

## Effects of short-term hyperglycemia on the vasoconstriction of the aorta

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Received: 25.07.2013 • Accepted: 23.12.2013 • Published Online: 24.10.2014 • Printed: 21.11.2014

**Background/aim:** To investigate vasoconstrictor responses of healthy blood vessels to short-term hyperglycemic conditions such as postprandial hyperglycemia, gestational diabetes mellitus, and steroid-induced diabetes.

**Materials and methods:** Female Wistar rat aorta rings were incubated in normal (11 mM) and high (22 mM and 44 mM) glucose concentrations for 4 h. Responses of vasoconstriction were measured in reaction to serotonin (10–5 M), phenylephrine (10–6 M), and KCl (60 mM) compared to the ambient condition, including different glucose concentrations.

**Results:** While the responses of vasoconstriction to KCl were increased in the presence of Krebs' solution with high glucose, no statistically significant changes were observed in the reaction to serotonin and phenylephrine. In addition, malondialdehyde levels were increased in hyperglycemic conditions.

**Conclusion:** Short-term hyperglycemia may lead to augmented contractile response in aorta rings through several mechanisms, and our results showed that oxidative stress is probably one of them.

**Key words:** Short-term hyperglycemia, vasoconstriction, oxidative stress

### 1. Introduction

Hyperglycemia includes a number of different parameters (acute, chronic, and postprandial hyperglycemia), all of which play a role in the vascular damage caused by high glucose levels (1). Short-term hyperglycemia is regarded as a significant risk factor for several diseases (2). Having high glucose levels during an acute cardiovascular event is relevant to a worse prognosis, even in nondiabetic states (3,4). One of the mechanisms of hyperglycemia-induced vascular damage is associated with the increased production of oxygen free radicals from endothelial cells, such as superoxide anion, which deactivate nitric oxide (NO) (5–7). Depletion of NO results in augmented contractility and proliferation of vascular smooth muscle cells with increased vasomotor tone (6), platelet hyperactivity (8), change of the adhesive properties of the endothelium (9), and elevated production of cytokines (10). Each of these mechanisms produce reactive oxygen species, reflected in an overall increased state of cellular oxidative stress (11).

It is well known that exposure to hyperglycemia is common in nondiabetic people in cases such

as postprandial hyperglycemia, gestational diabetes mellitus, steroid-induced diabetes, or impaired oral glucose tolerance tests. However, it is unclear how short-term hyperglycemia affects vascular contractility in healthy subjects. It can be claimed that this is the first experimental research investigating differences in response to some vasoconstrictor agents in short-term hyperglycemia. We assessed rat aorta contractility responses to both normoglycemic Krebs' solution and hyperglycemic Krebs' solution in an isolated organ bath.

### 2. Materials and methods

Our experimental protocol was approved by our institutional review board. Nine female Wistar rats were used in this study, weighing 200–250 g. The animals were sacrificed via cervical dislocation, and thoracic aortas were dissected immediately and were placed in oxygenated normal Krebs' solution. Arterial segments were cut into rings of about 3 mm, and the adhering perivascular tissue was carefully removed. Two triangular steel strings were placed into the lumen to determine tension alterations.

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One of the steel strings was fixed to the bottom of an in vitro chamber, and the other was attached to a tension transducer. The solutions were kept at 37 °C and were gassed with 95% air and 5% CO<sub>2</sub>. The vessels were superfused with normal Krebs' solution (composition in mmol/L: CaCl<sub>2</sub> 2.5, KCl 4.7, MgSO<sub>4</sub> 1.5, NaCl 119, NaHCO<sub>3</sub> 25, KH<sub>2</sub>PO<sub>4</sub> 1.2, and glucose 11) under 1.5 g tension for 1 h to obtain stabilization. The presence of intact endothelium was verified in each ring by indicating normal relaxation to acetylcholine (10<sup>-5</sup> M) after a submaximal contraction to KCl. Following that, rings were washed with normal Krebs' solution, and they were allowed to stabilize for 30 min. Vessels were then made to contract by adding serotonin (5-HT) (10<sup>-5</sup> M); afterwards, they were washed so that the tension returned to the basal level. The stimuli were then repeated again by adding phenylephrine (PE) (10<sup>-6</sup> M) and KCl (60 mM). After this, the aorta rings were placed in one of the following groups in separate repeating experiments. Experiments were carried out in 3 groups. The aortic rings in each group were incubated for 4 h in the following media: 1st group, normoglycemia (NG), 11 mM glucose (198 mg/dL) in Krebs' solution (12); 2nd group, high glucose 1 (HG-1), 22 mM glucose (396 mg/dL) in Krebs' solution; and 3rd group, high glucose 2 (HG-2), 44 mM glucose (792 mg/dL) in Krebs' solution (12). The incubation media were changed every 30 min. After 4 h of incubation, rings in each group were contracted by 5-HT (10<sup>-5</sup> M), PE (10<sup>-6</sup> M), and KCl (60 mM), respectively, and contractile responses were recorded by ProtoWin v 1.0 software.

### 2.1. Determinations of lipid peroxidation level

Tissue was homogenized using a motor-driven tissue homogenizer with phosphate buffer (pH 7.4). Unbroken cells, cell debris, and nuclei were sedimented at 2000 × g for 10 min, and the supernatant was collected into plastic tubes and stored at 70 °C until being assayed.

Malondialdehyde (MDA) levels, an indicator of free radical generation that increases at the end of lipid peroxidation, were estimated using the double heating method of Draper and Hadley (13). The main principle behind this method is the spectrophotometric measurement of the color generated by the reaction of thiobarbituric acid (TBA) with MDA. For this objective, 2.5 mL of 100 g/L trichloroacetic acid solution was added in each centrifuge tube containing 0.5 mL of supernatant, and the tubes were placed in a boiling water bath for 15 min. After the tubes were cooled in tap water, they were centrifuged at 1000 × g for 10 min, and 2 mL of the supernatant was added to 1 mL of 6.7 g/L TBA solution in a test tube. It was then incubated in a boiling water bath for 15 min. After the solution was cooled under tap water, its absorbance was measured using a spectrophotometer (Shimadzu UV-1601, Japan) at 532 nm. The concentration

of MDA was evaluated by the absorbance coefficient of the MDA-TBA complex (absorbance coefficient: 1.56 × 10<sup>5</sup> cm/M) and was represented as nanomoles per gram of wet tissue.

### 2.2. Statistics

The results were shown as mean ± standard error of the mean (SEM) for each group. Analysis of variance (ANOVA) was performed between the groups to determine the statistically significant differences. Differences with a P-value of lower than 0.05 were accepted as significant. GraphPad Prism 5 (trial) software was used for statistical calculations.

## 3. Results

### 3.1. Effects of hyperglycemia on KCl-induced contractions in aortic rings

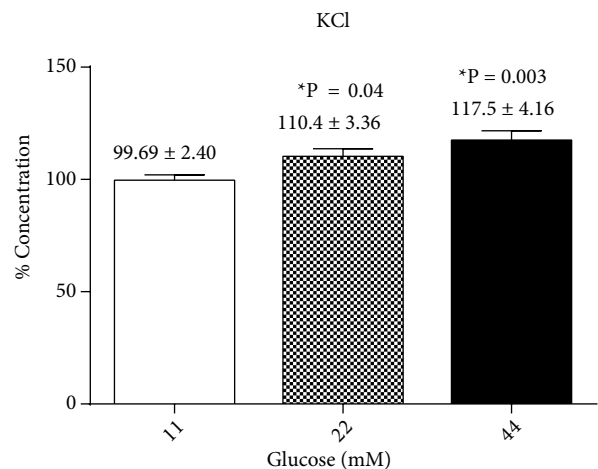
Compared with the NG group, incubation with HG-1 and HG-2 for 4 h increased the KCl-induced contraction of rings, and statistically significant changes were observed (Figure 1).

### 3.2. Effects of hyperglycemia on 5-HT-induced contractions in aortic rings

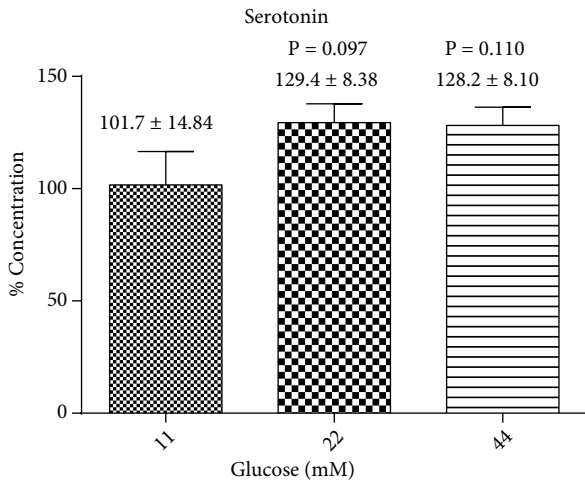
Compared with the NG group, incubation with HG-1 and HG-2 for 4 h increased the 5-HT-induced contraction of rings, but no statistically significant changes were observed (Figure 2).

### 3.3. Effects of hyperglycemia on PE-induced contractions in aortic rings

Compared with the NG group, incubation with HG-1 for 4 h decreased the PE-induced contractions, while they slightly increased with HG-2. Neither change was statistically significant (Figure 3).



**Figure 1.** Effects of hyperglycemia on KCl-induced contractions in aortic rings. Each bar and line represent the mean ± SEM. n = 5 in each group. \*: P < 0.05 vs. 11 mM glucose.



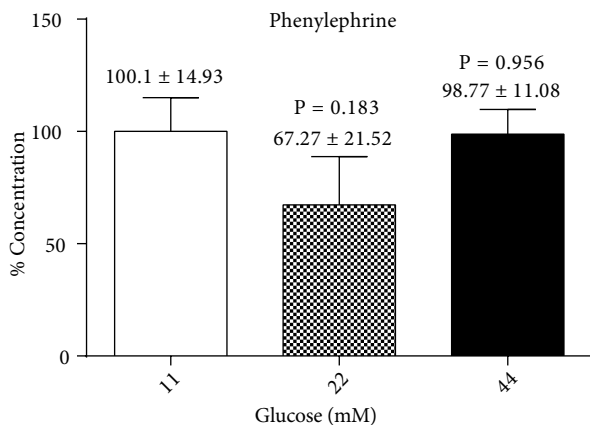
**Figure 2.** Effects of hyperglycemia on 5-HT-induced contractions in aortic rings. Data are expressed as mean ± SEM. n = 5 in each group.

### 3.4. MDA levels

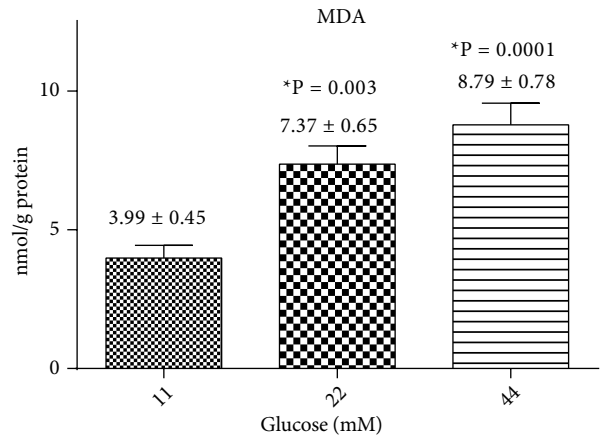
The MDA level was significantly increased with HG-1 and HG-2 compared to NG (Figure 4).

### 4. Discussion

In this study, the contractile responses to KCl were recorded in rat aortas with different glucose concentrations. The contractile responses increased in hyperglycemic conditions, and the increases were statistically significant. In addition, contractile responses to 5-HT in hyperglycemic conditions increased, but the responses were not significant. Contractile responses to PE in 22 mM glucose concentration diminished, but the decrease was not significant. Although there are many studies about chronic exposure to high concentrations



**Figure 3.** Effects of hyperglycemia on PE-induced contractions in aortic rings. Data are expressed as mean ± SEM. n = 5 in each group.



**Figure 4.** MDA levels in rat aortic tissue. Data are expressed as mean ± SEM. n = 5 in each group. \*: P < 0.05 vs. 11 mM glucose.

of glucose in rat aortas, limited studies have been done about short-term hyperglycemia so far. This is one of the first experimental studies on the response differences of some vasoconstrictor agents of short-term hyperglycemia.

The occurrence of hyperglycemia may happen as follows. Oxidative stress may appear in the postprandial phase. Triglycerides play an important role in this appearance. Subsequently, it may result in low-density lipoprotein oxidation, platelet activation, endothelial function disorders, and, finally, cardiovascular events (14).

There are not enough studies about the effects of hyperglycemia on the contractile response of animal vessels (15,16). Different results have been obtained from different studies. Houben et al. found that the contractile responses to norepinephrine on vessels of healthy people, who were treated using the forearm perfusion techniques, were not changed after short-term hyperglycemia (1 and 7 h) (17,18). When the contractile responses to 80 mmol/L KCl in high glucose concentration (11 mM Tyrode solution) were compared to the contractile responses in normoglycemic conditions (5.5 mM Tyrode solution), Nava et al. found that the contractile responses decreased in hyperglycemic conditions (15). In addition, Hamaty et al. reported that contractile responses to KCl and norepinephrine did not change the response of the tail artery of rat after it had been incubated at a high glucose concentration (20 mM) for 2 h (16). In our study, the increase of the contractile responses to KCl (60 mM) was statistically significant in rat aortas at high glucose concentrations (22 and 44 mM). On the other hand, there was not any significant change in the contractile responses to 5-HT ( $10^{-5}$  M) or PE ( $10^{-6}$  M).

The reasons for the different results are not known, but they may be connected to the following factors:

- The species and age of animal used in the experiment,

- The material and methods used in the experiment,
- The type of the vessel used in the experiment,
- The incubation period of vessels in hyperglycemic solution,
- The calculation methods for contractile responses,
- Whether there is undamaged endothelium on the vessel rings.

When vessel tension is evaluated, stable vasodilatation is predominantly observed in healthy animals and humans who have normal endothelial function and normal blood pressure. Many studies have revealed that the endothelial function of the vessel is impaired during hyperglycemia. Production of vasodilator substances, including NO, endothelium-derived hyperpolarizing factor, and prostaglandin I<sub>2</sub>, decreases, or degradation of these substances increases when the endothelial function is impaired. In this condition, the balance of hemodynamic stability is changed as vasoconstrictor substances become dominant (19). As a consequence of the balance changes, an increase of myogenic tension in vascular smooth muscle and hypertrophy are expected in the long-term period (20).

In our study, the reason for an increase in contractile responses to KCl and 5-HT can be explained with a relative increase of the efficiency of vasoconstrictor substances in comparison with vasodilator substances. The contractile response to KCl is mainly mediated by voltage-dependent Ca<sup>2+</sup> influx (21), and it may be partly due to Ca<sup>2+</sup> sensitization via activation of protein kinase C (PKC) (22). Hyperglycemia can activate PKC by increasing diacylglycerol production (23). This mechanism may be responsible for the increase of contractile responses to KCl in our study.

The contractile responses to PE were decreased under HG-1 conditions in our study. This is similar to the study performed by Meng et al., in which acute exposure of blood vessels to high glucose for more than 3 h showed decreased PE-induced contractions (24). On the other hand, the contractile responses to PE under HG-2 conditions were not statistically significant in our study. This is similar to the study performed by Houben et al., in which the contractile responses to norepinephrine were not changed by short-term hyperglycemia (17).

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Oxidative stress may occur in the body via many mechanisms during acute hyperglycemia (25). We examined the levels of MDA, which is the last product of lipid peroxidation, to show whether there was oxidant damage or not. MDA levels increased significantly ( $P < 0.05$ ) with both hyperglycemic solutions (HG-1 and HG-2) when compared to the NG solution. With this result, one of the responsible mechanisms for the increase of contractile responses in aorta rings in hyperglycemic solution may be explained as endothelial impairment caused by oxidant damage. Williams et al. indicated that endothelium-dependent vasodilatation was diminished by acute hyperglycemia in healthy, nondiabetic humans in vivo (26). Therefore, the effect of vasoconstrictor substances on vascular tension may be increased and contractile responses may be augmented.

Evliyaoglu et al. recently reported that CPK-MB/CPK levels, an indicator of an acute myocardial infarction, increased in hyperglycemia (27). In hyperglycemic conditions, both destruction of heart muscle cells (27) and oxidative stress and vasoconstrictor response to vasoconstrictor agents like 5-HT increased, as in this study. We suggest that this may increase the risk of cardiovascular events such as myocardial infarction, unstable angina pectoris, temporal brain ischemia, and stroke.

Hyperglycemia most likely causes some changes in vascular responses and contractile activity in response to different normal physiological substances. According to our results, one of the mechanisms of this alteration may be the increase of oxidative stress in hyperglycemic conditions. When we compare our results with other knowledge from the literature, it is clear that protection against hyperglycemia is very important, even for healthy people, in order to prevent cardiovascular risk. It is also suggested that when the effects of a hyperglycemic condition on the responses of vascular dilatation are investigated in vitro or in vivo, it should first be evaluated whether or not there has been a change in vascular contractions under hyperglycemic conditions.

## Acknowledgment

The study was supported by the Süleyman Demirel University Scientific Research Projects Coordination Unit (Project no.: 1562-YL-07).

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