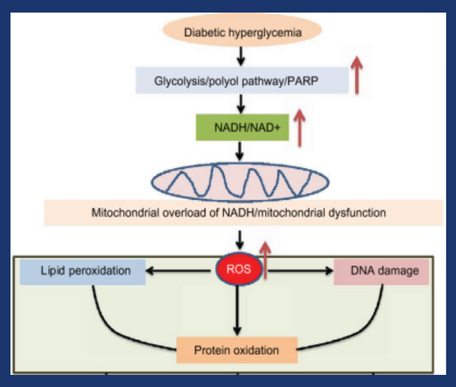
Sirt1 is essential for beneficial effect of resveratrol on HG-induced endothelial dysfunction

Mijiti M[1], Santini S Jr[1], Cordone V[1], Bignotti V[1], Falone S[1], Amicarelli F[1]
1 Dept. of Life, Health and Environmental Sciences, University of L`Aquila, Italy.

Introduction

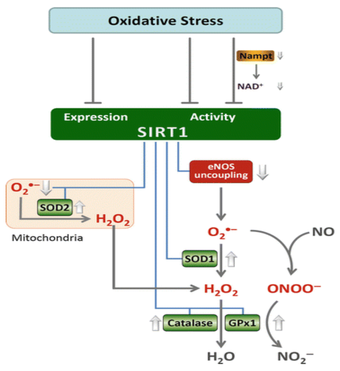
Hyperglycemia (HG) is a well-known oxidative stress (OS)-generating insult that may lead to endothelial dysfunction and, ultimately, to vascular complications of diabetes (DB) [1-2].
Resveratrol (RSV), a stilbene and non-flavonoid polyphenol produced by various plants, is known to have important anti-oxidant and anti-inflammatory properties, that might be exploited to prevent or minimize DB-dependent endothelial dysfunction [3]. Some biological actions of RSV are known to rely on the activation of sirtuins, NAD^+-dependent protein deacetylases, whose expression regulates key antioxidant cellular defence systems, both in extra- and intra-mitochondrial compartments. However, the detailed molecular mechanisms underlying the protective effect of RSV in the human endothelium upon hyperglycemia still remain unclear [4-5].



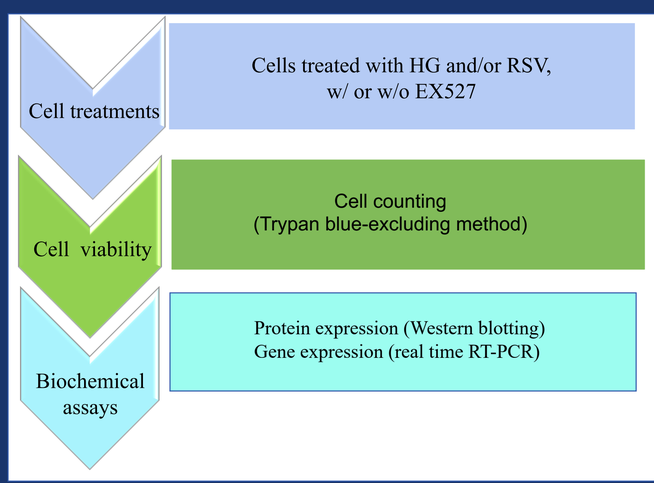
diabetic hyperglycemia and oxidative stress [6]

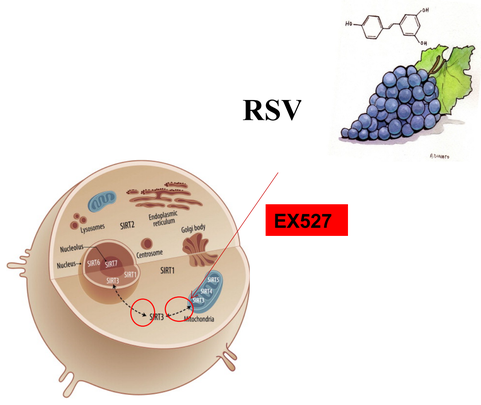
Objectives, Material and Methods

Aim : To investigate the involvement of SIRT1 (cytosolic/nuclear) and SIRT3 (mitochondrial) in the cytoprotective effect of RSV human umbilical vein endothelial cells (HUVECs) upon high glucose challenge.



The Role of SIRT1 in Diabetic Kidney Disease Schematic diagram [8]

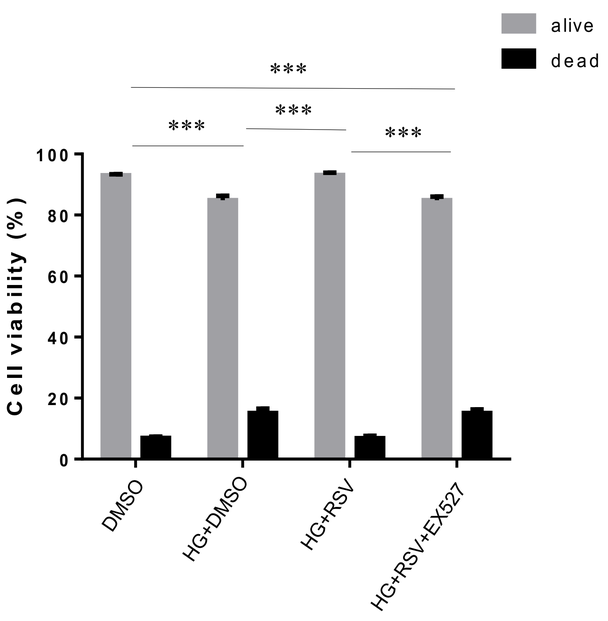




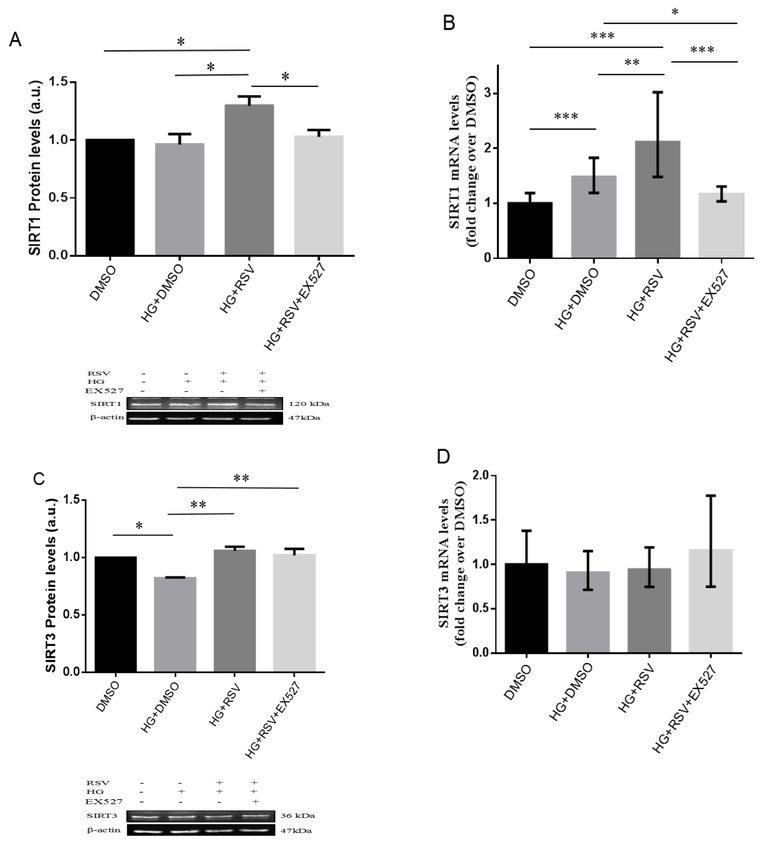
Cellular distribution of sirtuins [7]

Results

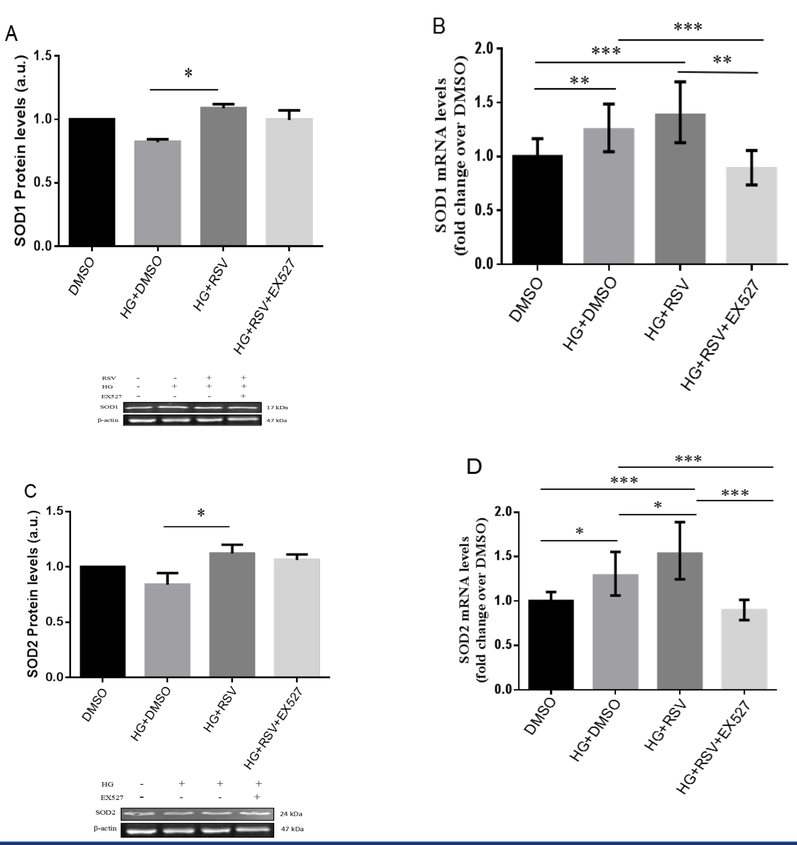
Cell viability



SIRT1 and SIRT3 regulation



SOD1 and SOD2 regulation



1. Cytoprotective effect of RSV on HG-induced toxicity in HUVECs
vehicle (DMSO), high glucose (HG+DMSO), high glucose + resveratrol (HG+RSV), or high glucose + resveratrol + EX527 (HG+RSV+EX527). Data was expressed as means (%) ± SEM, n=8. ***P<0.001 (One-way ANOVA with *post-hoc* Tukey tests).

2. Regulation of sirtuin 1 and sirtuin 3 expression
Expression analysis of SIRT1 (panel A, protein; panel B, mRNA) and SIRT3 (panel C, protein; panel D, mRNA) in HUVECs treated with vehicle (DMSO), high glucose (HG+DMSO), high glucose + resveratrol (HG+RSV), or high glucose + resveratrol + EX527 (HG+RSV+EX527). Representative Western blot images were reported in panels A and C. Protein data were expressed as means (arbitrary units) ± SEM, n=4. *P<0.05, **P<0.01, One-way ANOVA followed by *post-hoc* Tukey tests (Panels A and C). mRNA data were reported as means ± error estimation of the calculated ratio using the Taylor`s series. *P<0.05, **P<0.01, ***P < 0.001, One-way ANOVA (n=5; Panels B and D).

3. Effect of RSV on regulation of antioxidant system
Expression analysis of SOD1 (panel A, protein; panel B, mRNA), SOD2 (panel C, protein; panel D, mRNA) in HUVECs treated with vehicle (DMSO), high glucose (HG+DMSO), high glucose + resveratrol (HG+RSV), or high glucose + resveratrol + EX527 (HG+RSV+EX527). Representative Western blot images were reported (Panels A and C). Protein data were expressed as means (arbitrary units) ± SEM, n=4. *P<0.05 (One-way ANOVA followed by *post-hoc* Tukey tests) (Panels A and C). mRNA data were reported as means ± error estimation of the calculated ratio using the Taylor`s series. *P<0.05, **P<0.01, ***P<0.001 (One-way ANOVA; n=5, Panels B and D).

Conclusion and future perspective

- ✓ SIRT1, but not SIRT3, is required by RSV to elicit cytoprotective effect in HG-challenged HUVECs;
- ✓ SIRT1 is required by RSV to over-express both SOD1 and SOD2 at transcriptional level in HG-challenged HUVECs;
- ➢ In order to establish whether SIRT3 responds to HG challenge by modifying directly SOD2 mitochondial activity, the acetylation level of superoxide dismutase 2 will be investigated;
- ➢ In order to verify whether protein expression profiles reflect biochemical functions, the specific activities of SIRT1, SIRT3, and SODs will be evaluated.

Reference

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Contact Information
Email: Mmijiti@unite.it
Website:www.rep-eat.unite.it

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