How does chronic sildenafil prevent vascular oxidative stress in insulin resistant rats?

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• Insulin resistance, component of the metabolic syndrome, is a risk factor for endothelial dysfunction and subsequent cardiovascular diseases.

• Oxidative stress has been suggested to contribute to generalized endothelial dysfunction associated with insulin resistance.

• We have previously demonstrated that both endothelial dysfunction and oxidative stress associated with the metabolic syndrome can be reversed by a daily treatment with sildenafil and maintained 7 days after treatment in an experimental model of insulin resistance: the fructose fed rat (FFR).

*Behr-Roussel et al., Am J Hypertens, 2008
Oudot et al., Physiol Res, 2009.*
Since the mechanisms of action by which sildenafil exerts its antioxidant effects are still largely unknown, we aimed to investigate how a 3-week chronic sildenafil administration could impact oxidative stress in a validated rat model of insulin resistance induced by fructose overload: the FFR. By measuring:

1) Vascular endothelial NO release, and
2) Vascular endothelial superoxide release

CV risk (x3-4)  
ED risk

MOA ???

Insulin resistance

Oxidative stress 

endothelial dysfunction

Chronic Sildenafil

O

NO

O

2•
Experimental design

Wistar rats 6 weeks (200 g)

Control diet

Fructose-enriched diet (60% fructose + 5% fat)

Development of Insulin resistance

Control rats (CONT)

Saline sc (twice a day)

Fructose-fed rats (FFR)

Saline sc (twice a day)

FFR + SIL

Chronic daily sildenafil (20mg/kg, twice a day)

3 weeks of treatment

W0  W5  W8  W9

Wash-out 1 week

sildenafil: sc, 20mg/kg, twice a day, thus reaching clinically relevant plasma concentrations \textit{circa} 20nM unbound known to be effective in man (\textit{Pfizer Inc., data on file})

All experiments took place after 3 weeks of daily sildenafil and one week wash-out from sildenafil treatment.
Methods

- Unstimulated **vascular endothelial NO and O$_2^•$ production** were monitored ex-vivo in thoracic aortas segments using cell-permeable **specific fluorescent probes** (DAF-FM diacetate and DHE)

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Control, FFR, FFR+Sil aorta harvesting

Stabilization
60-90 min, Krebs-HEPES 37°C

+ DAF-FM 5µM
45 min, 37°C

+ DHE 20µM

± Sildenafil 20nM
± Rotenone
± DPI

NO
Endothelial fluorescence quantification
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**PELVI PHARM**
After one-week of wash-out, chronic administration of sildenafil (20 mg/kg twice a day) for 3 weeks prevented hyperglycemia, hyperinsulinemia and hypertriglyceridemia caused by the fructose-enriched diet.
In FFR rats, while 3 weeks of chronic sildenafil administration followed by a 1 week of washout had no effect either on $O_2^{•−}$ production or SOD/NADPH oxidase expression, acute sildenafil significantly inhibited superoxide production.
Results – effects on superoxide production (2)

In FFR rats, 3 weeks of chronic sildenafil administration followed by 1 week of washout restored the contribution of mitochondrial respiratory chain and NADPH oxidase in global endothelial superoxide production.

*P<0.05, **P<0.01 vs corresponding group without inhibitor, Student’s t-test
In FFR rats, 3 weeks of chronic sildenafil administration followed by 1 week of washout significantly increased endothelial NO production while acute sildenafil incubation did not.
In FFR rats treated with chronic sildenafil and after a 1 week wash-out, increased endothelial NO production is not linked to Akt-dependent eNOS phosphorylation but dependent on eNOS expression.

*P<0.05 vs FFR, Newman-Keuls post-test following P<0.01 1-way ANOVA
Discussion – Conclusion

• The present study showed that chronic sildenafil administration in insulin resistant rats produced **vascular antioxidant effects beyond PDE5 inhibition** since occurring even after treatment cessation by:

  (1) increasing eNOS expression leading to **increased NO production**, independently of Akt-dependent eNOS phosphorylation, and

  (2) by **restoring the equilibrium of superoxide production sources** including mitochondrial respiratory chain re-coupling and decreasing superoxide production by NADPH oxidase.

• Moreover, we identified a **potent inhibitory activity of acute sildenafil on vascular superoxide production** when incubated with aortic segments, which may be linked to the inhibition of vascular NADPH oxidase.

The present study supports therefore further investigations using chronic sildenafil administration in preventing cardiovascular alterations associated with oxidative stress.