Transient Elevation of Glucose Increases Arrhythmia Susceptibility in Non-Diabetic Rat Trabeculae With Non-Uniform Contraction

Masahito Miura, MD, PhD; Tetsuya Handoh; Yuhto Taguchi; Taiki Hasegawa; Yui Takahashi; Natsuki Morita; Ayana Matsumoto; Chiyohiko Shindoh, MD, PhD; Haruka Sato, MD, PhD

Background: In non-diabetic patients with acute coronary syndrome, stress hyperglycemia occasionally occurs and is related to their mortality. Whether transient elevation of glucose affects arrhythmia susceptibility in non-diabetic hearts with non-uniform contraction was examined.

Methods and Results: Force, intracellular Ca\textsuperscript{2+} ([Ca\textsuperscript{2+}]), and membrane potential were measured in trabeculae from rat hearts. Non-uniform contraction was produced by a jet of paralyzing solution. Ca\textsuperscript{2+} waves and arrhythmias were induced by electrical stimulation (2.0 mmol/L [Ca\textsuperscript{2+}]). The activity of Ca\textsuperscript{2+}/calmodulin-dependent protein kinaseII (CaMKII) was measured. An elevation of glucose from 150 to 400 mg/dL increased the velocity of Ca\textsuperscript{2+} waves and the number of spontaneous action potentials triggered by electrical stimulation. Besides, the elevation of glucose increased the CaMKII activity. In the presence of 1 μmol/L KN-93, the elevation of glucose did not increase the velocity of Ca\textsuperscript{2+} waves and the number of triggered action potentials. In addition, in the presence of 1 μmol/L autocamtide-2 related inhibitory peptide or 50 μmol/L diazo-5-oxonorleucine, the elevation of glucose did not increase the number of triggered action potentials. Furthermore, the elevation of glucose by adding L-glucose did not increase their number.

Conclusions: In non-diabetic hearts with non-uniform contraction, transient elevation of glucose increases the velocity of Ca\textsuperscript{2+} waves by activating CaMKII, probably through glycosylation with O-linked β-N-acetylglucosamine, thereby increasing arrhythmia susceptibility.

Key Words: Arrhythmia; Calcium wave; Glucose

Stress hyperglycemia is transient hyperglycemia during severe disease such as acute myocardial infarction or cerebrovascular events in patients without known diabetes and is caused by a complex interplay of catecholamines, growth hormone, cortisol, and cytokines. In such non-diabetic patients, admission blood glucose level is associated with increased in-hospital and 30-day mortality when suffering from acute myocardial infarction or unstable angina. Importantly, non-diabetic patients have a higher 30-day mortality than diabetic patients, and elevated admission glucose is more important than abnormal HbA\textsubscript{1c} level in predicting mortality. In addition, in non-diabetic patients with heart failure, admission glucose level is also associated with higher in-hospital and 60-day mortality. It has not yet been clarified, however, whether in past reports, the patients with stress hyperglycemia died suddenly of heart disease.

In patients with acute coronary syndrome, impaired muscle is distributed within their hearts and causes non-uniform muscle contraction due to its weaker contractile strength. Within the hearts with non-uniform contraction, impaired muscle is stretched by contractions of more viable neighboring muscle during the contraction phase and inversely shortened during the relaxation phase. Shortening of cardiac muscle dissociates Ca\textsuperscript{2+} from the myofilaments and causes arrhythmias by accelerating Ca\textsuperscript{2+} waves. Thus, it is important to investigate whether an elevation of glucose affects the velocity of Ca\textsuperscript{2+} waves and the occurrence of arrhythmias in the myocardium with non-uniform contraction.

Elevation of glucose causes glycosylation of serine or threonine residue on cytosolic, nuclear, and mitochondrial proteins by O-linked β-N-acetylgalactosamine (O-GlcNAc) and O-GlcNAcylation in the cardiovascular system, L-glutamine-D-fructose-6-phosphate amidotransferase 1 (GFAT1) channels a small fraction of glucose into the hexosamine biosynthetic pathway (HBP) and leads to modification of a variety of proteins by O-GlcNAcylation. In the case of diabetic hearts, long-lasting hyperglycemia causes O-GlcNAcylation of contractile proteins and decreases the
myofilament Ca\(^{2+}\) sensitivity.\(^{24-25}\) In addition, such hyperglycemia activates Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaMKII) by O-GlcNAcylation and increases the occurrence of arrhythmias by an increase in Ca\(^{2+}\) leak from the sarcoplasmic reticulum (SR).\(^{26}\) It has not yet been established, however, whether in non-diabetic patients with non-uniform contraction, transient hyperglycemia such as stress hyperglycemia also activates CaMKII by O-GlcNAcylation and affects the mortality by increasing arrhythmia susceptibility.

Therefore, in the present study, we focused on Ca\(^{2+}\) wave propagation in the myocardium with non-uniform contraction, investigating how transient elevation of glucose affects the occurrence of arrhythmias in non-diabetic hearts. Our results suggest that in the myocardium with non-uniform contraction, transient elevation of glucose increases the velocity of Ca\(^{2+}\) waves by activating CaMKII, probably through O-GlcNAcylation and thereby increases the occurrence of arrhythmias.

### Methods

See Expanded Methods in the Supplementary File.

**Measurements of Force, Membrane Potential, [Ca\(^{2+}\)], ROS, and CaMKII**

All experimental protocols were approved by the Tohoku University Animal Care and Use Committee (approval reference number: 2015-023, 2016-062). After rats were adequately anesthetized, trabeculae were obtained from the right ventricles of their hearts. Force, membrane potential, and [Ca\(^{2+}\)] \(^{i}\) were measured, as shown in Figure 1A.\(^{15-17}\)

To estimate changes in ROS, trabeculae were loaded with 2',7'-dichlorofluorescein (DCF), as previously described.\(^{27}\)

The CaMKII activity in cardiac muscle was measured using an ELISA-based kit (CycLex CaM Kinase II Assay Kit) according to the manufacturer’s protocol\(^{28,29}\) and was normalized using the volume of sample protein (Pierce™ BCA Protein Assay Kit). To create a non-uniform contraction model, trabeculae were regionally exposed to a jet of a solution containing 20 mmol/L 2,3-butanedione monoxime (BDM), as shown in Figure 1B.\(^{15-17}\)

When blebbistatin was used, fluorescence images were recorded a few minutes after excluding blebbistatin from the solution because blebbistatin has fluorescent properties.\(^{30,31}\)

### Experimental Protocol With Trabeculae

Ca\(^{2+}\) waves were induced by electrical stimulation (400-ms stimulus intervals for 7.5 s), and arrhythmias were induced by electrical stimulation (250-ms stimulus intervals for 15 s) in the presence of 0.2 \(\mu\)mol/L isoproterenol, as previously described.\(^{15-17}\)

To inhibit CaMKII, 1 \(\mu\)mol/L KN-93 or 1 \(\mu\)mol/L autocamtide-2 related inhibitory peptide (AIP) was added. In addition, to inhibit GFAT1, 50 \(\mu\)mol/L diazo-5-oxonorleucine (DON) was added. All measurements were performed at 24ºC. In the present study, we used D-glucose as “glucose”, unless otherwise mentioned.

### Statistical Analyses

All measurements were expressed as mean±SEM. Statistical analysis was performed with a paired t-test for 2-group comparisons and 1-way repeated-measures ANOVA with Bonferroni for multiple comparisons when the data were normally distributed. Otherwise, the Wilcoxon signed-ranks test was used for 2-group comparisons. These analyses were performed using software for statistical analysis (Ekuseru-Toukei 2012; Social Survey Research Information Co., Ltd, Tokyo, Japan). Values of P<0.05 were considered to be significant.

### Results

**Effect of Glucose on Force and Ca\(^{2+}\) Transients**

Effect of glucose concentration on the developed force and Ca\(^{2+}\) transients was examined at 0.7 mmol/L [Ca\(^{2+}\)] \(^{o}\) (2-s stimulus intervals) and 2.0 mmol/L [Ca\(^{2+}\)] \(^{o}\) (0.2 \(\mu\)mol/L isoproterenol, 2-s stimulus intervals). As shown in Figure 2A and B, the developed force and Ca\(^{2+}\) transients did not
Elevation of Glucose Increases Arrhythmias

The effect of glucose concentration on Ca$^{2+}$ waves and aftercontractions was first examined in the myocardium with non-uniform contraction. Under higher Ca$^{2+}$-load (2.0 mmol/L [Ca$^{2+}$]), 0.4-s stimulus), Ca$^{2+}$ waves were induced by electrical stimulation in trabeculae exposed to a BDM jet (Figure 3A). As shown in Figure 3A and B, 1 h after elevation of glucose from 150 to 400 mg/dL, the velocity of Ca$^{2+}$ waves and the amplitude of aftercontractions were increased. Conversely, 1 h after reduction of glucose from 400 to 150 mg/dL they were decreased. Because it has

**Figure 2.** Effect of glucose concentration on force and intracellular Ca$^{2+}$ ([Ca$^{2+}$]). (A) Representative recordings of force (Upper panel) and [Ca$^{2+}$] (Lower panel) during electrical stimulation (2-s stimulus intervals, 0.7 mmol/L [Ca$^{2+}$]). They were measured at 150 mg/dL glucose (black lines), 1 h after elevation of glucose from 150 to 400 mg/dL (red lines), and 1 h after reduction of glucose from 400 to 150 mg/dL (blue lines) (24°C, Exp.141008). (B) Summary data concerning the effect of glucose on the force (Upper left panel), the peak [Ca$^{2+}$] (Upper right panel), diastolic [Ca$^{2+}$] (Lower left panel), and the time constant of [Ca$^{2+}$] decline (Lower right panel) during electrical stimulation (2-s stimulus intervals, 0.7 mmol/L [Ca$^{2+}$]). (C) Representative recordings of force and [Ca$^{2+}$] during electrical stimulation (2-s stimulus intervals) at 2.0 mmol/L [Ca$^{2+}$] in the presence of 0.2 µmol/L isoproterenol (ISO). They were measured before (black lines) and 1 h after elevation of glucose from 150 to 400 mg/dL (red lines). The black arrow indicates an aftercontraction at 150 mg/dL glucose, and the red arrow indicates an aftercontraction 1 h after elevation of glucose to 400 mg/dL (24°C, Exp.191205). (D) Summary data concerning the effect of glucose on the force (Upper left panel), the peak [Ca$^{2+}$] (Upper right panel), diastolic [Ca$^{2+}$] (Lower left panel), and the time constant of [Ca$^{2+}$] decline (Lower right panel) during electrical stimulation (2-s stimulus intervals) at 2.0 mmol/L [Ca$^{2+}$] in the presence of 0.2 µmol/L isoproterenol. *P<0.05 vs. 150.
been reported that an elevation of glucose activates CaMKII by glycosylating it.²⁶ CaMKII activity was measured before and 1 h after elevation of glucose from 150 to 400 mg/dL. As shown in Figure 3C, the elevation of glucose to 400 mg/dL increased CaMKII activity. Also, in the presence of 1 μmol/L KN-93, an inhibitor of CaMKII, the velocity of Ca²⁺ waves and the amplitude of aftercontractions were not increased 1 h after elevation of glucose to 400 mg/dL (Figure 3D). These results suggest that an elevation of glucose increases the velocity of Ca²⁺ waves and the amplitude of aftercontractions by activating CaMKII.

Effect of Glucose on Arrhythmia Susceptibility
As the next step, we examined the effect of glucose concentration on the occurrence of arrhythmias in trabeculae with non-uniform contraction (0.2 μmol/L isoproterenol, 2.0 mmol/L [Ca²⁺]). As shown in Figure 4A, electrical stimulation induced action potentials; that is, arrhythmias in trabeculae exposed to a BDM jet at glucose concentration of 150 mg/dL. One hour after elevation of glucose to 400 mg/dL, these action potentials were increased, as shown in Figure 4A and C. In contrast, after the addition of KN-93 to the bath superfusate, action potentials induced by electrical stimulation were not increased 1 h after elevation of glucose from 150 to 400 mg/dL (Figure 4B). Figure 4C shows the summary data concerning the effects of KN-93, AIP, and DON, and the effect of the addition of 250 mg/dL L-glucose to 150 mg/dL glucose on the number of action potentials induced by electrical stimulation. The elevation of glucose did not increase the number of action potentials in the presence of KN-93, AIP, or DON. In addition, the increase in the osmotic pressure by the addition of L-glucose did not increase their number. Taken together, these results suggest that an elevation of glucose

![Figure 3](image-url)

**Figure 3.** Effect of glucose concentration on the velocity of Ca²⁺ waves and the amplitude of aftercontractions. (A) Representative recordings of force (Upper panels) and regional changes in [Ca²⁺] (Lower panels) during the last three electrical stimuli indicated by arrows with ST (0.4-s stimulus intervals) and the following aftercontractions (red arrows). In the Lower panels, Ca²⁺ waves (white arrows) appeared around the jet-exposed region (BDM Jet) after the train of electrical stimuli and propagated along the trabeculae. (a) The velocity of the Ca²⁺ wave was 1.7 mm/s at 150 mg/dL glucose. (b) One hour after elevation of glucose to 400 mg/dL, the velocity of the Ca²⁺ wave was increased to 2.2 mm/s with the higher aftercontraction. (c) One hour after reduction of glucose to 150 mg/dL, the velocity of the Ca²⁺ wave was decreased to 1.5 mm/s (2.0 mmol/L [Ca²⁺], 24.0°C, Exp.140730). (B) Summary data concerning the effect of glucose concentration on the velocity of Ca²⁺ waves (Left panel) and the amplitude of aftercontractions (AC force, Right panel). *P<0.05 vs. 150. (C) Summary data concerning the effect of glucose concentration on CaMKII activity. *P<0.05 vs. 150. (D) Summary data concerning the effect of glucose concentration on the velocity of Ca²⁺ waves (Left panel) and the AC force (Right panel) in the presence of 1 μmol/L KN-93.
Elevation of glucose increases arrhythmias suggesting that an elevation of glucose increases the velocity of Ca\textsuperscript{2+} waves not by affecting contractile strength.

It is probable that changes in contractile strength after elevation of glucose may affect the velocity of Ca\textsuperscript{2+} waves in the myocardium with non-uniform contraction because high glucose may decrease the myofilament Ca\textsuperscript{2+} sensitivity due to O-GlcNAcylation of contractile proteins.\textsuperscript{24,25} We thus examined the effect of glucose concentration on the velocity of Ca\textsuperscript{2+} waves under the condition of minimal contractile strength. As shown in Figure 5A and B, blebbistatin decreased the amplitude of developed force to less than 10% of its initial value and minimized the effect of contractile strength on the velocity of Ca\textsuperscript{2+} waves. Even in the presence of blebbistatin, the velocity of Ca\textsuperscript{2+} waves was increased 1 h after elevation of glucose from 150 to 400 mg/dL, suggesting that an elevation of glucose increases the velocity of Ca\textsuperscript{2+} waves not by affecting contractile strength.

**ROS Generation**

It has been reported that in a diabetic rat, ROS production is increased by activating CaMKII.\textsuperscript{32} Using DCF fluorescence, the effect of glucose concentration on ROS production was examined. As shown in Figure 5C, the slope of an increase in DCF fluorescence (DCF oxidation rate) was not increased 1 h after elevation of glucose from 150 to 400 mg/dL, suggesting that an elevation of glucose concentration does not increase ROS production within non-diabetic rat trabeculae.
than 150 mg/dL (mean 177 mg/dL) is related to increased risk of in-hospital mortality and that admission blood glucose higher than 152 mg/dL (mean 197 mg/dL) is related to increased death within 28 days.

Besides, it has been reported that admission blood glucose at 330 mg/dL predicted the highest 30-day mortality in patients with acute myocardial infarction. It has not yet been evaluated, however, when stress hyperglycemia occurs, how high and how long blood glucose was increased before death. In the present study, we thus elevated glucose concentration from 150 to 400 mg/dL and then evaluated the effect of glucose on arrhythmia susceptibility 1 h after elevation.

**Ca²⁺ Wave Propagation and Arrhythmias**

Glycosylation plays important roles in signal transduction and metabolism. Also, in the cardiovascular system, GFAT1 channels 2–5% of glucose into the HBP and leads to a series of enzyme-catalyzed reactions ending with

**Discussion**

The present study focused on Ca²⁺ wave propagation within trabeculae, investigating how an elevation of glucose affects the occurrence of arrhythmias in non-diabetic hearts with non-uniform contraction. To the best of our knowledge, it shows for the first time that in non-diabetic hearts with non-uniform contraction, transient elevation of glucose increases the velocity of Ca²⁺ waves by activating CaMKII through O-GlcNAcylation and thereby increases the occurrence of arrhythmias. These results suggest that transient hyperglycemia may affect arrhythmia susceptibility in non-diabetic patients with acute coronary syndrome.

**Admission Blood Glucose and Mortality**

In non-diabetic patients with acute myocardial infarction, it has been reported that admission blood glucose higher than 150 mg/dL (mean 177 mg/dL) is related to increased risk of in-hospital mortality and that admission blood glucose higher than 152 mg/dL (mean 197 mg/dL) is related to increased death within 28 days. Besides, it has been reported that admission blood glucose at 330 mg/dL predicted the highest 30-day mortality in patients with acute myocardial infarction. It has not yet been evaluated, however, when stress hyperglycemia occurs, how high and how long blood glucose was increased before death. In the present study, we thus elevated glucose concentration from 150 to 400 mg/dL and then evaluated the effect of glucose on arrhythmia susceptibility 1 h after elevation.

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**Figure 5.** Effect of glucose concentration on the velocity of Ca²⁺ waves in the presence of blebbistatin and on 2',7'-dichlorofluorescin (DCF) fluorescence. (A) Representative recordings of force (Upper panels) and regional changes in [Ca²⁺] (Lower panels) during the last three electrical stimuli in the myocardium with minimal contractile strength (0.4-s stimulus intervals, 2.0 mmol/L [Ca²⁺]o, 10 μmol/L blebbistatin). After the train of electrical stimuli indicated by arrows with ST, a Ca²⁺ wave (white arrow) appeared within the jet-exposed region (10 mmol/L Ca²⁺ jet) and propagated at a velocity of 1.6 mm/s at 150 mg/dL glucose (Left panel). One hour after elevation of glucose to 400 mg/dL, the velocity of the Ca²⁺ wave was increased to 5.6 mm/s (Right panel); (24.8°C, Exp.160627). (B) Summary data concerning the effect of glucose concentration on the velocity of Ca²⁺ waves in the myocardium with minimal contractile strength. *P<0.05 vs. 150. (C) Summary data concerning the effect of glucose concentration on the slope of an increase in DCF fluorescence (DCF oxidation rate) at 2-s stimulus intervals for 30 s (Upper panel) and 0.4-s stimulus intervals for 7.5 s (Lower panel).
formation of uridine diphosphate-N-acetylgalcosamine (UDP-GlcNAc). O-GlcNAC transferase adds O-GlcNAc modification to proteins using UDP-GlcNAc as the monosaccharide donor, and O-GlcNACase removes it from the proteins.\textsuperscript{22,23} It has been reported that a variety of proteins including CaMKII\textsuperscript{P} are modified by O-GlcNAcylation.\textsuperscript{18}

In the present study, an elevation of glucose to 400 mg/dL for 1 h increased both the velocity of Ca\textsuperscript{2+} waves and the amplitude of aftercontractions (Figure 3A, B), and further increased the number of action potentials induced by electrical stimulation (Figure 4A, C). It is reasonable to assume that these increases after elevation of glucose were caused by an increase in Ca\textsuperscript{2+} leak from the SR due to activation of CaMKII through O-GlcNAcylation for the following reasons. First, an elevation of glucose to 400 mg/dL activated CaMKII (Figure 3C), and it has been reported that activation of CaMKII increases Ca\textsuperscript{2+} release from the SR\textsuperscript{33} and Ca\textsuperscript{2+} uptake into the SR.\textsuperscript{44}

In diabetic hearts, the decrease in myofilament Ca\textsuperscript{2+} sensitivity plays an important role in the occurrence of arrhythmias caused by an elevation of glucose concentration.

Clinical Implications

In patients with acute myocardial infarction and unstable angina, [Ca\textsuperscript{2+}] increases within the ischemic region,\textsuperscript{25} and the weaker contractile strength within the ischemic region causes non-uniform muscle contraction in their hearts. As suggested in the present study, an elevation of glucose increases arrhythmia susceptibility by increasing the velocity of Ca\textsuperscript{2+} waves in non-diabetic hearts with non-uniform contraction. This means that in patients with acute coronary syndrome, transient hyperglycaemia may increase arrhythmia susceptibility in their hearts, explaining, at least in part, why admission blood glucose is associated with short-term mortality in non-diabetic patients with acute coronary syndrome.\textsuperscript{4,6,10}

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Disclosures

None.

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