 10.20959/wjpr20192-14036

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ABSTRACT

Background: The significance of cardiac autonomic neuropathy (CAN) has not been fully appreciated and there is no unified treatment algorithm. **Aim:** To investigate the effects of benfotiamine (BFT) on the heart rate variability (HRV) in patients with type 2 diabetes mellitus (T2DM) and CAN. **Patients and Methods:** 32 patients with T2DM and definite stage of CAN were allocated to two treatment groups: control (n = 15) received standard antihyperglycaemic therapy; group 2 (n = 17) - in addition BFT 300 mg/d for three months. The heart rate variability (HRV) parameters were analyzed. **Results:** It was found out that BFT contributed to increase of the sum of the squares of differences between adjacent normal-to-normal intervals, high-frequency component of HRV during the active and passive periods of the day. **Conclusions:** The positive influences of BFT suggests the feasibility of its administration to patients with T2DM and definite stage of CAN.

KEYWORDS: Type 2 diabetes mellitus, Cardiac autonomic neuropathy, Benfotiamine, Heart rate variability.

INTRODUCTION

The number of people with type 2 diabetes mellitus (T2DM) has been increasing worldwide. It was estimated that there were 415 million people with diabetes mellitus (DM) aged 20-79

years in 2015, and the number was predicted to rise to 642 million by 2040.[1] The majority of patients with long-term course of DM (mainly T2DM) are diagnosed with coronary heart disease (CHD) due to coronary vessels arterial sclerotic disease. Often the course of CHD is complicated by combination of hypertension, specific kidney arterial involvement, eyes and lower limbs affection. Metabolic alterations in the myocardium are combined with early coronary atherosclerosis. All these changes in heart occur out of prolonged duration of DM among middle age and elderly patients [coronary vessels affection, myocardium changes, diabetic cardiac autonomic neuropathy (CAN) and arterial sclerotic disease] are associated with the term "diabetic heart or diabetic cardiomyopathy".[2]

Based on the CAN Subcommittee of the Toronto Consensus Panel on Diabetic Neuropathy,[3] CAN is defined as the impairment of cardiovascular autonomic control among patients with established DM following the exclusion of other causes. Cardiac autonomic neuropathy among T2DM patients, is characterized by lesion of nerve fibers in the sympathetic and parasympathetic divisions of the autonomic nervous system (ANS), is diagnosed unsatisfactorily and may be accompanied by severe postural hypotension, decreased tolerance to the physical loadings, and cause of the cardiac arrhythmias, ischemia of coronary vessels, "silent" MI, sudden death syndrome.[4,5] CAN, especially at the early stages, can be subclinical and thus as the disease progresses, it becomes clinically evident.[6,7]

Therefore, the problem of effective treatment of CAN is particularly relevant. Pathogenetic treatment of CAN includes: balanced diet and physical activity; reducing insulin resistance; optimization of glycaemic control; treatment of dyslipoproteinaemia; correction of metabolic abnormalities in myocardium; prevention and treatment of thrombosis; use of aldose reductase inhibitors; γ-linolenic acid, acetyl-L-carnitine, antioxidants, use of omega-3 polyunsaturated fatty acids, vasodilators, fat-soluble vitamin B_1 (benfotiamine), aminoguanidine; symptomatic treatment of concomitant diseases and syndromes (hypertension, CHD, heart failure and arrhythmias) and others.[8-12]

Thus, we aimed to evaluate the effects of benfotiamine (a lipid-soluble thiamine derivative with higher bioavailability than thiamine) on the heart rate variability (HRV) parameters in patients with T2DM and definite stage of CAN.

MATERIALS AND METHODS

To explore the effectiveness of some above mentioned compounds we examined 32 patients with T2DM and definite stage of CAN, patients were aged between 50-59 years with disease duration 1-6 years and median glycated hemoglobin A1c (HbA1c) 7.1% ± 0.4%. Clinical characteristics of studied patients with T2DM and definite stage of CAN are given in Table 1.

Table 1: Baseline characteristics of patients included in this study.

Parameter	Patients with T2DM and definite stage of CAN (n = 32)	
	Control (n = 15)	Benfotiamine (n = 17)
	Group 1	Group 2
Age (years)	55.33±0.95	54.12±0.65
Gender		
Male (%)	8/53.3%	10/58.8%
Female (%)	7/46.7%	7/41.2%
Diabetes duration (years)	3.6 ± 0.42	4.06 ± 0.36
BMI (kg/m^2)	28.89 ± 0.16	26.66 ± 0.32
Medications		
ACE inhibitors (%)	12/80%	14/82.4%
β-blockers (%)	3/20%	4/23.5%
Metformin (%)	11/73.3%	11/64.7%
Sulfonylurea (%)	1/6.7%	1/5.9%
Combined hypoglycaemic therapy (%)	3/20%	5/29.4%
Hypertension (%)	12/80%	16/94.12%

T2DM: type 2 diabetes mellitus; CAN: cardiac autonomic neuropathy; BMI: body mass index; ACE: angiotensin-converting enzyme.

CAN was diagnosed according to previously proposed criteria.[3] The work was done according to the principles of the Helsinki Declaration II and was approved by the medical ethics committee of Danylo Halytsky Lviv National Medical University. All participants signed an informed consent prior to their inclusion in the study.

Patients with T2DM and definite stage of CAN were allocated to two treatment groups: first group received traditional antihyperglycaemic therapy (n = 15, control group); patients in group 2 (n = 17) received in addition to standard treatment-benfotiamine (BFT) 300 mg/d. The duration of the treatment was three months.

The concentration of glucose in the blood was determined by the glucose oxidase method while HbA1c level was assessed by using a highly sensitive method of ion exchange liquid chromatography with D-10 analyzer and BIO-RAD reagents (United States).

Resting 12-lead surface electrocardiography (ECG) with a paper speed of 25 mm/s and a signal size of 10 mm/mV was recorded in the morning period. We performed resting ECG analysis included measurement of following parameters: heart rhythm, heart rate, conduction intervals and Holter-ECG [(ECG "EC-3H" ("Labtech," Hungary)] analysis included measurement of 24 hours ECG, circadian indexes and following HRV parameters:[13] standard deviation of all NN intervals (SDNN), standard deviation of the means of all NN intervals for all 5-mins segments of the entire recording (SDANNi), the square root of the mean of the sum of the squares of differences between adjacent NN intervals (RMSSD), the square root of the mean of the sum of the squares of differences between adjacent NN intervals (pNN50,%), the high-frequency component of HRV (HF), the low-frequency component of HRV (LF), the very low-frequency component of HRV (VLF), ratio of low to high frequency power components [sympathetic/parasympathetic ratio (LF/HF)].

Statistical analysis

Statistical analysis was based on the variational method using statistical parametric t-test, nonparametric Wilcoxon t-test and Fisher's Pearson correlation coefficient. Data are presented as mean ± standard error of the mean (SEM). All tests were performed using the ANOVA (MicroCal Origin v. 8.0) software. Statistical significance was set at $P < 0.05$.

RESULTS

We found out that the HbA1c of patients with T2DM and definite stage of CAN was not statistically significant influenced by the treatment ($p > 0.05$).

The features of the time-domain HRV parameters in patients with T2DM and definite stage of CAN after BFT administration are given in Table 2.

Table 2: Changes of the time-domain heart rate variability parameters in patients with T2DM and CAN after 3-months of benfotiamine therapy (Δ%, Mean ± SEM).

Parameter	Patients with T2DM and definite stage of CAN (n = 32)			
	Groups	Baseline	After treatment	% change from baseline
SDNN (ms)	Control group (n = 15)	94.7 ± 4.84	91.3 ± 3.33	-0.82% ± 2.73%
	Benfotiamine (n = 17)	98.8 ± 2.73	103.8 ± 2.7	+5.3% ± 1.64%
SDANNi (ms)	Control group (n = 15)	72.3 ± 4.08	74.7 ± 3.33	+4.72% ± 2.67%
	Benfotiamine (n = 17)	81.8 ± 3.18	87.4 ± 2.78	+7.7% ± 2.33%
RMSSD (ms)	Control group (n = 15)	19.1 ± 1.36	18.7 ± 0.77	+0.96% ± 3.67%
	Benfotiamine (n = 17)	21.3 ± 1.42	23.1 ± 1.35	+9.48% ± 2.37%
pNN50 (%)	Control group (n = 15)	3.9 ± 0.42	3.7 ± 0.23	+7.1% ± 9.59%
	Benfotiamine (n = 17)	4.94 ± 0.55	**7.41 ± 1.06**[a]	+45.9% ± 7.91%

The results are presented as absolute values and as % change from baseline, (Δ%, Mean±SEM); [a]p < 0.05, compared to baseline. T2DM: type 2 diabetes mellitus; CAN: cardiac autonomic neuropathy; SDNN: standard deviation of all NN intervals; SDANNi: standard deviation of the means of all NN intervals for all 5-minute segments of the entire recording; RMSSD: the square root of the mean of the sum of the squares of differences between adjacent NN intervals; pNN50 - percentage of differences greater than 50 ms between adjacent sinus NN intervals.

Obtained results could prove that treatment with BFT among patients with T2DM and definite stage of CAN led to a significant decrease of the pNN50 and did not affect the SDNN, SDANNi and RMSSD parameters (Table 2).

Changes of the spectral heart rate variability parameters during the active period in patients with T2DM and CAN after 3-months of BFT therapy are given in Table 3.

Table 3: Changes of the spectral heart rate variability parameters during the active period in patients with T2DM and CAN after 3-months of benfotiamine therapy (Δ%, Mean ± SEM).

Parameter	Patients with T2DM and definite stage of CAN (n = 32)			
	Groups	Baseline	After treatment	% change from baseline
VLF (ms^2)	Control group (n = 15)	1113.6 ± 98.1	1103.9 ± 72.8	+1.81% ± 2.63%
	Benfotiamine (n = 17)	1128.4 ± 80.34	1241.2 ± 75.1	+11,5% ± 1,99%
LF (ms^2)	Control group (n = 15)	390.8 ± 21.98	388.5 ± 14.74	+1.6% ± 3.72%
	Benfotiamine (n = 17)	414.8 ± 21.0	474.9 ± 21.9	+16.8% ± 4.98%
HF (ms^2)	Control group(n = 15)	242.1 ± 18.5	244.5 ± 13.6	+4.1% ± 4.15%
	Benfotiamine (n = 17)	267.7 ± 17.61	**326.4 ± 16.96[a]**	+25.8% ± 5.58%
LF/HF	Control group (n = 15)	1.66 ± 0.06	1.63 ± 0.06	-1.6% ± 3.07%
	Benfotiamine (n = 17)	1.58 ± 0.04	1.47 ± 0.05	-6.3% ± 2.9%

The results are presented as absolute values and as % change from baseline, (Δ%, Mean ± SEM); [a]p < 0.05, compared to baseline. T2DM: type 2 diabetes mellitus; CAN: cardiac autonomic neuropathy; VLF: very low frequency power, total spectral power of all NN intervals between 0.003 and 0.04 Hz; LF: low frequency power, total spectral power of all NN intervals between 0.04 and 0.15 Hz; HF: high frequency power, total spectral power of all NN intervals between 0.15 and 0.4 Hz; LF/HF: sympathetic/parasympathetic ratio.

Obtained results of this study could prove that treatment with BFT is accompanied by significant increase of HF parameters during the active period compared to patients in control group (Table 3).

Changes of the spectral HRV parameters during the passive period in patients with T2DM and definite stage of CAN after 3-mo of BFT are given in Table 4.

Table 4: Changes of the spectral heart rate variability parameters during the passive period in patients with T2DM and CAN after 3-months of benfotiamine therapy (Δ%, Mean ± SEM).

Parameter	Patients with T2DM and definite stage of CAN (n = 32)			
	Groups	Baseline	After treatment	% change from baseline
VLF (ms^2)	Control group (n = 15)	1441.1 ± 106.7	1439.3 ± 84.7	+1.83% ± 2.47%
	Benfotiamine (n = 17)	1440.7 ± 90.9	1572.4 ± 85.1	+10.3% ± 1.98%
LF (ms^2)	Control group (n = 15)	509.1 ± 17.82	502.1 ± 13.0	-0.6% ± 2.55%
	Benfotiamine (n = 17)	524.1 ± 21.4	588.5 ± 25.8	+12.7% ± 3.31%
HF (ms^2)	Control group (n = 15)	340.5 ± 25.3	326.7 ± 19.01	-1.9% ± 3.38%
	Benfotiamine (n = 17)	332.5 ± 18.39	**396.6 ± 19.0[a]**	+21.1% ± 4.17%
LF/HF	Control group (n = 15)	1.57 ± 0.08	1.59 ± 0.07	+1.6% ± 1.92%
	Benfotiamine (n = 17)	1.61 ± 0.05	1.5 ± 0.04	-6.1% ± 2.75%

The results are presented as absolute values and as % change from baseline, (Δ%, Mean ± SEM); [a]$p < 0.05$, compared to baseline. T2DM: type 2 diabetes mellitus; CAN: cardiac autonomic neuropathy; VLF: very low frequency power, total spectral power of all NN intervals between 0.003 and 0.04 Hz; LF: low frequency power, total spectral power of all NN intervals between 0.04 and 0.15 Hz; HF: high frequency power, total spectral power of all NN intervals between 0.15 and 0.4 Hz; LF/HF: sympathetic/parasympathetic ratio.

It was found out that treatment with BFT is accompanied by significant increase of HF parameters during the passive period compared to patients in control group (Table 4).

As a result of our studies, it was found out that treatment with BFT contributed to a decrease in resting tachycardia [110 to 96 beats/min ($p < 0.05$)], improvement of subjective feeling and increase in tolerance of exercise loading. In addition in the majority of the patients with diabetic polyneuropathies (DPN) we observed the decrease and/or disappearance of pain, paresthesia, frequency of muscle cramps, improvement and/or restoration of tactile, vibration and temperature sensitivity.

DISCUSSION

Dysautonomia is a broad term that describes any disease or malfunction of the ANS. Evidence has been produced to indicate that various forms of mild to moderate vitamin deficiencies result in functional changes in the ANS. It is hypothesized that the predictable loss of efficiency in oxidative metabolism is the key to understanding the association of dysautonomia with many different diseases. Mild to moderate hypoxia and/or thiamine deficiency (TD) both give rise to exaggeration of centrally controlled mechanisms involved in all survival reflexes, mediated normally through a balanced reaction of the ANS and endocrine system. Together with dietary excesses, particularly in the universal ingestion of sugar, appears to be responsible for initiating long term disease related to the synthesis and use of cellular energy. Failure of ANS cholinergic neurotransmission might follow from TD and/or other cofactors involved in glucose metabolism, exposing the organism to adrenal medullary release of epinephrine.[14] Chronic exposure to moderate and severe hypoxia increases the activity of the sympathetic nervous system and adrenal medulla and TD induces an early functionally significant central muscarinic cholinergic lesion in rat studies.[15,16]

Diabetes might be considered as TD state, if not in absolute terms at least relative to the increased requirements deriving from accelerated and amplified glucose metabolism in non-insulin dependent tissues that, like the vessel wall, are prone to complications.[17,18] The conventional indicator of thiamine sufficiency, erythrocyte transketolase (TKT) activity, is masked in clinical diabetes by increased protein levels of thiamine transporter-1 and thiamin transporter-2. The deficiency of thiamine in clinical diabetes may increase the fragility of vascular cells to the adverse effects of hyperglycaemia and there by the increase of the risk of developing microvascular complications. A suppression of TKT activity, and subsequent down-regulation of the hexose monophosphate (HMP) shunt, resulting in accumulation of glyceraldehyde 3-phosphate (GA3P), fructose 6-phosphate (F6-P), and dihydroxyacetone phosphate (DHAP) may be at least one mechanism in the development of diabetes-induced vascular damage and other comorbidities.[19-22]

Restoring TKT activity via BFT or thiamin supplementation can increase the flux of glucose into HMP shunt, and also increase flux of GA3P, F6P, and DHAP into HMP shunt and away from hyperglycaemia-induced pathways that lead to vascular damage.[19,20] It is hypothesized that genetic variability in TKT might contribute to susceptibility to early DPN.[23,24] TKT is indeed pertinent to the pathogenesis of diabetic microangiopathic complications. When

activated in the cell by thiamine, TKT determines the diversion of F6-P and GA3P to the pentose-phosphates pathway (PPP), in this way it exercises a protective effect against three pathogenetic mechanisms of microvascular complications, i.e. the glucose-driven hexosamine, protein kinase C (PKC), and advanced glycation end products (AGE's) pathways.[25,23,24] The connection between genetic variability of TKT and nerve function measures in newly diagnosed diabetic patients were demonstrated, which can favour the identification of patients with a deficiency in TKT defensive mechanisms (and as such suitable candidates for therapeutic intervention).[23,24]

Thiamine and its derivatives have been demonstrated to prevent the activation of the biochemical pathways (increased flux through the polyol pathway, formation of AGE's, activation of PKC, and increased flux through the hexosamine biosynthesis pathway induced by hyperglycaemia in DM). Thiamine definitively plays a role in the diabetic endothelial vascular diseases (micro and macroangiopathy), lipid profile, retinopathy, nephropathy, cardiomyopathy, and neuropathy.[26,27] Thiamine acts as a coenzyme for TKT and for the pyruvate dehydrogenase and α-ketoglutarate dehydrogenase complexes, enzymes which play a fundamental role intracellular glucose metabolism. TKT and glucose-6-phosphate dehydrogenase, the rate-limiting enzymes of the PPP, are inhibited in the diabetic heart under basal conditions.[28]

Inhibition of AGE's proved to be more effective with thiamine pyrophosphate and pirydoxamin than aminoguanidine. The lipid-soluble derivate BFT is characterised by five times higher bioavailability compared to the water-soluble compound and was shown to be effective in the treatment of DPN.[29] Experiences from cardiology indicate that long-term increases in HRV and reduction in sudden cardiac death have only been shown with lipophilic agents that readily penetrate the blood nerve/blood brain barrier. In accordance with these observations experimental data indicate a preventive effect of BFT on the development of CAN.[30] High-dose therapy of thiamine and BFT suppressed AGE's accumulation in the peripheral nerve and reversed diabetic neuropathy potentially by reducing the levels of triose phosphates *via* activation of TKT.[31,29,18] Activation of AGE receptors in DM, found on glomerular endothelial cells, cardiomyocytes, pericytes, and podocytes, stimulates postreceptor signaling, intracellular reactive oxygen species formation, and altered gene expression, leading to vascular damage.[19,32,33]

The explanations about positive thiamine effects were confined to hydrophobic thiamine metabolites that fulfill an important function under oxidative stress (OS) and nitrosyl stress. Thiamine protects nervous tissue probably by inhibiting nitric oxide (NO)-dependent tyrosine nitration and subsequent formation of dityrosine and interprotein tyrosine-tyrosine crosslink.[18] Cardiac OS is involved in heart failure that is induced by thiamine deprivation in rats. These findings suggest that thiamine modulates OS.[27] Nitric oxide synthase (NOS) is an enzyme that is involved in the synthesis of NO, which regulates a variety of important physiological responses, including cell migration, the immune response, and apoptosis. Endothelial NOS (eNOS) and NO may play an important role in attenuating cardiac remodeling and apoptosis. Benfotiamine reduces OS and activates eNOS to enhance the generation and bioavailability of NO, and it subsequently improves the integrity of vascular endothelium to prevent sodium arsenite-induced experimental vascular endothelial dysfunction.[34,27]

The identification of the association of polymorphisms related to the genes of thiamine and TKT with DPN might be a first step in defining a DPN genetic risk profile with potential therapeutic repercussions. There is moderate evidence from preclinical experimental models that high-dose thiamine and BFT (1) inhibit the HMP, AGE's formation, and diacylglycerol-PKC through the TKT activation; (2) target at various surrogate markers of hyperglycaemia-induced pathological processes and (3) can delay the progression of microangiopathic complications.[35,24]

Benfotiamine treatment counters diabetes-induced cardiac mechanical dysfunction at the cellular level, associated with reduction in OS but not AGE’s formation or cardiac protein carbonyl formation. This apparent discrepancy in BFT-elicited action on AGE’s formation and OS (the oxidized/reduced glutathione ratio) seems to indicate that other mechanism(s) may predominantly contribute to diabetes-induced OS and cardiac contractile dysfunction in current experimental setting. Possible candidates may include alteration in glucose metabolism and PKC activation), although further study is warranted to verify involvement of these signaling pathways and beneficial effects of BFT against diabetic complications.[36,37,32]

CONCLUSION

In conclusion, the positive influences of BFT on increase of pNN50; HF parameters during the active and passive periods of the day by us are partly confirmed by its neurotropic,

cardioprotective and angioprotective properties; suggests the feasibility of its usage in the complex treatment of patients with T2DM and definite stage of CAN.

The mechanism of BFT influence on diabetic CAN pathogenesis is not well-known. Thus, further investigations aimed to understand the mechanism of action and confirmation of the beneficial effect of BFT on biochemical parameters, dynamics of independent cardiovascular tests, daily monitoring of electrocardiography, arterial wall stiffness parameters among patients with T2DM and definite stage of CAN and its associated comorbidities may be needed to validate this clinical findings.

ACKNOWLEDGMENTS

Conflict of interests: There are no ethical/legal conflicts involved in the article. There are no conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject of their manuscript.

Authorship: All the listed authors contributed significantly to conception and design of study, acquisition, analysis and interpretation of data and drafting of manuscript, to justify authorship.

FUNDING

No any grant or funds have been received for this study.

REFERENCES

1. Ogurtsova K, da Rocha Fernandes LD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, et al. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes Res Clin Pract., 2017; 128(2): 40-50. doi: 10.1016/j.diapres.2017.03.024.
2. Valensi P, Cosson E. Diabetic cardiovascular autonomic neuropathy. Eur J Endocrinol, 2007; (2): 69-71. doi: 10.17925/EE.2007.00.02.69.
3. Spallone V, Ziegler D, Freeman R, Bernardi L, Frontoni S, Pop-Busui R, et al. Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management. Diabetes Metab Res Rev., 2011; 27(7): 639-653. DOI: 10.1002/dmrr.1239.
4. Dimitropoulos A, Tahrani AA, Stevens MJ. Cardiac autonomic neuropathy in patients with diabetes mellitus. World J Diabetes, 2014; 1(5): 17-39. doi: 10.4239/wjd.v5.i1.17.

5. Pop-Busui R, Boulton AJM, Feldman EL, Bril V, Freeman R, Malik RA, et al. Diabetic neuropathy: A position statement by the American Diabetes Association. Diabetes Care., 2017; 40(1): 136-154. doi: 10.2337/dc16-2042.
6. Vinik AI, Erbas T, Casellini CN. Diabetic cardiac autonomic neuropathy, inflammation and cardiovascular disease. J Diabetes Investig, 2013; 4(1): 4-18. doi: 10.1111/jdi.12042.
7. Vinik AI, Nevoret ML, Casellini C, Parson H. Diabetic neuropathy. Endocrinol Metab Clin North Am., 2013; 42(4): 747-787. doi: 10.1016/j.ecl.2013.06.001.
8. Serhiyenko VA, Serhiyenko AA. Diabetic cardiac autonomic neuropathy: Do we have any treatment perspectives? World J Diabetes, 2015; 6(2): 245-258. doi: 10.4239/wjd.v6.i2.245.
9. Serhiyenko VA, Serhiyenko AA. Omega-3 polyunsaturated fatty acids in the treatment of diabetic cardiovascular autonomic neuropathy: A review. In: Moore SJ (eds.). Omega-3: Dietary sources, biochemistry and impact on human health, New York; Nova Science Publishers, 2017; 79-154. ISBN: 978-1-53611-824-7. print. ISBN: 978-1-53611-839-1. e-book.
10. Deli G, Bosnyak E, Pusch G, Komoly S, Feher G. Diabetic neuropathies: diagnosis and management. Neuroendocrinology, 2013; 98(45): 267-280. doi: 10.1159/000358728.
11. Serhiyenko VA, Serhiyenko AA. Cardiac autonomic neuropathy: Risk factors, diagnosis and treatment. World J Diabetes, 2018; 9(1): 1-24. doi: 10.4239/wjd.v9.i1.1.
12. Serhiyenko V, Hotsko M, Snitynska O, Serhiyenko A. Benfotiamine and type 2 diabetes mellitus. MOJ. Public Health, 2018; 7(1): 00200. doi: 10.15406/mojph.2018.07.00200.
13. Adamec J, Adamec R. ECG Holter: Guide to electrocardiographic interpretation. New York; Springer-Verlag US, 2008. doi: 10.1007/978-0-387-78187-7 1.
14. Lonsdale D. Dysautonomia, a heuristic approach to a revised model for etiology of disease. Evid Based Complementary Altern Med., 2009; 6(1): 3-10. doi: 10.1093 / ecam / nem064.
15. Barclay LL, Gibson GE, Blass JP. Impairment of behavior and acetylcholine metabolism in thiamine deficiency. J Pharmacol Exp Ther., 1981; 217(3): 537-543. PMID: 7229989.
16. Johnson TS, Young JB, Landsberg L. Sympathoadrenal responses to acute and chronic hypoxia in the rat. J Clin Invest., 1983; 71(5): 1263-1272. PMID: 6853714 PMCID: PMC436987.
17. Berrone E, Beltramo E, Solimine C, Ape AU, Porta M. Regulation of intracellular glucose and polyol pathway by thiamine and benfotiamine in vascular cells cultured in high glucose. J Biol Chem., 2006; 281(14): 9307-9313. doi: 10.1074/jbc.M600418200.

18. Rašić-Milutinović Z, Gluvić Z, Peruničić-Peković Z, Miličević D, Lačković M, Penčić B. Improvement of heart rate variability with benfothiamine and alpha-lipoic acid in type 2 diabetic patients - Pilot study. J Cardiol Ther., 2014; 2(1): 1-7. doi: 10.12970/2311-052X.2014.02.01.9.
19. Babaei-Jadidi R, Karachalias N, Kupich C, Ahmed N, Thornalley PJ. High-dose thiamine therapy counters dyslipidaemia in streptozotocin-induced diabetic rats. Diabetologia, 2004; 47(12): 2235-2246. doi: 10.1007/s00125-004-1582-5.
20. Oh SH, Witek RP, Bae SH, Darwiche H, Jung Y, Pi L, Brown A, et al. Detection of transketolase in bone marrow-derived insulin-producing cells: Benfotiamine enhances insulin synthesis and glucose metabolism. Stem Cells Dev., 2009; 18(1): 37-45. doi: 10.1089/scd.2007.0255.
21. Pácal L, Kuricová K, Kaňková K. Evidence for altered thiamine metabolism in diabetes: is there a potential to oppose gluco- and lipotoxicity by rational supplementation? World J Diabetes, 2014; 5(3): 288-295. doi: 10.4239/wjd.v5.i3.288.
22. Stirban A, Pop A, Tschoepe D. A randomized, double-blind, crossover, placebo-controlled trial of 6 weeks benfotiamine treatment on postprandial vascular function and variables of autonomic nerve function in Type 2 diabetes. Diab Med., 2013; 30(10): 1204-1208. doi: 10.1111/dme.12240.
23. Ziegler D, Schleicher E, Strom A, Knebel B, Fleming T, Nawroth P, et al. Association of transketolase polymorphisms with measures of polyneuropathy in patients with recently diagnosed diabetes. Diabetes Met Res Rev., 2016; 33(4): e2811. doi: 10.1002/dmrr.2811.
24. Spallone V. Might genetics play a role in understanding and treating diabetic polyneuropathy? Diabetes Met Res Rev., 2017; 33(4): 2882-2886. doi: 10.1002/dmrr.2882.
25. Rabbani N, Thornalley PJ. Glyoxalase in diabetes, obesity and related disorders. Semin Cell Dev Biol., 2011; 22(3): 309-317. doi: 10.1016/j.semcdb.2011.02.015.
26. González-Ortiz M, Martínez-Abundis E, Robles-Cervantes JA, Ramírez-Ramírez V, Ramos-Zavala MG. Effect of thiamine administration on metabolic profile, cytokines and inflammatory markers in drug-naïve patients with type 2 diabetes. Eur J Nutr., 2011; 50(2): 145-149. doi: 10.1007/s00394-010-0123-x.
27. Luong KV, Nguyen LT. The impact of thiamine treatment in the diabetes mellitus. J Clin Med Res., 2012; 4(3): 153-160. doi: 10.4021/jocmr890w.
28. Katare RG, Caporali A, Emanueli C, Madeddu P. Benfotiamine improves functional recovery of the infarcted heart via activation of pro-survival G6PD/Akt signaling pathway

and modulation of neurohormonal response. J Mol Cell Cardiol., 2010; 49(4): 625-638. doi: 10.1016/j.yjmcc.2010.05.014.

29. Stracke H, Gaus W, Achenbach U, Federlin K, Bretzel RG. Benfotiamine in diabetic polyneuropathy (BENDIP): Results of a randomised, double blind, placebo-controlled clinical study. Exp Clin Endocrinol Diabetes, 2008; 116(10): 600-605. doi: 10.1055/s-2008-1065351.
30. Aronson D. Pharmacologic modulation of autonomic tone: implications for the diabetic patient. Diabetologia, 1997; 40(4): 476-481. doi: 10.1055/s-2008-1065351.Thornalley PJ. The potential role of thiamine (vitamin B(1) in diabetic complications. Cur Diabetes Rev., 2005; 1(3): 287-298. doi: 10.2174/157339905774574383.
31. Ceylan-Isik AF, Wu S, Li Q, Li SY, Ren Y. High-dose benfotiamine rescues cardiomyocyte contractile dysfunction in streptozotocin-induced diabetes mellitus. J Appl Physiol., 2006; 100(1): 150-156. doi: 10.1152/japplphysiol.00988.2005.
32. Du X, Edelsteinv D, Brownlee M. Oral benfotiamine plus alpha-lipoic acid normalises complication-causing pathways in type 1 diabetes. Diabetologia, 2008; 51(10): 1930-1932. doi: 10.1007/s00125-008-1100-2.
33. Verma S, Reddy K, Balakumar P. The defensive effect of benfotiamine in sodium arsenite-induced experimental vascular endothelial dysfunction. Biol Trace Elem Res., 2010; 137(1): 96-109. doi: 10.1007/s12011-009-8567-7.
34. Pacal L, Tomandl J, Svojanovsky J, Krusova D, Stepankova S, Rehorova J, et al. Role of thiamine status and genetic variability in transketolase and other pentose phosphate cycle enzymes in the progression of diabetic nephropathy. Nephrol Dial Transplant, 2011; 26(4): 1229-1236. doi: 10.1093/ndt/gfq550.
35. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature., 2001; 414(6865): 813-820. doi: 10.1038/414813a.
36. Wu S, Ren J. Benfotiamine alleviates diabetes-induced cerebral oxidative damage independent of advanced glycation end-product, tissue factor and TNF-alpha. Neurosci Lett., 2006; 394(2): 158-162. doi: 10.1016/j.neulet.2005.10.022.