Hyperglycemia is associated with enhanced thrombin formation, platelet activation and fibrin clot resistance to lysis in patients with acute coronary syndrome

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Running title: Prothrombotic effects of hyperglycemia in ACS

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**Objective:** Acute hyperglycemia on admission for acute coronary syndrome (ACS) worsens the prognosis in patients with and without known diabetes mellitus. Postulated mechanisms of this observation include prothrombotic effects. The aim of this study was to evaluate the effect of elevated glucose levels on blood clotting in ACS patients.

**Research Design and Methods:** We studied 60 ACS patients within the first 12 hours after pain onset, including 20 subjects with type 2 diabetes, 20 with no diagnosed diabetes but with glucose levels above 7.0 mmol/L, and 20 with glucose levels below 7.0 mmol/L. We determined generation of thrombin-antithrombin complexes (TAT) and soluble CD40 ligand (sCD40L), a platelet activation marker, at the site of microvascular injury, together with ex vivo plasma fibrin clot permeability and lysis time.

**Results:** The ACS patients with no prior diabetes but elevated glucose levels had increased maximum rates of formation and total production of TAT (by 42.9%, p<0.0001 and 25%, p<0.0001, respectively) as well as sCD40L release (by 16.2%, p=0.0011 and 16.3%, p<0.0001, respectively) compared with those with normoglycemia, while DM patients had the highest values of the TAT and sCD40L variables (p<0.0001 for all comparisons). Patients with hyperglycemia, with no previously diagnosed diabetes, had longer clot lysis time (by ~18%, p<0.0001) similarly to diabetic subjects, but not lower clot permeability, compared with normoglycemic subjects.

**Conclusions:** Hyperglycemia in ACS is associated with enhanced local thrombin generation and platelet activation, as well as unfavorably altered clot features in patients with and without a previous history of diabetes.
Acute hyperglycemia occurs in up to 50% of all ST-segment-elevation myocardial infarctions (STEMI), while patients with diabetes mellitus represent approximately 25% of STEMI patients (1). When glucose tolerance testing is performed, even 65% of patients with myocardial infarction (MI) and a negative history of diabetes can be diagnosed with diabetes or impaired glucose tolerance (2). Acute hyperglycemia on admission has been reported to worsen the prognosis in MI patients with and without known diabetes (3), including increased risk of in-hospital mortality in both groups (4).

Cardiovascular stress induces a release of catecholamines, cortisol, and glucagon leading to increases in glucose and free fatty acids that enhance hepatic gluconeogenesis and diminish peripheral glucose uptake. Unfavorable effects of high blood glucose levels in MI involve impaired left ventricular function, increased incidence of the no-reflow phenomenon and tendency to arrhythmias (5). Several mechanisms implicated in the detrimental impact of hyperglycemia during acute myocardial ischemia have been postulated, ie, enhanced oxidative stress, the activation of blood coagulation and platelets, stimulation of inflammation, and endothelial cell dysfunction (5). All of them have also been reported in type 2 diabetes (6,7).

Evidence for prothrombotic effects of acute hyperglycemia in vivo is scanty. Exposure to 24-hour selective hyperglycemia in healthy volunteers results in increased tissue factor procoagulant activity (8). Acute hyperglycemia activates platelet aggregation, enhances thrombin generation, and activates coagulation factor VII (9). It is not known whether acute hyperglycemia during MI is potent enough to influence hemostasis. Moreover, hyperglycemia, both in diabetic patients and under in vitro conditions, is linked to unfavorably altered fibrin clot properties and reduced fibrinolysis compared with the results at normoglycemia (10,11). Recently, we have showed that in patients with acute MI, a history of type 2 diabetes is associated with impaired plasma clot permeability and fibrinolysis (12). The effect of hyperglycemia on clot properties in acute MI subjects with no history of diabetes has not been investigated yet.

The aim of the study was to evaluate potential prothrombotic alterations in acute MI patients in relation to hyperglycemia, including thrombin formation, platelet activation, and fibrin network structure/function.

METHODS

Patients: Patients with acute MI admitted to the coronary care unit within the first 12 hours after the onset of chest pain were enrolled in the study. We recruited 20 consecutive acute MI patients with a history of type 2 diabetes, who self-reported taking insulin or oral hypoglycemic drugs on a regular basis (the DM group) and 20 patients with a negative history of DM, who had the serum glucose level of 7 mmol/L or more on admission (the HG group). Twenty patients with glucose levels <7 mmol/L (the NG group) served as a reference group. Inclusion criteria were typical chest pain and elevated cardiac troponin levels. Changes in ECG recordings such as either ST-segment elevation ≥0.1 mV or ST-segment depression ≥0.1 mV in at least two contiguous ECG leads, or normal ECG were allowed. Exclusion criteria were as follows: cardiogenic shock, any acute illness, cancer, hepatic or renal dysfunction, a history of venous thromboembolism or stroke, anticoagulant therapy, recent MI within the previous 3 months. All subjects received aspirin 300 mg 2 to 8 hours before the study. Major adverse coronary events were recorded within the first 30 days after enrolment.
All subjects enrolled provided written, informed consent. The University Ethical Committee approved the study.

**Laboratory investigations**: Blood samples were obtained from an antecubital vein using a 21-gauge butterfly needle within 15 minutes upon admission. Lipid profile, C-reactive protein, glucose, creatinine, platelet count, and cardiac troponin T were determined using routine laboratory methods. HbA1c was analyzed by HPLC using a Variant II Analyzer (Bio-Rad Laboratories, Hercules, CA). A human-specific radioimmunoassay kit (Linco Research Inc., St. Charles, MO) was used to measure plasma insulin levels. Fibrinogen was determined using the Clauss method. High-sensitivity C-reactive protein (CRP) was measured by latex nephelometry (Dade Behring, Marburg, Germany). Blood samples for thrombin and platelet markers were centrifuged at 2,500 g for 15 minutes and plasma was stored at -80°C. Using commercially available ELISAs, we determined in plasma: interleukin-6 (IL-6; R & D Systems, Abingdon, UK); thrombin-antithrombin complexes (TAT) and prothrombin 1.2 fragments (F1.2), markers of thrombin formation (Enzygnost, Dade Behring, Marburg, Germany), and sCD40L, a marker of platelet activation (R & D Systems, Abingdon, UK). Routine laboratory data and hemostatic variables were also obtained after 30 days from the event.

**Model of vascular injury**: Measurements were performed in blood collected at 60-second intervals from the standardized skin incision, made using a Simplate IR device (Organon Teknika, Durham, NC) at the inflation of the sphygmomanometer cuff at 40 mm Hg, as previously described (13-15). Blood was collected by means of heparinized tubes (Kabe Labortechnik, Numbrecht-Elsenroth, Germany) into Eppendorf tubes containing anticoagulants as described (14,15). After centrifugation at 3,000 g at 4°C for 20 minutes, supernatants were frozen at -80°C. Both TAT (Dade Behring) and sCD40L (R & D Systems) were measured in the samples. Interassay and intraassay coefficients of variation were 5 to 7%. Thrombin formation and platelet activation were described as maximum velocity of both processes and total amounts of each marker produced within the first 6 minutes of bleeding (using the trapezoid rule) (14,15).

**Clot permeability**: Permeation properties of fibrin clots were assessed according to Mills et al. (16). Briefly, tubes containing plasma clots formed upon addition of calcium chloride and human thrombin (Sigma) were connected via plastic tubing to a reservoir of 0.05 mol/L Tris-HCl and its volume flowing through the gels was measured within 60 minutes. A permeation coefficient (Kₜ), which indicates the pore size, was calculated from the equation, as described (16). The interassay coefficient of variation was 9.2 %.

**Plasma clot lysis assay**: To determine lysis time, we used an assay by Lisman (17) with some modifications. Briefly, citrated plasma were mixed (1:1) with HEPES buffer, containing calcium chloride, diluted recombinant tissue factor (Innovin, Dade Behring), phospholipid vesicles, and rtPA (Boehringer Ingelheim). Turbidity of this mixture (100 μL) was measured at 405 nm at 37°C in a Spectramax 340 kinetic microplate reader (Molecular Devices Corp.). Clot lysis time was defined as the time from the midpoint of the baseline clear to maximum turbid transition, to the final plateau phase. The interassay and intraassay coefficients of variation were 8.1 and 6.2 %, respectively.

**Statistical analysis**: The study was powered to have an 80% chance of detecting a 10% intergroup difference in maximum rate of TAT generation at the site of microvascular injury using a p-value of 0.05, based on mean values in the published papers (13-15). In order to demonstrate such a difference or
greater, 12 patients were required in each group. A corresponding number of patients for local sCD40L release was calculated to be 12.

Continuous data are presented as mean±SD or as median and interquartile range. The Kolmogorov-Smirnov test was used to determine normal distribution. The significance of between-group differences was tested by analysis of variance (ANOVA) with Scheffe’s adjustment. Post-hoc comparisons were made using Tukey’s test. The χ² test or Fisher’s exact test were used to compare categorical variables. Pearson’s correlations were used to identify associations between variables. A two-sided p-value <0.05 was considered statistically significant.

RESULTS

All the three MI groups did not differ with regard to demographic and clinical variables (Table 1). All three patient groups were enrolled after 5.2±0.3 hours after chest pain onset (p=0.9). Patients with diabetes were treated either with insulin (n=8; 40%) or with oral hypoglycemic agents (n=12; 60%). Duration of the disease ranged from 0.5 to 11 (median, 5) years. As expected, glucose levels were higher in both hyperglycemic groups and patients with normoglycemia, while serum insulin and HbA1c were elevated in the DM group, with no difference between the HG and NG groups (Table 1). Higher cardiac troponin T was observed in the DM group than in the NG group (Table 1). In contrast to CRP, IL-6 levels were elevated by 86% both in the DM and HG groups compared with the normoglycemic patients. Fibrinogen levels were 29% higher in the DM group than in the NG group, with similar values in both hyperglycemic groups (Table 1).

Bleeding time did not differ among the 3 groups (Table 1). The total volume of blood collected from wounds was similar in all groups (data not shown).

Thrombin formation: Plasma TAT and F1.2 concentrations did not differ between the DM and HG groups. However, diabetic patients with acute MI, but not those from the HG group, had higher plasma levels of F1.2 (by 27.5%) and TAT (by 30%) than those observed in the NG group (Table 1).

Time courses of TAT generation at the site of injury were similar regardless of the presence or absence of hyperglycemia (Figure 1A). Maximum TAT levels were found at 6 minutes, with the highest values in the DM group (112.6±10.4 nmol/L) and the lowest in the NG group (89.7±9.1 nmol/L; p=0.006). There was no difference between maximum TAT levels in bleeding-time blood in the HG (96.1±5.9 nmol/L) and NG groups (p=0.3). A peak rate of TAT formation following vascular injury was higher in hyperglycemia (0.36±0.03 for the DM group and 0.3±0.03 nmol/L/s for the HG group, respectively) compared with patients with normoglycemia (0.21±0.03 nmol/L/s; p<0.0001 for both comparisons). However, TAT was also faster generated in the DM group than in the HG group (p<0.0001).

Total amounts generated following injury within 6 minutes were increased by 24.3% in diabetic patients with acute MI compared to those with elevated glucose levels, without a history of diabetes (p<0.0001) as well as by 55.4% compared with those with normoglycemia during acute MI (p<0.0001; Figure 2A which is available at http://care.diabetesjournals.org).

None of the variables describing TAT formation at the site of vascular injury showed no associations with plasma TAT levels, glycemia, insulinenia, age, or other clinical or laboratory variables in the three groups studied. Total formation of TAT within the first 6 minutes was associated with triglycerides in the HG group, but not in the two remaining ones (r=0.48; p=0.03). Maximum rate of TAT generation and TAT levels tended to be higher in patients whose
blood was drawn after longer time from pain onset only in the DM group (r=0.38; p=0.1 for both). Other variables showed no correlation with time from pain onset (data not shown).

**Platelet activation:** Plasma sCD40L levels were similar in the DM and HG groups. Compared to the normoglycemic patients, both in the DM and HG groups displayed higher plasma sCD40L levels by 120% and 82.5%, respectively (Table 1). Profiles of sCD40L release, reflected in its levels in blood obtained from bleeding-time wounds, shared common kinetics in acute MI patients, with the steepest increase in diabetic subjects (Figure 1B). The highest local sCD40L value of 23.4±2.6 ng/mL was observed in the DM group. A lower maximum sCD40L level of 20.3±1.9 ng/mL (p<0.001) was found in the HG group. Maximum rates of sCD40L release were higher in DM (0.087±0.009 ng/mL/s) and HG patients (0.086±0.01 ng/mL/s) compared to individuals with normoglycemia (0.074±0.012 ng/mL/s; p<0.001 for both comparisons). There was no difference in this variable between the DM group and HG groups (p=0.2). The velocity of sCD40L increase in shed blood was increased in the latter group compared with the NG group (p=0.011).

The total release of sCD40L within the first 6 minutes was similar in the DM and HG groups. Both these groups characterized by increased amounts of sCD40L measured following injury (by 28% and 16.3%, p<0.001, respectively) compared with the NG group (Figure 2B).

In the DM group, maximum rate of the sCD40L release showed no association with the duration of diabetes, insulin administration, age, or other clinical or laboratory variables with two exceptions. It was correlated with glucose (r=0.56; p=0.01) and plasma TAT levels (r=0.53; p=0.02). No similar associations were observed in the two other groups. Total release of sCD40L within the first 6 minutes was associated with total cholesterol (r=0.47; p=0.036) and plasma sCD40L levels (r=0.48; p=0.03) but only in the HG group. Variables describing local sCD40L release showed no significant correlations with time from pain onset (data not shown).

**Clot permeability:** Lower clot permeability was found in patients with prior history of diabetes compared with subjects from both the HG and NG groups (Table 2). However, Ks was similar in the HG and NG groups. Ks was correlated with fibrinogen in all the groups (r from -0.36 to -0.51; p<0.05). Ks was inversely associated with CRP only in the DM group (r=0.42, p=0.03), but showed no associations with lipids or thrombin or platelet parameters both in venous and bleeding-time blood in either group.

**Fibrinolysis:** Clot lysis time was the longest in the diabetes patients admitted for acute MI, and significantly shorter in the HG group than in subjects with normoglycemia (Table 2). Lysis time showed correlations only with CRP in all the 3 groups (r from 0.35 to 0.49; p<0.05). No associations between lysis time and glucose or insulin levels in any of the groups were observed. There were no correlations of lysis time with thrombin generation or platelet activation in the all patients and the 3 groups as well as with time from the onset of MI symptoms or troponin levels (data not shown).

**Short-term outcomes:** During a 30-day follow-up, there were 3 cardiovascular deaths (2 in the DM group and 1 in the NG group). Recurrent myocardial ischemia were observed in 6 patients, two in each group. No intergroup differences in major adverse cardiovascular events were observed. Glucose levels determined one month after enrollment revealed that all NG subjects had still normoglycemia, while 3 subjects from the HG group had glycemia >7 mmol/L; exclusion of these patients did not alter the results of hemostatic variables (data not shown).
DISCUSSION

The current study shows that elevated glucose levels are associated with significantly augmented thrombin formation and platelet protein secretion in response to vascular injury not only in patients with type 2 diabetes, but also in those with no prior history of diabetes and hyperglycemia during acute MI. Moreover, we demonstrated that hyperglycemia observed in acute MI results in hypofibrinolysis, regardless of a history of type 2 diabetes or not, while reduced clot permeability was found only in patients previously diagnosed with diabetes compared with normoglycemic individuals. Our findings indicate that not only diabetes, but also hyperglycemia occurring in acute MI patients with no prior diagnosis of diabetes, produce several prothrombotic effects that may contribute to an increased risk for thrombotic complications following an acute coronary event. The impact of hyperglycemia in MI patients appeared potent enough to be detected despite strong prothrombotic effects of coronary plaque injury during MI. Our findings may also help explain a recent observation that glucose-insulin-potassium therapy, resulting in increased glucose levels, could be harmful within the first days of acute MI (18).

Since efficient hemostasis occurs only at vascular lesions where tissue factor is exposed and platelets rapidly aggregate, measurements of hemostatic markers at the site of vascular injury are more sensitive than those in venous blood in the assessment of local thrombotic reactions (13, 14, 19). We did not observe elevated levels of thrombin or platelet markers in venous blood in diabetic patients compared with those from the HG group; the differences were detectable at the site of injury. Probable mechanisms of this effect of hyperglycemia involve enhanced activation of proinflammatory transcription factors that can increase tissue factor expression (20). Augmented local thrombin production in MI patients with glucose >7.0 mmol/L was accompanied by increased platelet activation, reflected by elevated sCD40L in venous plasma and bleeding-time blood. Of several soluble platelet activation markers, including β-thromboglobulin or P-selectin, sCD40L has been extensively studied in hyperglycemic subjects (8, 9, 21) and measured at the site of injury (19,22). Approximately 95% of circulating sCD40L is platelet-derived (11, 23). For these reasons, sCD40L has been chosen as the platelet activation marker in the current study. Importantly, a similar increase in sCD40L release correlated with thrombin formation has been reported in patients with the metabolic syndrome (24).

Fibrin clot analysis revealed reduced lysis time in the DM and HG groups compared to that with glycemia <7 mmol/L, without any intergroup differences in clot permeability except for significantly higher permeability in diabetic subjects. Glycation of the fibrinogen molecules is largely responsible for altered fibrin clot features found at elevated glucose levels (10, 11). We extended previous observations by showing a potent impact of diabetes on fibrin properties, easily detectable also in MI patients despite the fact that acute myocardial ischemia itself is associated with deleterious clot alterations similar to those described in diabetic patients (12). A short-term increase in glucose levels does not modify fibrin structure, which explains similar permeability observed in the HG and NG groups. Reduced lysis efficiency in the HG and DM groups indicates the presence of some glucose-mediated rapid mechanisms impairing fibrinolysis even if the extent of glycation is negligible. This effect could be explained by elevated plasminogen activator inhibitor 1 (PAI-1) observed in hyperglycemia (5,6). It might be speculated that altered fibrin in hyperglycemia leads to lower binding affinity of both t-PA and
plasminogen toward fibrin (11) and in consequence, impaired clot lysis in our assay.

One might suspect that insulin or oral hypoglycemic agents taken only by diabetic patients confounded the data interpretation. However, there is no evidence that in MI patients such therapy alters thrombin formation or platelet activation. In terms of fibrin-modifying properties, insulin, gliclazide and metformin have been shown to enhance clot lysis (25). We might speculate that susceptibility to lysis is likely even weaker in untreated diabetic patients with MI. Another potential drug-mediated effect could be mediated by statins that were taken by a significantly lower percentage of the HG patients prior to MI. Since statins can reduce thrombin generation (13) and platelet activation (20) following injury in stable patients, both processes might have been relatively more vigorous in the HG group than in the DM and NG groups. However, no data support the view that statins are potent enough to suppress massive activation of hemostasis observed in patients with acute MI (26).

This study has limitations. First, the number of the patients studied is limited. However, we well matched MI patients with and without elevated glucose levels as well as those with normoglycemia. Second, our analysis was based on a determination of each variable at a single time point. Third, results of oral glucose test after MI were not analyzed. However, lack of significant differences in HbA1c between the HG and NG groups speaks against the possibility that patients with diabetes not diagnosed with this disease prior to the acute event were enrolled in the HG group. Finally, statistical associations reported here do not necessarily mean the cause-effect relationships. Further studies are needed to elucidate this issue.

In conclusion, our findings demonstrate that acute hyperglycemia in acute MI patients without a previous history of diabetes is associated with increased thrombin generation and platelet activation at the site of vascular injury as well as greater resistance to fibrinolysis. This study provides further insights into the relationship between hyperglycemia and thrombosis in MI patients.

ACKNOWLEDGEMENTS
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References
Table 1. Comparisons of laboratory variables in the three groups of patients with acute coronary syndrome, based on a history of diabetes and glucose levels on admission.

<table>
<thead>
<tr>
<th></th>
<th>DM group (n=20)</th>
<th>HG group (n=20)</th>
<th>p</th>
<th>NG group (n=20)</th>
<th>p*</th>
<th>p**</th>
</tr>
</thead>
<tbody>
<tr>
<td>age, (years)</td>
<td>61±10</td>
<td>60±9</td>
<td>NS</td>
<td>61±7</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>men, n(%)</td>
<td>14(70)</td>
<td>16(80)</td>
<td>NS</td>
<td>11(55)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>STEMI, n(%)</td>
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<td>11(55)</td>
<td>NS</td>
<td>12(60)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>hypertension, n(%)</td>
<td>14(70)</td>
<td>13(65)</td>
<td>NS</td>
<td>14(70)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>previous PCI, n(%)</td>
<td>9(45)</td>
<td>4(20)</td>
<td>NS</td>
<td>7(35)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>current smokers, n(%)</td>
<td>14(70)</td>
<td>13(65)</td>
<td>NS</td>
<td>14(70)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>hypoglycaemic drugs, (%)</td>
<td>20(100)</td>
<td>0(0)</td>
<td>&lt;0.0001</td>
<td>0(0)</td>
<td>&lt;0.0001</td>
<td>NS</td>
</tr>
<tr>
<td>statins, n(%)</td>
<td>16(80)</td>
<td>5(25)</td>
<td>0.0002</td>
<td>11(55)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>β-blockers n(%)</td>
<td>17(85)</td>
<td>16(80)</td>
<td>NS</td>
<td>16(80)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ACEI, n(%)</td>
<td>15(75)</td>
<td>11(55)</td>
<td>NS</td>
<td>14(70)</td>
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<td>NS</td>
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<td>aspirin, n(%)</td>
<td>20(100)</td>
<td>20(100)</td>
<td>NS</td>
<td>20(100)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>diuretics, n(%)</td>
<td>8(40)</td>
<td>6(30)</td>
<td>NS</td>
<td>7(35)</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>glucose, (mmol/L)</td>
<td>9.74±2.34</td>
<td>8.58±0.87</td>
<td>NS</td>
<td>4.69±0.68</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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<tr>
<td>platelets, 10^3/mm³</td>
<td>258.9±49.3</td>
<td>249.5±45.8</td>
<td>NS</td>
<td>253.5±41.0</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>CRP, (mg/L)</td>
<td>2.72(0.9-6.5)</td>
<td>1.45(0.77-4.11)</td>
<td>NS</td>
<td>1.9(1.01-2.32)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>IL-6, (ng/mL)</td>
<td>3.13±1.19</td>
<td>3.13±1.06</td>
<td>NS</td>
<td>1.68±0.56</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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<tr>
<td>TnT, (ng/mL)</td>
<td>2.98±2.07</td>
<td>1.53±1.42</td>
<td>0.028</td>
<td>2.92±2.7</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>TnT max, (ng/mL)</td>
<td>5.9(2.7-13.2)</td>
<td>28.9(5.9-49.7)</td>
<td>0.019</td>
<td>24.9(7.5-41.9)</td>
<td>0.013</td>
<td>NS</td>
</tr>
<tr>
<td>fibrinogen, (g/L)</td>
<td>4.1±1.08</td>
<td>3.17±0.8</td>
<td>0.004</td>
<td>3.04±0.7</td>
<td>0.002</td>
<td>NS</td>
</tr>
<tr>
<td>TC, (mmol/L)</td>
<td>6.0±1.08</td>
<td>5.42±0.99</td>
<td>NS</td>
<td>5.42±1.14</td>
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<tr>
<td>LDL-C, (mmol/L)</td>
<td>3.75±1.06</td>
<td>3.31±0.73</td>
<td>NS</td>
<td>3.41±0.99</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>HDL-C, (mmol/L)</td>
<td>1.28±0.7</td>
<td>1.21±0.37</td>
<td>NS</td>
<td>1.23±0.15</td>
<td>NS</td>
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<tr>
<td>TG, (mmol/L)</td>
<td>1.97±1.46</td>
<td>1.68±1.06</td>
<td>NS</td>
<td>1.56±0.44</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>sCD40L, (pg/mL)</td>
<td>747.75±283.72</td>
<td>619.95±306.55</td>
<td>NS</td>
<td>339.75±92.97</td>
<td>&lt;0.0001</td>
<td>0.0003</td>
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<tr>
<td>TAT, (μg/L)</td>
<td>6.58±1.67</td>
<td>5.86±1.73</td>
<td>NS</td>
<td>5.06±1.84</td>
<td>0.0095</td>
<td>NS</td>
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<tr>
<td>F1.2, (nmol/L)</td>
<td>1.16(0.97-1.89)</td>
<td>1.04(0.88-1.19)</td>
<td>NS</td>
<td>0.91(0.78-1.13)</td>
<td>0.016</td>
<td>NS</td>
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<tr>
<td>HbA₁c, (%)</td>
<td>6.81±0.37</td>
<td>5.5±0.36</td>
<td>&lt;0.0001</td>
<td>5.47±0.27</td>
<td>&lt;0.0001</td>
<td>NS</td>
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<tr>
<td>Insulin (pmol/L)</td>
<td>154.985±53.47</td>
<td>96.825±31.12</td>
<td>0.0002</td>
<td>86.7±31.15</td>
<td>&lt;0.0001</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are shown as the mean±SD or median (interquartile range). Abbreviations – STEMI, ST elevation myocardial infarction; PCI, percutaneous coronary intervention; ACEI, angiotensin-converting enzyme inhibitors; CRP, C-reactive protein; IL-6, interleukin-6; TnT, cardiac troponin T; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; sCD40L, soluble CD40 ligand in plasma of venous blood; TAT, thrombin-antithrombin complexes in plasma of venous blood; F1.2, prothrombin fragment 1.2; HbA₁c, glycated hemoglobin. NS, non-significant. p* denotes a comparison (ANOVA, post-hoc analysis) between the group of patients with DM (the DM group) and that with glucose levels below 7.0 mmol/L (the NG group). p** denotes a comparison (ANOVA, post-hoc analysis) between the group with no known history of DM, but elevated glucose levels on admission for an acute event (the HG group), and the group with glucose levels below 7.0 mmol/L (the NG group).
Table 2
Fibrin clot permeability ($K_s$) and lysis time ($t$) in acute coronary patients with a previous history of diabetes mellitus (the DM group), patients with a negative history of DM, but elevated glucose levels on admission for an acute event (the HG group), and patients with serum glucose levels below 7 mmol/L (the NG group).

<table>
<thead>
<tr>
<th></th>
<th>DM group (n=20)</th>
<th>HG group (n=20)</th>
<th>p</th>
<th>NG group (n=20)</th>
<th>p*</th>
<th>p**</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_s$, $10^{-9}$ cm$^2$</td>
<td>6.1(5.3-7.9)</td>
<td>7.5(6.9-8.9)</td>
<td>0.02</td>
<td>7.6(7.1-9.1)</td>
<td>0.006</td>
<td>NS</td>
</tr>
<tr>
<td>$t$, min</td>
<td>127.9(98.3-137.4)</td>
<td>116.1(77.9-120.3)</td>
<td>0.001</td>
<td>98.5(73.4-111)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are given as median (interquartile range). NS, non-significant.
$p^*$ denotes a comparison between the DM and NG groups; $p^{**}$ denotes a comparison between the HG and NG groups.
Legends to Figures

**Figure 1.** Thrombin formation and platelet activation at the site of microvascular injury in patients with acute coronary syndrome. (A) Concentrations of thrombin-antithrombin (TAT) complexes in the 60-second bleeding-time blood samples in 20 patients with documented diabetes (closed circles), 20 patients with no history of diabetes, but elevated glucose levels, (open circles) and 20 patients with normoglycemia during the acute event (closed triangles). (B) Concentrations of soluble CD40 ligand (sCD40L) in the 60-second bleeding-time blood samples in 20 patients with diabetes (closed circles), 20 patients with no history of diabetes, but elevated glucose levels, (open circles) and 20 patients with normoglycemia during the acute event (closed triangles). Values are plotted as mean±SEM.