The Role of MRP8 in *in stent* Restenosis in the Diabetic Rat
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**Introduction**
The prevalence of type 2 diabetes mellitus is reaching pandemic proportions. The most common cause of death in diabetes mellitus is cardiovascular disease. Patients frequently undergo vascular interventions such as stenting, as a therapy for this disorder. While the occurrence of *in stent* restenosis has been reduced by the use of drug eluting stents, this is less effective in diabetes mellitus than in non-diabetic subjects. In addition, drug eluting stents may impair endothelial recovery post-stenting which may lead to an increased risk of *in stent* thrombosis, a condition with mortality of approximately 40%. Novel approaches to the prevention of *in stent* restenosis which do not impair endothelial function are required.

**Materials and Methods**
*In vivo*: The Zucker fatty rat has been chosen as model of type 2 diabetes for its metabolic characteristics. Stent implant was performed in fatty rats and lean controls in the left internal carotid artery: the animals were sacrificed before stenting, 1 day, 3 days and 14 days post surgery. Samples from day 1 and 14 were plastic embedded for histomorphometry; unstented carotid arteries and 3 day post surgery arteries have been used for RNA isolation.

*In vitro*: Rat Aortic Endothelial Cells (RAOEC) were cultured in normal glucose (5.5mM) and high glucose (22mM) for 48hr before performing cell count, apoptosis, proliferation and migration assays.

**Results**
The Zucker fatty rat demonstrated exaggerated intimal hyperplasia 14 days post surgery in comparison to lean rats (30% vs 15%). Microarray analysis showed MRP8 mRNA was upregulated 8-fold in the carotid artery of the fatty rats versus lean controls. Quantitative PCR confirmed this result. RAOEC incubated in high glucose showed a reduction in the cell number (approx 30%) as a result of increased apoptosis and reduced proliferation. Migration of rat aortic endothelial cells (RAOECs) was impaired (50% reduction) when the cells are exposed to high glucose. Interestingly endothelial cells (ECs) incubated in high glucose displayed a 75-fold upregulation of MRP8. MRP8 was cloned and a shRNA designed targetting it: RAOEC overexpressing MRP8 showed similar migration impairment to RAOECs exposed to high glucose; the migratory capacity was partially restored when the high glucose cells were treated with the shRNA against MRP8.

**Conclusions**
We show that MRP8 has an active role in endothelial cell dysfunction in diabetes. MRP8 is overexpressed in the arteries of diabetic rats and its expression is augmented in endothelial cells exposed to high glucose. MRP8 reduces the migration of endothelial cells. Endothelial cell proliferation and migration are fundamental to re-endothelialization of implanted stents, and thus limiting restenosis. Blocking MRP8 expression using shRNA we have been able to restore the migrational capacity of endothelial cells inhibited by high glucose incubation. MRP8 could be considered a good target to reduce in stent restenosis in diabetes.

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