# Metformin Impairs Endothelialization After Placement of Newer Generation Drug Eluting Stents

**Anwer Habib, MD**, **Vinit Karmali, MA**, **Rohini Polavarapu, BA**, **Hirokuni Akahori, MD, PhD**, **Kim Pachura, BS**, and **Aloke V. Finn, MD**

Department of Internal Medicine, Emory University School of Medicine, Atlanta, Georgia

## Abstract

**Objectives**—Metformin impairs endothelialization of drug eluting stents (DES) due to convergent signaling at the mammalian target of rapamycin (mTOR) pathway. We assessed whether metformin will continue to adversely affect stent endothelialization despite design improvements in newer generation DES.

**Methods**—Rabbit iliac artery stenting with newer generation DES was performed followed by 14 days of either normal chow diet or one with metformin (100 mg/kg/day) added. Scanning electron microscopy was used to assess stent endothelialization after sacrifice.

**Results**—In the metformin-treated group there was significantly less endothelialization compared to the placebo-treated group. Paclitaxel-eluting stents in placebo-treated group had the greatest degree of endothelialization with significantly less in its metformin-treated counterpart and all –limus eluting stent groups.

**Conclusions**—Metformin inhibited stent endothelialization in newer generation DES despite improvements in stent design. By impairing stent endothelialization, metformin may increase the risk for thrombotic complications after newer generation DES placement.

### Keywords

Metformin; Vascular Endothelium; Drug Eluting Stents

## Introduction

Patients with diabetes are at increased risk for the development of coronary artery disease. Treatment of symptomatic coronary artery disease with drug eluting stents (DES) in the diabetic population represents a significant advance by preventing stent restenosis which is a major limitation of this procedure (1,2). However, in diabetic patients, the long term safety profile of DES is limited by an increased risk of late stent thrombosis, which is thought to be

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due to poor stent endothelialization (1,3). While an explanation for this increased risk is unknown, recent studies have suggested mammalian target of rapamycin (mTOR) inhibitors, which are eluted from most DES, share convergent molecular signaling with commonly used diabetic medication such as metformin which results in impaired endothelialization (4). However, it remains unknown whether this same interaction applies to newer generation DES which are characterized by improvements in stent design and polymer biocompatibility while utilizing either newer mTOR inhibitors based agents or paclitaxel (5). The objective of this study was to compare stent endothelialization in newer generation DES with and without metformin treatment in a pre-clinical animal model of arterial stenting.

## Methods

Twenty-six New Zealand White Male rabbits from 3.0 to 3.5 kg underwent iliac artery injury and stenting with either paclitaxel-eluting stents (PES, Ion Paclitaxel Eluting Coronary Stent, Boston Scientific, $3.0 \times 12$ mm, Boston, MA, USA), everolimus-eluting stents (EES, Promus-Element, $3.0 \times 12$ mm, Boston Scientific) or zotarolimus-eluting stents (ZES, Resolute-Integrity, $3.0 \times 12$ mm, Medtronic, Minneapolis, MN, USA) deployed at a target stent-to-artery ratio of 1.3:1 as previously described (4). All animals were anti-coagulated with aspirin (40 mg/day) given orally 24-hours before catheterization with continued dosing throughout the study while single dose intra-arterial heparin (150 IU/kg) was administered at the time of catheterization. Rabbits were randomized equally and fed standard chow diet or one with Metformin (100 mg/kg/day *per os*) added for fourteen days after stenting (6). Metformin dosing was based on equivalent dosing of 2 gm/day in humans (4). Point of care testing for blood glucose (Roche, Basel, Switzerland) was performed with no incidence of hypoglycemia in either group before or after stent placement. Animals were sacrificed at the end of the study, perfused with normal saline followed fixing with 4% paraformaldehyde and processed for scanning electron microscopy. The study protocol was in compliance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health and approved by the Emory University Institutional Animal Care and Use Committee. Stents were analyzed by scanning electron microscopy and percent endothelialization was calculated as previously described (4). Statistical analysis was performed JMP Pro v10 (SAS, Cary, NC). All data was expressed as mean $\pm$ SD. Differences were evaluated using two-way analysis of variance (ANOVA) for multiple comparisons followed by Tukey HSD post-hoc test when the F-test was significant. A p-value of $< 0.05$ was considered statistically significant.

## Results

There was significant inhibition of stent endothelialization in Metformin-fed animals compared with standard chow diet among all stent types ($p = 0.01$) (figure 1 and table 1). The PES-standard chow diet had the greatest stent endothelialization at 14 days which compared with all –limus based groups ($p < 0.0001$) and the PES-Metformin group ($p = 0.038$) (table 1). When comparing stent types, animals receiving –limus based stents had less endothelialization when compared with PES ($p < 0.0001$) and stent endothelialization was the least with EES and ZES groups treated with Metformin. There was no significant interaction term between stent type and treatment group ($p = 0.544$).

## Discussion

Metformin is the most commonly prescribed anti-diabetic medication with increasing evidence suggesting that it also has anti-proliferative properties through its action on the mammalian target of rapamycin (mTOR) pathway (4,7). This study suggests that metformin in combination with newer generation –limus and paclitaxel DES acts to impair stent

endothelialization despite improvements in stent design. We have previously shown that metformin and mTOR inhibitors, such as sirolimus, converge on S6 kinase, a downstream target of the mTOR complex 1, leading to suppression of key regulators of the S/G1 cycle progression such as cyclin D1 (4,8). Paclitaxel, a taxane, impairs microtubule formation suppressing the mitotic phase and creating a cytotoxic environment by promoting increased apoptosis (8). Metformin in combination with paclitaxel likely suppresses endothelialization through its combined effect on apoptosis (4,8). Overall these findings, as well as recent clinical trials, suggest that non-insulin dependent diabetic patients treated with both metformin and current generation DES may potentially be at increased risk of impaired stent endothelialization, stent thrombosis and poorer outcomes (9,10). Our study is limited by the use of a non-diabetic animal model and likely leads to an overall underestimation of the degree of endothelial inhibition as diabetes may further impair endothelialization in the setting of injury (4). Given this increased risk, prolonged dual anti-platelet therapy beyond the indicated period may be beneficial in diabetic patients on chronic metformin therapy (11).

## Acknowledgments

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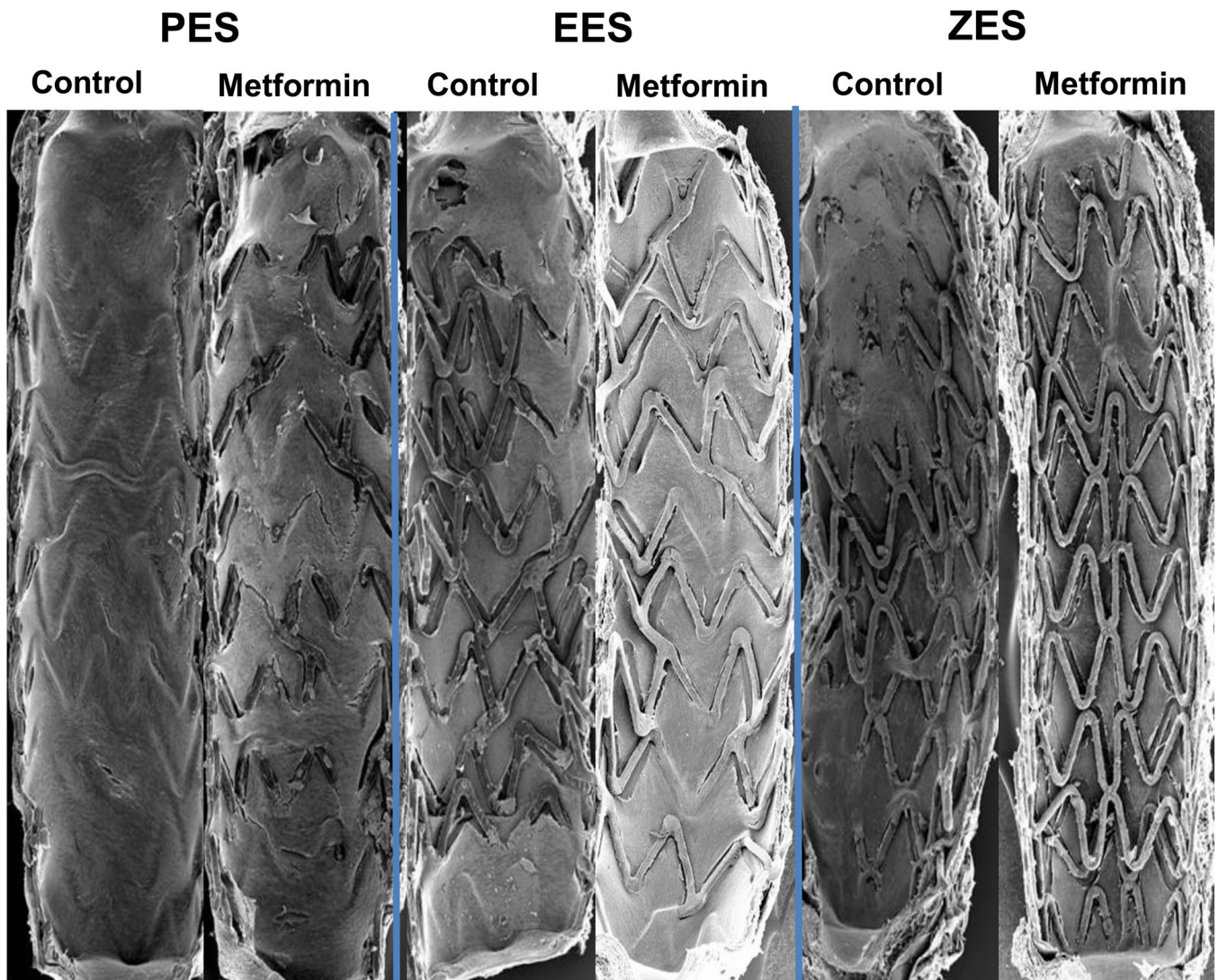
**Figure 1. Endothelial Stent Strut Endothelialization is Impaired in Newer Generation Drug Eluting Stents (DES) in Combination with Oral Metformin in the Rabbit Iliac Model of Arterial Stenting**

Representative scanning electron micrographs (15×) of 14-day paclitaxel eluting stents (PES), everolimus- and zotarolimus-eluting stents (EES, ZES) in the presence or absence of oral Metformin (100 mg/kg/day) treatment.

**Table 1**

Evaluation of % Endothelialization at 14-day by Scanning Electron Microscopy

|  | Ion[**],[†] (n=8) | Promus Element (n=8) | Endeavor Resolute (n=10) |
|---|---|---|---|
| Control [†] (n=13 animals) | 67.7 ± 30.5 | 24.3 ± 16.1 | 16.3 ± 16.2 |
| Metformin [*] (n=13) | 48.2 ± 23.5 | 10.4 ± 10.2 | 10.0 ± 13.4 |

Data expressed as mean ± standard deviation.

[*] Metformin is significantly different from Control (p = 0.01).

[**] Ion is significantly different from Promus Element and Endeavor Resolute stent (p < 0.0001).

[†] Ion-Control is significantly different than Ion-Metformin (p = 0.038).