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Acute Hyperglycemia Does Not Affect the Reactivity of Coronary Microcirculation in Humans

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Objective: There is some evidence that acute hyperglycemia (H) may cause vascular dysfunction in normal subjects. This study investigates whether acute, short-term H affects coronary vasodilatory function in healthy subjects.

Design: Diastolic peak flow velocity in the left anterior descending coronary artery was measured at rest and after dipyridamole (0.56 mg/kg over 4 min) using transthoracic color Doppler echocardiography in 13 healthy men. Coronary flow reserve (CFR) was defined as the ratio of dipyridamole-induced coronary peak diastolic to resting peak diastolic flow velocity. CFR was measured both in euglycemia (E) and after 3 h H (~14 mmol/liter) by a variable infusion of glucose and octreotide (0.4 mg/h) to prevent increase in insulin concentration.

Results: Fasting plasma glucose increased to 14.3 ± 0.33 mmol/liter during the study and maintained variability within less than 10%. Plasma insulin remained nearly stable during H. Resting diastolic flow velocity was 18.5 ± 0.6 cm/sec in E and increased to 20.0 ± 0.7 cm/sec during H ($P < 0.005$). Dipyridamole infusion produced a marked increase in coronary flow velocity, which reached values of 50.8 ± 2.9 cm/sec in E and 51.8 ± 2.1 cm/sec in H (P = not significant). CFR was 2.78 ± 0.16 in E and 2.59 ± 0.12 in H (P = not significant).

Conclusion: Our study indicates that short-term hyperglycemia does not affect the vasodilatory response of coronary microcirculation in healthy subjects. **(*J Clin Endocrinol Metab* 90: 3871–3876, 2005)**

CHRONIC HYPERGLYCEMIA PLAYS a key role in the development of both microvascular and macrovascular complications in patients with diabetes (1, 2). However, evidence is accumulating that even acute, short-term hyperglycemic spikes may cause vascular dysfunction in normal subjects (3–6). In addition, elevated glucose levels during an acute cardiovascular event (so-called stress hyperglycemia) are associated with a worse prognosis also in the absence of diabetes (7, 8). One of the mechanisms underlying hyperglycemia-induced vascular damage is related to the increased production of oxygen free radicals from endothelial cells, such as superoxide anion, which inactivate nitric oxide (NO) (9–11). Loss of NO results in enhanced contractility and proliferation of vascular smooth muscle cells with increased vasomotor tone (10), platelet hyperreactivity (12), alteration of the adhesive properties of the endothelium (13), and increased production of cytokines (14). Several studies have shown that a transient increase in plasma glucose is associated with endothelial dysfunction in the forearm vascular bed of healthy subjects and that this abnormality is restored by a variety of antioxidants (3–6). However, this finding was not confirmed in other studies using the model of both local and systemic hyperglycemia (15, 16). These discrepant results can, at least in part, be explained by methodological differences with regard to the technique used to assess endothelial function but also to the degree, duration, and pattern of hyperglycemia (16). Moreover, vascular responses in different vascular regions are heterogeneous, and findings obtained in the forearm cannot be extrapolated to the coronary vessels.

Transthoracic echo-Doppler echocardiography with harmonic mode has emerged as a reliable, noninvasive technique to measure coronary flow and estimate coronary vasodilatory capacity (17, 18). There is an increasing number of studies showing reduced coronary blood flow reserve (CFR) in diabetic patients even in the absence of epicardial coronary artery stenosis (19, 20). However, it is unknown whether acute, short-term hyperglycemia affects coronary microcirculation function in healthy subjects, in the absence of other vascular risk factors. The present study was undertaken to explore this issue. We assessed coronary flow velocities at rest and their vasodilator response both in euglycemia and 3 h after exposure to hyperglycemia experimentally induced through exogenous glucose infusion.

Subjects and Methods

Subjects

We studied 13 young, normotensive, normal-weight men (age, 31 ± 2 yr; body mass index, 25 ± 1 kg/m^2) who were recruited among students and employees of our department on a voluntary basis, under a protocol approved by the Ethics Committee of University Federico II. None of them had a history of hypertension, diabetes mellitus, hyperlipidemia, or endocrinopathies, which may affect coronary microcirculation. Three subjects were light smokers (<10 cigarettes per day). All subjects underwent physical examination, routine biochemical analysis, and maximal stress electrocardiogram to demonstrate ability to perform maximal exercise, indirectly indicating good fitness. None of them was on pharmacological treatment. All participants abstained from caffeine, smoking, and strenuous exercise 24 h before the study.

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Abbreviations: BP, Blood pressure; CFR, coronary flow reserve; HR, heart rate; LV, left ventricle; NO, nitric oxide; NS, not significant.

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Study protocol

Studies were performed at 0830 h after a 12–14 h overnight fast. A 20-gauge cannula was inserted into an antecubital vein for infusion of the test substances, and a second cannula was inserted into an ipsilateral distal vein for blood sampling. The antebrachium was placed into a heated box for arterialization of venous blood. After blood sampling for baseline analysis, all participants underwent blood pressure (BP) measurement, standard echocardiographic exam, and determination of coronary blood flow velocities before and after dipyridamole. Then, blood glucose concentration was rapidly raised and clamped to approximately 14 mmol/liter through a primed continuous infusion of 33% glucose at a variable rate. The glucose infusion rate was adjusted according to blood glucose measured every 10–20 min. To prevent an increase in insulin concentration, which is known to have NO-dependent vasodilatory activity, octreotide infusion (0.4 mg/h) was given 10 min before glucose infusion and continued at a constant rate throughout the study. In addition, regular insulin was administered at a rate of 0.15 mU/kg·min to replace basal insulin concentration. In so doing, the potential confounder of insulin increase is eliminated, and the observed effect can be attributed to hyperglycemia. Noteworthy is that no vasoactive effect of octreotide was demonstrated in previous studies using similar or even higher doses of the drug (21, 22). After 3 h of hyperglycemia, blood pressure and cerebral blood flow were reevaluated as at baseline, and two blood samples were taken at 15 min intervals for blood analysis. CFR obtained during euglycemia and hyperglycemia were compared to determine the effect of acute hyperglycemia on coronary microcirculation.

To account for possible changes of coronary flow velocities and CFR induced by volume expansion throughout the experimental period, three healthy volunteers (age, 41 ± 2 yr; body mass index, 25 ± 0.2 kg/m^2) repeated CFR measurements in basal conditions and after 3 h of saline infusion at a rate of 200 ml/h, corresponding to the same amount of fluids infused in the hyperglycemic study.

Determination of left ventricular function

Echocardiographic examinations were performed with subjects in partial left decubitus position by Vingmed Sytem FiVe AB Sound machine (GE, Horten, Norway) equipped with a 2.5 MHz phased-array transducer. M-Mode tracings were recorded, and the measurements were performed as reported previously in detail (23). Left ventricular (LV) mass was normalized for height in meters$^{2.7}$. LV systolic function was evaluated as both endocardial and midwall fractional shortening (24). Relative diastolic wall thickness was determined as the ratio of the sum of septal and posterior wall thickness to LV internal end-diastolic diameter. Transmitral pulsed Doppler was obtained in the apical four-chamber view, and early and atrial peak velocities (meters per second) and their ratio, early velocity deceleration time (milliseconds), and isovolumic relaxation time (milliseconds) were measured according to standard procedures (25).

Assessment of CFR ratio

A color-guided pulsed Doppler recording of coronary blood velocities in the distal left anterior descending artery was performed at rest and after low-dose dipyridamole administration (0.56 mg/kg over 4 min). Heart rate (HR), BP, and electrocardiogram were monitored during dipyridamole test. When imaging of color Doppler was not considered optimal, a contrast agent (Levovist, SHU-508A; Schering AG, Berlin, Germany) was infused into the right cubital vein (at a concentration of 300 mg/ml and an infusion rate of 1 ml/min) to enhance Doppler signal (17, 18). Methodological details and reproducibility of CFR evaluation by transthoracic Doppler echocardiography in our laboratory have been reported previously in detail (26). Briefly, Doppler sample volume was placed on the color signal of the distal left anterior descending artery, and spectral pulsed Doppler signal was recorded to examine the characteristic biphasic flow pattern, including systolic and diastolic velocities, both at rest and after dipyridamole. All images were recorded onto a magneto-optical disk and analyzed offline by two observers who were blinded to the sequence of examinations, after mixing up recordings in a random sequence together with other CFR exams. To calculate CFR ratio, coronary diastolic peak velocities were measured by averaging the highest three spectral Doppler signals, both at rest and after dipyridamole infusion. CFR was defined as the ratio of hyperemic to resting diastolic peak velocities.

Analytical methods

Total serum cholesterol, triglycerides, and high-density lipoprotein cholesterol were measured by commercially available kits. Serum low-density lipoprotein was calculated with the Friedewald formula. Plasma insulin was determined by ELISA.

Statistical analysis

Statistical analysis was performed by SPSS for Windows release 8.0 (SPSS, Inc., Chicago, IL). A paired, two-tailed Student *t* test was used to make comparisons between euglycemic and hyperglycemic state and between resting and hyperemic conditions. Statistical significance of differences was defined at a value of $P < 0.05$ (two-tailed). Results are expressed as mean ± SEM.

Results

Clinical, metabolic, and echocardiographic data

Baseline characteristics of the participants are summarized in Table 1. BP and lipid profile were within normal limits. All subjects had normal LV systolic and diastolic function, as evidenced by the echocardiographic parameters reported in Table 1. Glucose concentrations were raised to a mean value of 14.3 ± 0.33 mmol/liter during the hyperglycemic clamp, with a coefficient of variation less than 10%. Insulin concentrations during hyperglycemia were maintained at an average value of 12 ± 2 mU/liter. The amount of glucose required to maintain hyperglycemia throughout the study was approximately 300 g.

CFR measurement

None of the 13 subjects complained of major side effects during low-dose dipyridamole test, during both euglycemia and hyperglycemia. The administration of the contrast agent Levovist (both at rest and after dipyridamole infusion) was needed in two subjects to optimize Doppler signal and visualize adequately the coronary flow velocity profile.

Hemodynamic data during CFR measurements are listed in Table 2. HR was slightly lower during hyperglycemia compared with euglycemia both at rest and during dipyrid-

TABLE 1. Clinical and echocardiographic characteristics of the study group

Number (sex)	13 (males)
Age (yr)	31 ± 2
BMI (kg/m^2)	25 ± 1
Familial diabetes	5 of 13
Familial hypertension	2 of 13
Smoking	3 of 13
Total cholesterol (mmol/liter)	4.39 ± 0.25
HDL cholesterol (mmol/liter)	0.98 ± 0.20
Triglycerides (mmol/liter)	1.05 ± 0.16
Systolic BP (mm Hg)	117 ± 3
Diastolic BP (mm Hg)	76 ± 2
Fasting plasma glucose (mmol/liter)	4.7 ± 0.2
Fasting plasma insulin (mU/liter)	7 ± 1
LV mass index (m/g$^{2.7}$)	35.8 ± 5.3
Endocardial fractional shortening (%)	30.1 ± 4.6
Midwall fractional shortening (%)	18.7 ± 0.7
Peak velocity E/A ratio	1.58 ± 0.29

Values are mean ± SEM. E/A ratio, Peak velocity early to atrial ratio; BMI, body mass index; HDL, high-density lipoproteins.

TABLE 2. Hemodynamic data during CFR measurement

	Rest		Dipyridamole	
	Euglycemia	Hyperglycemia	Euglycemia	Hyperglycemia
HR (beats/min)	68 ± 3	64 ± 3[a]	89 ± 3	83 ± 2[a]
SBP (mm Hg)	116 ± 2	117 ± 3	117 ± 3	121 ± 3
DBP (mm Hg)	76 ± 2	75 ± 3	75 ± 3	74 ± 4
RPP (mm Hg × beats/min)	7,971 ± 360	7,494 ± 372	10,509 ± 524	10,035 ± 395

Values are mean ± SEM. SBP, Systolic blood pressure; DBP, diastolic blood pressure; RPP, rate-pressure product.
[a] $P < 0.05$ *vs.* euglycemia.

amole ($P < 0.05$), whereas there was no significant change in systolic and diastolic BP between euglycemia and hyperglycemia both at rest and after dypiridamole-induced vasodilation. The rate-pressure product at rest was similar in euglycemia and hyperglycemia and increased to a similar extent during hyperemia. Mean BP was 90 ± 2 mm Hg in euglycemia and 89 ± 2 mm Hg in hyperglycemia and remained substantially unchanged during dipyridamole in both experimental conditions (89 ± 2 mm Hg in euglycemia and 90 ± 3 mm Hg in hyperglycemia). Diastolic coronary flow velocity at rest was 18.5 ± 0.6 and 20.0 ± 0.7 cm/sec in euglycemia and hyperglycemia, respectively ($P < 0.005$). Dipyridamole infusion produced a marked increase in coronary diastolic peak velocity, which reached similar values in euglycemia (50.8 ± 2.9 cm/sec) and in hyperglycemia (51.8 ± 2.1 cm/sec) [P = not significant (NS)]. Individual values for these variables are displayed in Fig. 1. CFR ratio was 2.78 ± 0.16 in euglycemia and remained unchanged (2.59 ± 0.12; P = NS) in hyperglycemia (Fig. 2). Analysis of individual values shows that, in most subjects, CFR ratio remained constant during hyperglycemia; a substantial reduction (24%) occurred in only one subject who is to be considered as an outlier on the basis of resting CFR value. After exclusion of this subject from the analysis, mean CFR ratio was 2.64 ± 0.10 in euglycemia and 2.52 ± 0.11 in hyperglycemia.

In the three healthy volunteers who underwent CFR measurements before and after saline infusion, CFR ratio was 2.10 ± 0.2 at baseline and 2.03 ± 0.18 after 3-h saline infusion (P = NS), coronary peak velocities being 17.9 and 18.3 cm/sec at rest and 37.4 and 37.6 cm/sec after dipyridamole, respectively (Fig. 2).

Discussion

Although several studies have examined the role of hyperglycemia on endothelium-dependent and -independent vasodilation in humans, this is the first study that examines the impact of short-term hyperglycemia on coronary vasoreactivity. The present data show that acute, transient hyperglycemia does not affect the vasodilatory response of coronary microcirculation to dipyridamole in healthy subjects. Our findings apparently contradict previous studies that demonstrate a detrimental effect of acute hyperglycemia on peripheral vascular bed. Those studies have shown that endothelium-dependent vasodilation is impaired during short-term hyperglycemia in healthy subjects and that this alteration may be prevented by the administration of antioxidants (vitamins C and E), implying a role of oxidative stress in the genesis of vascular dysfunction (11–14). In addition, Beckman *et al.* (6) showed that local hyperglycemia, achieved through glucose infusion directly into the brachial artery, attenuates the vasodilatory response of the forearm microcirculation to endothelial agonists (11, 14). In contrast

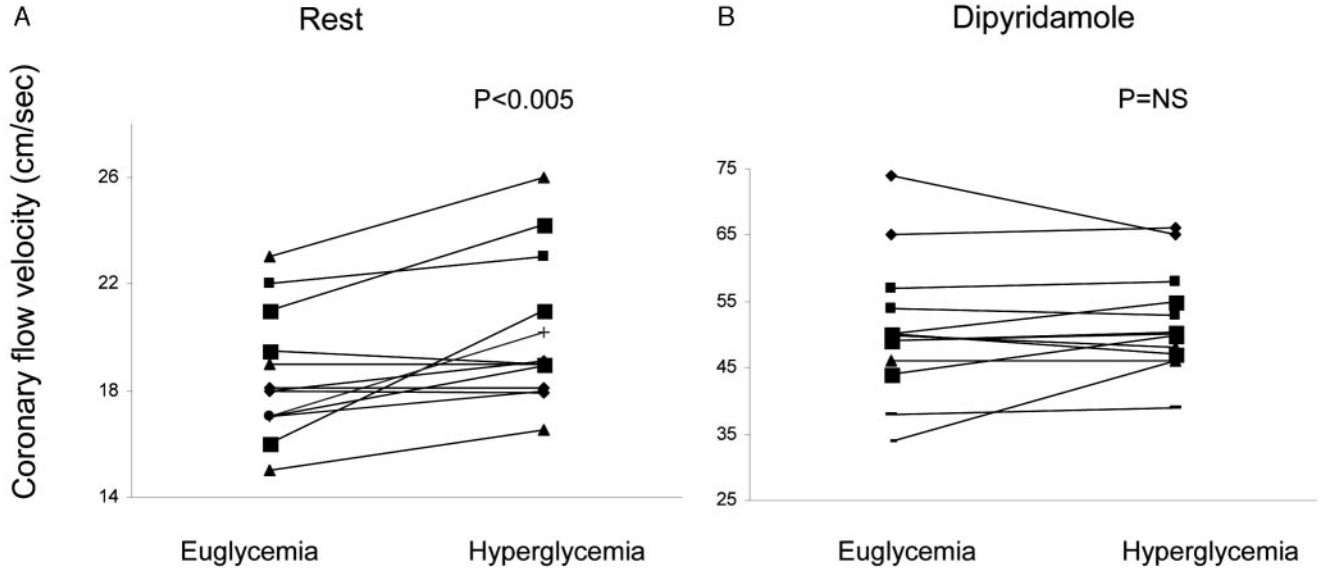

FIG. 1. Coronary flow velocity in the left anterior descending coronary artery at rest and after dipyridamole infusion in euglycemia and hyperglycemia.

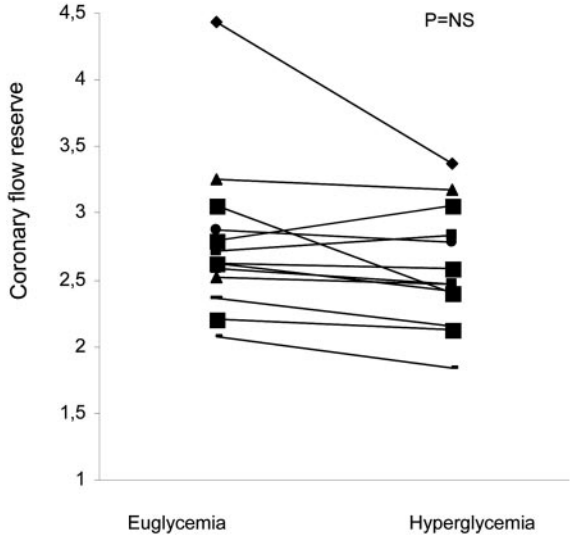

FIG. 2. CFR in healthy subjects in euglycemia and 3 h after hyperglycemia.

to these studies, Houben *et al.* (15) and, more recently, Reed *et al.* (16) could not find any impairment of vascular function in healthy subjects during either local or systemic hyperglycemia. These inconsistent results can, at least in part, be explained by methodological differences with regard to the technique used to assess endothelial function but also to the degree, duration, and pattern of hyperglycemia (16).

Very little is known on the impact of hyperglycemia on coronary vasoreactivity, and the few data available refer to animal models. In isolated rat hearts, 2-h exposure to severe hyperglycemia increases significantly the coronary perfusion pressure, implying an increased vascular tone (27). In instrumented dogs, acute hyperglycemia increases myocardial reactive oxygen species production and attenuates the vasodilatory response of LV descending coronary artery to graded infusion of acetylcholine. When animals are pretreated with tempol, a superoxide dismutase mimetic, the unfavorable effect of hyperglycemia is totally reversed (27). Noteworthy is the fact that, in these studies, hyperglycemia ranged from 350–600 mg/dl (27, 28). In contrast to these animal experiments, in the present study, we did not find any appreciable effect of short-term hyperglycemia on the vasoreactivity of coronary microcirculation in healthy subjects. To our knowledge, there are no other studies examining the impact of hyperglycemia on coronary vessels in humans and, therefore, our results cannot be compared in a larger context. However, there are pathophysiological considerations that make these findings interesting. First of all, vascular responses are heterogeneous in humans and often characteristic of the vascular bed in different regions; thus, the effect examined in one vessel region cannot be automatically extrapolated to other ones (29). For instance, local infusion of acetylcholine induces vasorelaxation in the forearm muscle compartment, whereas it produces vasoconstriction in the skin microcirculation (15). Similarly, insulin exerts a vasodilatory effect in the limb microcirculation (30) but not in large conduit arteries in which, in contrast, modest hyperinsulinemia impairs flow-mediated vasodilation (31). Because the response to stimuli seems to be vessel specific, it is conceivable that coronary microcirculation may respond differently to acute hyperglycemia with respect to peripheral microcirculation, also considering the peculiarity of coronary blood flow regulation. This is consistent with the great ability of coronary circulation to self-regulate blood flow across a wide spectrum of metabolic requirements and pressure gradients (32). Another factor contributing to explaining our finding is the duration of hyperglycemia. We cannot exclude that a more prolonged exposure and/or a higher degree of hyperglycemia, as induced in animal studies, could induce abnormalities of coronary vasomotor function, thus altering the hyperemic response to dipyridamole. Moreover, dipyridamole-induced dilation represents an integrated measure of endothelial function and vascular smooth muscle relaxation. The response to dipyridamole is not strictly comparable with that elicited in peripheral vessels by specific endothelial agonists, such as acetylcholine, because coronary vasodilation due to dipyridamole is primarily endothelium independent. However, the increment in coronary blood flow may trigger additional flow-induced vasodilation, which is indeed endothelium dependent (33).

It is important to underline that our findings, obtained during short-term hyperglycemia, cannot be extrapolated to pathophysiological conditions, such as diabetes mellitus or the metabolic syndrome, in which altered glucose levels are associated with a cluster of cardiovascular risk factors. In these states, manifest abnormalities of vascular function have been documented in both coronary and peripheral circulation (34). Interestingly, abnormal coronary vasomotor function, tested by low-dose dipyridamole, has been demonstrated recently in prediabetic subjects with insulin resistance, independent of the influence of body weight (35).

We found a mild elevation in resting coronary diastolic flow velocity during hyperglycemia. Under the assumption that the epicardial coronary artery caliper does not change substantially under the dose of dipyridamole we used (36), the increase in flow velocity can be produced by reduction of coronary resistance (a parameter not directly measurable by noninvasive tools) or by increase in blood flow. A vasodilator effect of insulin is unlikely because insulin levels during the hyperglycemic clamp were suppressed and the vasodilator effect of the hormone occurs at plasma concentration above 30 mU/liter (30). The possibility that some volume expansion due to the fluid infused during the study may have contributed to increase basal coronary flow can be excluded because we did not find any difference between coronary flow velocities measured at baseline and after saline infusion in a group of three normal volunteers. Another hypothesis is that such increase is consequent to plasma hyperosmolarity induced by hyperglycemia. This is supported by previous studies in healthy subjects showing that basal forearm blood flow increased to a similar extent either in hyperglycemia or during mannitol infusion designed to achieve a similar increase in osmolarity (11). Finally, noteworthy is the fact that the slight increase in resting coronary flow velocity was paralleled by a slight decrease in HR, suggesting that acute hyperglycemia may influence the sympathovagal balance. However, no information is available in the literature on the effect of acute hyperglycemia on the

autonomic system balance. Thus, it can be speculated that acute, short-term hyperglycemia affects resting coronary flow, whereas a more prolonged exposure to hyperglycemia is required to alter vasoreactivity of coronary microcirculation.

Limitations of the study

CFR ratio determined by transthoracic Doppler echocardiography presents some limitations that need to be highlighted. First, Doppler approach does not measure absolute flow but flow velocity gradient of left anterior descending artery, which is a reliable index of coronary flow provided that vessel diameter remains constant, especially during hyperemia. In this view, invasive studies with intracoronary Doppler ultrasound have shown that variations of epicardial vessel diameter in response to hyperemic stimuli are negligible (within 5%) (37). Second, according to the CFR model proposed by Hoffman (36), we have to consider that, in pathological conditions, CFR is lost first in the subendocardium and later on in the subepicardial layer of the myocardium. This differentiation cannot be unmasked by the current Doppler echocardiographic technique that explores flow velocities of distal left anterior descending artery, very proximal to subepicardial coronary microcirculation. Accordingly, subtle changes occurring during hyperglycemia at the subendocardial level of the myocardium could not have been detected in the present study. However, because we assessed healthy young subjects free of cardiovascular risk factors, an involvement of subendocardial microvessels is highly unlikely. Finally, we used dipyridamole at low dose (0.56 mg/kg) to measure hyperemic coronary flow and thus CFR. Although the standardized protocol for CFR assessment is still controversial, the use of low-dose dipyridamole might be considered insufficient to achieve maximal coronary vasodilation. This could have produced lower values of CFR ratio than those expected in a population of young healthy individuals. The choice to use low dose was based on the consideration that our subjects were free of coronary artery disease, and, in addition, high dose (0.84 mg/kg total dose) produces more marked decrease in BP and increase in both HR and myocardial contractility. The changes of these variables would make interpretation of data even harder. An additional reason to prefer low dose was that we decided not to use aminophylline to rapidly relieve symptoms to avoid possible interference of this substance on subsequent hemodynamic measurements. However, it is important to emphasize that coronary flow velocity during dipyridamole increased to the same extent in euglycemia and hyperglycemia, indicating that the response of coronary microcirculation to hyperemic, albeit not maximal, stimulus is well preserved.

In conclusion, moderate, short-term hyperglycemia does not affect the vasodilatory response of coronary microcirculation to low-dose dipyridamole in healthy humans.

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References

1. **Diabetes Control and Complications Trial Research Group** 1993 The effect of intensive treatment of diabetes on the development and progression of long term complications in insulin-dependent diabetes mellitus. N Engl J Med 329:977–986
2. **UK Prospective Diabetes Study (UKPDS) Group** 1998 Intensive blood glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 352:837–845
3. **Williams SB, Goldfine AB, Timimi FK, Ting HH, Roddy MA, Simonson DC, Creager MA** 1998 Acute hyperglycemia attenuates endothelium-dependent vasodilation in humans in vivo. Circulation 97:1695–1701
4. **Kawano H, Motoyama T, Hirashima O, Hirai N, Myiao Y, Sakamoto T, Kugiyama K, Ogawa H, Yasue H** 1999 Hyperglycemia rapidly suppresses flow-mediated endothelium-dependent vasodilation of brachial artery. J Am Coll Cardiol 34:146–154
5. **Title LM, Cummings PM, Giddens K, Nassar BA** 2000 Oral glucose loading acutely attenuates endothelium-dependent vasodilation in healthy adults without diabetes: an effect prevented by vitamins C and E. Am J Coll Cardiol 36:2185–2191
6. **Beckman JA, Goldfine AB, Gordon MB, Creager MA** 2001 Ascorbate restores endothelium-dependent vasodilation impaired by acute hyperglycemia in humans. Circulation 103:1618–1623
7. **Umpierrez GE, Isaacs SD, Bazargan N, You X, Thaler LM, Kitabchi AE** 2002 Hyperglycemia: an independent marker of in-hospital mortality in patients with undiagnosed diabetes. J Clin Endocrinol Metab 87:978–982
8. **Capes SA, Hunt D, Malmberg K, Gerstein H** 2000 Stress hyperglycemia and increased risk of death after myocardial infarction in patients with and without diabetes: a systematic overview. Lancet 355:773–778
9. **Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, Brownlee M** 2000 Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature 404:787–790
10. **Gutterman DD** 2002 Vascular dysfunction in hyperglycemia. Circ Res 90:5–11
11. **Cosentino F, Eto M, De Paolis P, Van der Loo B, Bachschmid M, Ullrich V, Kouroedov A, Delli Gatti C, Joch H, Volpe M, Luscher TF** 2003 High glucose causes upregulation of cyclooxygenase-2 and alters prostanoid profile in human endothelial cells: role of protein kinase C and reactive oxygen species. Circulation 107:1017–1023
12. **Gresele P, Guglielmini G, De Angelis M, Ciferri S, Ciofetta M, Falcinelli E, Lalli C, Ciabattoni G, Davì G, Bolli GB** 2003 Acute, short-term hyperglycemia enhances shear stress-induced platelet activation in patients with type II diabetes mellitus. J Am Coll Cardiol 41:1013–1020
13. **Marfella R, Esposito K, Giunta R, Coppola G, De Angelis L, Farzati B, Paolis G, Giugliano D** 2000 Circulating adhesion molecules in humans role of hyperglycemia and hyperinsulinemia. Circulation 101:2247–2251
14. **Esposito K, Nappo F, Marfella R, Giugliano G, Giugliano F, Ciotola M, Quagliaro l, Cervello A, Giugliano D** 2002 Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans. Role of oxidative stress. Circulation 106:2067–2072
15. **Houben AJHM, Schaper NC, De Hann CHA, De Mann CH, Huvers FC, Slaaf DW, De Leeuw PW, Nieuwenhuijzen Kruseman C** 1996 Local 24-h hyperglycemia does not affect endothelium-dependent or -independent vasoreactivity in humans. Am J Physiol 270:H2014–H2020
16. **Reed AS, Charkoudian N, Vella A, Shah P, Rizza RA, Joyner MJ** 2004 Forearm vascular control during acute hyperglycemia in healthy humans. Am J Physiol 286:E472–E480
17. **Lambertz H, Tries HP, Stein T, Lethen H** 1999 Noninvasive assessment of coronary flow reserve with transthoracic signal-enhanced Doppler echocardiography. J Am Soc Echocardiogr 12:186–195
18. **Caiati C, Montaldo C, Zedda N, Bina A, Iliceto S** 1999 A new noninvasive method for coronary flow reserve assessment: contrast-enhanced transthoracic second harmonic echo Doppler. Circulation 99:771–778
19. **Nasher Jr PJ, Brown RE, Oskarsson H, Winniford MD, Rossen JD** 1995 Maximal coronary flow reserve and metabolic coronary vasodilation in patients with diabetes mellitus. Circulation 91:635–640
20. **Strauer BE, Motz W, Vogt M, Schwartzkopff B** 1997 Impaired coronary flow reserve in NIDDM: a possible role for diabetic cardiopathy in humans. Diabetes 46 (Suppl 2):S119–S124
21. **Capaldo B, Santoro D, Riccardi G, Perrotti N, Saccà L** 1986 Direct evidence for a stimulatory effect of hyperglycemia per se on peripheral glucose disposal in type II diabetes. J Clin Invest 77:1285–1290
22. **Moller N, Bagger JP, Schmitz O, Jorgensen JO, Moller J, Alberti KG, Orskov H** 1995 Somatostatin enhances insulin-stimulated glucose uptake in the perfused human forearm. J Clin Endocrinol Metab 80:1789–1793
23. **de Simone G, Scalfi L, Galderisi M, Cementano A, Di Biase G, Tammaro P, Garofano M, Mureddu GF, DeDivitiis O, Contaldo F** 1994 Cardiac abnormalities in young women with anorexia nervosa. Br Heart J 71:287–292
24. **de Simone G, Devereux RB, Roman MJ, Ganau A, Saba PS, Alderman MH, Laragh JH** 1994 Assessment of left ventricular function by the midwall function

shortening/end-systolic stress relation in human hypertension. J Am Coll Cardiol 23:1444–1451
25. **Galderisi M, Paolisso G, Tagliamonte MR, Alfieri A, Petrocelli A, De Divitiis M, Varricchio M, De Divitiis O** 1997 Is insulin action a determinant of left ventricular relaxation in uncomplicated essential hypertension? J Hypertens 15:745–750
26. **Galderisi M, Cicala S, Caso P, De Simone L, D'Errico A, Petrocelli A, De Divitiis O** 2002 Coronary flow reserve and myocardial diastolic dysfunction in arterial systemic hypertension. Am J Cardiol 90:860–864
27. **Ceriello A, Quagliaro L, D'Amico M, Di Filippo C, Martella R, Nappo F, Berrino L, Rossi F, Giugliano D** 2002 Acute hyperglycemia induces nitrotyrosine formation and apoptosis in perfused heart from rat. Diabetes 51:1076–1082
28. **Gross ER, LaDisa Jr JF, Weihrauch D, Olson LE, Kress TT, Hettrick DA, Pagel PS, Warltier DC, Kersten JR** 2003 Reactive oxygen species modulate coronary wall shear stress and endothelial function during hyperglycemia. Am J Physiol Heart Circ Physiol 284:H1552–H1559
29. **Arcaro G, Solini A, Monauni T, Cretti A, Brunato B, Lechi A, Fellin R, Caputo M, Cocco C, Bonora E, Muggeo M, Bonadonna RG** 2000 ACE genotype and endothelium-dependent vasodilation of conduit arteries and forearm microcirculation in humans. Arterioscler Thromb Vasc Biol 21:1313–1319
30. **Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD** 1996 Obesity/insulin resistance is associated with endothelial dysfunction: implication for the syndrome of insulin resistance. J Clin Invest 97:2601–2610
31. **Arcaro G, Cretti A, Balzano S, Lechi A, Muggeo M, Bonora E, Bonadonna RC** 2002 Insulin causes endothelial dysfunction in humans. Sites and mechanisms. Circulation 105:576:582
32. **Driscol TE, Moir TW, Eckstein RW** 1964 Autoregulation of coronary blood flow: effect of interarterial pressure gradients. Circ Res 15:103–111
33. **Drexler H, Zeiber AM, Wollschlager H, Meinertz T, Just H, Bonzel T** 1989 Flow-dependent coronary artery dilation in humans. Circulation 80:466–474
34. **Calles-Escandon J, Cipolla M** 2001 Diabetes and endothelial dysfunction: a clinical perspective. Endocr Rev 22:36–52
35. **Quinones MJ, Hernandez Pampaloni M, Schelbert H, Bulsen-Enriquez I, Jimenez X, Hernandez G, De La Rosa R, Chon Y, Yang H, Nicholas SB, Modilevsky T, Yu K, Van Herle K, Castellani LW, Elashoff R, Hsueh WA** 2004 Coronary vasomotor abnormalities in insulin-resistant individuals. Ann Inter Med 140:700–708
36. **Hoffman JIE** 2000 Problems of coronary flow reserve. Ann Biomed Eng 28: 884–896
37. **Sudhir K, MacGregor JS, Barbant SD, Barbant SD, Foster E, Fitzgerald PJ, Chatterjee K, Yock PG** 1993 Assessment of coronary conductance and resistance vessel reactivity in response to nitroglycerin, ergonevine and adenosine: in vivo studies with simultaneous intravascular two-dimension and Doppler ultrasound. J Am Coll Cardiol 21:1261–1268

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