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Diabetic dyslipidaemia is associated with alterations in eNOS, caveolin-1 and endothelial dysfunction in streptozotocin treated rats

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Abstract

Background: Diabetes is a complex progressive disease characterised by chronic hyperglycaemia and dyslipidaemia associated with endothelial dysfunction. Oxidised LDL (Ox-LDL) is elevated in diabetes and may contribute to endothelial dysfunction. The aim of this study was to relate the serum levels of Ox-LDL with endothelial dysfunction in streptozotocin (STZ)-diabetic rats and to further explore the changes in endothelial nitric oxide synthase (eNOS) and caveolin-1 (CAV-1) expression in primary aortic endothelial cells (ECs).

Methods: Diabetes was induced with a single intraperitoneal injection of STZ in male Wistar rats. During the hyperglycaemic diabetes state serum lipid markers, aortic relaxation and aortic ECs eNOS and CAV-1 protein expression was measured.

Results: Elevated serum Ox-LDL (STZ 1486 ± 78.1 pg/ml vs control 732.6 ± 160.6 pg/ml, $p < 0.05$) was associated with hyperglycaemia (STZ 29 ± 0.9 mmol/L vs control: 7.2 ± 0.2 mmol/L, $p < 0.001$) and hypertriglyceridemia (STZ 9.0 ± 1.5 mmol/L vs control: 3.0 ± 0.3 mmol/L, $p < 0.01$) in diabetic rats. A significant reduction was observed in STZ-diabetic aortic endothelial cell eNOS and CAV-1 of 40% and 30% respectively, accompanied by a compromised STZ-diabetic carbachol-induced vasodilation (STZ $29.6 \pm 9.3\%$ vs control $77.2 \pm 2.5\%$, $p < 0.001$).

Conclusions: The elevated serum Ox-LDL in hyperglycaemic STZ-diabetic rats may contribute to diabetic endothelial dysfunction, possibly through downregulation of endothelial CAV-1 and eNOS.

Key words:

diabetes, oxidative LDL, endothelial nitric oxide synthase, caveolin-1, endothelial dysfunction

Abbreviations:

AGEs, advanced glycation end products;

STZ, streptozotocin;

Ox-LDL, oxidative low density lipoprotein

HDL, high density lipoprotein

TGs, triglycerides

eNOS: endothelial nitric oxide synthase

CAV-1: caveolin-1

CC: carbachol

ECs: endothelial cells

NA: noradrenaline

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The incidence of diabetes mellitus is continuously increasing, and numbers are expected to reach 592 million globally by the year 2035 [1]. Diabetes complications contribute to a huge economic burden to the health system and society, such as endothelial dysfunction where endothelium-dependent vasodilation is compromised hence rendering diabetics vulnerable to limb infections and end-organ damage such as nephropathy, neuropathy as well as retinopathy [2].

As yet, there are no biomarker predictors to indicate endothelial dysfunction in diabetes. Oxidised LDL (Ox-LDL) is a key component in the development of atherosclerotic lesions, is cytotoxic to various cell types including endothelial cells (ECs) and therefore is believed to contribute to endothelial dysfunction [3].

Caveolae form highly organised microdomains in the ECs plasma membrane providing docking sites for numerous signalling molecules including endothelial nitric oxide synthase (eNOS) [4]. Caveolin-1 (CAV-1) is a principal protein and marker found in the endothelial caveolae and is co-localised with eNOS in cultured bovine aortic ECs [5, 6]. Disruption of endothelial caveolae leads to eNOS uncoupling compromising vasodilation in coronary arterioles of diabetic patients [7].

Here we show for the first time that the development of hyperglycaemia increases Ox-LDL, cholesterol and triglycerides (TGs) serum biomarkers, reduces expression of eNOS and CAV-1 proteins and leads to vascular dysfunction in streptozotocin (STZ)-induced diabetic rats.

Development of dyslipidaemia and endothelial dysfunction in STZ diabetic rats.

STZ-injected male Wistar rats developed significant blood glucose elevations within one week (hyperglycaemia) (STZ-diabetic: 29 ± 0.9 mmol/L; control: 7.2 ± 0.2 mmol/L $***p < 0.001$, versus control), and the hyperglycaemia was associated with a significant increase in serum Ox-LDL, TGs and total cholesterol (Figure 1A-C). This increase in serum Ox-LDL, TGs and cholesterol was accompanied by a compromised endothelial function in STZ-diabetic rats' aortic rings characterised by a significant decrease in vascular relaxant response to carbachol ($EC_{50} = 0.8 \pm 0.4 \mu\text{M}$ & $E_{\text{max}} = 29.6 \pm 9.3\%$, $*** P < 0.001$) compared with control rat aorta ($EC_{50} = 0.6 \mu\text{M} \pm 0.2 \mu\text{M}$ & $E_{\text{max}} = 77.2 \pm 2.5\%$, Figure 1D).

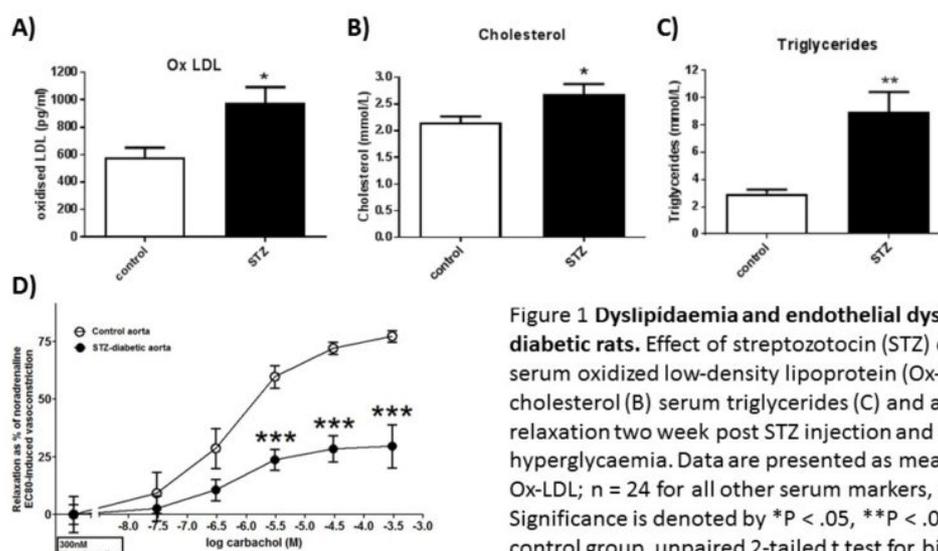


Figure 1 Dyslipidaemia and endothelial dysfunction in STZ diabetic rats. Effect of streptozotocin (STZ) diabetes on (A) serum oxidized low-density lipoprotein (Ox-LDL); (B) serum total cholesterol (C) and aortic ring vessel relaxation two week post STZ injection and development of hyperglycaemia. Data are presented as mean \pm SEM ($n = 4$ for Ox-LDL; $n = 24$ for all other serum markers, $n = 5-6$ aortic rings). Significance is denoted by * $P < .05$, ** $P < .01$, *** $P < .001$ versus control group, unpaired 2-tailed t test for biomarkers and Bonferroni 2-way repeated measures ANOVA for aortic vessel relaxation. (Data adapted from Shamsaldeen et al., *Diabetes Metab Res Rev.* 34(5), 2018)

Downregulation of Endothelial nitric oxide synthase (eNOS) and Caveolin-1 (CAV-1) in STZ-diabetic rat aortic endothelial cells.

As nitric oxide is considered as a major vasodilator, we investigated the expression pattern of eNOS in rats' primary aortic ECs. We isolated primary ECs from control and STZ diabetic rat aortic rings and measured the level of expression of eNOS in ECs through laser scanning confocal microscopy. Cultured ECs were incubated with a selective ECs marker, acetylated-LDL and a selective eNOS antibody. As shown in Figure 3 eNOS expression was significantly reduced by 40% in STZ-rats' aortic ECs.

Caveolae are 50- to 100-nm diameter lipid raft invaginations in the ECs' membrane, and form approximately 95% of the ECs surface invaginations where it provides a signalling platform that facilitates interactions of signalling molecules such as eNOS [6, 8, 9]. Disruption of endothelial caveolae can lead to eNOS uncoupling compromising vasodilation in coronary arterioles of diabetic patients [7]. CAV-1 is a major protein component of the endothelial caveolae and is co-localised with eNOS in cultured bovine aortic ECs [5, 6]. Since eNOS expression was downregulated, we measured CAV-1 expression to investigate whether eNOS downregulation correlates with CAV-1 downregulation. As shown in Figure 2 CAV-1 expression was significantly reduced by 30% in STZ-rats' aortic ECs.

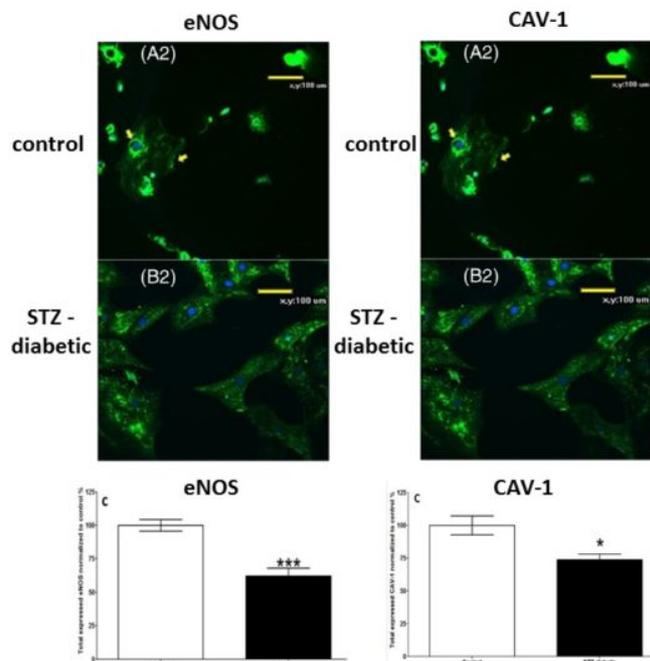


Figure 2 Downregulation of Endothelial nitric oxide synthase (eNOS) and Caveolin-1 (CAV-1) in STZ-diabetic rat aortic endothelial cells. eNOS and CAV-1 expression in primary aortic endothelial cells (ECs) and from control and STZ diabetic rat aorta under laser scanning confocal microscopy. Primary antibodies probed with secondary fluorescence antibody showed distinct distribution around the nucleus and at the edge of plasma membrane in control aortic ECs (A2, yellow arrows). STZ-diabetic ECs showed disrupted eNOS and CAV-1 distribution with less fluorescence light emission (B2). C, Quantitative analysis of eNOS and CAV-1 immunoreactivity. Data are mean ± SEM (N = 5-6) of total eNOS / CAV-1 expression (% of control). Significance is represented as *P<0.05, ***P <0.001 versus control, 2 tailed unpaired student t test. (Data adapted from Shamsaldeen et al., Diabetes Metab Res Rev. 34(5), 2018)

Our findings reveal that STZ-injected rats develop significant hyperglycaemia and elevated Ox-LDL, TGs and total cholesterol serum concentrations, associated with significant endothelial dysfunction characterised by eNOS and CAV-1 downregulation. Ox-LDL molecules are cholesterol acceptors that deplete caveolar from cholesterol causing eNOS displacement and hence eNOS inactivation (Figure 3). The mechanisms underlying CAV-1 and eNOS downregulation, reduced eNOS activity and contribution to macrovascular disease in diabetes needs further investigation.

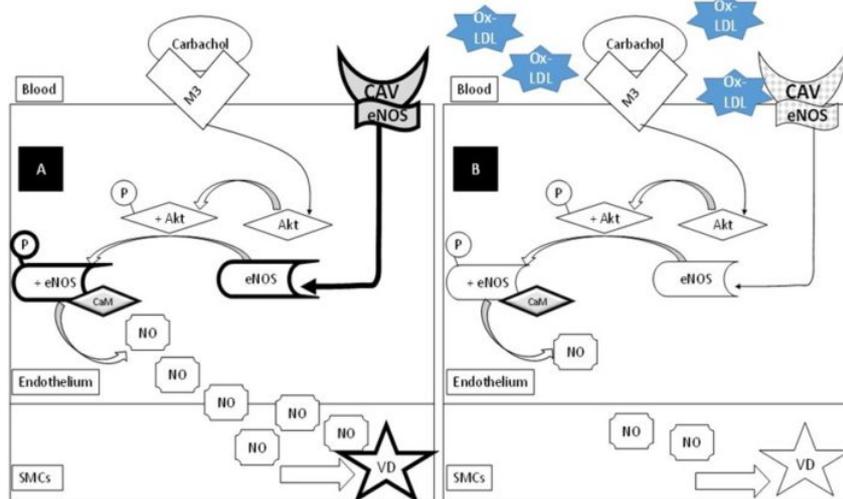


Figure 3 Schematic depiction of Ox-LDL effects and potential mechanisms underlying diabetic vascular dysfunction. A, Cholinergic-induced aortic endothelium dependent vasodilation. B, Dysfunctional cholinergic-induced aortic endothelial-dependent vasodilation following STZ. NO, nitric oxide; CAV, caveolin-1; eNOS, endothelial nitric oxide synthase; Ox-LDL, oxidized low-density lipoprotein; VD, vasodilation; CaM, calmodulin; SMCs, smooth muscle cells (Data adapted from Shamsaldeen et al., Diabetes Metab Res Rev. 34(5), 2018)

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