Improvement of Heart Rate Variability with Benfothiamine and Alpha-Lipoic Acid in Type 2 Diabetic Patients - Pilot Study

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Abstract: According to hypothesis that the lower level of bioactive thiamine, vitamin B1, is partially responsible for microvascular complications in type 2 diabetes patients (T2DM), we investigated whether the supplementation with benfothiamine and alpha-lipoic acid (ALA) could improve cardiac autonomic neuropathy (CAN) in those patients. Heart rate variability (HRV) was used as a tool for the assessment of equilibrium in the activity of the autonomic nervous system (ANS) in the control of heart rate in T2DM patients, without serious co-morbidity. Blood samples for glucose, HbA1c, triglycerides, cholesterol, and HDL-C were obtained before and at the end of treatment. High-frequency ln (HFln), was significantly lower ($p< 0.01$) and low- frequency/ high frequency ln (LF/HFln) was significantly higher ($p<0.01$) in T2DM. After 12 weeks of supplementation, very low-frequency ln (VLFln), HFln and LF/HFln indices were changed significantly ($p=0.03$; $p=0.01$; $p=0.04$, respectively), and we concluded that benfothiamine associated with ALA could participate in repairing cardiac autonomic dysfunction in T2DM.

Keywords: Cardiac autonomic neuropathy, heart rate variability, type 2 diabetes, benfothiamine, alpha-lipoic acid.

INTRODUCTION

Based on current data, glucose intolerance, even at a pre-diabetic stage, is associated with progressive development of a variety of abnormalities that adversely affect survival and pre-dispose sudden cardiac death. A very serious and common complication of T2DM is diabetic autonomic neuropathy (DAN), particularly CAN, with a wide spectrum of adverse cardiovascular outcomes [1-4].

CAN results from damage to the autonomic nerve fibers of heart, and the earliest indicator of CAN is a shift of autonomic function toward sympathetic over-activity, with disturbed of some heart rate variability (HRV) indices. Therefore, HRV has been proposed as a marker of cardiovascular risk [5]. The Hoorn Study has shown that five of frequency domain indices were associated with all-cause mortality during the 9-year follow-up period, in patients with T2DM and CAN, compared to those without CAN [6]. Another report derived from the Atherosclerosis Risk in Communities (ARIC) study, after 8-years of follow-up period, have shown that the patients with diabetes from lowest quartile of HF power was associated with incident MI, CHD, fatal CHD, and fatal non-CHD deaths with the hazard ratios ranging from 1.27 to 2.03 [7]. We established that reduced HRV, particularly HFln and increased LF/HFln, as a marker of autonomic dysfunction, were present in persons with metabolic syndrome, before the development T2DM [8].

Thiamine is required at several stages of anabolic and catabolic intermediary metabolism, such as the intracellular glucose metabolism (glycolysis, Krebs cycle, pentose-phosphate cycle), and is also a modulator of neuronal and neuromuscular transmission, probably through its activation of ionic chloride channel [9]. Benfothiamine, fat-soluble analogue of thiamine/vitamin B1, activates transketolase, an enzyme converting fructose-6 phosphate into pentose-5 phosphates thus eliminating potentially damaging metabolites from the cytosol. Diabetes might be considered a thiamine-deficient 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 [10]. Recently, it has been shown that high-dose therapy of thiamine and benfothiamine suppressed AGE accumulation in the peripheral nerve [11] and reversed diabetic neuropathy potentially by reducing the levels of triose phosphates via activation of transketolase [12].

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Alpha-lipoic acid is potent antioxidant that inhibits TNF-alpha induced NF-*k*B activation and acts as a direct free-radical scavenger [13,14]. Zhang and Frei demonstrated the ALA prevents TNF-alpha-induced NF-*k*B activation and adhesion molecule expression in vascular endothelial cells [15], suggesting that ALA has atheroprotective effects. It is currently employed in clinical practice to treat patients with distal diabetic neuropathy [16].

Because our knowledge about the treatment of CAN in T2DM is still obscured, we investigated potential beneficial effects of benfothiamine and alpha-lipoic acid on reverse of early stages of CAN in well-controlled T2DM.

METHODS

Study Design

This pilot, cross-sectional and follow-up dietary intervention study, was carried out in outpatients departments of two university hospitals. Regarding inclusion criteria, the patients were randomly selected from T2DM population to be treated with benfothiamine and ALA or to be the control patients with disease. Such inclusion criteria were: diabetes duration ≤5 years, BMI ≤30kg/m^2, fasting glucose level <7.5 mmol/l, HbA1c ≤8%, with prehypertension or hypertension stage one (JNC VII), normal physical examination and ECG, without diabetic retinopathy, nephropathy, or other serious co-morbidity. Study involved ten healthy control subjects, who did not suffer from diabetes or other serious disease as well as obesity (BMI ≤30kg/m^2).

Evaluation of Autonomic Function

All patients were tested in the Neurocardiology Unit using comprehensive three steps protocol for the assessment of autonomic nervous system status. The three steps protocol included: 1) five standard Ewing's clinical autonomic function tests, 2) short-term ECG, and 3) long-term ECG and long-term HRV analysis.

Long-Term ECG and Long-Term HRV Analysis

Twenty-four-hour ambulatory ECG monitoring was obtained in all patients and control subjects, using a 3-channel ECG Holter monitor (ArguSys). Long-term ECG was repeated in all patients, after 12 week. ECG signals were digitized and stored using a commercially available PC-based system. Power spectral analysis of HRV was carried out by fast Fourier transformation. Heart rate variability is currently used as a tool for the assessment of equilibrium in the activity of the sympathetic and parasympathetic branches of the ANS in the control of heart rate. For the evaluation of HRV, the time domain and frequency domain analyses (power spectral analysis) were used. For time domain analysis two HRV indices were measured: the SD of all normal-to-normal RR intervals in the entire recording (SDNN) (milliseconds) and the square root of the average sum of squares of difference between adjacent filtered RR intervals (rMSSD) (milliseconds). SDNN represents joint sympathetic and parasympathetic modulation of heart rate, whereas rMSSD is thought to represent parasympathetic modulation of heart rate. From frequency domain analysis, three different frequencies were reported in addition to total power (TP): high-frequency power (HF) (0.15–0.4 Hz), low-frequency power (LF) (0.04–0.15 Hz), and very low-frequency power (VLF) (0.003-0.04 Hz). LF power is thought to reflect both sympathetic and parasympathetic activity, whereas HF is determined solely by parasympathetic activity. An LF-to-HF ratio was calculated as a measure of sympathovagal balance that would reflect any shift toward sympathetic or parasympathetic activation [5].

Study Population

We consecutively recruited 16 T2DM patients, 9 females and 7 males; mean age 55.36 (4.63) years) with shorter duration of disease [median age 3 (interquartile range 2-3.5) years], without serious co-morbidity. The patients were divided into two groups, eight were recruited for special treatment, and another 8 were determined as control patients. Ten control subjects were also included in the study, 6 females and 4 males, mean age 48.50 (12.80), without diabetes and other serious disease. All subjects were selected carefully and referred from endocrinologist to Neurocardiologycal Unit. All patients received an optimal diet therapy, adequate oral antidiabetic agents, and those with dyslipidemia received hypolipemic agents, preferably fibrates.

Recommended antihypertensive drug were ACE inhibitors and/or angiotensin II receptor antagonists.

Treatment

The patients were forced to continue diet already-in-use, and medication prescribed by endocrinologist. Additionally, the patients from the first group were treated with subsequent treatment regime: at first 10

days alpha-lipoic acid (Thiogamma N 600mg in 250 ccm of 0.9% saline solution) *via iv route* and benfothiamine (Milgamma N: 100mg thiamine-chloride + 100mg pyridoxine-chloride + 0.1mg cyancobalamine) *via im route*, and furthermore with alpha-lipoic acid 600mg/d *via oral route* with benfothiamine 100mg + pyridoxine-chloride 100mg tid *via oral route*, until the control visit 12 weeks after.

The blood samples were taken for determination of metabolic variables: glucose, HbA1c, triglycerides, cholesterol, HDL-C, in all patients before the beginning and at the end of treatment period of 12 weeks. Blood samples were analysed with a convential autoanalyzer. At the same time-points, anthropometric measures (BMI and waist circumferenece) were assessed. The institutional review and ethical board approved the protocol, and all participants gave written informed consent.

Statistical Analysis

Statistical tests were conducted using SPSS ver.17 (IBM, Inc.,Chicago,IL, USA). Data are expressed as means ± SD. Because of skewed distribution of the HRV measurements, the values were log-transformed and a normal distribution was confirmed by the Kolmogorov-Smirnov test (p>0.15). Multiple time points variables were analysed by ANOVA, and *post hoc* multiple comparisons were performed using LSD test when ANOVA testing was significant (p < 0,05). Correlation analysis was performed by calculating Pearson's correlation coefficient.

RESULTS

Demographic and metabolic features of study patients, as well as control subjects, are shown in Table **1**. The mean levels of fasting glucose, HbA1c, and triglycerides were significantly higher in T2DM patients than in controls (p=0.01; p=0.01 and p=0.05, respectively). The mean values of BMI, waist circumference, HDL-cholesterol, systolic and diastolic blood pressure and the presence of hypertension did not differ between the groups (Table **1**). We analyzed the results of autonomic testing in both group of participants, and the presence of total autonomic dysfunction, according to Ewing tests, was significantly higher in T2DM (46.2% *vs*. 12.9%, p=0.03), as well as the presence of at least two positive tests for parasympathetic dysfunction (71.4% *vs*. 25.7%, p=0.01). Sympathetic dysfunction was present frequently in those with T2DM (76.9% *vs*. 32.9%, p=0.04), as we expected. The results of long term HRV indices in controls, and T2DM patients before treatment, are presented in Table **2**. The values of HRV indices were log-transformed. There was no significant difference between T2DM and controls for any of time domain. Mean Total PowerIn, VLFIn, and LFIn did not deffer in T2DM patients. Only mean value of HFIn was significantly lower and LF/HFIn significantly higher in T2DM patients, before treatment (Table **2**). At the

Table 1: Demographic, Clinical, and Laboratory Data from Controls or T2DM Patients, before and after Treatment

Variable	Control Group	T2DM-before Treatment	T2DM-after Treatment	*p*1,2/*p*1,3/*p*2,3
Female (male)	6(4)	5(3)		0,19
Age (year)	48.50(12.80)	55.36(4.63)		0,47
Disease duration (year)		3(2-3.5)		
BMI (kg/m^2)	26,59(1,75)	27,77(2,90)	26,88 (3.68)	0,23;0,0,91;0,88
Waist circumference (cm)	95,30(7,55)	94,40(13,00)	93,70(11,30)	0,27;0,19;0,07
Fasting glucose (mmol/L)	5,02(0,54)	6,49(1,14)	6,10(0,65)	0,01;0,01;0,40
HbA1c (%)	4,90	7,20	6,70	0,01;0,01;0,08
Cholesterol (mmol/L)	5,94(0,74)	5,40(0,79)	5,56(1,16)	0,81;0,54;0,87
HDL-cholesterol (mmol/L)	1,28(0,34)	1,12(0,37)	1,10 (0,46)	0,06;0,05;0,67
Triglycerides (mmol/L)	1,55(0,90)	2,13(0,59)	2,19(0,68)	0,05;0,06;0,28
sBP (mmHg)	128,43(16,35)	129,23(15,52)	126,35(12,00)	0,72;0,41;0,08
dBP (mmHg)	78,47(8,42)	79,08(12,13)	78,90 (8,05)	0,78;0,56;0,88
Hypertension, n(%)	20,6	25	25	0,51;0,51;

Results are presented as Means (SD).

Table 2: 24 Hours HRV Analysis of Controls and T2DM Patients before and after Initiation of Treatment

24h HRV indices	controls	T2DM-before	*p*
SDNN ln	4,72(0,44)	4,69 (0,09)	0,51
rMSSD ln	2,98(0,93)	2,97(0,18)	0,08
Total power ln	7,51(0,41)	5,56(0,98)	0,06
VLF RRI ln	5,88(0,75)	4,75 (1,95)	0,08
LF RRI ln	5,72(0,82)	4,82(0,82)	0,08
HF RRI ln	6,72(0,89)	3,98(1,63)	0,01
LF/HF ln	0,99(0,28)	1,37(0,74)	0,01

Results are presented as Means (SD).

Table 3: 24 Hours HRV Analysis of Two T2DM Patient Groups, before and after 12 Weeks of Initiation of Treatment

	T2DM-without treatment	T2DM-without treatment	T2DM-before treatment	T2DM-after treatment
24 HRV indices	**week 0**	**week 12**	**week 0**	**week 12**
SDNN ln	4,38(0,52)	4,26(0,48)	4,69 (0,09)	4,67 (0,13)
rMSSD ln	3,20(0,51)	3,05(0,65)	2,97(0,18)	3,10(0,19)
VLF RRI ln	5,31(1,10)	5,57(0,10)	4,75 (1,95)	6,09(0,25)*
LF RRI ln	4,66(1,12)	4,90(1,18)	4,82(0,82)	5,65(0,79)*
HF RRI ln	3,81(1,22)	3,73(0,90)	3,98(1,63)	5,40(0,72)*
LF/HF ln	1,32(0,85)	1,41(1,03)	1,37(0,74)	1,04(0,07)*

Results are presented as Means (SD); *$p < 0.05$ comparing the mean difference between the two T2DM groups (without and with treatment).

begining, no differences existed between two patient groups, for any HRV measure. After 12 weeks-treatment period, VLFln, LFln and HFln became higher, and LF/HFln ratio decreased (Table **3**). If we compered the changes between two diabetic groups, treated or untreated, we found significant differences for the same parameters, VLFln, LFln (with borderline significance), HFln and LF/HFln (Table **3**). The differences between the values of HRV inidices in T2DM patients, with or without treatment, as well as according to controls have shown on Figure **1**. Anthropometric and metabolic features (BMI, waist circumference, HbA1c, HDL-cholesterol, triglycerides, systolic and diastolic blood pressure) of T2DM patients did not change significantly beteen two measurements (we didn't show that data).

DISCUSSION

Our pilot-study data reveal positive evidences in regard to improvement of autonomic dysfunction with increased sympathetic activity in T2DM patients obtained *via* concomitant use of benfothiamine and ALA. Despite the fact of small study sample (limitation factor) and sparse reference data about the topic, we can conclude that benfothiamine and ALA possibly could correct simpathetic overactivity in T2DM. The results of previous studies confirmed beneficial effects of ALA alone on distal diabetic polyneuropathy [14-17]. Numerous studies elucidated how the oxidative stress affected functional, metabolic, and morphological neuronal properties specific for distal diabetic polyneuropathy. In such studies, ALA was used as "universal" antioxidant and confirmed as beneficial and very potent [18, 19]. It is noteworthy to emphasize the ability of ALA to regenerate other antioxidants such as GSH, ascorbate, α-tocopherol, catalase, glutathione peroxidase.

The therapeutic effect of thiamine against diabetic complications is in concordance with the evidence of some thiamine deficiency (moderate to severe) in diabetes patients. That was explained with excessive oxidative stress [20, 21], as well as with higher renal clearance of thiamine [22]. Hyperglycemia favored increase in ROS production. Such species could oxidize thiamine and made it insufficiently active. Some oxidized thiamine metabolites (thiochrome and oxydihydrothiochrome) could also play role in diabetes complications' pathogenesis [23]. Furthermore, it was

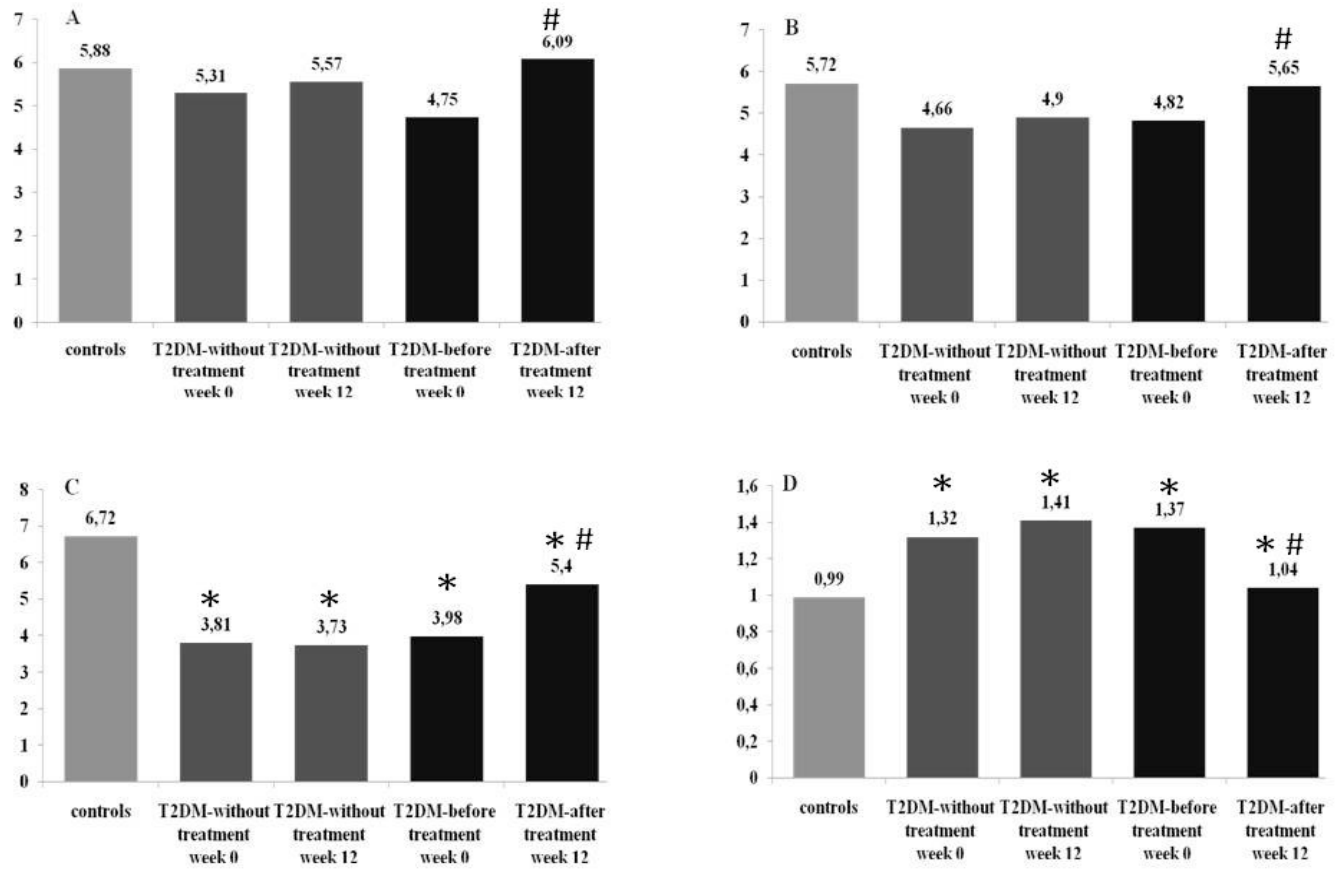

Figure 1: VLFln (panel **A**), LFln (panel **B**), HFln (panel **C**) and LF/HFln (panel **D**) in 24h ECG from controls and T2DM, treated or untreated with Benfotiamine and ALA; *p<0.05 *vs*. control group, #p<0.05 *vs*. corresponding T2DM-before treatment group.

shown that renal clearance of thiamine was increased 24-fold in type 1 diabetes and 16-fold in type 2 diabetes [24]. The findings of Shan WU *et al*. [25] revealed that benfothiamine antagonizes oxidative stress of brain cortex in STZ-induced diabetic mice *via* mechanisms that are unlikely dependent on AGE formation or effects of TNF-alpha or tissue factor. The protective effect of high-dose thiamine on detrusor contractility and on progression of diabetic cystopathy in STZ-diabetic rats was some of the findings directed to the effect of thiamine on diabetic autonomic neuropathy [26]. The explanations about positive thiamine effects were confined to hydrophobic thiamine metabolites that fulfill an important function under oxidative and nitrosyl stress. Thiamine protects nervous tissue probably by inhibiting NO-dependent tyrosine nitration and subsequent formation of dityrosine and interprotein tyrosine–tyrosine crosslink [27].

Performed study confirmed the presence of total autonomic dysfunction in 46.2%, with parasympathetic dysfunction in 71.4% and sympathetic dysfunction in 76.9% study-enrolled patients using standard Ewing's reflex tests. By use of short-term, and particularly, long-term analysis of HRV in T2DM group, reduced HRV, significantly lowered HFln, and increased LF/HFln ratio was revealed. The obtained results pointed out on imbalance of autonomic control, with shift towards increased sympathetic activity. Reduction of HRV, as the earliest indicator of CAN and established cardiovascular risk, is sugested as a new treatment strategy by Task Force [5, 28]. Beside poor glycemic control, a lot of other factors are involved in the pathogenesis of diabetic neuropathy. It was learnt from DCCT study that better metabolic control could delay or improve autonomic nervous system function in T1DM [29]. To be added, it is very likely that combined management directed toward various components and stages of the pathogenic pathways may be required. In the case of T2DM, it was shown that intensive multifactorial management, targeting hyperglycemia, hypertension, dyslipidemia and microalbuminuria reduced the risk of developing autonomic neuropathy. We are the culprits of numerous trials, which investigate a potential positive effect of study-drug on diabetes-caused autonomic nerve dysfunction (antiglycation and neurotrophic agents, Protein kinase C inhibitors) [30].

Our pilot study, was an attempt to overcome sympathetic over-activity in T2DM patients by increasing thiamine serum levels and improving thiamine dependent transketolase activity. To reduce an oxidative stress, presented in diabetes patients, ALA was added to benfothiamine, which is currently recommended for treatment distal diabetic neuropathy [16-18]. Our results were encouraged, because frequency domene, VLFln, HFln, LF/HFln ratio, of treated patients significantly differed compared to controls (untreated diabetes patients). Statistical methods showed that lowered HFln value reached the most significant increase after treatment, together with reduction of LF/HFln ratio. Hence, we can suggest that benfothiamine with ALA is responsible for shift of simpathovagal balance toward vagal activity.

There are several potential limitations that should be considered when interpreting the results of this study. First, we did not examine potential thiamine deficiency before the treatment, as was suggested by Thornalley *et al.* [24], and second, this study was conducted with limited number of patients.

In conclusion, the mechanism of benfothiamine influence on autonomic neuropathy pathogenesis is not well-known. Despite some limitations of our study, we showed beneficial effects of double therapy by thiamine and ALA on autonomic dysfunction in T2DM. Another extensive intervention study must be performed to confirm our results.

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