# Effects of low- and high-advanced glycation endproduct meals on macro- and microvascular endothelial function and oxidative stress in patients with type 2 diabetes mellitus[1–3]

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## ABSTRACT

**Background:** An advanced glycation endproducts (AGEs)–rich diet induces significant increases in inflammatory and endothelial dysfunction markers in type 2 diabetes mellitus (T2DM).

**Objective:** The aim was to investigate the acute effects of dietary AGEs on vascular function in T2DM patients.

**Design:** Twenty inpatients with T2DM [$\bar{x}$ (±SEM) age: 55.4 ± 2.2 y; glycated hemoglobin: 8.8 ± 0.5%] were investigated. In a randomized crossover design, the effects of a low-AGE (LAGE) and high-AGE (HAGE) meal on macrovascular [by flow-mediated dilatation (FMD)] and microvascular (by Laser-Doppler flowmetry) function, serum markers of endothelial dysfunction (E-selectin, intracellular adhesion molecule 1, and vascular cell adhesion molecule 1), oxidative stress, and serum AGE were assessed. The meals had identical ingredients but different AGE amounts (15.100 compared with 2.750 kU AGE for the HAGE and LAGE meals, respectively), which were obtained by varying the cooking temperature and time. The measurements were performed at baseline and 2, 4, and 6 h after each meal.

**Results:** After the HAGE meal, FMD decreased by 36.2%, from 5.77 ± 0.65% (baseline) to 3.93 ± 0.48 (2 h), 3.70 ± 0.42 (4 h), and 4.42 ± 0.54% (6 h) ($P < 0.01$ for all compared with baseline). After the LAGE meal, FMD decreased by 20.9%, from 6.04 ± 0.68% (baseline) to 4.75 ± 0.48% (2 h), 4.69 ± 0.51% (4 h), and 5.62 ± 0.63% (6 h), respectively ($P < 0.01$ for all compared with baseline; $P < 0.001$ for all compared with the HAGE meal). This impairment of macrovascular function after the HAGE meal was paralleled by an impairment of microvascular function (−67.2%) and increased concentrations of serum AGE and markers of endothelial dysfunction and oxidative stress.

**Conclusions:** In patients with T2DM, a HAGE meal induces a more pronounced acute impairment of vascular function than does an otherwise identical LAGE meal. Therefore, chemical modifications of food by means of cooking play a major role in influencing the extent of postprandial vascular dysfunction. *Am J Clin Nutr* 2007;85:1236–43.

**KEY WORDS** Nutrition, diet, glycation, advanced glycation endproducts, oxidative stress, endothelium

## INTRODUCTION

The main cause of mortality in persons with type 2 diabetes mellitus (T2DM) is cardiovascular disease (CVD). In patients with T2DM, the prevalence of CVD is 2- to 5-fold that observed in individuals without diabetes (1, 2). In recent years, increasing attention has been paid to the role of endothelium in the triggering and development of CVD. Endothelial dysfunction is now considered to be the major initial step in the development of atherosclerosis (3, 4). However, the mechanisms leading to endothelial dysfunction in diabetes are still controversial.

Abnormalities occurring in the postprandial state have an important contribution to the development and progression of atherosclerosis, both in subjects with (5) or without diabetes (6). It has been shown in healthy and T2DM subjects that postprandial hyperglycemia and hypertriglyceridemia lead to oxidative stress and endothelial dysfunction and that these effects are additive (7).

Dietary factors other than glucose and lipids can also modulate endothelial function, and among them advanced glycation endproducts (AGEs) have been intensively studied in recent years. AGEs, best known in the context of DM, are a heterogeneous group of molecules resulting from the nonenzymatic glycation of proteins, lipids, and nucleic acids. T2DM patients have significantly higher serum AGE concentrations than do healthy control subjects (8). AGEs have significant proinflammatory and prooxidant effects, thus playing an important role in the development of diabetes complications and in changes associated with aging (9, 10). Nutrition is an important source of exogenous AGEs, with the highest content found in heat-treated foods (11), especially lipid- and protein-rich foods, typical of Western diets (12). The AGE content of ingested foods depends on nutrient composition, temperature, method and duration of heat application (12). Approximately 10% of ingested AGEs are absorbed, and about two-thirds of them are deposited in tissues, where they

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remain biologically active and exert their pathological effects (13). It has become increasingly evident that dietary AGEs represent an important source of circulating and tissue AGEs and manifest similar pathogenic effects to their endogenous counterparts (13–18).

In T2DM subjects, a high-AGE (HAGE) diet over 6 wk caused a significant increase in serum AGEs, markers of inflammation [C-reactive protein (CRP) and tumor necrosis factor $\alpha$ (TNF-$\alpha$)] and endothelial dysfunction [vascular cell adhesion molecule 1 (VCAM-1)], whereas a low-AGE (LAGE) diet led to suppression of all these markers (19). Moreover, LDL pooled from patients on a HAGE diet was more glycated and more oxidized and markedly stimulated necrosis factor $\kappa$B (NF-kB) activity, thus enhancing LDL vascular toxicity (20).

Our current study tested the hypothesis that a single "real-life" HAGE meal acutely induces more pronounced vascular dysfunction than does a low-AGE matched meal. We also postulated that this dysfunction exceeds the effects of postprandial hyperglycemia and hypertriglyceridemia. To this aim, we compared the effects of 2 meals with identical ingredients but different AGE amounts on micro- and macrocirculation, as well as on serum lipids, markers of inflammation and endothelial dysfunction, oxidative stress, and serum AGEs.

## SUBJECTS AND METHODS

### Patients

Between August 2004 and July 2005, 20 subjects (14 men, 6 women) with T2DM aged 41–71 y were recruited among inpatients from the Diabetes Center NRW, Bad Oeynhausen, Germany. The patients' mean (±SEM) characteristics were the following: age: 55.4 ± 2.2 y; diabetes duration: 8.5 ± 2.0 y; diabetes therapy: oral ($n$ = 14), oral+insulin ($n$ = 4), insulin ($n$ = 2); glycated hemoglobin (Hb $A_{1c}$): 8.8 ± 0.5%; body mass index (BMI; in kg/m$^2$): 29.5 ± 0.9; and smoker ($n$ = 3) or nonsmoker ($n$ = 17). Three patients had nonproliferative retinopathy, 2 had microalbuminuria, 8 had peripheral neuropathy, and 16 had arterial hypertension. Other medications included aspirin ($n$ = 15), angiotensin-converting enzyme inhibitors ($n$ = 9), angiotensin receptor blockers ($n$ = 4), $\beta$ blockers ($n$ = 7), diuretics ($n$ = 6), calcium channel blockers ($n$ = 3), and 3-Hydroxy-3-methylglutaryl-coenzyme A inhibitors ($n$ = 9).

Exclusion criteria were Hb $A_{1c} \geq 13\%$, pregnancy, heart failure [New York Heart Association classes III-IV], history of stroke, myocardial infarction, unstable angina, peripheral arterial vascular disease stage ≥IIB, renal failure (serum creatinine >1.2 mg/dL), cancer, chronic alcohol abuse, severe hypo- or hypertension (resting blood pressure <90/50 mm Hg or >180/110 mm Hg), therapy with >3 antihypertensives, therapy with nitrates, and severe diabetes complications (proliferative diabetic retinopathy, macroalbuminuria, painful diabetic peripheral neuropathy, or diabetic foot syndrome). The local ethics committee approved the study protocol, and the study was carried out according to the principles from the Declaration of Helsinki. Written informed consent was obtained from all subjects.

### Study design

The subjects were given a standardized diabetes diet over the 6 d of the study. On days 4 and 6, the effects of LAGE and HAGE meals on vascular function were studied in an investigator-blinded, randomized, crossover design. Vascular function was assessed after an 8-h overnight fast, then 2, 4, and 6 h after the test meal.

The following variables were assessed at every investigation: serum glucose; lipid metabolism (total, HDL, and LDL cholesterol and triacylglycerols); markers of inflammation [fibrinogen, CRP, and interleukin 6 (IL-6)], endothelial function [intracellular adhesion molecule 1 (ICAM-1) and VCAM-1], and oxidative stress [thiobarbituric acid-reactive substance (TBARS)]; and serum AGEs (methylglyoxal and carboxymethyllysine). E-selectin was measured at baseline and 4 h postprandially.

### HAGE and LAGE meals

The 2 meals were isocaloric, had identical ingredients, and differed only by the temperature and time of cooking. Each meal consisted of 200 g chicken breast, 250 g potatoes, 100 g carrots, 200 g tomatoes, and 15 g vegetable oil and provided 580 kcal, 54 g protein, 17 g fat, 48 g carbohydrates, 60 mg cholesterol, and 10 g fibers. The HAGE meal (15.100 kU AGE) was prepared by frying or broiling at 230 °C for 20 min, whereas the LAGE meal (2750 kU AGE) was prepared by steaming or boiling at 100 °C for 10 min. The subjects were instructed to eat the test meal within 30 min.

### Assessment of vascular function

The macrocirculatory function was assessed by measuring flow-mediated dilatation (FMD), whereas the functional assessment of microcirculation was performed by Laser-Doppler flowmetry.

#### *Flow-mediated dilatation*

The investigations started at 0700, after an 8-h overnight fast, and were performed in a dark, quiet, temperature-controlled room (22–24 °C). All medications were withheld for ≥12 h before the first investigation, but they were kept constant throughout the study. The subjects were asked not to exercise and not to smoke and were only allowed to drink mineral water until the last investigation (6 h) was performed.

FMD was measured by using the methodology described by Celermajer et al (21). Measurements of brachial artery diameter were performed with a high-resolution, 2-dimensional ultrasound imaging system (ATL HDI 5000; Advanced Technology Laboratories, Bothell, WA) by using B-mode, ECG-triggered ultrasound images obtained with a 7–15 MHz linear-array transducer.

The subjects laid at rest for ≥10 min before the first scan was taken and remained in a recumbent position throughout the investigation. To avoid movement artifacts, the subject's arm was immobilized in a foam cast. The artery was scanned longitudinally at 3–10 cm above the elbow. Once a satisfactory image was located, the ultrasound probe was kept stable at that point, which was marked on the skin, and all subsequent measurements were made at this defined location. Depth and gain settings were not changed throughout the investigations for the same patient. Arterial flow velocity was measured in the center of the vessel with a pulsed Doppler signal.

After measurements at baseline, a blood pressure cuff placed around the forearm was inflated to 250 mm Hg for 4.5 min and then deflated. Pulse Doppler was recorded for the first 15 s after

cuff release, followed by a 2-dimensional scan for 120 s. At baseline and 4 h postprandially, 10 min were allowed for vessel recovery, then a further resting scan was taken. Sublingual glycerotrinitrate spray (0.4 mg) was administered, and 5 min later the last scan was performed (as a measure of endothelium-independent vasodilatation).

All continuous image sequences were saved on a personal computer, where measurements of arterial diameter were performed by using an automated edge-detection software (HDI Lab, Advanced Technology Laboratories Ultrasound 1.91). All measurements were made at the end-diastole, coincident with the R wave on the ECG. For the endothelium-dependent vasodilatation, diameter measurements were taken 60 s after cuff deflation (maximal arterial diameter after reactive hyperemia). Four cardiac circles were analyzed for each scan and the measurements averaged. FMD and endothelium-independent vasodilatation were calculated as the percentage increase of the baseline diameter value.

Both the investigator and the person who performed the measurements were unaware of the study phase the patient was in. Testing for reproducibility of the FMD measurements showed a CV of 5.41%.

*Laser Doppler (microlightguide spectrophotometer)*

The skin microcirculation was assessed non-invasively, simultaneously with the FMD, using a microlightguide spectrophotometer (O2C; LEA Medizintechnik, Giessen, Germany) (22). The Laser-Doppler probe was fixed on the hypothenar surface of the right hand. It transmits continuous wave laser light (830 nm and 30 mW) and white light (20W, 500–800 nm, 1 nm resolution) to the tissue, where it is scattered and collected on the skin surface at fibers in the probe. The collected light is converted into an electrical signal, which is digitized and recorded on a personal computer and allows thus the measurement of the skin blood flow (BF).

Given the great inter- and intraassay variability, we used a reproducible alternative of this technique, which assesses the increase in skin blood flow of the hand after 4.5 min ischemia of the forearm (23). The BF [expressed in arbitrary units (22)] was measured in 2-mm depth before and after the 4.5-min forearm occlusion.

BF was continuously measured over 2 min before compression, and measurements were averaged. The measurement was repeated after decompression, and maximal BF was recorded. Reactive hyperemia (RH) was calculated as the ratio between the maximal postischemia and baseline BF.

### Biochemical measurements

Blood was collected with minimal stasis, with the subjects resting in a supine position. To avoid endothelial and platelet activation, no indwelling canulas were used, but a single-use blood collecting system (Venofix, size 0.8 mm-21G; BBraun, Melsungen, Germany). Plasma and serum were obtained by centrifugation of blood at 4 °C and 1500 × *g* for 20 min. Aliquots of 750 $\mu$L were stored at −80 °C.

Serum glucose was measured by the glucose-oxidase method (Architect ci8200; Abbott Diagnostics, Wiesbaden, Germany); Hb $A_{1c}$ was assessed by HPLC (Menarini, Berlin, Germany). Serum cholesterol, triacylglycerols, and LDL and HDL cholesterol were measured by using the Architect ci8200 analyzer (Abbott Diagnostics, Wiesbaden, Germany).

Commercially available assays were used to measure TBARS (Alexis Biochemicals, Grünberg, Switzerland), fibrinogen, CRP (Architect ci8200; Abbott Diagnostics), TNF-$\alpha$, IL-6 (ImmuLite 2000; DPC Biermann, Bad Nauheim, Germany). Plasma concentrations of VCAM-1, ICAM-1, and E-selectin were measured by using commercially available immunosorbent kits (R&D Systems, Wiesbaden, Germany; Technoclone, Wien, Austria).

Serum AGE concentrations were measured by CML-sensitive enzyme-linked immunosorbent assay (ELISA) (24). Total serum methylglyoxal derivatives were assessed by ELISA by using a monoclonal anti- methylglyoxal -BSA antibody (MG3D11) raised against methylglyoxal-modified BSA (22 methylglyoxal–modified Arg/mol BSA, by HPLC, obtained from Dr Y Al-Abed, The Picower Institute, Manhasset, NY). MG3D11 was found to be strongly immunoreactive against methylglyoxal-ovalbumin and AGE-BSA, but not with carboxymethyllysine-BSA or unmodified BSA (25).

### Statistical analysis

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 the HAGE and LAGE meals on FMD, laser-Doppler parameters, and serum variables. Meal and time effects, as well as meal × time interactions, were tested by using 2-factor repeated-measures analysis of variance, with Bonferroni's correction for multiple comparisons. When the study meal × time interaction was significant, a 2-tailed paired *t* test was used to assess differences at individual time periods, with Bonferroni's correction for multiple comparisons. Statistical significance was defined as $P < 0.05$. Statistical analysis was performed by using SPSS version 12.0 (SPSS Inc, Chicago, IL). Unless otherwise stated, data are expressed as means ± SEMs. The sample size was chosen to detect a change of FMD of ≥30% in a crossover design, with a power of 0.90 (26, 27).

## RESULTS

### Macrocirculation (flow-mediated vasodilatation)

FMD decreased after both meals, but significantly more after the HAGE meal than after the LAGE meal, and the maximal effects were present after 4 h (**Figure 1**). FMD impairment after the HAGE meal was >1.5 times that observed after the LAGE meal (max change: 36.2% compared with 20.9%, respectively).

Vasodilatation of the brachial artery was noted postprandially after both meals, without differences between the LAGE and HAGE meals. Brachial artery diameter changed after the HAGE meal from 3.89 ± 0.13 mm (baseline) to 3.97 ± 0.13 at 2 h, 3.99 ± 0.13 at 4 h, and 4.14 ± 0.15 at 6 h ($P < 0.01$ for all, in a comparison with baseline) and after the LAGE meal from 3.91 ± 0.13 mm (baseline) to 4.02 ± 0.14 at 2 h, 4.01 ± 0.14 at 4 h, and 4.09 ± 0.14 at 6 h ($P < 0.01$ for all, in a comparison with baseline). The maximal postocclusive diameter showed no significant differences between the test meals (data not shown).

The endothelium-independent vasodilatation had no significant changes, either after each meal or between them. After the HAGE meal, vasodilation went from 16.25 ± 1.14% at baseline to 16.10 ± 1.27% at 4 h, and after the LAGE meal, it went from 16.46 ± 1.38% at baseline to 16.07 ± 1.29% at 4 h ($P$ = not significant).

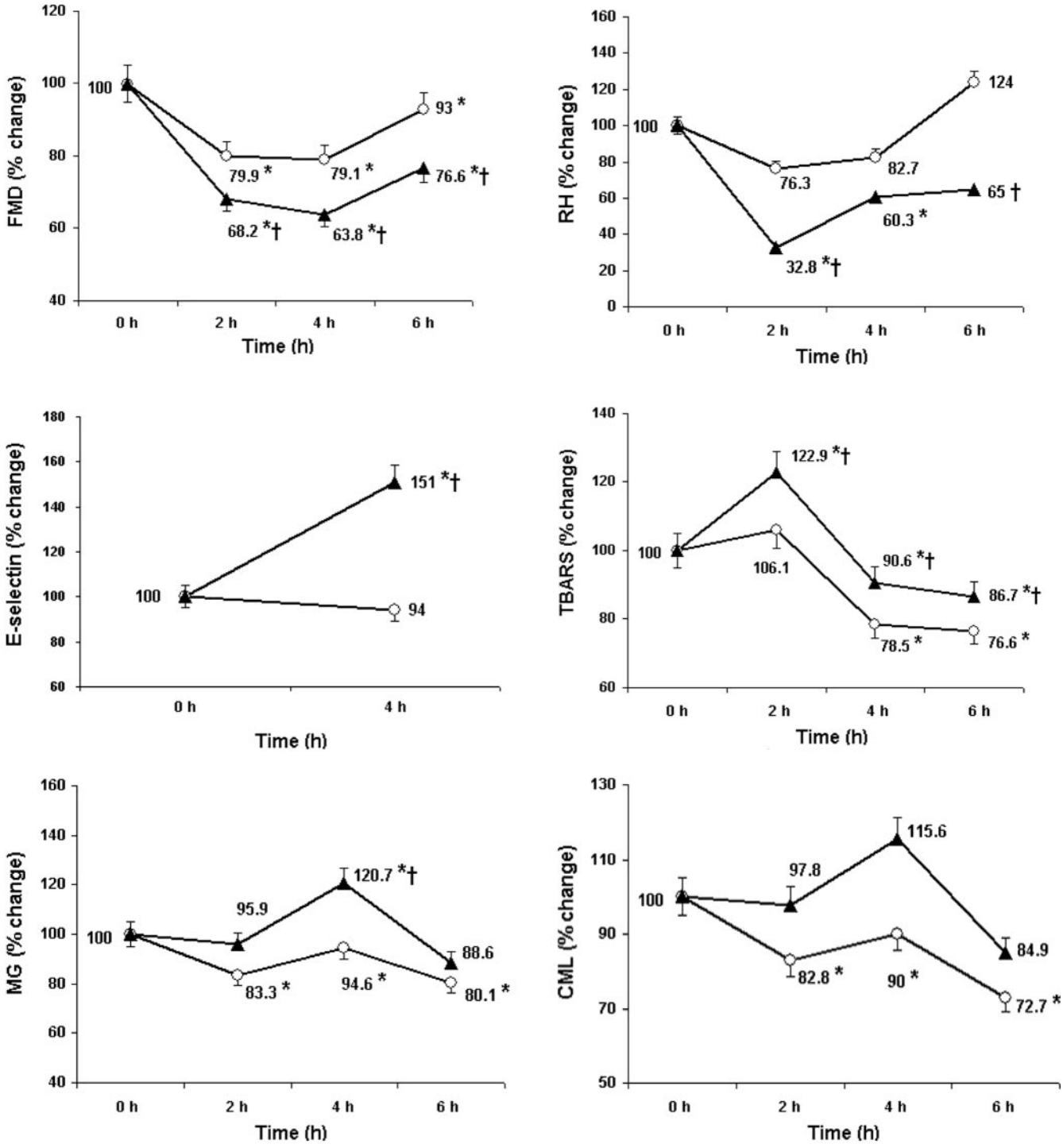


**FIGURE 1.** Mean (±SEM) changes in clinical and laboratory variables in response to low-advanced glycation endproducts (LAGE) and high-AGE (HAGE) meals. ○, LAGE; ▲, HAGE. $n = 20$. FMD, flow-mediated dilation; RH, reactive hyperemia, TBARS, thiobarbituric acid–reactive substances; MG, methylgloxal; CML, carbosymethyllisine. $P$ values for the effects of meal (M) and time (T) and the meal × time (M × T) interaction were obtained with 2-factor repeated-measures ANOVA with post hoc Bonferroni's correction for multiple comparisons. FMD—baseline values: HAGE = 5.77 ± 0.65%, LAGE = 6.04 ± 0.68%; M, $P < 0.0001$; T, $P < 0.0001$; M × T interaction, $P = 0.002$. RH—baseline values: HAGE = 3.72 ± 0.89%, LAGE = 2.66 ± 0.45%; T, $P = 0.007$; M × T interaction, $P = 0.032$. Plasma E-selectin—baseline values: HAGE = 37.2 ± 2.8 ng/mL, LAGE = 40.7 ± 4.1 ng/mL; M, $P = 0.039$; M × T interaction, $P = 0.008$. TBARS—baseline values: HAGE = 7.57 ± 0.36 nmol/mL, LAGE = 8.16 ± 0.38 nmol/mL; T, $P < 0.0001$; M × T interaction, $P = 0.003$. Serum MG—baseline values: HAGE = 2.99 ± 0.35 nmol/mL, LAGE = 3.12 ± 0.34 nmol/mL; T, $P = 0.032$, M × T interaction, $P = 0.048$. Serum CML—baseline values: HAGE = 9.51 ± 1.13 U/mL, LAGE = 10.2 ± 1.25 U/mL; T, $P = 0.032$. *Significantly different from baseline, $P < 0.01$ (paired Student's $t$ test, with post hoc Bonferroni's correction for multiple comparisons). †Significantly different from the LAGE meal, $P < 0.05$ (paired Student's $t$ test).

**TABLE 1**
Effects of the low- (LAGE) and high- (HAGE) advanced glycation endproducts meals on laboratory variables[1]

| | Time after meal | | | | $P$[2] | | |
|---|---|---|---|---|---|---|---|
| | 0 h | 2 h | 4 h | 6 h | Time | Meal | Time × Meal |
| Serum glucose (mg/dL) | | | | | 0.001 | — | — |
| LAGE | 150.2 ± 9.9[3] | 167.3 ± 9.9 | 111.6 ± 5.7 | 102.1 ± 5.4 | | | |
| HAGE | 152.0 ± 9.9 | 174.7 ± 12.9 | 123.7 ± 8.7 | 112.1 ± 6.6 | | | |
| TG (mg/dL) | | | | | NS | NS | NS |
| LAGE | 136.1 ± 15.3 | 141.9 ± .16.8 | 153.9 ± 19.1 | 153.1 ± 15.7 | | | |
| HAGE | 126.2 ± 11.1 | 134.2 ± 13.2 | 145.7 ± 13.3 | 145.1 ± 12.6 | | | |
| ICAM-1 (ng/mL) | | | | | — | 0.028 | 0.0001 |
| LAGE | 236.5 ± 5.9 | 223.2 ± 6.9[4] | 224.7 ± 9.7 | 228.0 ± 8.4 | | | |
| HAGE | 228.3 ± 8.3 | 239.6 ± 8.5[4,5] | 236.1 ± 9.9[5] | 229.9 ± 7.7 | | | |
| VCAM-1 (ng/mL) | | | | | 0.049 | 0.048 | 0.006 |
| LAGE | 568.4 ± 43.7 | 542.1 ± 44.8[4] | 485.4 ± 32.0[4] | 516.4 ± 37.2 | | M = 0.048 | |
| | | | | | | T=0.049 | |
| HAGE | 541.5 ± 39.4 | 645.2 ± 57.6[4,5] | 576.3 ± 56.1[5] | 535.9 ± 37.9 | | M × T=0.006 | |

[1] $n = 20$. No significant differences were observed in the baseline concentration of the measured variables between the meals. TG, triacylglycerols; IL-6, interleukin 6; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1.
[2] Two-factor repeated-measures ANOVA with post hoc Bonferroni's correction for multiple comparisons.
[3] $\bar{x}$ ± SEM (all such values).
[4] Significantly different from baseline (paired Student's $t$ test with post hoc Bonferroni's correction for multiple comparisons), $P < 0.001$.
[5] Significantly different from LAGE (paired Student's $t$ test), $P < 0.05$.

### Microcirculation (reactive hyperemia)

Similar changes to FMD were observed in the microcirculation: RH was impaired after both meals, but significantly more after the HAGE than after the LAGE meal, with a maximal decrease already observed after 2 h (Figure 1). RH impairment after the HAGE meal was almost 3 times that observed after the LAGE meal (−67.2% compared with −23.7%, respectively).

### Physiologic variables

Systolic blood pressure decreased significantly 2 and 4 h after the HAGE meal, from 130.5 mm Hg (baseline) to 125.8 mm Hg at 2 h, 125.9 mm Hg at 4 h, and 130.1 mm Hg at 6 h, whereas after the LAGE meal it remained unchanged: 131.7 mm Hg at baseline, 131.4 mm Hg at 2 h, 130.7 mm Hg at 4 h, and 131.6 mm Hg at 6 h. No significant changes in diastolic blood pressure and heart rate were noted (data not shown).

### Laboratory variables

Changes in laboratory variables are presented in **Table 1**.

### Glucose and lipid parameters

No significant differences in serum glucose changes or values were observed between the meals. Serum triacylglycerols also increased similarly after both meals. Total cholesterol decreased after the LAGE meal (167.5 ± 9.5 mg/dL at baseline, 162.1 ± 8.6 mg/dL at 6 h; $P < 0.05$) and remained unchanged after the HAGE meal (164.5 ± 7.9 and 162.8 ± 8.0 mg/dL, respectively). HDL cholesterol remained unchanged (data not shown), whereas LDL cholesterol decreased after both meals (LAGE: 108.6 ± 8.4 mg/dL at baseline compared with 103.6 ± 7.7 mg/dL at 6 h, $P < 0.05$; HAGE: 106.9 ± 6.9 mg/dL at baseline compared with 104.5 ± 7.1 mg/dL at 6 h, $P < 0.05$).

No significant correlation between baseline values of FMD and either baseline glucose or triacylglycerols was found. Moreover, no correlation between FMD change and the changes of these variables was found, except for the change in FMD from baseline to 2 h, which correlated inversely with the change in triacylglycerols ($r = -0.686$, $P = 0.001$).

No significant correlation between baseline values of RH and either baseline glucose or triacylglycerols was present. The same applied for the change of these variables.

### Insulin

Plasma insulin values at baseline and 2, 4, and 6 h postprandially were comparable during the HAGE (9.9 ± 2.5, 29.5 ± 4.5, 14.9 ± 3.1, 10.4 ± 1.9 pmol/L, resepectively) and LAGE (9.1 ± 1.1, 31.2 ± 4.7, 13.2 ± 2.9, 9.8 ± 1.9 pmol/L, respectively) meals. The values at 2 h were significantly higher ($P < 0.05$) than those measured at baseline.

### Inflammatory markers

Inflammatory markers were in the normal range for all patients, and the baseline values were comparable between study days (HAGE meal compared with LAGE meal: CRP: 2.7 ± 3.3 and 3.5 ± 4.7 mg/dL; fibrinogen: 298.4 ± 57.6 and 296.4 ± 59.2 mg/dL; TNF-$\alpha$: 6.6 ± 1.9 and 6.6 ± 2.0 pg/mL, respectively). None of these variables changed significantly after either meal (data not shown). IL-6 decreased after the LAGE meal (3.5 ± 0.8, 2.9 ± 0.4, 3.1 ± 0.4, and 4.0 ± 0.6 pg/mL for baseline and 2, 4, and 6 h after the meal, respectively) and increased after the HAGE meal (2.9 ± 0.6, 3.9 ± 1.1, 3.4 ± 0.6, and 5.8 ± 1.2 pg/mL for baseline and 2, 4, and 6 h after the meal, respectively), without significant differences between meals.

### Markers of endothelial dysfunction

Plasma E-selectin did not significantly change after the LAGE meal and increased significantly 4 h after the HAGE meal, so that the difference between the meals after 4 h was significant (Figure 1). ICAM-1 decreased 2 h after the LAGE meal and increased 2 h after the HAGE meal, with significant differences between the

meals at 2 and 4 h (Table 1). VCAM-1 persistently decreased after the LAGE meal and increased after the HAGE meal, with significant differences between the meals again after 2 and 4 h (Table 1).

### Markers of oxidative stress

Serum TBARS increased significantly 2 h after the HAGE meal (21.3%) and nonsignificantly 2 h after the LAGE meal (5.3%) (Figure 1).

### Serum AGEs (methylglyoxal and carboxymethyllysine)

After the HAGE meal, serum methylglyoxal increased after 4 h (significantly when compared with baseline and to the LAGE meal) and decreased significantly after the LAGE meal at all time points (Figure 1). The change in serum methylglyoxal concentrations 4 h after the HAGE meal was significantly correlated with the change in FMD ($r = -0.462$, $P = 0.035$).

Serum carboxymethyllysine increased 4 h after the HAGE meal, but the changes did not reach significance. After the LAGE meal, a decrease in serum carboxymethyllysine was noted at 6 h (Figure 1).

## DISCUSSION

Our study showed for the first time that the cooking method of a meal influences decisively the extent of postprandial vascular dysfunction in patients with T2DM. It showed that a single "real-life" HAGE meal induces a profound impairment of both macro- and microvascular function (−36.2% and −67.2%, respectively). These changes are significantly greater than those induced by a meal containing the same ingredients but with a five-fold lower AGE concentration (LAGE meal).

These results are consistent with previous studies that show postprandial FMD reduction in T2DM patients due to independent and cumulative contributions of hyperglycemia and hypertriglycerolemia (7, 28, 29) and add AGEs and dicarbonyls (methylglyoxal) as new factors coresponsible for these effects. To our knowledge, the current study is the first that has compared the acute effects of HAGE and LAGE meals on vascular function in patients with T2DM.

Endothelium-independent vasodilatation did not change significantly after both meals, suggesting that vascular smooth muscle cells remained nitric oxide (NO)–sensitive. Thus, the observed FMD impairment is due either to a decreased endothelial NO production or secretion or to an increased NO scavenging [eg, by AGEs or reactive oxygen species (ROS)]. Decreased endothelial NO production seems likely, because the postprandial impairment in endothelial function after ingestion of the HAGE meal was accompanied by an increase in markers of endothelial dysfunction (E-selectin, ICAM-1, and VCAM-1). Oxidative stress, through an increased generation of ROS, has been shown to increase acutely after glucose intake (30) and postprandially (31–33). The amplified postprandial oxidative stress in the present study was confirmed by the increase in serum TBARS, which was significantly higher after the HAGE than after the LAGE meal. This could have further contributed to the observed vascular dysfunction through increased NO scavenging, direct cytotoxic effects on endothelial cells, and promotion of endogenous AGE synthesis. Partly due to technical reasons, we decided to assess TBARS and not nitrotyrosine as a measure of oxidative stress. TBARS represent a well-established method for measuring lipid peroxidation and is a frequently used parameter in studies assessing oxidative stress in patients with DM, given the importance of peroxidized lipids in the pathogenesis of atherosclerosis in this patient collective (34, 35).

Vasodilatation of the brachial artery was observed after both meals and was significant when compared with the baseline diameter, but this vasodilation was comparable between the meals. Nevertheless, the observed impairment of FMD cannot be due only to the brachial artery vasodilatation, because an increase in markers of endothelial dysfunction also occurred. An explanation for the observed postprandial vasodilatation could be the insulin secretion induced by the meal. Plasma insulin concentrations increased both after the HAGE and LAGE meals, and significantly after 2 h, but this increase was comparable between the meals. The increase in insulin concentrations can enhance NO production, leading to vasodilation at the macrovascular level (36). Plasma nitrite and nitrate as a measure of NO were not assessed; instead, the concentrations of adhesion molecules (VCAM-1 and ICAM-1) and of E-selectin were determined; they reflect directly the endothelial dysfunction and have been shown to correlate with plasma nitrite and nitrate concentrations, at least under controlled dietary intake (37, 38).

Given the fact that no significant differences in serum glucose and triacylglycerol changes were noted between the test meals, the more pronounced impairment of macro- and microcirculation induced by the HAGE meal than by the LAGE meal results, at least partly, from differences in factors other than glycemia or triglyceridemia. Similar changes to FMD were observed in the microcirculation, but here the maximal impairment was noted already after 2 h. The explanation for the more rapid deterioration of microvascular function resides probably in more complex regulatory mechanisms, which involve not only NO regulation, but also prostaglandin-mediated effects (39).

The HAGE meal induced a significant increase in serum methylglyoxal concentrations (a highly reactive dicarbonyl intermediate) after 4 h, an effect not seen after the LAGE meal. Moreover, the change in serum methylglyoxal 4 h after the HAGE meal ingestion was negatively correlated with the FMD change, suggesting that FMD impairment was at least partly due to the increase in serum methylglyoxal. Carboxymethyllysine increased after the HAGE meal, but the change did not reach statistical significance, probably due to the limited sample size. An oxidative environment is known to accelerate AGE formation, and indeed TBARS increased maximally already after 2 h, preceding methylglyoxal and carboxymethyllysine maximal increase; it can therefore be speculated that the observed increase in serum AGEs resulted partly from an increased endogenous generation due to oxidative stress and partly from intestinally absorbed AGEs.

Koschinsky et al (13) examined the oral absorbtion and the renal clearance kinetics of dietary AGEs in patients with diabetes mellitus. They have proven that, in diabetic patients with microalbuminuria, serum AGE concentrations peaked at 8 h postingestion and returned to baseline no later than 24 h postmeal. Urinary AGE concentrations peaked at 10–12 h and returned to baseline no later than 40–48 h. The subjects in our study were (with 2 exceptions) normoalbuminuric, and the interval between the test meals was 48 h. Therefore, it is almost certain that serum AGEs had been cleared out and and did not contribute to the serum AGEs after the next test meal.

The LAGE meal also impaired vascular function, albeit significantly less than the HAGE meal. These data are consistent with the findings of Bierhaus et al (40), who showed that, in T2DM patients, both the LAGE and HAGE standardized meals induced a postprandial proinflammatory response through activation of NF-kB. The postprandial activation of NF-kB and the subsequent activation of inflammation after a mixed meal was also shown in other studies (41). NF-kB plays an essential role in the expression of several cytokines, adhesion molecules, factors regulating vascular tone and the AGE receptor (RAGE). Therefore, sustained activation of the NF-kB leads to an up-regulation of RAGE. Postprandial hyperglycemia rapidly induces the synthesis of dicarbonyls (42), highly reactive AGE precursors, which bind to RAGE. Moreover, dietary AGEs are absorbed gastrointestinally (13). Therefore, people with T2DM, who are known to have an over-expression of RAGE (9) and also a higher dietary AGE load than do healthy subjects, are particularly vulnerable to the abnormalities of the postprandial state.

The test meals in our study had identical ingredients (and identical macronutrient composition), with differences only in the AGE content, which obtained by modifying the cooking methods. A limitation of the study is the fact that heat treatment of foods can induce changes other than variations in AGE content, such as inactivation of vitamins and antioxidants (43) or generation of other toxic compounds (44). Therefore, the contribution of substances other than AGEs to the observed effects cannot be completely excluded. This limitation could have been avoided by replacing the meals with products with a standardized AGE content, but we wanted a food study that was close to "real life" as possible. Thus, the main message of the study, namely that the processing method can decisively influence the effect of a meal on postprandial endothelial function, remains unaltered.

In animal models, restriction of AGE intake offers significant protection against atherosclerosis, postinjury restenosis, and nephropathy and improves wound healing and insulin sensitivity (14, 45–47). Recently, evidence from animal studies suggests that dietary restriction of AGEs extends the median life span to a similar extent as marked caloric restriction (−40%), which was until now considered the only method for prolonging survival (18).

One of the strengths of our study was that the crossover design eliminated the possibility that the observed effects were due to differences related to patient characteristics or medications used. However, because we only investigated patients with T2DM, it is clear that the results cannot be extrapolated to individuals without diabetes.

In conclusion, the current study showed that the same meal cooked under different conditions had different effects on vascular function and oxidative stress in patients with T2DM. A single HAGE meal induced significant acute endothelial dysfunction and oxidative stress. We speculate that a chronic HAGE diet, like most Western diets are, could lead over weeks or months to a persistent endothelial dysfunction and thus contributes to the development of the micro- and macrovascular complications of DM. As a logical consequence, a simple dietetic intervention, which does not necessarily mean deprivation of certain foods, but only the preferred use of low AGE-producing culinary techniques (boiling, poaching, or stewing), could represent an attractive prevention alternative to pharmacologic approaches (48). Testing this hypothesis in further studies is warranted. Moreover, it was beyond the scope of the current study to answer the questions related to the exact cellular and molecular mechanisms of the observed effects; this could also represent a challenging direction for future research.

The authors' responsibilities were as follows: MN designed the study, recruited the patients, performed the investigations, collected and performed the statistical processing of the data, and wrote the manuscript; AS designed the study, recruited the patients, performed the investigations, collected and performed the statistical processing of the data, and wrote the manuscript; TK designed the study; DT designed the study; BS processed the blood samples and performed part of the laboratory investigations; TG processed the blood samples and performed a part of the laboratory investigations; TH prepared the meal recipes and was responsible for the nutritional counseling of the patients; MM-R prepared the meal recipes and was responsible for the nutritional counseling of the patients; CG measured the endothelial dysfunction and oxidative stress markers; KK measured the endothelial dysfunction and oxidative stress markers; JU performed the AGE assessments; and HV performed the AGE assessments. All authors read and revised the manuscript. The authors have no conflicts of interest to disclose.

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