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

ARNO GREYLING[1,2], TIM H.A. SCHREUDER[1], THIJS LANDMAN[1], RICHARD DRAIJER[2], REBECCA J.H.M. VERHEGGEN[1], MARIA T.E. HOPMAN[1], DICK H.J. THIJSSEN[1,3]

[1]*Department of Physiology*,

Radboud University Medical Center, Nijmegen, the Netherlands

[2]Unilever R&D Vlaardingen, The Netherlands

[3]*Research Institute for Sports and Exercise Sciences, Liverpool John Moores University,*

United Kingdom

Running head: Shear, FMD and hyperglycemia

Figures: 3

Table: 3

Word count text: 5741

Word count abstract: 250

Author for correspondence:

Prof. Dick H.J. Thijssen, Department of Physiology, Radboud University Medical Center, Nijmegen, the Netherlands. Tel: +31243614222, Email: Dick.Thijssen@radboudumc.nl

ABSTRACT

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

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

INTRODUCTION

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

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

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

METHODS

Participants

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

Experimental Design

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

Experimental Measures

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

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

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

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

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

Brachial Artery Diameter and Blood Flow Analysis

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

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

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

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

Statistical analysis

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

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

RESULTS

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

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

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

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

Impact of hyperglycemia and heating on brachial artery FMD

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

DISCUSSION

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

Hyperglycemia may affect endothelial function via different pathways. NO bioavailability is decreased through inhibition of eNOS and increased production of reactive oxygen species (ROS). Moreover, hyperglycemia may increase production of vasoconstrictor prostanoids such as prostaglandin H_2 and thromboxane A_2 (3, 36). Transient damage to the endothelial glycocalyx may also occur. This luminal surface layer serves as a mechanosensor of shear stress to mediate

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

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

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

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

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

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

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

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

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

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

GRANTS:

DHJT is financially supported by the Netherlands Heart Foundation (2009T064).

DISCLOSURES:

AG and RD are employed by Unilever R&D Vlaardingen B.V. This work was financially supported by a grant from Unilever R&D Vlaardingen B.V. No conflicts of interest, financial or otherwise, are declared by the remaining authors.

AUTHORS' CONTRIBUTION TO MANUSCRIPT:

DHJT, AG and RD designed the study. DHJT, TL, RJHMV, and TS performed the experiments. TS and TL analyzed the data. TS, MTEH, DHJT, AG and RD wrote the manuscript. DHJT had primary responsibility for final content. All authors read and approved the final manuscript.

REFERENCES:

1. **Akbari CM, Saouaf R, Barnhill DF, Newman PA, LoGerfo FW, and Veves A**. Endothelium-dependent vasodilatation is impaired in both microcirculation and macrocirculation during acute hyperglycemia. *J Vasc Surg* 28: 687-694, 1998.

2. **Atkinson G, and Batterham AM**. Allometric scaling of diameter change in the original flow-mediated dilation protocol. *Atherosclerosis* 226: 425-427, 2013.

3. **Ballard KD, Mah E, Guo Y, Pei R, Volek JS, and Bruno RS**. Low-fat milk ingestion prevents postprandial hyperglycemia-mediated impairments in vascular endothelial function in obese individuals with metabolic syndrome. *J Nutr* 143: 1602-1610, 2013.

4. **Bau PF, Bau CH, Naujorks AA, Rosito GA, and Fuchs FD**. Diurnal variation of vascular diameter and reactivity in healthy young men. *Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas / Sociedade Brasileira de Biofisica [et al* 41: 500-503, 2008.

5. **Bauer TA, Reusch JE, Levi M, and Regensteiner JG**. Skeletal muscle deoxygenation after the onset of moderate exercise suggests slowed microvascular blood flow kinetics in type 2 diabetes. *Diabetes care* 30: 2880-2885, 2007.

6. **Ceriello A, Taboga C, Tonutti L, Quagliaro L, Piconi L, Bais B, Da Ros R, and Motz E**. Evidence for an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on endothelial dysfunction and oxidative stress generation: effects of short- and long-term simvastatin treatment. *Circulation* 106: 1211-1218, 2002.

7. **Charkoudian N**. Mechanisms and modifiers of reflex induced cutaneous vasodilation and vasoconstriction in humans. *J Appl Physiol (1985)* 109: 1221-1228, 2010.

8. **Chen XM, Zhang Y, Shen XP, Huang Q, Ma H, Huang YL, Zhang WQ, and Wu HJ**. Correlation between glucose fluctuations and carotid intima-media thickness in type 2 diabetes. *Diabetes research and clinical practice* 90: 95-99.

9. **Creager MA, Luscher TF, Cosentino F, and Beckman JA**. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: Part I. *Circulation* 108: 1527-1532, 2003.

10. **Duckworth W, Abraira C, Moritz T, Reda D, Emanuele N, Reaven PD, Zieve FJ, Marks J, Davis SN, Hayward R, Warren SR, Goldman S, McCarren M, Vitek ME, Henderson WG, and Huang GD**. Glucose control and vascular complications in veterans with type 2 diabetes. *The New England journal of medicine* 360: 129-139, 2009.

11. **Hambrecht R, Adams V, Erbs S, Linke A, Krankel N, Shu Y, Baither Y, Gielen S, Thiele H, Gummert JF, Mohr FW, and Schuler G**. Regular physical activity improves endothelial function in patients with coronary artery disease by increasing phosphorylation of endothelial nitric oxide synthase. *Circulation* 107: 3152-3158, 2003.

12. **Johnson BD, Mather KJ, and Wallace JP**. Mechanotransduction of shear in the endothelium: basic studies and clinical implications. *Vasc Med* 16: 365-377, 2011.

13. **Johnson JM, and Kellogg DL**. Local thermal control of the human cutaneous circulation. *J Appl Physiol (1985)* 109: 1229-1238, 2010.

14. **Jones H, Green DJ, George K, and Atkinson G**. Intermittent exercise abolishes the diurnal variation in endothelial-dependent flow-mediated dilation in humans. *Am J Physiol Regul Integr Comp Physiol* 298: R427-432.

15. **Jones H, Lewis NC, Thompson A, Marrin K, Green DJ, and Atkinson G**. Diurnal variation in vascular function: role of sleep. *Chronobiology international* 29: 271-277.

16. **Kawano H, Motoyama T, Hirashima O, Hirai N, Miyao Y, Sakamoto T, Kugiyama K, Ogawa H, and Yasue H**. Hyperglycemia rapidly suppresses flow-mediated endothelium-dependent vasodilation of brachial artery. *J Am Coll Cardiol* 34: 146-154, 1999.

17. **Kelly R, Ruane-O'Hora T, Noble MI, Drake-Holland AJ, and Snow HM**. Differential inhibition by hyperglycaemia of shear stress- but not acetylcholine-mediated dilatation in the iliac artery of the anaesthetized pig. *The Journal of physiology* 573: 133-145, 2006.

18. **Kiely C, O'Connor E, O'Shea D, Green S, and Egana M**. Haemodynamic responses during graded and constant-load plantar flexion exercise in middle-aged men and women with type 2 diabetes. *J Appl Physiol (1985)* 2014.

19. **Kihara T, Biro S, Imamura M, Yoshifuku S, Takasaki K, Ikeda Y, Otuji Y, Minagoe S, Toyama Y, and Tei C**. Repeated sauna treatment improves vascular endothelial and cardiac function in patients with chronic heart failure. *Journal of the American College of Cardiology* 39: 754-759, 2002.

20. **Kim SH, Park KW, Kim YS, Oh S, Chae IH, Kim HS, and Kim CH**. Effects of acute hyperglycemia on endothelium-dependent vasodilation in patients with diabetes mellitus or impaired glucose metabolism. *Endothelium* 10: 65-70, 2003.

21. **Lalande S, Gusso S, Hofman PL, and Baldi JC**. Reduced leg blood flow during submaximal exercise in type 2 diabetes. *Medicine and science in sports and exercise* 40: 612-617, 2008.

22. **Langille BL, and O'Donnell F**. Reductions in arterial diameter produced by chronic decreases in blood flow are endothelium-dependent. *Science* 231: 405-407, 1986.

23. **Laughlin MH, Newcomer SC, and Bender SB**. Importance of hemodynamic forces as signals for exercise-induced changes in endothelial cell phenotype. *J Appl Physiol* 104: 588-600, 2008.

24. **Lopes-Virella MF, Carter RE, Gilbert GE, Klein RL, Jaffa M, Jenkins AJ, Lyons TJ, Garvey WT, and Virella G**. Risk factors related to inflammation and endothelial dysfunction in the DCCT/EDIC cohort and their relationship with nephropathy and macrovascular complications. *Diabetes care* 31: 2006-2012, 2008.

25. **Luscher TF, Creager MA, Beckman JA, and Cosentino F**. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: Part II. *Circulation* 108: 1655-1661, 2003.

26. **Mah E, Noh SK, Ballard KD, Matos ME, Volek JS, and Bruno RS**. Postprandial hyperglycemia impairs vascular endothelial function in healthy men by inducing lipid peroxidation and increasing asymmetric dimethylarginine:arginine. *J Nutr* 141: 1961-1968, 2011.

27. **Naylor LH, Carter H, FitzSimons MG, Cable NT, Thijssen DH, and Green DJ**. Repeated increases in blood flow, independent of exercise, enhance conduit artery vasodilator function in humans. *American journal of physiology* 300: H664-669, 2011.

28. **Newcomer SC, Thijssen DH, and Green DJ**. Effects of exercise on endothelium and endothelium/smooth muscle cross talk: role of exercise-induced hemodynamics. *J Appl Physiol (1985)* 111: 311-320, 2011.

29. **Nieuwdorp M, van Haeften TW, Gouverneur MC, Mooij HL, van Lieshout MH, Levi M, Meijers JC, Holleman F, Hoekstra JB, Vink H, Kastelein JJ, and Stroes ES**. Loss

of endothelial glycocalyx during acute hyperglycemia coincides with endothelial dysfunction and coagulation activation in vivo. *Diabetes* 55: 480-486, 2006.

30. **Petrofsky J, Berk L, Alshammari F, Lee H, Hamdan A, Yim JE, Patel D, Kodawala Y, Shetye G, Chen WT, Moniz H, Pathak K, Somanaboina K, Desai R, Dave B, Malthane S, Alshaharani M, Neupane S, Shenoy S, Nevgi B, Cho S, and Al-Nakhli H**. The effect of moist air on skin blood flow and temperature in subjects with and without diabetes. *Diabetes Technol Ther* 14: 105-116, 2012.

31. **Petrofsky J, Lee H, Trivedi M, Hudlikar AN, Yang CH, Goraksh N, Alshammari F, Mohanan M, Soni J, Agilan B, Pai N, Chindam T, Murugesan V, Yim JE, and Katrak V**. The influence of aging and diabetes on heat transfer characteristics of the skin to a rapidly applied heat source. *Diabetes Technol Ther* 12: 1003-1010, 2010.

32. **Pitocco D, Zaccardi F, Tarzia P, Milo M, Scavone G, Rizzo P, Pagliaccia F, Nerla R, Di Franco A, Manto A, Rocca B, Lanza GA, Crea F, and Ghirlanda G**. Metformin improves endothelial function in type 1 diabetic subjects: a pilot, placebo-controlled randomized study. *Diabetes Obes Metab* 15: 427-431, 2013.

33. **Rammos G, Peppes V, and Zakopoulos N**. Transient insulin resistance in normal subjects: acute hyperglycemia inhibits endothelial-dependent vasodilatation in normal subjects. *Metab Syndr Relat Disord* 6: 159-170, 2008.

34. **Schreuder TH, Duncker DJ, Hopman MT, and Thijssen DH**. Randomized controlled trial using bosentan to enhance the impact of exercise training in subjects with type 2 diabetes mellitus. *Experimental physiology* 99: 1538-1547, 2014.

35. **Schreuder TH, Maessen MF, Tack CJ, Thijssen DH, and Hopman MT**. Life-long physical activity restores metabolic and cardiovascular function in type 2 diabetes. *European journal of applied physiology* 114: 619-627, 2014.

36. **Sena CM, Pereira AM, and Seica R**. Endothelial dysfunction - a major mediator of diabetic vascular disease. *Biochim Biophys Acta* 1832: 2216-2231, 2013.

37. **Sokolnicki LA, Strom NA, Roberts SK, Kingsley-Berg SA, Basu A, and Charkoudian N**. Skin blood flow and nitric oxide during body heating in type 2 diabetes mellitus. *J Appl Physiol (1985)* 106: 566-570, 2009.

38. **ter Avest E, Holewijn S, Stalenhoef AF, and de Graaf J**. Variation in non-invasive measurements of vascular function in healthy volunteers during daytime. *Clin Sci (Lond)* 108: 425-431, 2005.

39. **Thijssen DH, Atkinson CL, Ono K, Sprung VS, Spence AL, Pugh CJ, and Green DJ**. Sympathetic Nervous System Activation, Arterial Shear Rate and Flow Mediated Dilation. *J Appl Physiol (1985)* 2014.

40. **Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, Parker B, Widlansky ME, Tschakovsky ME, and Green DJ**. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. *American journal of physiology* 300: H2-12, 2011.

41. **Thijssen DH, Dawson EA, Black MA, Hopman MT, Cable NT, and Green DJ**. Brachial artery blood flow responses to different modalities of lower limb exercise. *Medicine and science in sports and exercise* 41: 1072-1079, 2009.

42. **Tinken TM, Thijssen DH, Hopkins N, Black MA, Dawson EA, Minson CT, Newcomer SC, Laughlin MH, Cable NT, and Green DJ**. Impact of shear rate modulation on vascular function in humans. *Hypertension* 54: 278-285, 2009.

43. **Title LM, Cummings PM, Giddens K, and Nassar BA**. Oral glucose loading acutely attenuates endothelium-dependent vasodilation in healthy adults without diabetes: an effect prevented by vitamins C and E. *Journal of the American College of Cardiology* 36: 2185-2191, 2000.

44. **Tuttle JL, Nachreiner RD, Bhuller AS, Condict KW, Connors BA, Herring BP, Dalsing MC, and Unthank JL**. Shear level influences resistance artery remodeling: wall dimensions, cell density, and eNOS expression. *American journal of physiology* 281: H1380-1389, 2001.

45. **Tyldum GA, Schjerve IE, Tjønna AE, Kirkeby-Garstad I, Stølen TO, Richardson RS, and Wisløff U**. Endothelial dysfunction induced by post-prandial lipemia: complete protection afforded by high-intensity aerobic interval exercise. *J Am Coll Cardiol* 53: 200-206, 2009.

46. **WHO**. Diabetes Fact Sheet http://www.who.int/mediacentre/factsheets/fs312/en/index.html. [03 august, 2011].

47. **Williams SB, Goldfine AB, Timimi FK, Ting HH, Roddy MA, Simonson DC, and Creager MA**. Acute hyperglycemia attenuates endothelium-dependent vasodilation in humans in vivo. *Circulation* 97: 1695-1701, 1998.

48. **Woodman RJ, Playford DA, Watts GF, Cheetham C, Reed C, Taylor RR, Puddey IB, Beilin LJ, Burke V, Mori TA, and Green D**. Improved analysis of brachial artery ultrasound using a novel edge-detection software system. *J Appl Physiol* 91: 929-937, 2001.

49. **Zhang L, Gong D, Li S, and Zhou X**. Meta-analysis of the effects of statin therapy on endothelial function in patients with diabetes mellitus. *Atherosclerosis* 223: 78-85, 2012.

FIGURE CAPTIONS

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

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

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

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

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 ± SD.

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

539 † One erroneous measurement from the control group was excluded

540

Table 2. Brachial artery diameter and skin temperature before (0) and 60, 120 and 150 minutes after 75-gr oral glucose load in the 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 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)				
	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)	0.09	0.88	0.85	0.61
	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 (ºC)									
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)*				
	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)	**<0.001**	**<0.001**	**<0.001**	0.09
	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)				

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

Table 3. Brachial artery flow-mediated dilation before (0) and after 75-gr oral glucose (60, 120 and 150 minutes) in the 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 presented 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	*Heated*	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
	Non-heated	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	*Heated*	0.18 (0.12; 0.24)	0.26 (0.20; 0.32)*†	0.21 (0.15; 0.27)	0.23 (0.17; 0.29)				
	Non-heated	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	*Heated*	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
	Non-heated	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	*Heated*	3.7 (2.5; 5.1)	5.4 (4.0; 6.7)*	4.3 (2.9; 5.7)	5.1 (3.8; 6.4)				
	Non-heated	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} ($\times 10^3$ s^{-1})									
Controls	*Heated*	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
	Non-heated	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	*Heated*	25.9 (20.8; 31.0)	29.3 (24.2; 34.4)	27.7 (22.6; 32.8)	27.1 (22.0; 32.2)
	Non-heated	24.6 (19.5; 29.7)	27.0 (21.9; 32.1)	22.6 (17.5; 27.7)	23.4 (18.3; 28.5)

FMD; Flow Mediated Dilation. SR_{AUC}; Shear Rate Area Under the Curve. *Post hoc significantly different from baseline at P<0.05 (paired *t*-test). †Post-hoc significantly different from non-heated arm at P<0.05 (unpaired *t*-test)

552