Short Report: Treatment

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

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Abstract

Aims In a pilot study we suggested that benfotiamine, a thiamine prodrug, prevents postprandial endothelial dysfunction in people with Type 2 diabetes mellitus. The aim of this study was to test these effects in a larger population.

Methods In a double-blind, placebo-controlled, randomized, crossover study, 31 people with Type 2 diabetes received 900 mg/day benfotiamine or a placebo for 6 weeks (with a washout period of 6 weeks between). At the end of each treatment period, macrovascular and microvascular function were assessed, together with variables of autonomic nervous function in a fasting state, as well as 2, 4 and 6 h following a heated, mixed test meal.

Results Participants had an impaired baseline flow-mediated dilatation (2.63 ± 2.49%). Compared with the fasting state, neither variable changed postprandially following the placebo treatment. The 6 weeks’ treatment with high doses of benfotiamine did not alter this pattern, either in the fasting state or postprandially. Among a subgroup of patients with the highest flow-mediated dilatation, following placebo treatment there was a significant postprandial flow-mediated dilatation decrease, while this effect was attenuated by benfotiamine pretreatment.

Conclusions In people with Type 2 diabetes and markedly impaired fasting flow-mediated dilatation, a mixed test meal does not further deteriorate flow-mediated dilatation or variables of microvascular or autonomic nervous function. Because no significant deterioration of postprandial flow-mediated dilatation, microvascular or autonomic nervous function tests occurred after placebo treatment, a prevention of the postprandial deterioration of these variables with benfotiamine was not feasible.


Introduction

Endothelial dysfunction, an early, reversible stage of atherosclerosis, is exacerbated in people with diabetes mellitus [1], and flow-mediated dilatation, a measure of endothelial dysfunction, represents a prognostic factor for cardiovascular events [2–4]. An improvement in flow-mediated dilatation occurs with therapies that decrease cardiovascular risk [5,6], suggesting that restoration of flow-mediated dilatation promotes cardiovascular health. As marked postprandial endothelial dysfunction occurs in people with Type 2 diabetes mellitus [7,8], possibly representing the link between postprandial dysmetabolism and cardiovascular disease [9,10], postprandial endothelial dysfunction prevention represents a meaningful treatment target.

Benfotiamine (a vitamin B1 prodrug), blocks four hyperglycaemia-induced pathways [11,12], exerts direct antioxidant effects [13] and is used for the treatment of diabetic neuropathy [14]. We have shown in a pilot study that benfotiamine prevents postprandial microvascular and macrovascular endothelial dysfunction in people with Type 2 diabetes [15].

The present study tested in a larger population of people with Type 2 diabetes and in a double-blind, placebo-controlled, randomized, crossover way, whether 6 weeks of treatment with high doses (900 mg/day) of benfotiamine can prevent the detrimental effects of a heated mixed test meal on postprandial flow-mediated dilatation, microvascular and autonomic nervous function tests.
What’s new?

- This study investigates the effects of 6 weeks of benfotiamine therapy on postprandial vascular function.
- The magnitude of the postprandial response is dependent on the baseline vascular function: when fasting vascular function is already markedly impaired, no further significant postprandial deterioration is possible and therefore effects of potential beneficial interventions are not obvious. This might have implications for further study designs.
- In people with diabetes and marked impairment of vascular function, either long-term therapies are necessary to restore vascular function, or there is a point of no return. Both hypotheses deserve further investigation.

Methods

People from the Heart and Diabetes Center NRW provided written informed consent and the study followed the requirements of the Declaration of Helsinki and Good Clinical Practice. The study protocol was approved by the ethics committee of the Ruhr-University Bochum.

This was a double-blind, placebo-controlled, randomized, crossover trial investigating the effects of 6 weeks of therapy with benfotiamine 900 mg/day or a matching placebo in 36 people with Type 2 diabetes (data of 31 participants were used for the per protocol analysis) without recent (6 months) major pathology. The sequence of investigation in 16 participants was: (6 weeks benfotiamine treatment)—(test meal day)—(6 weeks washout phase)—(6 weeks placebo treatment)—(test meal day). Fifteen participants started with the placebo treatment.

On the test meal days, macrovascular (flow-mediated dilatation) and microvascular (laser Doppler reactive hyperaemia) function, in addition to autonomic nervous function tests, were assessed fasting, as well as 2, 4 and 6 h following a heated mixed test meal (200 g chicken breast, 250 g potatoes, 100 g carrots, 200 g tomatoes and 15 g vegetable oil; providing 580 kcal, 54 g protein, 17 g fat, 48 g carbohydrates, 60 mg cholesterol and 10 g fibre, prepared by frying or broiling at 230°C for 20 min; calculated advanced glycation end product content: 15.100 kU advanced glycation end products [15]. Throughout the study, dietary recommendations and medication were kept unchanged.

The primary endpoint was the difference between treatments in postprandial flow-mediated dilatation change; secondary endpoints were the difference between treatments in fasting and postprandial changes of reactive hyperaemia and autonomic nervous function tests.

Demographic characteristics were [mean (minimum–maximum): age 56 (44–69) years, BMI 30 (23–42) kg/m², diabetes duration 7 (1–22) years, HbA1c 57 (41–87) mmol/mol [7.4 (5.9–10.1)]%; flow-mediated dilatation 2.86 (−1.09 – 6.06)%]. Co-morbidities were: sensorimotor neuropathy (Neuropathy Disability Score ≥ 3 and Neuropathy Symptom Score ≥ 5 or Neuropathy Disability Score ≥ 6, n = 8), autonomic neuropathy (≥ 2 abnormal values of autonomic nervous function tests, n = 3), nephropathy [micro- or macroalbuminuria and/or glomerular filtration rate (GFR) < 90 ml/min, n = 12], retinopathy (at least non-proliferative retinopathy, n = 5), hypertension (n = 30), treated dyslipidaemia (n = 13) and history of coronary artery disease (n = 6).

Flow-mediated dilatation

Flow-mediated dilatation was assessed at the right brachial artery as previously described [7]. Endothelium-independent vasodilatation was measured at 4 h, 5 min after sublingual administration of glycerotrinitrate spray (0.4 mg).

Laser Doppler flowmetry

Laser Doppler flowmetry (O2C; LEA Medizintechnik, Giessen, Germany) was used to assess blood flow with the probe on the dorsal thenar right-hand area as previously described [7].

Variables of heart rate variability

Variables of heart rate variability (Table 1) (Suess Medizin-technik, Aue, Germany) were used for autonomic nervous function test assessments [16] over 5 min, following 10 min rest, in fasting state and 4 h postprandially.

Laboratory analyses

Laboratory analyses were performed with commercially available methods [15].

After database lock and unblinding, paired, two-tailed t-tests were used to compare fasting and postprandial variables following the two treatments. The difference between the maximum postprandial change and the fasting value and the area under the curve of variables from fasting (0 h) to 6 h were calculated. We calculated that 30 participants are sufficient to detect a difference of 2% in flow-mediated dilatation between treatments (80% power).

A post-hoc analysis was performed for flow-mediated dilatation by dividing the whole population into two subgroups (16 and 15 participants, respectively) according to their fasting flow-mediated dilatation values. The level of significance was set at 0.05. Values are presented as mean ± standard deviation if not otherwise stated.
Table 1: Study variables

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Benfotiamine</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>2 h</td>
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<tr>
<td>Flow-mediated dilatation (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole group</td>
<td>2.86 ± 1.88</td>
<td>3.33 ± 2.37</td>
</tr>
<tr>
<td>Subgroup 1</td>
<td>1.45 ± 1.42</td>
<td>2.29 ± 1.86</td>
</tr>
<tr>
<td>Subgroup 2</td>
<td>4.37 ± 0.97</td>
<td>3.80 ± 2.62</td>
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| Endothelium-independent dilatation (glycerotrinlate) (%) |              | 14.1 ± 1.4   |              |              |
| BFbi† (AU)                | 30.7 ± 18.8 | 30.8 ± 19.4  | 31.3 ± 23.9  | 25.8 ± 15.8  |
| Maximal blood flow‡ (AU)  | 93.6 ± 35.6 | 95.6 ± 34.7  | 89.6 ± 38.6  | 87.2 ± 30.7  |
| Area under the curve of blood flow§ (AU × min)          | 7520 ± 3180 | 7684 ± 3235  | 7239 ± 3577  | 7073 ± 2980  |
| Reactive hyperaemiaª     | 279 ± 178   | 311 ± 207    | 264 ± 143    | 295 ± 156    |
| Heart rate (ms)         | 863 ± 132   | 849 ± 138    | 875 ± 149    | 848 ± 102    |
| Standard deviation (SD)  | 34.3 ± 35.4 | 30.1 ± 19.9  | 34.7 ± 38.8  | 32.9 ± 38.8  |
| Variation coefficient     | 3.83 ± 3.52 | 3.43 ± 2.00  | 3.86 ± 3.87  | 3.92 ± 4.65  |
| RMSSD                    | 36.7 ± 53.3 | 30.1 ± 29.6  | 37.3 ± 57.2  | 34.7 ± 60.9  |
| Low frequency            | 705 ± 1402  | 466 ± 609    | 751 ± 2062   | 703 ± 1785   |
| High frequency           | 724 ± 2295  | 286 ± 726    | 830 ± 3418   | 728 ± 2706   |
| Low frequency/high frequency ratio | 4.9 ± 4.3  | 5.2 ± 5.3    | 4.3 ± 3.7    | 5.3 ± 5.0    |
| E-selectin (ng/ml)       | 52.6 ± 19.2 | 50.2 ± 18.4  | 53.6 ± 20.9  | 52.2 ± 21.6  |
| Systolic blood pressure (mmHg) | 127 ± 13    | 122 ± 12*    | 124 ± 11     | 125 ± 12     |
| Diastolic blood pressure (mmHg) | 79 ± 7      | 75 ± 8       | 78 ± 7       | 78 ± 8       |
| Pulse (bpm)              | 69 ± 10     | 73 ± 11*     | 71 ± 10      | 68 ± 11      |
| Plasma blood glucose (mg/dl) | 153 ± 38    | 171 ± 50*    | 132 ± 41*    | 116 ± 32*    |

*P < 0.05 vs. fasting.
†BFbi = Blood flow before forearm ischemia.
‡Maximal blood flow before forearm ischemia.
§Area under the curve of blood flow 2 min following forearm ischemia (arbitrary units × s).
ªReactive hyperaemia = Percent increase in blood flow after forearm ischemia compared to the blood flow before forearm ischemia.
Variances (variation coefficient), standard deviation (StdDev), the absolute value in milliseconds (HR_ms) as well as the square root of the mean square differences in successive RR intervals (RMSSD). Calculation by Fast Fourier Transformation: very low frequency (VLF) component (specific for several physiologic processes), low frequency (LF) component (sympathetic modulation), high frequency (HF) component (vagal modulation) and the LF/HF ratio as a measure of the sympatho-vagal balance.
Results

The values of the investigated variables are listed in Table 1. No significant differences in laboratory variables were observed at the end of treatments (data not shown). No significant postprandial change in flow-mediated dilatation, reactive hyperaemia or of autonomic nervous function tests occurred following the test meal (Table 1). This pattern remained unchanged following benfotiamine treatment.

We divided our participants into two subgroups according to their fasting flow-mediated dilatation following placebo: subgroup 1 with the lowest flow-mediated dilatation (1.45 ± 1.42%) and subgroup 2 with the highest flow-mediated dilatation (4.37 ± 0.97%). For subgroup 1, flow-mediated dilatation following placebo tended to increase at 2 h, to decrease at 4 h (P = not significant for all) and to significantly increase at 6 h, while after benfotiamine treatment there was a constant tendency for flow-mediated dilatation to increase (significant at 6 h). For subgroup 2, following placebo, flow-mediated dilatation was significantly impaired at 4 h, an effect reduced by benfotiamine pretreatment (Table 1 and Fig. 1).

The therapy was well tolerated; no drug-related serious adverse event occurred.

Conclusions

In people with Type 2 diabetes and impaired flow-mediated dilatation, a heated mixed test meal did not alter postprandial flow-mediated dilatation, reactive hyperaemia and variables of autonomic nervous function tests and this pattern was not influenced by a middle-term, high-dose benfotiamine treatment (Table 1).

Our study was powered for a postprandial flow-mediated dilatation decrease of at least 2% (flow-mediated dilatation units) and a difference of 2% between treatments. As in this study no impairment in postprandial flow-mediated dilatation occurred, our data cannot either support or exclude effects of benfotiamine on postprandial flow-mediated dilatation in this population.

At a first glance, these data are in contradiction with our previously reported data [15], although the two studies cannot be directly compared: the duration of therapy was 3 days in the previous study and 6 weeks in the present study, and the population in the previous study had a better flow-mediated dilatation than the population investigated here (flow-mediated dilatation: 6.39 vs. 2.86%).

The finding that the test meal had no significant effect on postprandial flow-mediated dilatation and reactive hyperaemia was surprising. In previous studies [7,15], using a similarly prepared meal and similar assessment methods, significant effects on both macro- and microvascular function were shown. Again, in the present study, fasting flow-mediated dilatation was markedly decreased, suggesting that this population had a more advanced vascular disease and an increased cardiovascular risk [17]. We therefore believe that the lack of vascular effects of the test meal is attributable to an already maximally impaired vascular function in the fasting state that cannot be further decreased postprandially. Indeed, our participants had a mean fasting flow-mediated dilatation of 2.86%, which is even lower than the lowest postprandial flow-mediated dilatation value seen in the previous study (flow-mediated dilatation decreased from 6.39% fasting to a minimum of 4.15% 4 h postprandially) [15]. Moreover, in the present study, a significant negative correlation (r = −0.48, P < 0.05) existed between fasting flow-mediated dilatation and postprandial flow-mediated dilatation changes from fasting to 4 h, suggesting that the better the fasting flow-mediated dilatation, the more can it be reduced postprandially.

In our study, a 6-week therapy with high-dose benfotiamine (900 mg/day) did not influence fasting variables of macrovascular and microvascular function, suggesting that, in people with markedly impaired flow-mediated dilatation, a critical level of endothelial damage might be reached that cannot be reversed by 6 weeks of therapy. Further studies are warranted to demonstrate whether a middle-term therapy with benfotiamine is able to restore flow-mediated dilatation in people with a better baseline flow-mediated dilatation, or a longer therapy is needed in people with a marked flow-mediated dilatation impairment.

To assess the hypothesis that the postprandial flow-mediated dilatation regulation might differ between participants with different stages of flow-mediated dilatation impairment, we divided our participants into two subgroups according to their fasting flow-mediated dilatation. Subgroup 1 (with the lowest flow-mediated dilatation) showed no postprandial flow-mediated dilatation decrease. In contrast, in subgroup 2 (with the highest flow-mediated dilatation), following placebo, a significant flow-mediated dilatation decrease

![FIGURE 1 Subgroup analysis of postprandial flow-mediated dilatation (%)](image-url)
occurred at 4 h, an effect attenuated by benfotiamine treatment. Even though this was a post-hoc analysis and the study was not powered for subgroup analyses, these data are in line with our previous result [15] and suggest that benfotiamine exerts beneficial effects on postprandial flow-mediated dilatation only in people with minor impaired flow-mediated dilatation and not in those with markedly impaired flow-mediated dilatation. Further studies are warranted to prove this hypothesis. The fact that the magnitude of postprandial flow-mediated dilatation excursions is dependent on the preprandial value is important for the design of further studies.

The results of the present study, together with the data of our previous pilot study [15], suggest that earlier interventions with benfotiamine or other agents with protective vascular effects could be more beneficial among patients with a less pronounced impairment of flow-mediated dilatation than in those with advanced vascular damage and advocate for early interventions.

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Competing interests

AS received consultancy fees from Woerwag Pharma GmbH & Co KG. AP and DS have nothing to declare.

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References