Experimental Studies

Optical Coherence Tomography Assessment of Glucose Fluctuation Impact on the Neointimal Proliferation After Stent Implantation in a Diabetic/Hypercholesterolemic Swine Model

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SUMMARY

The aim of the present study was to investigate the effects of glucose fluctuation on neointimal proliferation after stent implantation by optical coherence tomography (OCT) in a diabetic/hypercholesterolemic (DM/HC) swine model. A total of 24 everolimus-eluting stents (EES) were implanted in the right coronary artery (RCA) of the animals using a 20% overstretch ratio. The 24 swines were divided into a DM-high glucose fluctuation (HGF) group (n = 8), DM-low glucose fluctuation (LGF) group (n = 8), and a control group (n = 8). Percent diameter stenosis (%DS), late loss (LL), percent area stenosis (%AS), and neointimal thickness (NIT) were analyzed. The differences in neointimal characteristics and circulating oxidative stress and inflammation biomarkers were assessed and measured.

At 28 days, the highest values of %DS, LL, %AS, and NIT were achieved in the HGF group followed by the LGF group (P < 0.05) and the control group (P < 0.05). The highest frequency of the heterogeneous pattern was in the HGF group followed by the LGF group (P < 0.05) and the control group (P < 0.05). This was also the case for the oxidative stress and inflammation biomarkers.

DM might have a deleterious impact on neointimal proliferation after EES implantation in this DM/HC swine model. The extent of glucose fluctuation may be related to the degree of neointimal proliferation and this needs to be further confirmed by long-term follow-up and histology. (Int Heart J 2017; 58: 608-614)

Key words: Glucose variability, Diabetes mellitus, Intravascular imaging, Intima

A n extensive amount of research has focused on how to improve outcomes after drug eluting stent (DES) implantation. Dyslipidemia and high blood pressure have been recognized as the most important promoters of stent restenosis. A large number of clinical trials have reported the beneficial effects of lipid lowering and blood pressure lowering for secondary prevention and improved all-cause mortality. However, the limited ability of risk reduction associated with lipid-lowering and blood pressure lowering therapy has attracted attention to the unmet need for residual clinical risk management.

Diabetes mellitus (DM) in the form of chronic sustained hyperglycemia is thought to be associated with worse outcome after DES implantation. There is increasing evidence that glucose fluctuation produces more detrimental effects on the coronary arteries than chronic sustained hyperglycemia. Epidemiological studies have suggested that glucose fluctuation may be a marker of increased progression of coronary disease. However, it remains unclear whether glucose fluctuations may affect vessel healing after EES implantation and the detailed impact of glucose fluctuation on neointimal proliferation remains elusive. The related preclinical data is lacking.

Optical coherence tomography (OCT) is emerging as a very important imaging device for the evaluation of coronary plaque characteristics and neointimal proliferation after stenting due to its high-resolution.1,2 The human-like porcine model of DM/HC is practical and highly relevant for translational research in stent restenosis and imaging. The present study aimed to investigate the effects of glucose fluctuation on neointimal proliferation after EES implantation by OCT in the DM/HC swine model.

METHODS

H C and DM/HC swine model: The study protocol is shown in Figure 1. A total of 24 Chinese experimental male minipigs (obtained from the breeding factory of China Agricultural University, Beijing, China) were assigned to the HC control group (n = 8) and DM/HC group (n = 16). The minipigs were 4 months old. The control group was fed a high-fat diet (2% cholesterol, 20% lard and 1.5% cholate) for 18 weeks.3 The DM/HC group was fed a high-fat and high-sucrose diet (2% cholesterol, 20% lard, 37% sucrose and 1.5% cholate) for 18
After stent implantation, the previous diet was continued. The amount of daily food given was 2.5% of body weight. The pigs were fed at different times during the day. The 8 control group pigs were fed equal portions 4 times a day at 7 am, 11 am, 3 pm, and 7 pm. The 16 DM/HC group pigs were divided into two subgroups: a high glucose fluctuation (HGF) group (n = 8) and a low glucose fluctuation (LGF) group (n = 8). The HGF group pigs were fed equal portions twice (7 am, 7 pm) daily and the LGF group pigs were fed equal portions 4 times (7 am, 11 am, 3 pm and 7 pm) daily.

**Study design:** At the beginning of 19 weeks (baseline), 24 coronary sites located in the right coronary artery (RCA) in 24 pigs were selected after angiography and quantitative coronary analysis (QCA) for stent implantation. A total of 24 EES were implanted targeting up to 20% overstretch. All animals were followed up for 28 days (19-22 weeks). Angiography and OCT were performed at 28 days (the end of 22 weeks) after EES implantation.

**Experimental procedures:** The research was approved by the Animal Research Councils and the Ethical Council, Health Center, Xuanwu Hospital, Capital Medical University, China. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The mean weight at 18 weeks was 46 kg. All the pigs were premedicated with dual antiplatelet agents (75 mg of clopidogrel and 300 mg of aspirin) at least 12 hours before the procedure. After anesthesia, they were intubated and given mechanical ventilation. Arterial carotid access was achieved using a cut-down technique. Intravenous heparin (5000–10,000 U) was administered to maintain the activated clotting time of 250-300 seconds. Following coronary angiography, the RCA were sized for proper stent implantation by QCA. After stent implantation, final angiography was performed. The animals were then extubated. They were allowed to recover from the procedure and then returned to the animal care facilities by the research team. At 28 days after stent implantation, the animals were anesthetized and prepared in the same fashion as described above. Angiography and OCT were performed.

**Quantitative coronary analysis (QCA):** QCA analysis was performed by QAngio XA Software version 7.1.14.0 (Medis Medical Imaging Systems, Leiden, The Netherlands). Reference vessel diameters (RVD) were taken from the proximal and distal portions of the treated segments using the guiding catheter as a standard for measurement. The in-stent minimal luminal diameter (MLD) and overstretch ratio were measured and calculated. Percent diameter stenosis (%DS) at 28 days follow-up was calculated as: (1-[MLD/RVD])×100%. LL was calculated as MLD at baseline by subtracting MLD at 28 days of follow-up.

**OCT imaging protocol, analysis, and OCT classification of neointimal type:** OCT images were obtained using the C7-XROCT imaging system (Light-Lab Imaging, Inc., St. Jude Medical, St. Paul, MN, USA), and was performed using a non-occlusive technique with contrast flush injection via the guiding catheter to permit imaging in a blood-free environment. Motorized OCT pullbacks were performed at a rate of 20 mm/s. All images were acquired at 100 frames per second. This study involved choosing 3 cross-sectional images per pullback: proximal stent, mid-stent, and distal stent. Integrated OCT image analysis software developed by Light Lab Imaging, Inc.
was used for measurements. The lumen area (LA) and stent area (SA) were measured. Neointimal area (NIA) was calculated by subtracting SA from LA. Neointimal thickness (NIT) was achieved by determining the distance between the centre of each strut and the luminal border in the direction of the centre of gravity. The percent area stenosis (%AS) was calculated by $[1 - (LASA)] \times 100$.

Neointimal tissue was evaluated qualitatively in cross-sections over 20 mm of mean NIT using a recently published OCT classification, which is based on tissue structure and backscatter. A homogeneous pattern is defined as neointimal tissue with uniform optical properties without focal variation in the backscattering pattern. The heterogeneous pattern refers to those with focally changing optical properties and various backscattering patterns, and the layered pattern has concentric layers with different optical properties. The frequencies of the homogeneous, heterogeneous, and layered patterns were analyzed and compared among the 3 groups.

**Blood samples:** Blood samples were drawn at 0 weeks and at 18 weeks to measure total cholesterol and overnight fasting blood glucose. Blood glucose levels were measured 5 times per day at 6 am, 9 am, 1 pm, 5 pm, and 9 pm for 1 week (19 weeks) after stent implantation in both the HGF group and LGF group. The standard deviation in blood glucose levels in both the HGF group and LGF group was analyzed.

Blood samples for determining oxidative stress and inflammation biomarkers were drawn at baseline (beginning of 19 weeks) and at 28 days of follow-up (end of 22 weeks). The incremental value was calculated by subtracting the baseline (beginning of 19 weeks) from the value at day 28 of follow-up (end of 22 weeks). The increment was compared among the 3 groups. Circulating oxidative stress, measured as the plasma concentration of 8-isoprostaglandin, was analyzed using an enzyme-linked immunosorbent assay (ELISA) commercial kit (Uscnlife, Missouri, TX, USA). The plasma levels of malondialdehyde (MDA) were measured using colorimetric kits (Nanjing Jiancheng Institute of Bio-engineering, China). Circulating inflammation, plasma tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) were quantified using commercially available ELISA kits (Uscnlife, Missouri, TX, USA), according to the manufacturer’s protocol.

**Statistical analysis:** Descriptive analyses were used. Continuous variables are expressed as the mean ± standard deviation and were compared with the $t$-test for two groups and ANOVA for more than two groups. Categorical variables are expressed as percentages that were compared with the $\chi^2$ test or Fisher’s exact test, as appropriate. A $P$ value $< 0.05$ was considered significant.

## Results

### Quantitative coronary analysis (QCA):
A summary of the QCA results is shown in Table I. The reference vessel diameters and overstretch ratio at baseline (beginning of 19 weeks) were not significantly different among the 3 groups. At 28 days (end of 22 weeks), the highest degrees of %DS and LL were achieved by the HGF group followed by the LGF group ($P < 0.05$) and the control group ($P < 0.05$). The lowest degree of in-stent MLD was achieved by the HGF group followed by the LGF group ($P = 0.02$) and control group ($P < 0.001$).

### OCT analysis:
OCT analysis at 28 days (end of 22 weeks) is presented in Table II. The stent area was not significantly different among the 3 groups. The highest degree of NIA, %AS, and NIT were achieved by the HGF group followed by the LGF ($P < 0.05$) and control groups ($P < 0.001$). The lowest degree of LA was achieved by the HGF group followed by the LGF group ($P = 0.045$) and control group ($P < 0.001$). The representative vessels with angiography and OCT are presented in Figure 2.

The different neointimal characteristics were assessed among the 3 groups. A total of 2175 frames (control 733 frames, LGF 717 frames, and HGF 725 frames) were analyzed. The highest frequency of a homogeneous pattern was in the control group (513/733; 70%) followed by the LGF group (427/717; 59.6%) ($P < 0.05$) and the HGF group (382/725; 52.7%) ($P < 0.05$). The highest frequency of a heterogeneous pattern was in the HGF group (236/725; 32.5%) followed by

### Table I. Summary of Quantitative Coronary Analysis in All Treated Vessels

<table>
<thead>
<tr>
<th></th>
<th>HGF $n = 8$</th>
<th>LGF $n = 8$</th>
<th>Control $n = 8$</th>
<th>$P$</th>
<th>HGF versus LGF</th>
<th>HGF versus Control</th>
<th>LGF versus Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>RVD (mm)</td>
<td>2.87 ± 0.56</td>
<td>2.98 ± 0.51</td>
<td>3.06 ± 0.61</td>
<td>0.872</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overstretch ratio</td>
<td>1.17 ± 0.1</td>
<td>1.16 ± 0.1</td>
<td>1.17 ± 0.1</td>
<td>0.545</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In-stent MLD (mm)</td>
<td>1.58 ± 0.33</td>
<td>2.18 ± 0.41</td>
<td>2.78 ± 0.49</td>
<td>0.022</td>
<td>0.02</td>
<td>&lt; 0.001</td>
<td>0.044</td>
</tr>
<tr>
<td>% Diameter stenosis</td>
<td>45.1 ± 9.8</td>
<td>29.1 ± 7.6</td>
<td>12.5 ± 9.4</td>
<td>0.006</td>
<td>0.01</td>
<td>&lt; 0.001</td>
<td>0.007</td>
</tr>
<tr>
<td>Late loss (mm)</td>
<td>1.23 ± 0.38</td>
<td>0.75 ± 0.21</td>
<td>0.48 ± 0.19</td>
<td>0.024</td>
<td>0.022</td>
<td>0.002</td>
<td>0.042</td>
</tr>
</tbody>
</table>

HGF indicates high glucose fluctuation; LGF, low glucose fluctuation; RVD, reference vessel diameter; and MLD, minimal luminal diameter.

### Table II. Summary of OCT in All Treated Vessels at 28 Days Follow Up

<table>
<thead>
<tr>
<th></th>
<th>HGF $n = 8$</th>
<th>LGF $n = 8$</th>
<th>Control $n = 8$</th>
<th>$P$</th>
<th>HGF versus LGF</th>
<th>HGF versus Control</th>
<th>LGF versus Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen area (mm²)</td>
<td>3.29 ± 0.52</td>
<td>3.87 ± 0.34</td>
<td>5.49 ± 0.79</td>
<td>&lt; 0.001</td>
<td>0.045</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Stent area (mm²)</td>
<td>6.49 ± 0.96</td>
<td>6.56 ± 0.85</td>
<td>6.48 ± 1.02</td>
<td>0.644</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neointimal area (mm²)</td>
<td>3.20 ± 0.47</td>
<td>2.69 ± 0.24</td>
<td>1.29 ± 0.61</td>
<td>&lt; 0.001</td>
<td>0.04</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Percent area stenosis (%)</td>
<td>49.3 ± 6.8</td>
<td>41.2 ± 4.5</td>
<td>20.1 ± 7.9</td>
<td>&lt; 0.001</td>
<td>0.035</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>NIT (mm)</td>
<td>0.61 ± 0.14</td>
<td>0.45 ± 0.09</td>
<td>0.25 ± 0.05</td>
<td>&lt; 0.001</td>
<td>0.04</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

OCT indicates optical coherence tomography; HGF, high glucose fluctuation; LGF, low glucose fluctuation; and NIT, neointimal thickness.
the LGF group (191/717; 26.7%) ($P < 0.05$) and the control group (149/733; 20.3%) ($P < 0.05$). Both the HGF (107/725; 14.8%) and LGF (98/717; 13.7%) groups had a higher frequency of the layered pattern than the control group (70/733; 9.6%) ($P < 0.05$), but there was no significant difference between the HGF and LGF groups ($P = 0.67$).

**Blood sample analysis:** At 18 weeks, total cholesterol was significantly increased compared to that at 0 week in both the DM/HC group (4.3 ± 1.1 mmol/L versus 2.9 ± 0.8 mmol/L) and control group (4.8 ± 1.2 mmol/L versus 3.2 ± 0.9 mmol/L) ($P < 0.05$). Total cholesterol at 0 weeks (2.9 ± 0.8 mmol/L versus 3.2 ± 0.9 mmol/L) and 18 weeks (4.3 ± 1.1 mmol/L versus 4.8 ± 1.2 mmol/L) and overnight fasting blood glucose at 0 weeks (4.1 ± 1.3 mmol/L versus 3.8 ± 0.6 mmol/L) were not significantly different between the DM/HC group and control group ($P > 0.05$). At 18 weeks, overnight fasting blood glucose was significantly increased in the DM/HC group compared to the control group (7.9 ± 1.9 mmol/L versus 4.2 ± 0.8 mmol/L) ($P < 0.05$).

At 19 weeks, mean blood glucose levels were not significantly different between the HGF group (11.8 ± 4.0 mmol/L) and LGF group (11.5 ± 1.4 mmol/L) ($P = 0.68$). However, the standard deviation was 4.0 in the HGF group and 1.4 in the LGF group. The 1 week mean glucose values are presented in Figure 3.

On day 28 of follow-up (end of 22 weeks), the highest degree of oxidative stress increment was achieved by the HGF group followed by the LGF group ($P < 0.05$) and the control group ($P < 0.05$) (8-isoprostaglandin: 21.5 ± 2.4 pg/mL versus 15.3 ± 2.5 pg/mL versus 6.2 ± 1.5 pg/mL; MDA: 1.15 ± 0.28 nmol/L versus 0.67 ± 0.24 nmol/L versus 0.32 ± 0.16 nmol/L); the highest degree of inflammation increment was achieved by the LGF group followed by the LGF group ($P < 0.05$) and the control group ($P < 0.05$) (TNF-α: 45.3 ± 6.2 pg/mL versus 31.7 ± 5.3 pg/mL versus 18.2 ± 3.7 pg/mL; IL-6: 55.3 ± 7.2 pg/mL versus 49.2 ± 6.1 pg/mL versus 25.5 ± 4.2 pg/mL).

**Discussion**

The present study investigated the effects of glucose fluctuation on neointimal proliferation after EES implantation in a DM/HC swine model. The main findings are: 1) DM was related to a higher degree of neointimal proliferation compared to the control group; 2) in the DM subgroup, higher glucose fluctuation was associated with a higher degree of neointimal proliferation and a higher frequency of a heterogeneous pattern; and 3) a higher glucose fluctuation was also associated with a higher degree of oxidative stress and inflammation.

In this study, we established a DM/HC swine model successfully by using a high-fat and high-sucrose diet program. At 18 weeks the total cholesterol level was significantly increased compared to that at 0 week in the DM/HC model and the overnight fasting blood glucose level was significantly increased compared to the HC group. In a previous study in a swine model, DM was commonly induced with a chemical drug to achieve a consistently high blood glucose level.67) Llano, et al68) successfully established a DM/HC swine model with strepto-
zotocin to achieve a consistently high blood glucose level and then maintain it with a HC diet for 20 weeks. However, chemical drugs such as streptozotocin are expensive and may cause damage to vital organs or even death. The pig model established by using a high-fat and high-sucrose diet is safe and less expensive. We also established a different glucose fluctuation model successfully by feeding the animals at different times daily. There were differences in the mean blood glucose fluctuation range and standard deviation of glucose between HGF and LGF. This is supported by a previous study from Gordon, et al who used apoE-deficient mice fed maltose twice daily as a model of repetitive postprandial glucose spikes to assess the role of glucose fluctuations on atherogenesis.

We observed that DM caused a higher degree of neointimal proliferation than controls in the DM/HC swine model in our study. Several human studies focusing on diabetes and DES have had varied results with respect to angiographic outcome and adverse clinical outcomes. Sakata, et al found that neointima suppression by EES was comparable in patients with and without DM in a human IVUS study. Masamichi, et al found that the average neointimal thickness and neointimal coverage treated with EES did not differ between DM and non-DM patients in a human OCT study examining 159 coronary artery lesions in 123 patients. Moreover, previous IVUS studies suggested that greater residual plaque burden at reference vessel segments was associated with edge stenosis in the SES, PES, and BMS cohorts. From these findings, in-segment restenosis may be frequently exhibited in DM, which may suggest that DM is still one of the risk factors for target lesion revascularization, even in the DES era.

We found that higher glucose fluctuation was associated with a higher degree of neointimal proliferation (higher degree of %DS, LL, NIA, % AS and NIT; lower degree of in-stent MLD and LA). From Figure 3, we found that although mean blood glucose was similar between the HGF group and LGF group, the HGF group had a higher glucose fluctuation than the LGF group. Thus, we speculate that glucose fluctuation but not mean blood glucose has a deleterious impact on neointimal proliferation. This is supported by a previous study that showed angiographic LL and NIA were significantly greater in DM/HC arteries than non-DM/HC arteries in a DM/HC porcine model of advanced coronary atherosclerosis. In recent years there is increasing evidence that glucose fluctuation produces more detrimental effects on the coronary arteries than chronic sustained hyperglycemia. In a recent clinical study, Kuroda, et al found that the mean amplitude of glycemic excursion (MAGE) had the strongest effect on the percentage of necrotic core and MAGE was the only independent predictor of the presence of thin-cap fibroatheroma. They also found that MAGE had the strongest effect on variability in NIT and the percentage of uncovered struts. In a recent study in Wistar rats, Joubert, et al found that in the context of experimental myocardial ischaemia/reperfusion, glycaemic variability might have a potentially deleterious impact on myocardial outcomes beyond the classical glucose metrics.

We also found that HGF was associated with a higher frequency of a heterogeneous pattern. OCT is emerging as a very important imaging device for the evaluation of coronary plaque characteristics and neointimal proliferation after stenting due to its high-resolution. Recent animal and human data validated this tool in the evaluation of peri-strut neointimal formation and coverage. In addition, recent studies suggest that the different characteristics of neointimal tissue seen by OCT may correlate with clinical events such as late stent thrombosis. Kim, et al compared different OCT morphological characteristics with different in-stent neointimal tissue types analyzed by histology and found that the homogeneous pattern of neointimal formation appeared to correlate with

![Figure 3. HGF and LGF model (n = 8 each group). HGF indicates high glucose fluctuation; LGF, low glucose fluctuation; D, day. At 19 weeks, mean blood glucose levels were not significantly different between the HGF group (11.8 ± 4.0 mmol/L) and LGF group (11.5 ± 1.4 mmol/L) (P = 0.68). The standard deviation was 4.0 in the HGF group and 1.4 in the LGF group.](image-url)
less neointimal formation and more favorable vessel healing characteristics when compared with the other optical patterns; the heterogeneous pattern correlated more with the presence of fibrin deposits than the other patterns; the layered pattern correlated with the higher incidence of peri-strut inflammation, neovascularization, and external elastic lamina rupture, which might lead to a higher degree of neointimal proliferation. In a clinical study, Tanaka, et al.15 concluded that in DM patients, the heterogeneous pattern was observed with high frequency. Neointimal coverage and neointimal thickness were also higher in DM patients as compared with non-DM patients. Our study investigated the relationship between glucose fluctuation and the frequency of neointimal pattern. We observed that both HGF and LGF were associated with a higher frequency of layered pattern than the control group; HGF was associated with a higher frequency of the heterogeneous pattern. It suggests that DM-HGF may be related to poor outcomes with more fibrin deposits and higher degree of neointimal proliferation. Our findings need to be further confirmed by histology.

Plasma 8-iso prostaglandin and MDA are useful biomarkers of oxidative stress, TNF-α and IL-6 are classic biomarkers of inflammation. We found that DM was related to a higher degree of oxidative stress and inflammation compared with the control group; higher glucose fluctuation was associated with a higher degree of oxidative stress and inflammation that are consistent with previous studies.12,23,24 Increasing evidence suggests that diabetes and glucose fluctuation lead to the deterioration of vascular complications related to oxidative stress and the inflammatory system.12,23,24 Our results indicate that higher glucose fluctuation is likely to induce more severe oxidative stress and inflammation and may be more harmful to coronary endothelial cells.

Limitations: There are some limitations to our study. First, this DM/HC swine model might reflect some of the disease characteristics, but their exact interpretation is still unclear. No animal model can reproduce all of the complex characteristics of human disease. Second, the study included a small sample size. Third, we did not do a histological evaluation. The next step for us will be to carry out a long-term follow-up study that includes a full histological evaluation.

Conclusion: DM might have a deleterious impact on neointimal proliferation after EES implantation in this DM/HC swine model; the extent of glucose fluctuation may be related to the degree of neointimal proliferation. Glucose fluctuation may become a new target for evaluation of the prognosis and selection of therapeutic strategies after DES implantation in clinical practice.

DISCLOSURE

Conflict of interest: The authors declare that they have no competing interests.

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