Benfotiamine Prevents Macro- and Microvascular Endothelial Dysfunction and Oxidative Stress Following a Meal Rich in Advanced Glycation End Products in Individuals With Type 2 Diabetes

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OBJECTIVE — Diabetes is characterized by marked postprandial endothelial dysfunction induced by hyperglycemia, hypertriglyceridemia, advanced glycation end products (AGEs), and dicarbonyls (e.g., methylglyoxal [MG]). In vitro hyperglycemia-induced MG formation and endothelial dysfunction could be blocked by benfotiamine, but in vivo effects of benfotiamine on postprandial endothelial dysfunction and MG synthesis have not been investigated in humans until now.

RESEARCH DESIGN AND METHODS — Thirteen people with type 2 diabetes were given a heat-processed test meal with a high AGE content (HAGE; 15.100 AGE kU, 580 kcal, 54 g protein, 17 g lipids, and 48 g carbohydrates) before and after a 3-day therapy with benfotiamine (1,050 mg/day). Macrovascular flow-mediated dilatation (FMD) and microvascular reactive hyperemia, along with serum markers of endothelial disfunction (E-selectin, vascular cell adhesion molecule-1, and intracellular adhesion molecule-1), oxidative stress, AGE, and MG were measured during both test meal days after an overnight fast and then at 2, 4, and 6 h postprandially.

RESULTS — The HAGE induced a maximum reactive hyperemia decrease of −60.0% after 2 h and a maximum FMD impairment of −35.1% after 4 h, without affecting endothelium-independent vasodilatation. The effects of HAGE on both FMD and reactive hyperemia were completely prevented by benfotiamine. Serum markers of endothelial dysfunction and oxidative stress, as well as AGE, increased after HAGE. These effects were significantly reduced by benfotiamine.

CONCLUSIONS — Our study confirms micro- and macrovascular endothelial dysfunction accompanied by increased oxidative stress following a real-life, heat-processed, AGE-rich meal in individuals with type 2 diabetes and suggests benfotiamine as a potential treatment.

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Abbreviations: AGE, advanced glycation end product; CML, carboxymethyllysine; CRP, C-reactive protein; FMD, flow-mediated dilatation; HAGE, high AGE content; HAGE+BT, HAGE plus benfotiamine; ICAM, intracellular adhesion molecule; IL, interleukin; MG, methylglyoxal; TBARS, thiobarbituric acid reacting substance; TNF, tumor necrosis factor; VCAM, vascular cell adhesion molecule.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Endothelial dysfunction is an early marker of atherosclerosis and accompanies states showing a high cardiovascular risk, such as smoking (1), dyslipidemia (2), arterial hypertension (3), obesity (4), coronary artery disease (5), congestive heart failure (6), and type 1 (7) and type 2 (8) diabetes. Postprandial endothelial dysfunction has been proposed as the link between postprandial dysmetabolism and atherosclerosis (9) and occurs not only in patients with cardiovascular disease (10) or diabetes (11) but even in healthy subjects (12). Distinctive and cumulative (11) effects of hyperglycemia (13) and hypertriglyceridemia (14) on postprandial endothelial dysfunction have been shown. Since the postprandial state covers most of our daytime, interventions aimed at reducing postprandial endothelial dysfunction might play a decisive role in prevention of atherosclerosis. Several therapeutic approaches have been suggested for the treatment of postprandial endothelial dysfunction, including insulin, folic acid, tetrahydrobiopterin, vitamins C and E, and statins (11). These approaches aim at reducing postprandial oxidative stress (vitamins C and E, statins, and partly folic acid), postprandial hyperglycemia (insulin), postprandial hypertriglyceridemia (statins), or have a direct effect on endothelial nitric oxide (NO) production (folic acid, insulin, and tetrahydrobiopterin) (15–17).

Recent data suggests that advanced glycation end products (AGEs) might also play a role in the development of endothelial dysfunction (18), leading to the long-term complications of diabetes and aging (19). AGEs are a heterogeneous group of moieties, one of the most representative being carboxymethyllysine (CML). Diet is a major source of exogenous AGEs, and the food's AGE content is highly dependent on food nutrient composition, as well as on temperature and method and duration of heat application during cooking (20). About 10% of in-

gested AGEs are rapidly absorbed and partly retained in the body, where they exert different pathological effects (21) including binding with and activation of receptors for AGE (22). AGE precursors such as methylglyoxal (MG) can also activate receptors for AGE. Endogenous MG synthesis increases in parallel with hyperglycemia in vivo (23). Postprandially, the absorbed and endogenously generated AGEs and MG act synergistically to decrease vascular function through direct NO scavenging or increase in oxidative stress. Part of these effects might be counteracted by benfotiamine, a liposoluble vitamin B_1 with much higher bioavailability than thiamine (24). Benfotiamine, commonly used in the treatment of diabetic neuropathy (25), is a transketolase activator that directs glucose substrates to the pentose phosphate pathway. Thus, it blocks several hyperglycemia-induced pathways, one of them being endogenous AGEs and dicarbonyls formation (26). Benfotiamine was shown to prevent experimental diabetic retinopathy (26) and in vitro hyperglycemia-induced endothelial dysfunction (27,28). The effects of benfotiamine on in vivo endothelial function remained unknown.

In this study, we investigated the effects of a real-life, cooked, AGE-rich meals on endothelial function with and without benfotiamine pretreatment in 13 individuals with type 2 diabetes. Endothelial function was assessed at the macrovascular level with high-resolution ultrasound measurements of the flow-mediated dilatation (FMD) and at the microvascular level with laser Doppler assessment of reactive hyperemia. Simultaneously, we measured serum markers of endothelial function (vascular cell adhesion molecule [VCAM]-1, intracellular adhesion molecule [ICAM]-1, and soluble E-selectin), inflammation (tumor necrosis factor [TNF]-α, C-reactive protein [CRP], interleukin [IL]-6, IL-8, and fibrinogen), oxidative stress (thiobarbituric acid reacting substances [TBARSs]), serum AGEs, and MG.

RESEARCH DESIGN AND METHODS

— Thirteen adults with type 2 diabetes, aged 56.9 ± 2.8 years, without a history of acute cardiovascular events within the previous 6 months were recruited from the metabolic ward of the Heart and Diabetes Center NRW. We chose patients with type 2 diabetes because they represent the majority of individuals with diabetes (>90%), and their number is continuously increasing (29), and because of their known susceptibility for cardiovascular complications (30). Sample size calculation was performed according to previous published data (31). Patients' characteristics were (means ± SE): diabetes duration 9.6 ± 2.5 years, BMI 30.3 ± 0.9 kg/m^2, HbA_{1c} (A1C) 8.5 ± 0.5%, systolic blood pressure 136.5 ± 6.0 mmHg, diastolic blood pressure 79.1 ± 3.7 mmHg, heart rate 67.9 ± 3.1/min, smokers/nonsmokers 3/10, 4 patients had nonproliferative retinopathy, 2 had nephropathy (microalbuminuria), 5 had peripheral neuropathy, and 10 had arterial hypertension. Medications included oral/oral plus insulin (n = 9/4), aspirin (n = 11), ACE inhibitors (n = 9), angiotensin receptor blockers (n = 1), hydroxymethylglutaryl-CoA inhibitors (n = 6), β-blockers (n = 5), diuretics (n = 5), and calcium channel blockers (n = 3).

Exclusion criteria also included A1C ≥13%, pregnancy, heart failure New York Heart Association III-IV, history of myocardial infarction, unstable angina, stroke, peripheral arterial vascular disease stadium IIB or higher, renal failure (serum creatinine >1.2 mg/dl), cancer, chronic alcohol abuse, hypo- or hypertension (resting blood pressure <90/50 or >180/110 mmHg), therapy with more than three antihypertensives, therapy with nitrates, and severe diabetes complications (proliferative diabetic retinopathy, macroalbuminuria, painful diabetic peripheral neuropathy requiring morphine derivatives, and diabetic foot syndrome).

Subjects were studied after giving written informed consent. The local ethics committee approved the study, which was carried out according to the principles outlined in the Declaration of Helsinki.

Each subject was studied on two occasions in a crossover design, following an overnight fast. All medications were withdrawn for at least 12 h before every investigation, but they were kept constant throughout the study. Patients were on a standard diabetes diet for the 9-day study period. On day 4 (n = 6) or 6 (n = 7), we assessed the acute effects of a cooked test meal with a high AGE content (HAGE) on endothelial function and oxidative stress. Benfotiamine (Milgamma; Woerwag Pharma, Böblingen, Germany) was given orally on days 7, 8 (3 × 350 mg/day), and 9 (1.050 mg, 1 h before the intake of the cooked meal, i.e., HAGE plus benfotiamine [HAGE+BT]).

On each test meal day, micro- and macrovascular function were assessed in the fasting state (7:00 A.M.) and 2, 4, and 6 h following the test meal. Venous blood was drawn on each occasion. Between tests, patients were allowed to stand up and walk but were prevented from participating in any major physical activity. After the test meal, patients were allowed to drink only mineral water at a maximal rate of 50 ml/h until the last test (6 h) was performed.

Test meal

At both visits, subjects received a cooked test meal with a HAGE, which they were asked to eat within 30 min (until 8:30 A.M.). The meal consisted of 200 g chicken breast, 250 g potatoes, 100 g carrots, 200 g tomatoes, and 15 g vegetable oil; had an energy content of 580 kcal; and provided 54 g protein, 17 g fat, and 48 g carbohydrates. The meal was fried/broiled at 230°C for 20 min in order to obtain a high concentration of AGEs (15.100 kU AGE/meal). The AGE content was calculated according to recently published data (20).

Measurement of endothelial function

The FMD was assessed at the right brachial artery (using a protocol first described by Celermajer et al. [32]) by measuring the arterial diameter response to reactive hyperemia causing endothelium-dependent dilatation. Measurements of arterial diameter were performed with a high-resolution, two-dimensional ultrasound imaging system, ATL HDI 5000 (Advanced Technology Laboratories, Bothell, WA), using B-mode, electrocardiogram-triggered ultrasound images obtained with a 7–15 MHz linear-array transducer.

Studies were performed at 22–24°C in a dark, quiet room. The study subject rested for at least 10 min before the first scan and remained in a recumbent position throughout the investigation. To avoid movement artifacts, the subject's arm was immobilized in a foam cast. Scanning of the brachial artery was performed 3–10 cm above the antecubital crease. A baseline measurement (before ischemia) was taken and digitally recorded. A pneumatic tourniquet, placed on the forearm of the subject, was then inflated at 250 mmHg for 4.5 min. Pulse Doppler was recorded for the first 15 s after the cuff release, followed by continuous digital B-mode recording of the arterial diameter for 120 s after deflation. At baseline and 4 h postprandially, further

10 min were allowed for vessel recovery and then a new baseline measurement was performed. Sublingual glycerotrinitrate spray (0.4 mg) was administered, and 5 min later, the last data acquisition was made.

Endothelium-dependent dilatation was defined as the percent change in arterial diameter following reactive hyperemia compared with the baseline diameter. The endothelium-independent dilatation was the percent increase in arterial diameter 5 min following glycerotrinitrate.

All recorded continuous image sequences were analyzed off-line by a skilled investigator (M.N.) blinded to the sequence of investigation. For the reactive hyperemia test, diameter measurements were taken 60 s after cuff deflation (maximal arterial diameter following reactive hyperemia). Four cardiac cycles were analyzed at the end of the diastole, and arterial diameter was automatically measured using special software (ATL Ultrasound version 1.91; HDI Lab) and then averaged. The same procedure was applied to the measurements of endothelium-independent vasodilatation. Repeated measurements showed a coefficient of variation of 5.41%.

Laser Doppler (microlightguide spectrophotometer)

The skin microcirculation was assessed simultaneously with the FMD, using a microlightguide spectrophotometer (O2C; LEA Medizintechnik, Giessen, Germany). The laser Doppler probe was applied on the thenar surface of the right hand. The laser Doppler transmits continuous-wave laser light (830 nm and 30 mW) to the tissue, where it is scattered and collected on the skin surface into the probe. The blood flow was measured in 2 mm depth and is expressed in arbitrary units. Given the great inter- and intra-assay variability of absolute blood flow values, a reproducible test was developed, which assesses the increase in blood flow following a 4.5-min ischemia (33). Reactive hyperemia was calculated as the ratio between the maximal postischemia and the baseline blood flow.

Blood sample collection and biochemical measurements

Blood drawing closely (10 min) followed each measurement of vascular function in the contralateral arm, and stasis was avoided if possible. Plasma or serum was obtained after centrifugation at 1,500*g* for 20 min at 4°C. Aliquots of 750 μl were stored at −80°C.

Serum glucose was measured by the glucose-oxidase method (Architect ci8200; Abbott Diagnostics, Wiesbaden, Germany); A1C was assessed by high-performance liquid chromatography (Menarini, Berlin, Germany). Serum cholesterol, triglycerides, and LDL and HDL cholesterol were measured using the Architect ci8200 analyzer (Abbott Diagnostics). Plasma concentrations of VCAM-1, ICAM-1, and soluble E-selectin were determined using commercially available immunosorbent kits (R&D Systems, Wiesbaden, Germany and Technoclone, Wien, Austria).

Commercially available assays were used to measure TBARS (Alexis Biochemicals, Grünberg, Switzerland), fibrinogen, CRP (Architect ci8200; Abbott Diagnostics), TNF-α, IL-6, and IL-8 (ImmuLite 2000; DPC Biermann, Bad Nauheim, Germany).

Serum AGE levels were measured by CML-sensitive enzyme-linked immunosorbent assay (34). Total serum MG derivatives were assessed by enzyme-linked immunosorbent assay using a monoclonal anti–MG-BSA antibody (MG3D11) raised against MG-modified BSA (22 MG-modified Arg/mol BSA, by high-performance liquid chromatography, obtained from Dr. Y. Al-Abed, The Picower Institute, Manhasset, NY). MG3D11 was found to be strongly immunoreactive against MG-ovalbumin and AGE-BSA but not with CML-BSA or unmodified BSA (35).

Statistical analysis

Data were analyzed using SPSS for Windows 12.0. Continuous variables are expressed as means ± SE unless otherwise indicated. The Shapiro-Wilk algorithm was used to determine whether each variable had a normal distribution. Paired Student's *t* test was used to compare the effects of HAGE and HAGE+BT on FMD, as well as laser Doppler parameters and serum variables. Postprandial changes in these variables after each meal were assessed by two-way, repeated-measures ANOVA. If differences reached statistical significance, a two-tailed paired *t* test was used to assess differences at individual time periods, with Bonferroni's correction for multiple comparisons. To assess the effects of benfotiamine, changes in variables were compared between the two test meal days. Univariate linear regression models were fitted to assess the relationship between the change in vascular parameters (FMD, reactive hyperemia, VCAM-1, ICAM-1, and E-selectin) and the postprandial change in plasma glucose and triglycerides. The level of significance was set at 0.05, and all tests were performed two sided.

RESULTS

Effects on macrocirculation

The HAGE induced a significant impairment in FMD from 6.39 ± 0.91 (baseline) to 4.40 ± 0.67 (2 h), 4.15 ± 0.53 (4 h), and 4.89 ± 0.79% (6 h) ($P < 0.01$ vs. baseline). The effect of HAGE was completely prevented by benfotiamine, the corresponding (HAGE+BT) changes were 6.21 ± 0.85 (baseline), 5.73 ± 0.71 (2 h), 5.84 ± 0.83 (4 h), and 6.30 ± 0.85% (6 h) ($P < 0.01$ vs. HAGE).

A vasodilatation of the brachial artery could be noted postprandially after HAGE, an effect prevented at 2 h by benfotiamine. Brachial artery diameter (before ischemia) changed after HAGE from 4.01 ± 0.14 mm (baseline) to 4.10 ± 0.14 (2 h), 4.11 ± 0.15 (4 h), and 4.29 ± 0.15 (6 h) ($P < 0.01$ vs. baseline) and after HAGE+BT from 4.00 ± 0.14 mm (baseline) to 4.04 ± 0.14 (2 h) ($P < 0.05$ vs. HAGE) and 4.07 ± 0.13 (4 h) and 4.17 ± 0.14 (6 h) ($P < 0.01$ vs. baseline). There were no differences in postprandial changes in maximal arterial diameter following reactive hyperemia on both study days (data not shown).

Endothelium-independent vasodilatation remained unchanged throughout the study during both occasions; values at baseline and 4 h were 14.51 ± 1.46 and 14.55 ± 1.68% (HAGE), respectively, and 14.86 ± 1.52 and 14.47 ± 1.53% (HAGE+BT), respectively.

Effects on microcirculation

Reactive hyperemia significantly decreased after HAGE from 2.87 ± 0.50 (baseline) to 1.15 ± 0.09 (2 h), 2.00 ± 0.41 (4 h), and 1.60 ± 0.27 (6 h) ($P < 0.05$ vs. baseline). The effect of HAGE was again counteracted by benfotiamine pretreatment; in this case (HAGE+BT), reactive hyperemia was 1.97 ± 0.31 (baseline), 1.93 ± 0.28 (2 h), 2.71 ± 0.81 (4 h), and 2.36 ± 0.44 (6 h) ($P < 0.05$ vs. HAGE).

Blood pressure and heart rate

Baseline systolic and diastolic blood pressure, as well as heart rate, was comparable between the two study days (data not

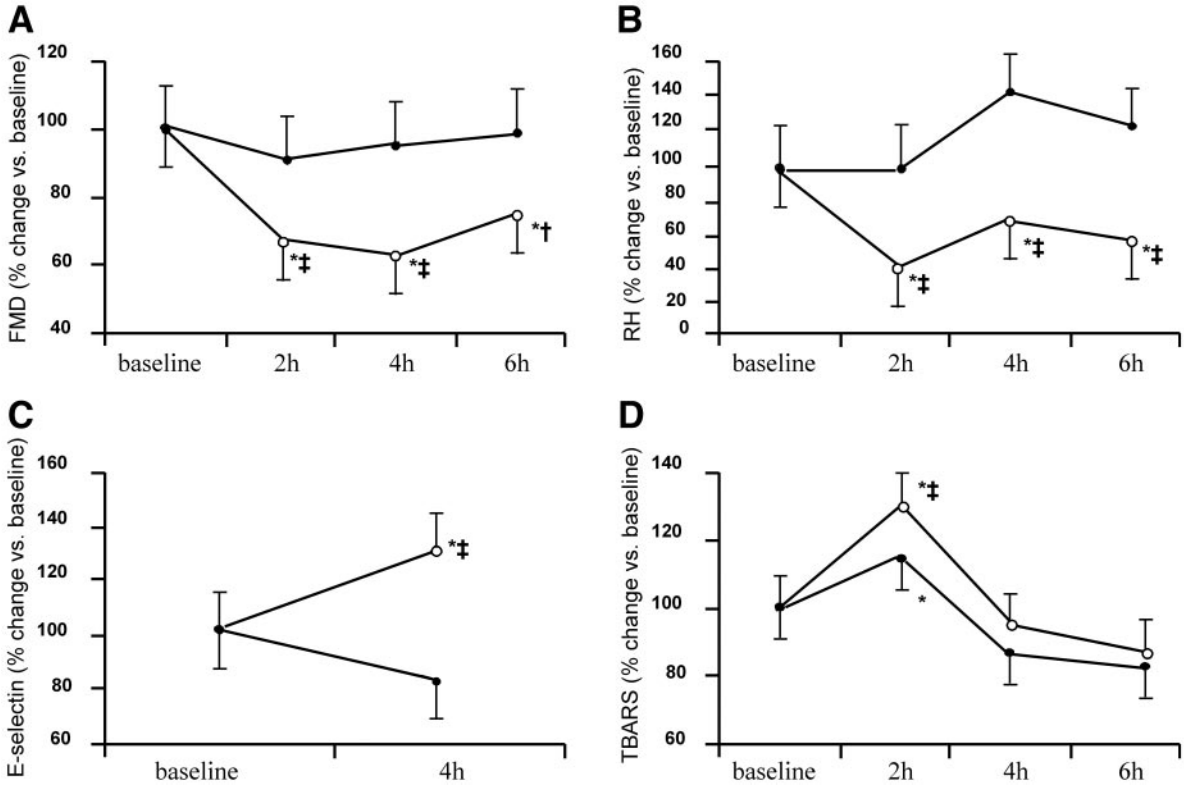

Figure 1—*A: Change in FMD following HAGE (○) and HAGE+BT (●). *P < 0.05 vs. baseline; †P < 0.01 and ‡P < 0.001 vs. HAGE+BT. B: Change in reactive hyperemia following HAGE (○) and HAGE+BT (●). *P < 0.05 vs. baseline; ‡P < 0.05 vs. HAGE+BT. C: Change in E-selectin following HAGE (○) and HAGE+BT (●). *P < 0.05 vs. baseline; ‡P < 0.05 vs. HAGE+BT. D: Change in TBARS following HAGE (○) and HAGE+BT (●). *P < 0.05 vs. baseline; ‡P < 0.05 vs. HAGE+BT.*

shown), and systolic blood pressure was significantly decreased 4 h after both meals by −6.8 ± 2.3 mmHg (HAGE) and −6.6 ± 2.55 mmHg (HAGE+BT). No changes in postprandial heart rate occurred.

Metabolic changes

Serum glucose values at baseline and at 2, 4, and 6 h postprandially during HAGE were 153 ± 14, 194 ± 19*, 136 ± 12, and 115 ± 9* mg/dl, respectively, and during HAGE+BT were 138 ± 13, 164 ± 14*, 117 ± 9, and 106 ± 6* mg/dl (*$P <$ 0.05 vs. baseline) (Fig. 1).

Triglyceride values at baseline and at 2, 4, and 6 h during HAGE were 153 ± 24, 162 ± 26, 176 ± 28*, and 172 ± 25* mg/dl, respectively, and during HAGE+BT were 138 ± 21, 146 ± 18, 157 ± 17*, and 151 ± 19* mg/dl (*$P <$ 0.05 vs. baseline). No significant differences with respect to the baseline and the postprandial blood glucose and triglyceride excursions were found between the two test meal days. No changes in total, LDL, and HDL cholesterol followed either meal.

Effects on serum markers of endothelial dysfunction

All circulating markers of endothelial dysfunction significantly increased after HAGE: E-selectin, 39.5 ± 3.6 (baseline) and 53.3 ± 5.2* ng/ml (4 h); ICAM-1, 213.9 ± 11.3 (baseline), 231.8 ± 11.5 (2 h), 228.5 ± 12.5* (4 h), and 221.6 ± 9.9 ng/ml (6 h); and VCAM-1, 607.4 ± 53.0 (baseline), 741.3 ± 95.9* (2 h), 650.1 ± 89.8 (4 h), and 575.4.6 ± 48.3 ng/ml (6 h) (*$P <$ 0.05 vs. baseline).

These effects were prevented by benfotiamine pretreatment: E-selectin, 44.1 ± 4.6 (baseline) and 35.9 ± 3.3* ng/ml (4 h); ICAM-1, 221.3 ± 15.4 (baseline), 220.0 ± 11.7 (2 h), 210.2 ± 10.4* (4 h), and 211.1 ± 9.9 ng/ml (6 h); and VCAM-1, 621.3 ± 61.3 (baseline), 611.4 ± 65.0* (2 h), 614.6 ± 52.8 (4 h), and 650.1 ± 76.3 ng/ml (6 h) (*$P <$ 0.05 vs. HAGE).

Effects on inflammatory markers

The HAGE significantly increased CRP from 0.260 ± 0.087 at baseline to 0.273 ± 0.092 mg/dl at 6 h ($P = 0.043$), while HAGE+BT resulted in no significant CRP change (0.254 ± 0.103 at baseline, 0.259 ± 0.101 mg/l at 6h; P = NS vs. baseline and vs. HAGE). We found no changes in fibrinogen, TNF-α, IL-6, and IL-8 (data not shown).

Effects on serum AGEs and dicarbonyls

CML significantly increased at 4 h after HAGE. Values were 9.46 ± 1.59 (baseline), 8.75 ± 1.49 (2 h), 13.28 ± 1.83* (4 h), and 7.09 ± 1.44 units/ml (6 h) (*$P <$ 0.05 vs. baseline). This effect was prevented by benfotiamine: 11.79 ± 1.48 (baseline), 12.35 ± 1.93 (2 h), 11.57 ± 1.90 (4 h), and 8.05 ± 2.16 units/ml (6 h) (P = NS vs. baseline and vs. HAGE for all). Similarly, MG increased 4 h following HAGE, changing from 3.41 ± 0.44 (baseline) to 3.04 ± 0.31 (2 h), 4.16 ± 0.42* (4 h), and 3.02 ± 0.45 nmol/ml (6 h) (*$P <$ 0.05 vs. baseline) but not after HAGE+BT: 3.76 ± 0.56 (baseline), 3.61 ± 0.38 (2 h), 3.51 ± 0.54 (4 h), and 2.68 ± 0.44 nmol/ml (6 h) (P = NS vs. baseline and vs. HAGE for all).

Effects on oxidative stress

The HAGE induced a significant increase in TBARS: 8.14 ± 0.47 (baseline) to 10.53 ± 0.97* (2 h), 7.57 ± 0.61 (4 h), and 7.02 ± 0.49 nmol/ml (6 h) (*$P <$ 0.05 vs. baseline). The effect was reduced by benfotiamine; the corresponding changes were 7.35 ± 0.41 (baseline), 8.62 ± 0.66 (2 h) ($P < 0.05$ vs. baseline; $P < 0.05$ vs. HAGE), 6.37 ± 0.46 (4 h), and 6.12 ± 0.31 nmol/ml (6 h) . All fasting parameters (FMD, reactive hyperemia, E-selectin, ICAM-1, VCAM-1, CRP, fibrinogen, CML, MG, and TBARS) were comparable between study days.

Correlations

We found significant correlations between changes in the following parameters: CML and reactive hyperemia (during HAGE, baseline to 4 h: $r = -0.782$, $P = 0.004$), FMD and triglycerides (during HAGE, baseline to 2 h: $r = -0.751$, $P = 0.005$, and during HAGE+BT: $r = -0.708$, $P = 0.010$), MG and CML (during HAGE, baseline to 2 h: $r = 0.726$, $P = 0.008$). A borderline significant, inverse correlation between the change in MG and FMD during HAGE (baseline to 4 h: $r = -0.532$, $P = 0.075$) was found. We also found a positive correlation between baseline values of MG and TBARS ($r = 0.701$, $P = 0.011$). No further correlations existed between either absolute values or changes in different parameters.

CONCLUSIONS — The main finding of our study is that benfotiamine is able to completely prevent micro- and macrovascular dysfunction induced by an AGE-rich test meal in patients with type 2 diabetes. We suggest reduction of endogenous AGE and dicarbonyls production, as well as reduction of oxidative stress as the main mechanisms.

FMD decrease following HAGE occurred already after 2 h, reached a maximum at 4 h, and lasted for at least 6 h postprandially. Pretreatment with benfotiamine not only prevented the FMD fall at every time point but completely changed its dynamic, since at 6 h, the FMD was fully recovered.

A short-term impairment in FMD can occur due to three different mechanisms: a decrease in endothelial NO synthesis, an increase in NO scavenging (e.g., by AGEs, dicarbonyls, reactive oxygen species), or a change in NO sensitivity of smooth muscle cells. We can exclude the latter mechanism since the glycerotrinitrate-induced vasodilation remained unchanged throughout the study. The simultaneous increase in serum markers of endothelial dysfunction (E-selectin, ICAM-1, and VCAM-1) suggests a postprandial impairment in endothelial function and hence subsequently reduced NO production. Since TBARS also increased postprandially, an increase in oxidative stress probably further promoted NO scavenging. Therefore, the postprandial FMD reduction can be the result of a combined effect of reduced NO production and increased scavenging, both decreasing NO bioavailability.

We found a postprandial vasodilatation after HAGE, which was significantly smaller following HAGE+BT only at 2 h. Similar postprandial changes in the arterial diameter following reactive hyperemia were noted on both study days. However, the postprandial impairment in FMD cannot be attributed solely to the vasodilatation (measured as arterial diameter before ischemia) since our study clearly showed a parallel increase in plasma markers reflecting endothelial dysfunction.

An explanation for the observed postprandial vasodilatation could be the insulin secretion induced by the meal. This increase of insulin level can enhance NO production, leading to vasodilation at the macrovascular level (36), and this can be only partially counteracted by the superoxide production due to HAGE. Benfotiamine, by preventing oxidative stress (decrease of TBARS after 2 h), can ameliorate insulin resistance. Consequently, after benfotiamine pretreatment, a decrease of insulin secretion and of the subsequent macrovascular vasodilation could be assumed (since serum insulin levels were not assessed).

Plasma nitrite and nitrate as a measure of NO were not assessed; instead, the levels of adhesion molecules (VCAM-1 and ICAM-1) and E-selectin were determined. They directly reflect the endothelial dysfunction and have been shown to correlate with plasma nitrite and nitrate levels, at least under controlled dietary intake (37,38). Even though the microcirculation reacted comparable with the FMD, it also showed some differences: reactive hyperemia was already maximally impaired 2 h after HAGE (FMD was maximally impaired after 4 h) and did not reach the baseline value after 6 h (similar to FMD). Benfotiamine again prevented reactive hyperemia impairment, and a full restoration of function occurred as soon as 4 h.

FMD is considered to be predominantly a measure of NO bioavailability (if glycerotrinitrate is assessed in parallel), while the regulation of microcirculation is a complex system where the involvement of vasodilatatory prostaglandins has been suggested by some authors (39) but questioned by others (40). The microcirculation seems to react more rapidly to postprandial changes than the macrocirculation, as shown by our finding of maximal reactive hyperemia impairment at 2 h, while FMD was lowest at 4 h. Moreover, pretreatment with benfotiamine had already led to a complete restoration of reactive hyperemia after 4 h, whereas FMD only normalized after 6 h.

Circulating markers of endothelial dysfunction, E-selectin, ICAM-1, and VCAM-1 increased significantly after HAGE. Pretreatment with benfotiamine for 3 days completely abolished these postprandial effects.

Although benfotiamine markedly changed the postprandial response, it did not improve any of the parameters measured in the fasting state: FMD, reactive hyperemia, E-selectin, VCAM-1, ICAM-1, CRP, or fibrinogen. This could be explained by the short duration of treatment, which was insufficient to induce baseline changes, but an alternative explanation is that the benfotiamine-mediated mechanisms protecting from postprandial endothelial dysfunction reside "outside" the endothelial cells. Benfotiamine probably acts by reducing mechanisms that induce endothelial dysfunction (such as oxidative stress, AGE, and dicarbonyls production) rather than directly improving endothelial function. A study by Beisswenger et al. (23), conducted in people with diabetes, showed that hyperglycemia rapidly induces in vivo synthesis of dicarbonyls, which are highly reactive AGE precursors such as MG and 3-deoxyglucosone. Benfotiamine decreased dicarbonyls formation in animal models (41). In our study, the postprandial circulating MG levels increased maximally at 4 h, an effect prevented by benfotiamine. Dicarbonyls can induce oxidative stress, promote inflammation (42), and induce endothelial apoptosis (43) and dysfunction (44).

Our data show that benfotiamine prevented postprandial increases in circulating MG and CML levels in humans. Unless it is shown that benfotiamine reduces AGE absorption, one should consider the possibility that not only MG but also endogenous CML production may

occur fast in the hyperglycemic state as previously suggested (45), and hence, benfotiamine could actually decrease both endogenous AGE and dicarbonyls formation. An oxidative environment is known to accelerate AGE formation, and indeed TBARS had already increased maximally after 2 h, preceding MG and CML maximal increase. Our hypothesis is that benfotiamine reduces postprandial CML and MG increase through two different mechanisms: by activating the enzyme transketolase (it redirects glucose away from the AGE-generating pathway) and by reducing oxidative stress (it also slows the reaction). By these means, benfotiamine might further reduce AGE and reactive oxygen species–dependent endothelial cell dysfunction and NO scavenging.

We found a significant inverse correlation between CML increase and microvascular function impairment and a borderline significant inverse correlation between the MG increase and FMD impairment. This suggests direct effects of CML and MG on vascular function, with CML playing a more important role in the regulation of the microcirculation and the MG in the regulation of macrocirculation. Further studies are needed to strengthen these hypotheses.

The inverse correlation between FMD and TG changes and the lack of correlation between FMD and BG changes, suggesting that hypertriglyceridemia has a greater influence on vascular function than hyperglycemia, probably by inducing a more pronounced oxidative stress. This hypothesis is strengthened by the finding that meal-induced vascular dysfunction is reversed by simvastatin treatment (11).

HAGE alone induced a raise in CRP values after 6 h, an effect that was prevented by benfotiamine. Direct or indirect anti-inflammatory properties of benfotiamine have been previously postulated (46) and are clearly suggested by our study.

Comparable baseline metabolic parameters characterized both study days. Postprandial periods after both meals were accompanied by a significant increase in blood glucose after 2 h and decrease after 6 h (compared with baseline). Triglycerides increased 4 and 6 h postprandially after both meals. Since both blood glucose and triglyceride excursions were comparable on the two study days, the different results cannot be attributed to these parameters.

In conclusion, our study demonstrated that a heat-processed test meal, with a HAGE, induces endothelial dysfunction and oxidative stress and increases serum AGE and MG concentration, effects that are prevented by benfotiamine. We suggest that benfotiamine might play an important role in atherosclerosis preventive therapy in patients with diabetes.

Limitations of the study

Our patients had rather high values of A1C (8.5 ± 0.5%) with relatively low levels of fasting glycemia. This could be explained by the postprandial blood glucose excursions, which were shown to account for ~50% of the A1C value for an A1C between 8.5 and 9.2% (47).

Although the number of studied subjects ($n = 13$) is certainly not large, it allowed us to establish statistically significance to our results. Moreover, this sample size was based on calculations previously published (31).

Heat treatment of foods can induce modifications other than changes in the content of AGEs, such as inactivation of vitamins and antioxidants (48) or generation of other toxic compounds (49). Therefore, the contribution of substances other than AGEs to the observed effects cannot be excluded. Still, the main message of the study remains unaltered, namely that a highly processed meal induces marked postprandial endothelial function and that these effects can be reversed by pretreatment with benfotiamine. Our study does not completely elucidate the mechanisms through which benfotiamine prevents postprandial vascular dysfunction but raises some hypotheses. Further studies are warranted to bring light into these subtle mechanisms.

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