Influence of Postprandial Hyperglycemic Conditions on Arterial Stiffness in Patients With Type 2 Diabetes

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Context: Patients with type 2 diabetes (T2D) are at an increased risk of cardiovascular disease.

Objective: The objective of the study was to determine whether postprandial hyperglycemia affects arterial function in T2D.

Design: A single-center, open-label study of three groups of men were studied: 1) T2D patients with albuminuria (n = 22), 2) T2D patients without albuminuria (n = 24), and 3) nondiabetic controls (n = 25). Patients were randomized to a two-period crossover study schedule, ingesting breakfast, with or without insulin lispro (to induce low or high postprandial glycemia).

Main Outcome Measures: Arterial stiffness was assessed by calculating pulse wave velocity (PWV) and augmentation index using applanation tonometry, and endothelial dysfunction was assessed using peripheral arterial tonometry, 30 minutes before breakfast and up to 240 minutes after breakfast.

Results: At baseline, arterial stiffness was increased in patients. When adjusted for age and body mass index, in a combined group of patients with and without albuminuria, brachial PWV was higher during low (P = .032) and high (P = .038) postprandial glycemia vs controls. These differences were driven by the albuminuria group vs controls during low (P = .014) and high (P = .018) postprandial glycemia. No differences were observed in aortic PWV, augmentation index, or peripheral arterial tonometry ratio between patients and controls. Endothelin-1 and IL-6 were higher, and superoxide dismutase was lower, during postprandial hyperglycemia in T2D patients vs controls.

Conclusions: In patients with T2D and albuminuria, brachial PWV was higher under postprandial hyperglycemic conditions, relative to controls. These data suggest that hyperglycemia induces an increase in stiffness of intermediate-sized arteries. We found no changes in other parts of the arterial bed. (J Clin Endocrinol Metab 101: 1134–1143, 2016)
Patients with type 2 diabetes (T2D) are at an increased risk of cardiovascular disease, which is likely to be mediated by various pathophysiological events including arterial stiffness (1–6). However, it is not known whether postprandial hyperglycemia alters arterial stiffness in these patients. This interaction may be of interest because postprandial hyperglycemia is associated with cardiovascular disease in patients with T2D (7–9).

Therefore, the aim of this study was to determine whether postprandial hyperglycemia affects arterial function (arterial stiffness and endothelial function) in patients with T2D. Because albuminuria is a sign of pronounced endothelial dysfunction and an additional risk factor for cardiovascular disease (10, 11), we included patients with and without albuminuria. In addition to assessing arterial function by measuring pulse waves, we also analyzed the levels of biomarkers of inflammation (serum C-reactive protein [sCRP], IL-6, asymmetric dimethylarginine [ADMA]), endothelial dysfunction (endothelin-1 [ET-1]), soluble vascular cell adhesion molecule-1 [sVCAM-1], soluble intercellular adhesion molecule-1 [sICAM-1]), oxidative stress (superoxide dismutase [SOD]), and glycated proteins (soluble extracellular domain of the human receptor for advanced glycation end products [sRAGE]) in the fasting and postprandial states.

Materials and Methods
This was an open-label study (clinicaltrials.gov identifier NCT01159938; https://www.clinicaltrials.gov/ct2/show/NCT01159938) conducted at a single site in Finland. A two-period crossover approach (from high to low postprandial glucose or vice versa) was used for T2D patients. Nondiabetic controls were studied for one period.

Subject eligibility
The study was performed using the following three subject groups: 1) T2D patients with albuminuria; 2) T2D patients without albuminuria (normal urinary albumin excretion rate [UAER], <20 µg/min or <30 mg per 24 h); in two patients in this group, serum creatinine levels were elevated (129 µmol/L and 164 µmol/L); and 3) a nondiabetic control arm.

Eligible subjects were male, aged 45–70 years, had not smoked in the 12 hours immediately before each study visit, and had not experienced a cardiovascular event such as coronary heart disease, stroke, or peripheral vascular disease. The T2D patients were diagnosed according to the American Diabetes Association classification (12) and had to have been on insulin therapy for 6 months or longer.

All subjects gave informed written consent before experiencing any study-related procedures. The study protocol was approved by the local ethics committee, and the study was conducted in accordance with the ethical principles in the Declaration of Helsinki and followed good clinical practice guidelines provided by the International Conference on Harmonisation.

Variables
In all three subject groups, the following indicators of arterial function were measured at the following time points: carotid intima-media thickness (CIMT) at screening; pulse wave velocity (PWV) and augmentation index (AIx) at baseline (fasting), ie, 30 minutes before breakfast, and 60, 120, 180, and 240 minutes after breakfast; peripheral arterial tonometry (PAT) ratio 30 minutes before breakfast and 120 and 240 minutes after breakfast; and blood glucose levels 30 minutes before breakfast and 50, 110, 170, and 230 minutes after breakfast. Biomarkers were measured 30 minutes before breakfast and 60, 120, 180, and 240 minutes after breakfast.

Study procedures
Insulin lispro (Humalog; Eli Lilly & Co), ie, a rapid-acting insulin, and a standard basal insulin (glargine, detemir, or NPH, whichever was part of the patient’s usual treatment regimen) were used during the study but only by the diabetes patients. Insulin dosing was based on the patient’s normal morning insulin dose and the energy content of the patient’s normal breakfast. No medication except insulin was taken in the morning of the clinical visits.

The study was performed over three single-day clinical visits. After screening during visit 1, the diabetes patients were randomized (stratified by the presence of albuminuria vs normal UAER) to the following treatment schedules: insulin lispro, injected sc and preprandially (before a standard breakfast [500 kcal, 60% carbohydrate, 20% fat, 20% protein]) during visit 2 (low postprandial glucose condition), and no rapid-acting insulin during visit 3 (high postprandial glucose condition) or vice versa. The randomization was done blinded and separately for each patient by a telephone call to an automatized tool.

If the standard breakfast was larger than the patient normally eats, the insulin lispro dose was increased accordingly by 20%–50% at the discretion of the investigator. Visit 2 occurred 60 days or sooner after visit 1, and visit 3 occurred 1 week or longer but 8 weeks or sooner after visit 2.

During visits 2 and 3, assessments of arterial function (ie, for PWV, AIx, and PAT ratio) were performed only if fasting blood glucose was between 5 and 9 mmol/L. If it was outside this range, the investigator adjusted the patient’s basal insulin regimen as necessary and rescheduled the visit.

All controls underwent a standard oral glucose tolerance test during screening. Otherwise the same measurements as for the diabetes patients were taken from controls before and after receiving the standard breakfast but only during one clinical visit (ie, there was no second crossover visit).

Subject characteristics
In total, 65 patients with T2D were screened for inclusion in the study (29 had albuminuria, 36 had normal UAER), and 33 subjects were screened for inclusion in the control group. Due to screening failures, 46 T2D patients were included in the study, 22 of whom had albuminuria (17 had microalbuminuria, five had macroalbuminuria), whereas the other 24 patients had normal UAER.

With the exception of the mornings of clinical visits, subjects were allowed to use concomitant medications. All patients with
albuminuria (n = 24), 96% of patients without albuminuria (n = 23), and 20% of controls (n = 5) were on antihypertensive medications. (Antihypertensive medications used by patients with albuminuria were angiotensin converting enzyme inhibitors [n = 11], angiotensin receptor blockers [ARBs; n = 7], β-blockers [n = 6], calcium channel blockers [n = 16], and diuretics [n = 4]. Patients without albuminuria used angiotensin converting enzyme inhibitors [n = 9], ARBs [n = 7], β-blockers [n = 9], calcium channel blockers [n = 9], and diuretics [n = 5]. Non-diabetic controls were on ARBs [n = 3], calcium channel blocker [n = 1], and diuretics [n = 1]. No patients without albuminuria had albuminuria before starting treatment with ARBs.) Cholesterol-lowering medications (ie, statins) were used by 73% of patients with albuminuria (n = 16), 92% of patients without albuminuria (n = 22), and 8% of controls (n = 2). Further subject characteristics are presented in Table 1.

### Measurement of arterial function

Using applanation tonometry (13), the pressure waveforms were recorded in the radial artery of the right arm with a high-fidelity micromanometer (SPC-301; Millar Instruments). A model of the central pressure waveform was synthesized with SphygmoCor software (SphygmoCor; Atcor Medical), and using a validated generalized transfer function, as previously described (14). The heart rate-adjusted AIx, a commonly used parameter to estimate arterial stiffness, was then calculated by the software.

To measure the arterial stiffness in the large (aortic) and intermediate-sized (brachial) arteries, carotid-femoral (aortic) and carotid-radial (brachial) PWV pressure waveforms were recorded sequentially at the carotid, femoral, and radial arteries. With a simultaneous electrocardiographic recording of the R wave as a reference frame, the software calculated the PWV (13).

### Table 1. Subject Characteristics at Screening, Insulin Dosages During the Study, and Vascular Parameters During Fasting (30 Minutes Preprandially)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Nondiabetic Controls (N = 25)</th>
<th>Patients With Albuminuria (N = 22)</th>
<th>Patients With Normal UAER (N = 24)</th>
<th>All Patients (N = 46)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, y</strong></td>
<td>58.8 (6.6)</td>
<td>61.2 (4.9)</td>
<td>63.7 (5.2)a</td>
<td>62.5 (5.2)a</td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>27.3 (3.3)</td>
<td>33.8 (5.3)a</td>
<td>32.2 (6.2)a</td>
<td>33.0 (5.8)a</td>
</tr>
<tr>
<td><strong>CIMT, left, µm</strong></td>
<td>666.1 (126.6)</td>
<td>715.5 (119.4)</td>
<td>761.1 (142.0)a</td>
<td>739.3 (132.2)a</td>
</tr>
<tr>
<td><strong>CIMT, right, µm</strong></td>
<td>680.8 (91.0)</td>
<td>709.2 (106.3)</td>
<td>735.4 (127.4)</td>
<td>722.8 (117.3)</td>
</tr>
<tr>
<td><strong>Disease duration, y</strong></td>
<td>N/A</td>
<td>12.1 (3.7)</td>
<td>16.2 (8.2)</td>
<td>14.2 (6.7)</td>
</tr>
<tr>
<td><strong>UAER, µg/min</strong></td>
<td>N/D</td>
<td>273.3 (476.7)</td>
<td>4.8 (4.5)</td>
<td>133.2 (352.8)</td>
</tr>
<tr>
<td><strong>HbA1c, %</strong></td>
<td>5.4 (0.3)</td>
<td>7.8 (1.3)a</td>
<td>7.4 (1.1)a</td>
<td>7.6 (1.2)a</td>
</tr>
<tr>
<td><strong>HbA1c, mmol/mol</strong></td>
<td>36 (3.3)</td>
<td>62 (14.2)a</td>
<td>57 (12.0)a</td>
<td>60 (13.1)a</td>
</tr>
<tr>
<td><strong>Total cholesterol, mmol/L</strong></td>
<td>5.3 (0.8)</td>
<td>4.1 (0.7)a</td>
<td>4.3 (0.9)a</td>
<td>4.2 (0.8)a</td>
</tr>
<tr>
<td><strong>HDL-cholesterol, mmol/L</strong></td>
<td>1.4 (0.3)</td>
<td>1.3 (0.3)</td>
<td>1.4 (0.5)</td>
<td>1.3 (0.4)</td>
</tr>
<tr>
<td><strong>LDL-cholesterol, mmol/L</strong></td>
<td>3.6 (0.7)</td>
<td>2.4 (0.7)a</td>
<td>2.5 (0.7)a</td>
<td>2.4 (0.7)a</td>
</tr>
<tr>
<td><strong>Triglycerides, mmol/L</strong></td>
<td>1.3 (0.5)</td>
<td>1.5 (0.8)</td>
<td>1.6 (1.5)</td>
<td>1.6 (1.2)</td>
</tr>
<tr>
<td><strong>White race</strong></td>
<td>25 (100)</td>
<td>22 (100)</td>
<td>24 (100)</td>
<td>46 (100)</td>
</tr>
<tr>
<td><strong>Smokers</strong></td>
<td>1 (4)</td>
<td>4 (18)</td>
<td>0</td>
<td>4 (9)</td>
</tr>
<tr>
<td><strong>Insulin dosages, IU, mean (±SD)</strong></td>
<td>N/A</td>
<td>13.4 (13.71)</td>
<td>11.2 (6.14)</td>
<td>12.2 (10.40)</td>
</tr>
<tr>
<td><strong>Insulin lispro</strong></td>
<td>N/A</td>
<td>80.1 (53.25)</td>
<td>60.8 (49.49)</td>
<td>69.8 (51.61)</td>
</tr>
<tr>
<td><strong>Basal insulin, high PP glucose</strong></td>
<td>N/A</td>
<td>77.7 (53.44)</td>
<td>61.3 (50.05)</td>
<td>69.1 (51.78)</td>
</tr>
<tr>
<td><strong>Basal insulin, low PP glucose</strong></td>
<td>N/A</td>
<td>77.7 (53.44)</td>
<td>61.3 (50.05)</td>
<td>69.1 (51.78)</td>
</tr>
<tr>
<td><strong>Aortic PWV during fasting, before, m/sec, mean (±SD)</strong></td>
<td>8.6 (2.12)</td>
<td>10.9 (3.52)a</td>
<td>10.7 (2.22)a</td>
<td>10.8 (2.88)a</td>
</tr>
<tr>
<td><strong>High PP glucose</strong></td>
<td>11.0 (4.01)a</td>
<td>10.6 (4.02)</td>
<td>10.8 (3.98)a</td>
<td></td>
</tr>
<tr>
<td><strong>Low PP glucose</strong></td>
<td>7.5 (1.21)</td>
<td>7.8 (1.30)</td>
<td>7.2 (1.88)</td>
<td>7.5 (1.64)</td>
</tr>
<tr>
<td><strong>Brachial PWV, during fasting, before, m/sec, mean (±SD)</strong></td>
<td>7.0 (1.46)</td>
<td>7.7 (1.46)</td>
<td>7.8 (1.45)</td>
<td></td>
</tr>
<tr>
<td><strong>High PP glucose</strong></td>
<td>17.2 (10.81)</td>
<td>22.6 (6.47)a</td>
<td>21.0 (6.26)</td>
<td>21.7 (6.34)a</td>
</tr>
<tr>
<td><strong>Low PP glucose</strong></td>
<td>20.7 (7.45)</td>
<td>21.6 (9.03)</td>
<td>21.2 (8.24)</td>
<td></td>
</tr>
<tr>
<td><strong>PAT ratio during fasting, before, %, mean (±SD)</strong></td>
<td>2.1 (0.53)</td>
<td>1.9 (0.52)</td>
<td>1.7 (0.33)a</td>
<td>1.8 (0.44)a</td>
</tr>
<tr>
<td><strong>High PP glucose</strong></td>
<td>2.1 (0.45)</td>
<td>2.1 (0.64)</td>
<td>2.0 (0.56)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; HbA1c, hemoglobin A1c; LDL, low-density lipoprotein; N, total number of subjects with analyzed data; n, number of subjects; N/A, not applicable; N/D, not determined; PP, postprandial.

a Values ranged between P < .001 and P = .037 vs nondiabetic controls, based on an ANOVA.

b Not analyzed for statistical significance.

c Not adjusted for age or BMI.
PAT, also known as pulse amplitude tonometry, is a non-invasive technique used to evaluate endothelial function by measuring digital pulse amplitude (15, 16). This was achieved using a biosensor (Endo-PAT2000; Itamar Medical) placed on the tip of each index finger.

The inflation pressure of the digital device was electronically set at 10 mm Hg below diastolic blood pressure (BP). After equilibration, baseline pulse amplitude was measured from the fingertips for 5 minutes. Arterial flow was interrupted for an additional 5 minutes by inflating the cuff at whichever occlusion pressure was higher: 60 mm Hg above systolic BP or 200 mm Hg. The cuff was then abruptly deflated to induce reactive hyperemia and the PAT signal from both hands was recorded for another 5 minutes. The PAT ratio was calculated as follows: first, the ratio of the postdeflation pulse amplitude 90–150 sec to the average baseline pulse amplitude was calculated, and this was divided by the corresponding ratio from the control finger, and the result was multiplied by a baseline correction factor to adjust for basal vascular tone.

CIMT was measured using a novel system (ArtLab) based on high-resolution echotracking technology (Wall Track system), using a 128 radiofrequency line multiaarray (17). The rough radiofrequency data were analyzed online, and the 6-second cine loops were stored without compression (120 Mb) for the offline analysis. CIMT was measured with a 21-μm resolution.

**Biomarkers**

Biomarkers were analyzed in the subjects’ blood samples using the following commercially available ELISAs, in accordance with each manufacturer’s instructions: endogenous ADMA (DLD Diagnostika GmbH), SOD (Cayman Chemical Co), human ET-1, sVCM-1, sICAM-1, and sRAGE (R&D Systems Inc). Chemiluminescence was detected using a Varioskan Flash analyzer (Thermo Electron Corp/Thermo Fischer Scientific Inc). IL-6 was analyzed using a chemiluminescent immunometric assay (IMMULITE 2000 IL-6; (Siemens) and IMMULITE 2000 Systems analyzer (Siemens), and sCRP was analyzed using a photometric immunoassay and a modular analyzer (Roche).

**Statistical analyses**

Continuous baseline (fasted) and demographic characteristics were compared between T2D patients and controls using an ANOVA model, with the group as factor, but unadjusted for age or body mass index (BMI).

The PWV was summarized by subject group, glycemic condition (ie, low or high), and time point (every 60 min after breakfast, up to 240 min). Postprandial PWV was compared in low glycemic vs high glycemic conditions in T2D patients at each time point, using a mixed-effects analysis of covariance (ANCOVA) model. (The model included age and BMI as covariates, the group, the glycemic condition, and the group-by-condition interaction as the fixed effects and the subject as the random effect.) The least squares (LS) means were reported for each condition and the group-by-condition combinations. The type III tests for the main effects of the condition and the group by condition were reported, and the significance was assessed at the two-sided 5% level.

The area under the curve (AUC) (AUC$_{0–240}$ minutes) of PWV was calculated for each subject group and was adjusted using LS means at baseline (fasting). The LS means were obtained from an ANCOVA model (the model included age and BMI as covariates and the group as factor), and the baseline adjustments were made using LS means from the control group.

The PWV and change from baseline (fasting) in PWV were compared between the T2D groups and the controls at each time point, and baseline-adjusted AUC$_{0–240}$ minutes of PWV was compared between the subject groups, using ANCOVA (the model included age and BMI as covariates and the group as factor). Separate analyses were performed for the low and high glycemic conditions. For PWV, and for change from baseline in PWV, sensitivity analyses without adjustment for age and BMI were also carried out.

The same ANCOVA model (the model included age and BMI as covariates and the group as factor) was used to compare the levels of biomarkers between the T2D patient groups and the controls, separately for the low and high glycemic conditions. Levels of biomarkers were measured at 60-minute intervals and are shown as AUC$_{0–240}$ minutes.

Whereas the T2D patients were studied during two periods (ie, under high and low postprandial glucose conditions), the controls were studied for only one period. However, for all results based on ANCOVA, the model-predicted mean data for the control group were different under the two glycemic conditions due to the effect of adjusting for covariates. For instance, different model-predicted mean levels of biomarkers are shown for the control group, depending on whether the comparison was with T2D patients under low or high postprandial glucose conditions (Table 2).

Between 69 and 73 subjects were planned to be included in the study, depending on the number of diabetes patients with albuminuria included. With 44 evaluable diabetes patients, the study would have at least 90% power to show a mean PWV change of 0.7 m/sec between low postprandial glycemia and high postprandial glycemia at the two-sided 5% level. Sample size was estimated assuming a PWV total SD of 1.2 m/sec, and an intra-subject SD of 1.0 m/sec.

**Results**

**Insulin dosages**

Rapid-acting insulin lispro was injected before breakfast but only during the visit when lower postprandial glucose levels were required. The mean doses of insulin lispro and basal insulin that were injected in the two patient groups are shown in Table 1.

**Blood glucose levels**

As shown in Figure 1, in the T2D patients, fasting blood glucose values were within the required window (5–9 mmol/L) for the arterial function to be assessed. In the two diabetes patient groups combined, the LS mean ± SE glucose level was 6.8 ± 0.2 mmol/L before breakfast (Figure 1A). In these patients, the LS mean ± SE postprandial glucose levels had increased when measured 50 minutes after breakfast, and the change was larger under high-glucose conditions (5.3 ± 0.3 mmol/L) compared with low-glucose conditions (3.3 ± 0.3 mmol/L, P < .001; Figure 1A). There were no differences in postprandial glucose...
levels between patients with or without albuminuria under high (Figure 1B) or low (Figure 1C) glucose conditions.

Measurement of arterial function

As shown in Table 1, at screening, CIMT tended to be greater in diabetes patients than in nondiabetic controls; this difference was statistically significant on the left side.

In addition, fasting aortic PWV and AIX were elevated in diabetes patients, particularly in those with albuminuria, compared with controls (P < .05) (Table 1). However, the patients were slightly older and had higher BMI than the controls. When adjusted for age and BMI, no statistically significant differences were observed in aortic PWV (see Figure 3), AIX (data not shown), or PAT ratio (data not shown) during fasting in patients vs controls. Hereafter we report PWV, AIX, and PAT ratio adjusted for age and BMI.

PWV, adjusted for age and BMI

There were no significant differences in brachial PWV between the three subject groups in the fasting state (Figure 2, A and B). However, postprandial (60, 180, 240 min) brachial PWV was faster (P = .003–.046) in diabetes patients with albuminuria than in controls, both during high (Figure 2A) and low (Figure 2B) postprandial glycemia. This increase in PWV in the albuminuria group was not statistically significantly different from the smaller changes in PWV observed in the patients without albuminuria (Figure 2, A and B). In addition, in the two groups of diabetes patients combined, there were no differences in brachial PWV under low vs high postprandial glucose conditions (Figure 2C).

Regarding PWV in the aorta, no differences were observed between the three subject groups in response to a meal (Figure 3, A and B) or under low vs high postprandial glucose conditions in the two groups of pooled diabetes patients combined (Figure 3C).

When adjusted for baseline using LS means from the controls (ie, the LS means during fasting shown in Figure 2, A and B), the AUC0–240 minutes of brachial PWV was higher in the T2D patients with albuminuria than in the controls, both during high (+3.67 mh/sec; P = .018) and low (+4.50 mh/sec; P = .014) postprandial glycemia. In T2D patients without albuminuria, the AUC0–240 minutes of brachial PWV was not significantly higher than in controls, both during high (+2.01 mh/sec; P = .179) and low (+2.62 mh/sec; P = .154) postprandial glycemia. When both diabetic groups were combined, the AUC0–240 minutes of brachial PWV was higher than in controls, both during high (+2.84 mh/sec; P = .038) and low (+3.56 mh/sec; P = .032) postprandial glycemia. No other significant differences were observed in the AUC of PWV between the subject groups.

AIX and PAT, adjusted for age and BMI

In diabetes patients with or without albuminuria, there were no differences in AIX or PAT ratio (both measured up to 240 min after breakfast) under low vs high postprandial glucose conditions (data not shown). The AIX and PAT ratio were also similar in the diabetes patients with or
without albuminuria, relative to controls, in both post-prandial states (data not shown).

**BP and heart rate**

In the fasting state, systolic/diastolic BP and heart rate were similar in controls (LS mean ± SE, 145 ± 4/79 ± 2 mm Hg; mean ± SD, 59 ± 9 bpm), patients with albuminuria (147 ± 4/81 ± 2 mm Hg; 70 ± 10 bpm), and patients without albuminuria (145 ± 4/79 ± 2 mm Hg; 67 ± 10 bpm). After breakfast, BP and heart rate remained unchanged in each subject group.

**Biomarkers**

In general, baseline levels of various biomarkers were not significantly different between the T2D patients and the controls. However, in T2D patients vs controls, the levels of IL-6 and ET-1 were consistently higher and SOD was consistently lower at postprandial time points under high- and low-glucose conditions, particularly in patients with albuminuria, as shown as AUC$_{0-240}$ minutes in Table 2. No consistent differences were observed in the other biomarkers (sCRP, sICAM-1, sVCAM-1, sRAGE, and ADMA).

**Discussion**

Our study demonstrates that patients with T2D and albuminuria experience higher brachial PWV and thus greater arterial stiffness, relative to nondiabetic controls, under post-prandial hyperglycemic conditions. This adverse reaction could be the consequence of pathological preconditioning of the vasculature due to the long-term diabetic state as well as being a consequence of postprandial conditions. Increased stiffness of these muscular, smaller arteries may have prognostic value for coronary calcification and cardiovascular outcome (18, 19). Because the stiffening appeared to be more pronounced in patients with albuminuria, it could represent another indicator of diabetic complications, endothelial dysfunction, and microvascular deterioration in T2D patients with albuminuria.

Our present results extend our earlier findings. Similar to the current data, in our previous study in patients with type 1 diabetes (T1D), acute hyperglycemia did not influence aortic PWV but did appear to induce brachial PWV that was markedly higher in patients with T2D and albuminuria vs...
nondiabetic controls (20). Therefore, we conclude that T1D and T2D are both associated with pathological changes in the smaller arteries, resulting in greater brachial arterial stiffness, relative to nondiabetic controls, under hyperglycemic conditions. However, the magnitude of the effect on brachial arterial stiffness appeared to be larger in T1D than in T2D patients. Also, unlike in the present study, the T1D patients had a statistically significant rise in AIx. There are several possible explanations for the differences between the two studies. First, during fasting, brachial PWV tended to be lower in the T1D patients but higher in the T2D patients vs controls. The T2D patients were older, with greater vascular aging, and thus probably had more advanced arterial stiffness than the T1D patients. Second, the meal-induced hyperglycemia in the current study (up to LS mean 12.4 mmol/L) was much lower than the elevation of circulating glucose (up to mean 17.9 mmol/L) achieved by glucose infusion in the previous study (20). Third, the route of glucose administration was different, i.e., iv infusion in the previous study vs the more natural route of oral ingestion of a meal in the current one. These differences in the patient populations and study conditions may explain the smaller effect of acute hyperglycemia on PWV and the lack of effect on AIx in T2D patients in the current study relative to the previous one in patients with T1D.

We assume that hyperglycemia is the main contributor to greater brachial stiffness in the T2D patients with albuminuria vs controls, similar to the finding in patients with T1D (20), although we cannot exclude a contribution from other nutritional components. For instance, low high-density lipoprotein (HDL)-cholesterol levels are associated with increased arterial stiffness (21). Thus, our findings may be partly related to postprandial changes in plasma lipoproteins.

We observed an increase in the inflammatory biomarker IL-6 in patients with T2D with albuminuria compared with the controls during the postprandial phase. Similarly, SOD was lower during postprandial hyperglycemia in the patients compared with the control subjects, indicating higher concentrations of reactive oxygen radicals. These results are in line with earlier findings in nondiabetic subjects as well as in patients with T1D.
showing in hyperglycemic clamp studies an inflammatory response to acute hyperglycemia (22, 23). Notably, in the current study, hyperglycemia was induced by a high carbohydrate meal instead of an iv glucose infusion.

During fasting, before adjustment for age and BMI, indices of arterial stiffness (AIx and aortic PWV) and endothelial dysfunction (PAT ratio) were significantly higher in patients with T2D than in controls. However, when the results were adjusted for age and BMI, which are known to increase arterial stiffness in diabetes, the differences during fasting were no longer significant. The lack of significant differences may be explained by the frequent use of renin-angiotensin blockers and statins in the T2D groups, which regulate BP and cholesterol. Hypertension (6) and low HDL-cholesterol levels (21) are both associated with increased arterial stiffness. Our data suggest that rigorous control of BP and cholesterol could mitigate the development of arterial stiffness.

At screening, CIMT was moderately greater in T2D patients vs controls. This is in line with numerous similar observations (24, 25). Interestingly, recent research suggests that greater CIMT is more likely to be a consequence of arterial stiffening than of chronic hyperglycemia (26).

Our study was limited by patients and controls not being completely matched for age and BMI, which were lower in the controls. When the data were analyzed, we were able to make appropriate adjustments to control for the differences in BMI and age. However, the lack of matching along with the aforementioned use of statins and antihypertensive drugs may have resulted in less arterial stiffness. These limitations may also explain the lack of changes in most biomarkers.

On the other hand, our study was thoroughly designed in several ways to gain reliable results. First, for the diabetes patients, a two-period crossover design was chosen to reduce variability between subjects and to limit bias, which could have been introduced by the open-label characteristic of the study. Second, we chose to study only men, thus avoiding the possibility that hormonal variations during the menstrual cycle might affect arterial stiffness. Thus, these observations are valid only for men. Third, the study was adequately powered to detect changes in PWV with excursions based on previous glucose clamp data (20, 27, 28). Fourth, nondiabetic controls were included as references to determine how close to

Figure 3. Comparisons of LS mean (±SE) aortic PWV, adjusted for age and BMI, between subject groups, in high postprandial glucose conditions (A); between subject groups in low postprandial glucose conditions (B); and in diabetes patients during high vs low postprandial glucose conditions (C). Whereas the T2D patients were studied during two periods (ie, under high and low postprandial glucose conditions), the nondiabetic controls were studied for only one period. The model-predicted mean data per time point for the control group were different between the two glycemic conditions due to the effect of adjusting for covariates.
normal vascular parameters were in T2D patients in the presence of low postprandial glycemia. Finally, by using albuminuria as an early cardiovascular disease risk marker, the vasculature would probably be more sensitive to react to the sophisticated tests used in this study. Thus, the probability of finding differences in early markers between the controls, patients with albuminuria, and patients with normal albumin excretion should have increased.

In summary, brachial PWV was higher in T2D patients with albuminuria during postprandial hyperglycemia vs nondiabetic controls. These data suggest that an elevation of postprandial PWV may serve as a marker for stiffness of intermediate-sized arteries in patients with T2D. We found no changes in other parts of the arterial bed.

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Disclosure Summary: I.P. and B.S.-H. are employees and V.K. is a contractor of Eli Lilly & Co, the manufacturer of Humalog. P.-H.G. has received lecture honoraria from Boehringer Ingelheim, Eli Lilly, Genzyme, Medscape, MSD, Novartis, and Novo-Nordisk and is on the advisory boards of Boehringer Ingelheim, Eli Lilly, Novartis, and Abbot/Abb/ Vie. P.-H.G. has received grants from Eli Lilly (for this study) and from Roche. The other authors have nothing to disclose.

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