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1 **ELEVATION IN BLOOD FLOW AND SHEAR RATE PREVENTS**
2 **HYPERGLYCEMIA-INDUCED ENDOTHELIAL DYSFUNCTION IN HEALTHY AND**
3 **TYPE 2 DIABETIC SUBJECTS**

4
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13
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22 **ABSTRACT**

23 Hyperglycemia, commonly present after a meal, causes transient impairment in endothelial
24 function. We examined whether increases in blood flow (BF) protect against the hyperglycemia-
25 mediated decrease in endothelial function in healthy subjects and patients with type 2 diabetes
26 mellitus (T2DM). Ten healthy subjects and 10 age- and sex-matched T2DM patients underwent
27 simultaneous bilateral assessment of brachial artery endothelial function by means of flow-
28 mediated dilation (FMD), using high-resolution echo-Doppler. FMD was examined before and
29 60, 120 and 150 minutes after a 75-gr oral glucose challenge. We unilaterally manipulated BF by
30 heating one arm between minute 30 and 60. Oral glucose administration caused a statistically
31 significant, transient increase in blood glucose in both groups ($P<0.001$). Forearm skin
32 temperature, brachial artery BF and shear rate significantly increased in the heated arm
33 ($P<0.001$), and to a greater extent compared to the non-heated arm in both groups (interaction-
34 effect, $P<0.001$). The glucose load caused a transient decrease in FMD% ($P<0.05$), whilst
35 heating significantly prevented the decline (interaction-effect: $P<0.01$). Also when correcting for
36 changes in diameter and shear rate, we found that the hyperglycemia-induced decrease in FMD
37 can be prevented by local heating ($P<0.05$). These effects on FMD were observed in both groups.
38 Our data indicate that non-metabolically driven elevation in BF and shear rate can similarly
39 prevent the hyperglycemia-induced decline in conduit artery endothelial function in healthy
40 volunteers and in patients with type 2 diabetes. Additional research is warranted to confirm that
41 other interventions increasing BF and shear rate equally protect the endothelium when
42 challenged by hyperglycemia.

43 **KEYWORDS:** cardiovascular risk; flow mediated dilation; hyperglycemia; endothelial function;
44 shear rate; blood flow

45 INTRODUCTION

46 Type 2 diabetes mellitus (T2DM) affects approximately 200 million people worldwide (46).
47 Whilst the inability to maintain appropriate glucose levels plays a central role in the etiology of
48 T2DM, mortality and morbidity in T2DM is largely related to the presence of cardiovascular
49 diseases and vascular complications (9, 10). In developing cardiovascular complications, the
50 presence of endothelial dysfunction plays a major role (24, 25). Various stimuli are identified
51 that potentially alter endothelial (dys)function (23, 28). For example, increased blood glucose
52 levels or hyperglycemia, which is frequently present in T2DM despite optimal pharmacological
53 treatment (8), induces a transient impairment in endothelial function (1, 16, 17, 33, 43, 47). The
54 detrimental impact of hyperglycemia on endothelial function has been found in several studies in
55 healthy adults (1, 33, 43, 47) as well as in patients with T2DM (16, 20). Given the frequent
56 exposure to hyperglycemia, these observations highlight the importance for strategies to prevent
57 or attenuate the impact of hyperglycemia on endothelial function.

58
59 Studies in animals (22, 44) and humans (11) suggest that elevation in shear rate, i.e. the frictional
60 force of blood upon the arterial wall, leads to improvements in vascular function and structure. A
61 recent series of studies in humans demonstrated that elevation in shear rate, induced by heating
62 of a forearm, can acutely (42) and chronically (27) improve vascular function. Moreover, we
63 recently found that increases in shear rate (via heating of the forearm) prevents the immediate
64 decline in brachial artery endothelial function during activation of the sympathetic nervous
65 system (39). In line with these recent observations, elevation in shear rate may prevent the
66 hyperglycemia-induced decline in endothelial function in healthy volunteers and in T2DM.

67

68 The primary objective of this study, therefore, was to examine whether increases in shear rate
69 (through heating of the skin) protect against the transient hyperglycemia-mediated decrease in
70 endothelial function in healthy subjects and patients with T2DM. For this purpose, we bilaterally
71 examined brachial artery flow mediated dilation (FMD) as a measure for endothelial function
72 before and after a 75-gr glucose load, which is demonstrated to cause marked elevations in blood
73 glucose and a transient decrease in endothelial function (1, 16, 20, 33, 43, 47). In addition, we
74 unilaterally manipulated shear rate in the brachial artery by heating the arm for 30-minutes, to
75 examine the hypothesis that (non-metabolically driven) increases in shear rate prevent the
76 decline in endothelial function.

77

78

79 **METHODS**

80 **Participants**

81 Ten male subjects with T2DM (age 63 ± 6 years) and 10 age-matched healthy male controls (57
82 ± 9 years) were included in our study. Individuals were excluded if they smoked, had past or
83 present cardiovascular disease, hypercholesterolemia or hypertension (>160 mmHg systolic
84 and/or >90 mmHg diastolic pressure). The subjects in the T2DM group had to be diagnosed with
85 type 2 diabetes mellitus at least two years ago. Subjects in this group were excluded if they had
86 vascular complications due to type 2 diabetes mellitus (e.g. diabetic foot ulcer). All participants
87 provided written informed consent before participation. The study procedures were approved by
88 the medical ethics committee of the region Arnhem-Nijmegen, the Netherlands and adhered to
89 the Declaration of Helsinki (2000). This study is registered at the Netherlands Trial Registry as
90 NTR4631.

91

92 Experimental Design

93 In this study, both groups reported to our laboratory once for assessment of glucose homeostasis
94 and brachial artery endothelial function. First, we performed simultaneous, bilateral assessment
95 of brachial artery FMD, immediately followed by the ingestion of 75-gr of glucose dissolved in
96 200 mL water. Thirty minutes after ingestion, we unilaterally heated one forearm for 30 minutes.
97 Heating of the arm was randomized between subjects. Subsequently, bilateral simultaneous
98 assessment of brachial artery FMD was repeated at 60, 120 and 150 minutes after ingestion of
99 the glucose load.

100

101 Experimental Measures

102 Ultrasound assessments were performed in a quiet temperature-controlled room (22°C).
103 Measurements in a single arm were always performed by the same sonographer for each
104 individual subject. All measurements were performed following a ≥ 6 hour fast, ≥ 18 hour
105 abstinence from coffee (and other products containing caffeine, including energy drinks),
106 alcohol, vitamin supplements, products with high levels of vitamin C, polyphenol-rich foods, and
107 at least 24 hours after strenuous physical activity. All glucose lowering and vasoactive
108 medication was also withheld on the morning of the measurement (40). We performed all tests
109 between 8 AM and 4 PM to control for variation in FMD between subjects (4, 14, 15, 38).

110

111 *Brachial artery FMD.* Measurements were performed by 2 well-experienced sonographers
112 following a resting period of at least 20 minutes in the supine position. We simultaneously
113 measured FMD in the right and left brachial arteries according to recent guidelines for

114 assessment of FMD as previously described by Thijssen *et al* (40). For this purpose, both arms
115 were extended and positioned at an angle of $\sim 80^\circ$ from the torso. A rapid inflation and deflation
116 pneumatic cuff (D.E. Hokanson, Bellevue, WA) was positioned on the forearm, immediately
117 distal to the olecranon process to provide a stimulus to forearm ischemia. A 10-MHz multi-
118 frequency linear array handheld probe, attached to a high-resolution ultrasound machine (T3000;
119 Terason, Burlington, MA) was then used to image the brachial artery in the distal one third of the
120 upper arm. When an optimal image was obtained, the probe was held stable and the ultrasound
121 parameters were set to optimize the longitudinal, B-mode image of the lumen-arterial wall
122 interface. Settings were identical between all assessments of the FMD. Continuous Doppler
123 velocity assessments were also obtained using the ultrasound and were collected using the lowest
124 possible insonation angle (always $<60^\circ$). Baseline images were recorded for 1 minute after which
125 the forearm cuff was inflated (>200 mmHg) for 5-minutes. Diameter and flow recordings
126 resumed 30 sec prior to cuff deflation and continued for 3 minutes thereafter, in accordance with
127 recent technical specifications (48).

128

129 *Forearm skin temperature.* During the complete protocol, forearm skin temperature of both
130 forearms was measured using iButtons® (Maxim Integrated, San Jose, CA). These data were
131 transferred to a computer and analyzed afterwards. Furthermore, forearm skin temperature was
132 also measured manually using a standard auricle thermometer before every FMD and every five
133 minutes during the heating process so that the researcher had a direct indication of the heating
134 progress.

135

136 *Venous blood.* In all individuals, a routine hematochemical check was performed by standard
137 methods before testing. A venous blood sample was taken at baseline for assessment of fasting
138 blood lipids, glucose and insulin levels. The subjects' degree of insulin resistance was assessed
139 by calculating the HOMA-IR index from fasting glucose and insulin levels. Furthermore, venous
140 blood was repeatedly taken to assess blood glucose levels at 60, 120 and 150 minutes after
141 glucose ingestion.

142

143 **Brachial Artery Diameter and Blood Flow Analysis**

144 Analysis of brachial artery diameter was performed using custom-designed edge-detection and
145 wall-tracking software, which is independent of investigator bias. Previous papers contain
146 detailed descriptions of our analysis approach (48). From synchronized diameter and velocity
147 data, blood flow (the product of arterial lumen cross-sectional area and Doppler velocity) were
148 calculated at 30 Hz. Shear rate (an estimate of shear stress without viscosity) was calculated as 4
149 times mean blood velocity/vessel diameter. Reproducibility of diameter measurements using this
150 semi-automated software is significantly better than manual methods and reduces observer error
151 significantly (48).

152

153 Baseline diameter and shear rate were calculated as the mean of data acquired across the 1
154 minute preceding the cuff inflation period. Following cuff deflation, peak diameter following
155 cuff deflation was automatically detected according to an algorithm which identified the
156 maximum bracket of data subsequent to performance of a moving window smoothing function.
157 This smoothing routine calculates the median value from 100 consecutive samples, before the
158 window shifts to the next bracket of data which shares 20% overlap with the preceding bracket.

159 The maximum value of all the calculated median values is then automatically detected and
160 chosen to represent the peak of the diameter curve.

161
162 FMD was calculated as the percentage rise of this peak diameter from the preceding baseline
163 diameter. Calculation of FMD was therefore observer-independent and based on standardized
164 algorithms applied to data which had undergone automated edge-detection and wall-tracking.
165 The post-deflation shear rate data, derived from simultaneously acquired velocity and diameter
166 measures at 30 Hz, was used to calculate the area under the shear rate curve (SR_{AUC}) for data up
167 to the point of maximal post-deflation diameter (FMD) for each individual. In addition, we
168 calculated the peak blood flow across a 10-second period after cuff release. Reproducibility of
169 the brachial artery FMD using this semi-automated software possesses a CV of 6.7-10.5%.

170

171 **Statistical analysis**

172 Statistical analyses were performed using SPSS 21.0 software (SPSS, Chicago, IL). Descriptive
173 statistics are presented as means and standard deviation (SD). All data are reported as LSmeans
174 (95%CI), unless reported otherwise and was considered statistically significant at $P < 0.05$.
175 Baseline differences between both arms were examined using a paired Student's *t*-test. A two-
176 way repeated measures ANOVA was used to assess difference in blood glucose levels after
177 glucose ingestion. To examine the impact of hyperglycemia and local heating on outcome
178 parameters between both groups, we performed a Linear Mixed Model. This model assessed
179 whether the baseline-adjusted changes in FMD over time (repeated, within-subject variable
180 'time': 0 vs 60 vs 120 vs 150 minutes) were altered by local heating of a forearm (repeated,
181 within-subject variable 'arm': heated vs non-heated), and whether such changes differ between

182 groups (between-subject factor ‘group’: controls vs T2DM). When a main effect or interaction-
183 effect was observed, post-hoc tests were performed to identify the differences. Additionally,
184 FMD data were also analyzed and are presented as covariate-controlled for baseline artery
185 diameter as this approach is more accurate for scaling changes in artery diameter than simple
186 percentage change in some cases (2).

187

188

189 **RESULTS**

190 Baseline characteristics are described in Table 1. T2DM patients had significantly higher glucose
191 and HOMA-IR compared to controls whereas the control group had significantly higher total-
192 and LDL cholesterol concentrations. There were no apparent differences in brachial artery
193 diameter, blood flow, shear rate and FMD between groups at baseline (all comparisons $P>0.05$).

194

195 **Impact of hyperglycemia and heating on blood flow, diameter and glucose**

196 In both groups, ingestion of 75-gr glucose resulted in a significant increase in blood glucose
197 levels at 60 minutes, which returned towards baseline levels within 150 minutes (“Time” effect,
198 $P<0.001$, Figure 1). The increase in blood glucose was significantly larger in T2DM patients
199 compared to controls (“Time*Group”-interaction effect, $P=0.002$, Figure 1). Forearm skin
200 temperature significantly increased in the heated arm during the experiment (“Time” effect,
201 $P<0.001$, Table 2) and to a greater extent compared to the non-heated arm (“Time*Arm”-
202 interaction effect, $P<0.001$). Both T2DM patients and controls demonstrated a comparable
203 change in skin temperature across time in both arms (“Time*Arm*Group”-interaction effect,
204 $P=0.09$).

205
206 Similar to skin temperature, brachial artery blood flow and shear rate significantly increased in
207 the heated arm during the experiment (both: “Time” effect, $P<0.001$) (Figure 2A-B) and to a
208 greater extent compared to the non-heated arm (both: “Time*Arm”-interaction effect, $P<0.001$).
209 T2DM patients and controls demonstrated a comparable change in blood flow and shear rate
210 across time in both arms (“Time*Arm*Group”-interaction effect, $P=0.15$ and 0.25 , respectively).
211 In both groups, post hoc analyses indicated a significant increase in blood flow and shear rate at
212 60 minutes in the heated arm which returned to baseline within 150 minutes. The increase in
213 blood flow and shear rate at 60 minutes was significantly greater in the controls compared to the
214 T2DM patients (both $P<0.05$). Baseline brachial artery diameter did not change during the
215 experiment and differed neither between arms nor healthy controls and T2DM patients (Table 2).

216

217 **Impact of hyperglycemia and heating on brachial artery FMD**

218 A significant Time*Arm-interaction was found ($P=0.01$). Post hoc analyses indicated that
219 hyperglycemia induced a significant decrease in FMD in the non-heated arm of 1.4% at 60
220 minutes ($P<0.05$). In contrast, the heated arm demonstrated an increase in FMD of 1.5% and
221 1.3% at 60 and 150 minutes, respectively, despite the presence of hyperglycemia ($P<0.05$). The
222 difference in FMD between the heated and non-heated arms was statistically significant at 60,
223 120 and 150 minutes ($P<0.05$, Figure 3). When comparing the responses between controls and
224 T2DM patients, we found no time*arm*group-interaction effect (Figure 3). These outcomes
225 were reinforced when we repeated our analyses using the absolute (mm) or allometrically scaled
226 FMD (Table 3). No changes across time or differences between arms or groups were evident for
227 baseline diameter (Table 2) or shear rate area-under-the-curve (Table 3).

228

229 **DISCUSSION**

230 The aim of this study was to examine whether non-metabolically driven increases in blood flow
231 and shear rate (through heating of the skin) protects the hyperglycemia-mediated decrease in
232 endothelial function in healthy subjects and patients with type 2 diabetes mellitus. For this
233 purpose, we performed bilateral assessment of brachial artery endothelial function which enabled
234 us to simultaneously study the effects of both a systemic challenge (i.e. hyperglycemia) and a
235 local intervention (i.e. increased blood flow through unilateral heating). This provides a number
236 of observations. First, we confirmed previous observations (1, 16, 17, 33, 43, 47) that
237 hyperglycemia, induced by a 75-gr glucose load, leads to a transient decline in brachial artery
238 endothelial function. Secondly, local heating of a forearm leads to a marked increase in blood
239 flow and shear rate, which effectively prevented the hyperglycemia-induced decline in brachial
240 artery endothelial function. Third, the ability of increases in blood flow and shear rate to prevent
241 brachial artery endothelial dysfunction after hyperglycemia is similarly present in healthy
242 middle-aged controls and type 2 diabetes mellitus patients. Taken together, these data suggest
243 that elevation in blood flow or shear rate can prevent the hyperglycemia-induced decline in
244 conduit artery endothelial function which typically occurs after a meal.

245

246 Hyperglycemia may affect endothelial function via different pathways. NO bioavailability is
247 decreased through inhibition of eNOS and increased production of reactive oxygen species
248 (ROS). Moreover, hyperglycemia may increase production of vasoconstrictor prostanoids such
249 as prostaglandin H₂ and thromboxane A₂ (3, 36). Transient damage to the endothelial glycocalyx
250 may also occur. This luminal surface layer serves as a mechanosensor of shear stress to mediate

251 shear-induced release of NO (17, 29). Consequently, a decline in (partly NO-mediated)
252 endothelial function is observed after a meal or glucose load in healthy volunteers (1, 26, 43),
253 with some suggestion of an exaggerated impairment in T2DM patients (6, 16).

254

255 Mechanistic work from Hambrecht and coworkers revealed that increases in shear stress, for
256 example through exercise (11), can improve NO-mediated vasodilator function, increases eNOS
257 expression and endothelial content of phospho-eNOS_{Ser1177}, Akt, and phospho-Akt. Other
258 mechanistic work demonstrated that elevation in shear also down-regulates expression of
259 vasoconstrictors, adhesion molecules and coagulation factors (12). Based on this mechanistic
260 work, we hypothesized that elevation in shear may attenuate or prevent the hyperglycemia-
261 induced decrease in FMD. After successfully increasing blood flow and shear rate in the heated
262 arm, we confirmed our hypothesis in that FMD in the heated arm showed no decrease. In fact, a
263 significant increase was observed, which may be explained by the marked increases in blood
264 flow during the heating intervention. Previous studies also found that (local) heating can acutely
265 and chronically increase brachial artery FMD% (27, 42). **In addition to the effects of shear**
266 **rate, sympathetic- and sensory nerve activity play a major role in vasodilatory responses to**
267 **local heat in the skin (7, 13). We cannot exclude that these responses in the skin could also**
268 **have affected our observations in the brachial artery.** Taken together, our observations
269 suggest that local heat-induced increases in shear stress protect the endothelial function under
270 hyperglycemic conditions.

271

272 Our study also allowed for the comparison between healthy volunteers and patients with T2DM.
273 The significantly larger increase in blood glucose levels after 75-gr glucose fits with the presence

274 of insulin resistance in the diabetic patients. Despite a substantially larger increase in blood
275 glucose in the T2DM patients, the attenuation in FMD in the non-heated arm was similar to that
276 in the healthy controls. We also found no correlation between changes in blood glucose and
277 FMD (data not shown), which is in agreement with two earlier studies in healthy subjects (1, 43).
278 Kawano et al. did however report a significant negative correlation between plasma glucose and
279 FMD, which may be due to the larger sample size and the inclusion of untreated T2DM patients
280 (16).

281
282 Additionally, heating resulted in comparable increases in FMD between the two groups.
283 Therefore, our results suggest that the ability of increases in shear to prevent hyperglycemia-
284 induced endothelial dysfunction is similarly present in T2DM patients and healthy, age-matched
285 controls. It is however interesting to note that the change in blood flow in response to heating
286 was less pronounced in our group of diabetic patients compared to the healthy controls. This
287 could indicate that decreased reactivity of resistance vessels and skin microcirculation is already
288 present in type 2 diabetics before overt changes in conduit artery endothelial function occur. This
289 observation however should be interpreted with caution since a statistically significant group
290 effect was not evident in our main analysis. A subsequent sub-group analysis on data from the
291 heated arm did indicate a significant time*group interaction at T = 60 minutes for both blood
292 flow and shear rate though (P=0.05 and 0.03 respectively, data not shown). This finding is also
293 supported by data from other groups who have demonstrated that diabetics have impaired skin
294 blood flow responses to both local (30, 31) and whole body heating (37). Moreover, impaired
295 muscle blood flow responses to exercise stimuli in diabetics compared to healthy controls have
296 also been demonstrated in a number of studies (5, 18, 21).

297
298 **An unexpected finding in our study is that control subjects and T2DM patients reveal no**
299 **difference in baseline brachial artery FMD. Although it is generally accepted that T2DM**
300 **patients demonstrate lower FMD compared to healthy peers (24, 25), this is not a universal**
301 **finding (34, 35). One potential explanation for our finding is that T2DM patients received**
302 **optimal pharmacological therapy, including statins (60%) and metformin (80%). These**
303 **drugs are associated with improvements in brachial artery FMD (32, 49) and may,**
304 **therefore, contribute to the lack of difference in FMD between groups, despite all**
305 **medication being withheld on the morning of the measurement. Nonetheless, it is important**
306 **to emphasize that both groups demonstrated distinct responses to the heat stimulus.**

307
308 *Limitations:* A potential limitation of our study is that we did not examine endothelium-
309 independent vasodilation. **Due to the prolonged effects of glyceryl trinitrate (unpublished**
310 **data), including repeated measurements of endothelium-independent dilation would**
311 **importantly compromised our study design and outcomes.** However previous studies have
312 found no indication that acute heating or hyperglycemia directly affect vascular smooth muscle
313 cell reactivity (19, 27). **Our study set-up did not allow us to explore specific mechanisms,**
314 **such as the evaluation of NO metabolites, to better understand the underlying mechanisms**
315 **of our findings.** Another potential limitation relates to our method of heating. We employed a
316 simple setup involving directed hot air. This inadvertently caused a small increase in ambient-
317 and skin temperature of the control arm (Table 2). As a result small (non-significant) increases in
318 blood flow and shear rate were evident in the non-heated arm (Figure 2). Therefore, although a

319 reduction FMD was still evident in the non-heated arm, it may have been attenuated to a small
320 extent.

321

322 *Clinical relevance:* A potential clinical relevance of our findings relates to the suggestion that
323 (repeated) exposure to transient periods of endothelial dysfunction contributes to the
324 development of atherosclerosis. Although speculative, prevention or attenuation of endothelial
325 dysfunction during hyperglycemia seems a potential target. Our observations suggest that non-
326 metabolically driven elevations in shear may be of use to prevent the presence to endothelial
327 dysfunction. Similarly, (high-intensity) exercise is demonstrated to prevent the post-prandial
328 decrease in endothelial function (45). As exercise is a strong stimulus for elevation in blood flow
329 (41), our observations warrant future studies to explore whether the immediate benefits of
330 physical activity or exercise to prevent post-prandial endothelial dysfunction relate to shear
331 stress-mediated mechanisms, and may contribute to preservation of endothelial function in
332 diabetic patients.

333

334 In conclusion, post-prandial hyperglycemia resulted in a transient impairment in brachial artery
335 FMD in healthy subjects and type 2 diabetics, whilst a heating induced increase in brachial artery
336 blood flow and shear rate countered the impairment in FMD. Therefore, our data suggest that
337 that interventions which are aimed at elevating blood flow or shear rate can prevent the
338 hyperglycemia-induced decline in conduit artery endothelial function which typically occurs
339 after a meal.

340

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343

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345 AG and RD are employed by Unilever R&D Vlaardingen B.V. This work was financially
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347 otherwise, are declared by the remaining authors.

348

349 **AUTHORS' CONTRIBUTION TO MANUSCRIPT:**

350 DHJT, AG and RD designed the study. DHJT, TL, RJH MV, and TS performed the experiments.

351 TS and TL analyzed the data. TS, MTEH, DHJT, AG and RD wrote the manuscript. DHJT had

352 primary responsibility for final content. All authors read and approved the final manuscript.

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508

509 **FIGURE CAPTIONS**

510 **FIGURE 1.** Blood glucose levels before (0) and after 75-gr oral glucose (60, 120 and 150
511 minutes) in patients with type 2 diabetes (n=10, **diamonds**) and healthy, age-matched
512 controls (n=10, circles). P-values refer to a two-way ANOVA with ‘group’ (diabetic vs
513 control) and ‘time’ (0, 60, 120 and 150 minutes) as fixed effects and subject as random
514 effect. Data are presented as LSmeans \pm SE corrected for baseline.*Post-hoc
515 significantly different from baseline at $P<0.05$. †Post-hoc significantly different from
516 control group at $P<0.05$

517
518 **FIGURE 2.** Brachial artery blood flow (A) and shear rate (B) before (0) and after 75-gr oral
519 glucose (60, 120 and 150 minutes) in the control arm (**solid symbols**) and the heated
520 arm (**open symbols**) in patients with type 2 diabetes (n=10, **diamonds**) and healthy,
521 age-matched controls (n=10, circles). P-values refer to a linear mixed model to assess
522 whether the change in blood flow and shear rate during the experiment (‘Time’)
523 differed between the heated and non-heated arm (‘Arm’) and/or between controls and
524 type 2 diabetes patients (‘Group’). Data are presented as LSmeans \pm SE corrected for
525 baseline. *Post-hoc significantly different from baseline at $P<0.05$.

526
527 **FIGURE 3.** Brachial artery flow mediated dilation (FMD) before (0) and after 75-gr oral
528 glucose (60, 120 and 150 minutes) in the control arm (**solid symbol**) and the heated
529 arm (**open symbol**) in patients with type 2 diabetes (n=10, **diamonds**) and healthy,
530 age-matched controls (n=10, circles). P-values refer to a linear mixed model to assess
531 whether the change in FMD during the experiment (‘Time’) differed between the

532 heated and non-heated arm ('Arm') and/or between controls and type 2 diabetes
533 patients ('Group'). Data are presented as LSmeans \pm SE corrected for baseline. *Post-
534 hoc significantly different from baseline at $P < 0.05$.

535

536 **Table 1.** Subject characteristics from patients with type 2 diabetes (n=10) and healthy, age-
 537 matched controls (n=10). P-value refers to an unpaired Student's *t*-test. Data are presented as
 538 mean \pm SD.

Characteristics	Controls	T2DM	P-value
	Mean \pm SD	Mean \pm SD	
Age (years)	57 \pm 9	63 \pm 6	0.14
Height (cm)	178 \pm 6	176 \pm 7	0.43
Weight (Kg)	85 \pm 10	90 \pm 13	0.35
Body Mass Index (Kg/m ²)	26.7 \pm 3.6	29.0 \pm 3.6	0.16
Systolic BP	132.4 \pm 14	138.2 \pm 17.3	0.42
Diastolic BP	79.0 \pm 6.2	79.9 \pm 6.7	0.77
Total Cholesterol (mmol/L)	6.2 \pm 1.1	4.7 \pm 1.1	<0.01
High-density lipoproteins (mmol/L)	1.5 \pm 0.2	1.3 \pm 0.3	0.07
Low-density lipoproteins (mmol/L)	4.0 \pm 1.0	2.7 \pm 1.0	<0.01
Triglycerides (mmol/L)	1.9 \pm 0.9	2.0 \pm 0.8	0.85
Insulin (mmol/L)†	6.0 \pm 6.1	10.3 \pm 5.7	0.13
Glucose (mmol/L)	4.9 \pm 0.5	7.1 \pm 1.0	<0.001
HOMA-IR†	1.4 \pm 1.6	3.3 \pm 1.9	0.04

539 † One erroneous measurement from the control group was excluded

540

541 **Table 2.** Brachial artery diameter and skin temperature before (0) and 60, 120 and 150 minutes after 75-gr oral glucose load in the
 542 non-heated control arm and the heated arm in patients with type 2 diabetes (n=10) and healthy, age-matched controls (n=10). Data are
 543 presented as LSmeans (95% CI) corrected for baseline (time = 0).

		Time				Linear Mixed Model			
Baseline D (mm)		0	60	120	150	Time	Arm	Time*Arm	Time*Arm *Group
Controls	Heated	4.3 (4.2; 4.5)	4.6 (4.4; 4.7)	4.6 (4.4; 4.7)	4.5 (4.3; 4.6)	0.09	0.88	0.85	0.61
	Non-heated	4.3 (4.2; 4.5)	4.5 (4.3; 4.6)	4.4 (4.2; 4.6)	4.4 (4.3; 4.6)				
T2DM	Heated	4.3 (4.2; 4.5)	4.3 (4.2; 4.5)	4.4 (4.2; 4.6)	4.4 (4.2; 4.5)				
	Non-heated	4.4 (4.2; 4.5)	4.4 (4.2; 4.6)	4.5 (4.3; 4.6)	4.4 (4.3; 4.6)				
Temperature (°C)									
Controls	Heated	31.8 (31.0; 32.5)	41.1 (40.3; 41.9)*†	33.8 (33.0; 34.6)*	32.9 (32.1; 33.7)*	<0.001	<0.001	<0.001	0.09
	Non-heated	31.6 (30.9; 32.4)	32.9 (32.1; 33.6)*	33.1(32.3; 33.8)*	32.5 (31.8; 33.3)				
T2DM	Heated	31.9 (31.2; 32.6)	39.4 (38.7; 40.2) *†	33.1 (32.4; 33.8)*	32.7 (32.0; 33.5)				
	Non-heated	31.8 (31.1; 32.5)	33.4 (32.7; 34.2)*	32.6 (31.8; 33.3)	32.3 (31.5; 33.0)				

544 D; diameter. *Post-hoc significantly different from baseline at P<0.05 (paired *t*-test). †Post-hoc significantly different from non-heated
 545 arm at P<0.05 (unpaired *t*-test)

546

547 **Table 3.** Brachial artery flow-mediated dilation before (0) and after 75-gr oral glucose (60, 120 and 150 minutes) in the non-heated
 548 control arm and the heated arm in patients with type 2 diabetes (n=10) and healthy, age-matched controls (n=10). Data are presented
 549 as LSmeans (95% CI) corrected for baseline (time = 0).

		Time				Linear Mixed Model			
FMD (mm)		0	60	120	150	Time	Arm	Time*Arm	Time*Arm *Group
Controls	<i>Heated</i>	0.20 (0.14; 0.25)	0.25 (0.20; 0.31)†	0.24 (0.18; 0.30)	0.26 (0.20; 0.31)	0.85	0.01	0.01	0.88
	<i>Non-heated</i>	0.19 (0.14; 0.25)	0.16 (0.10; 0.22)	0.17 (0.11; 0.23)	0.18 (0.12; 0.24)				
T2DM	<i>Heated</i>	0.18 (0.12; 0.24)	0.26 (0.20; 0.32)*†	0.21 (0.15; 0.27)	0.23 (0.17; 0.29)				
	<i>Non-heated</i>	0.22 (0.16; 0.28)	0.15 (0.09; 0.21)*	0.15 (0.09; 0.21)	0.16 (0.10; 0.22)				
Scaled FMD (%)									
Controls	<i>Heated</i>	4.0 (2.7; 5.3)	6.1 (4.8; 7.4)*†	5.5 (4.1; 6.8)	5.6 (4.3; 6.9)*	0.65	0.09	0.02	0.91
	<i>Non-heated</i>	4.3 (3.0; 5.6)	3.7 (2.4; 5.0)	3.8 (2.6; 5.1)	4.1 (2.8; 5.4)				
T2DM	<i>Heated</i>	3.7 (2.5; 5.1)	5.4 (4.0; 6.7)*	4.3 (2.9; 5.7)	5.1 (3.8; 6.4)				
	<i>Non-heated</i>	5.4 (4.0; 6.7)	3.9 (2.6; 5.2)	3.9 (2.6; 5.3)	4.0 (2.6; 5.3)				
SR_{AUC} (×10³ s⁻¹)									
Controls	<i>Heated</i>	27.7 (22.6; 32.9)	30.0 (24.9; 35.1)	26.3 (21.1; 31.4)	22.6 (17.5; 27.7)	0.1	0.09	0.82	0.82
	<i>Non-heated</i>	25.8 (20.7; 30.9)	25.7 (20.6; 30.8)	22.6 (17.5; 27.7)	22.9 (17.8; 28.0)				

T2DM	<i>Heated</i>	25.9 (20.8; 31.0)	29.3 (24.2; 34.4)	27.7 (22.6; 32.8)	27.1 (22.0; 32.2)
	<i>Non-heated</i>	24.6 (19.5; 29.7)	27.0 (21.9; 32.1)	22.6 (17.5; 27.7)	23.4 (18.3; 28.5)

550 FMD; Flow Mediated Dilation. SR_{AUC} ; Shear Rate Area Under the Curve. *Post hoc significantly different from baseline at $P < 0.05$

551 (paired t -test). †Post-hoc significantly different from non-heated arm at $P < 0.05$ (unpaired t -test)

