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

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ESSM Congress, Lyon- France
nov 16, 2009

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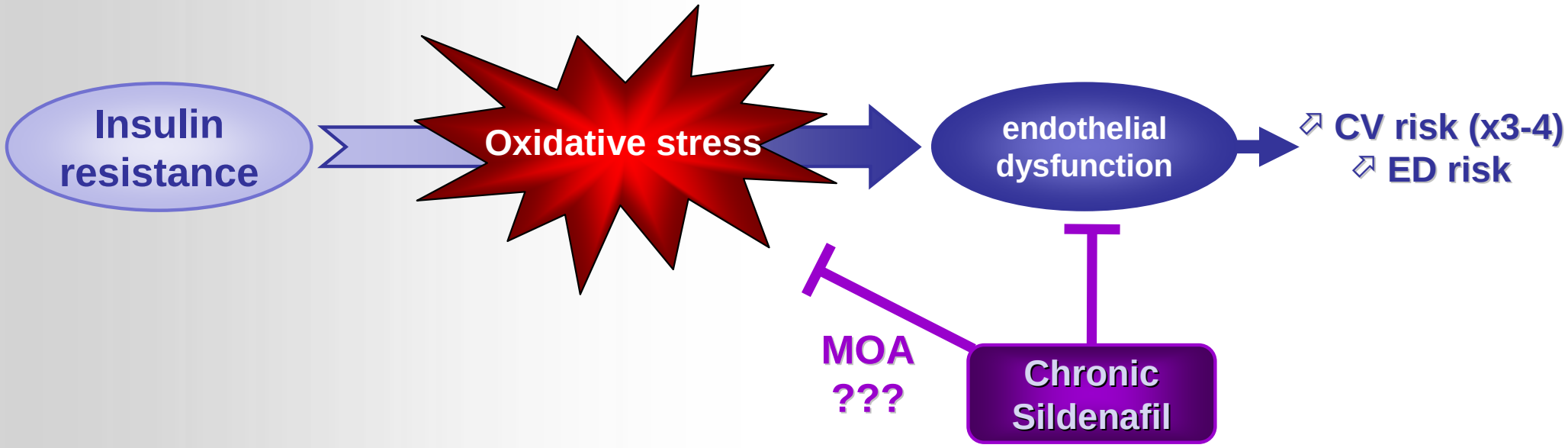
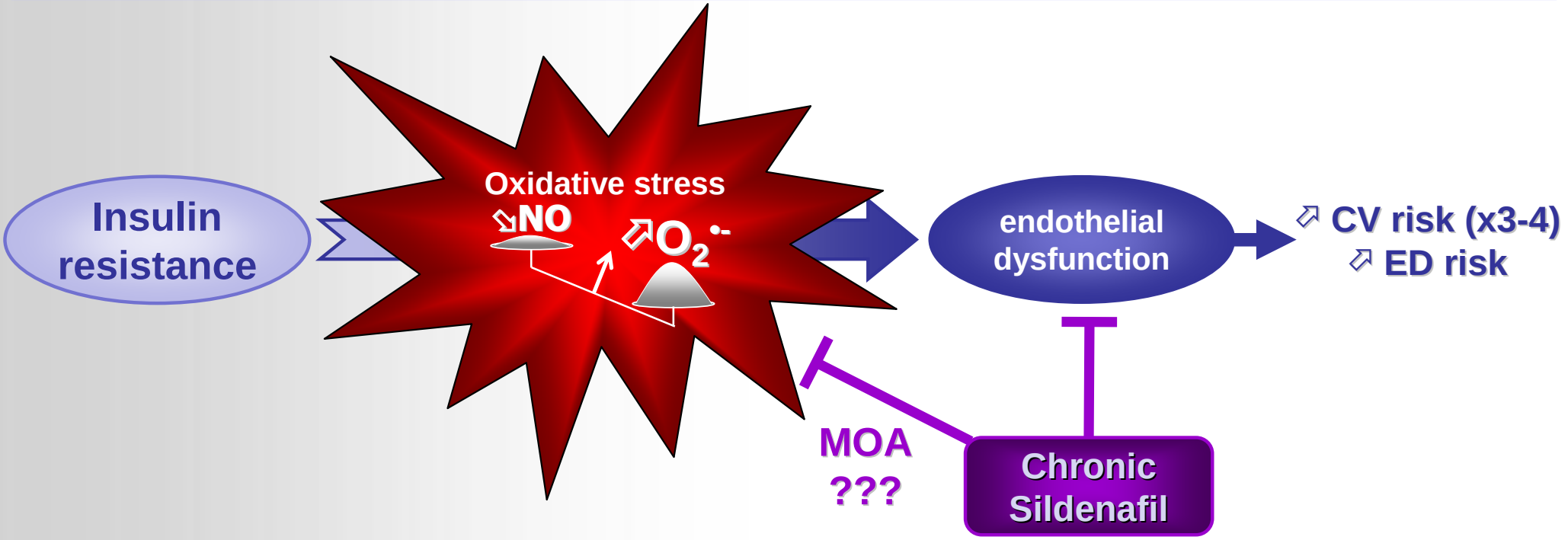
Rationale and objectives



- 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.

Rationale and objectives



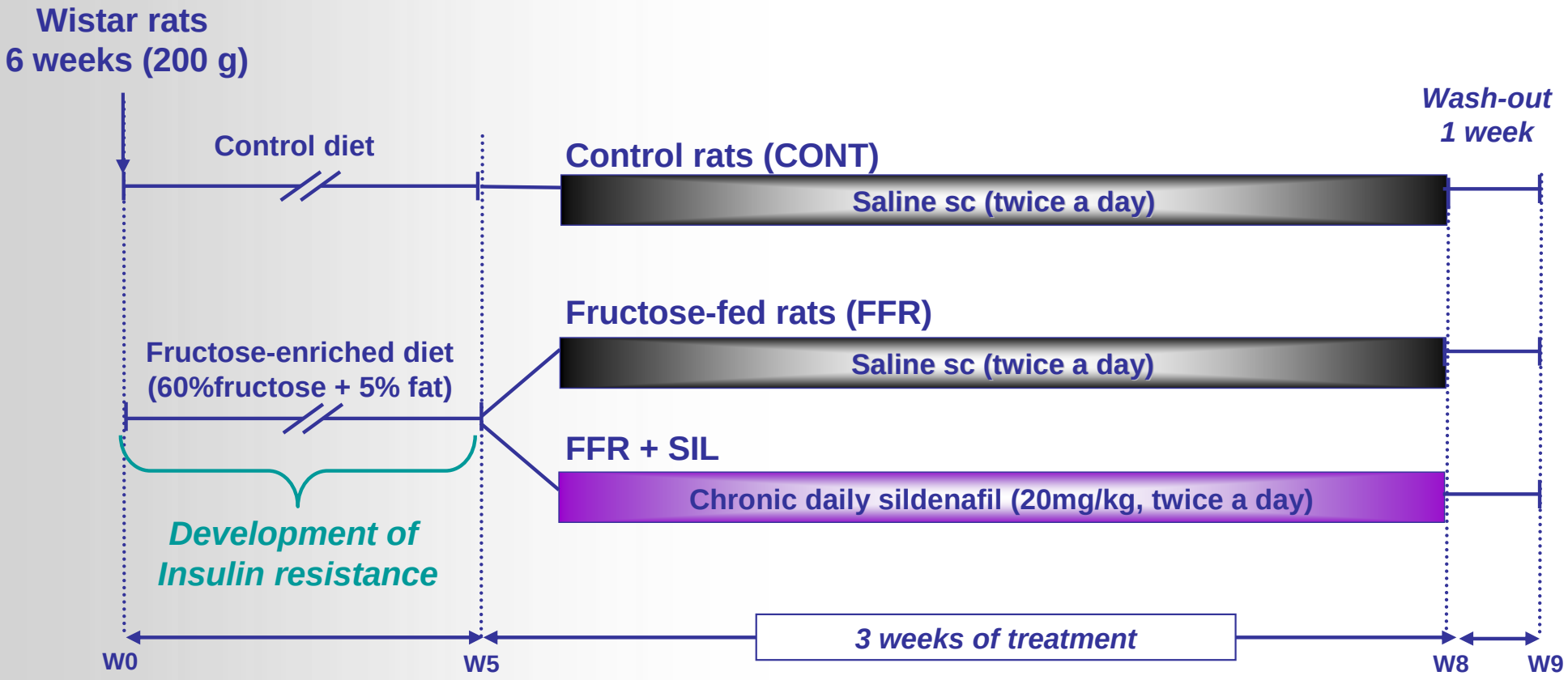
Since the mechanisms of action by which sildenafil exerts its antioxidant effects are still largely unknown,

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

Experimental design



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

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

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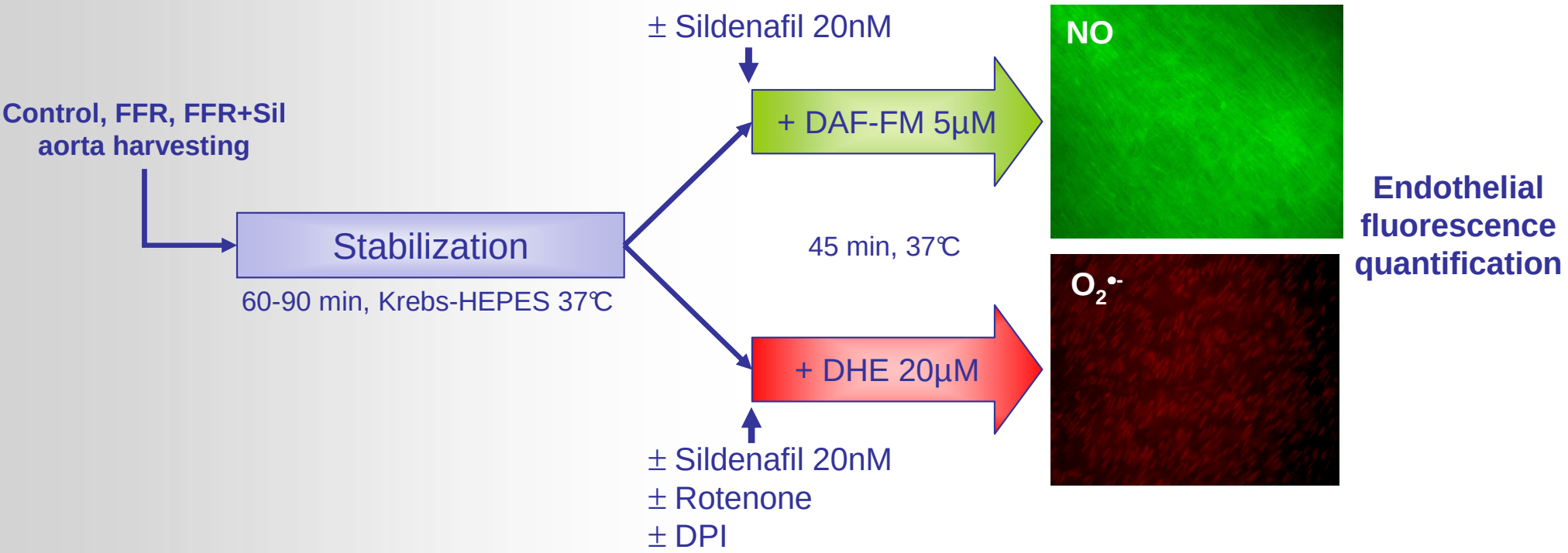
Methods

- Unstimulated **vascular endothelial NO and $O_2^{\bullet-}$ production** were monitored ex-vivo in thoracic aortas segments using cell-permeable **specific fluorescent probes** (DAF-FM diacetate and DHE)



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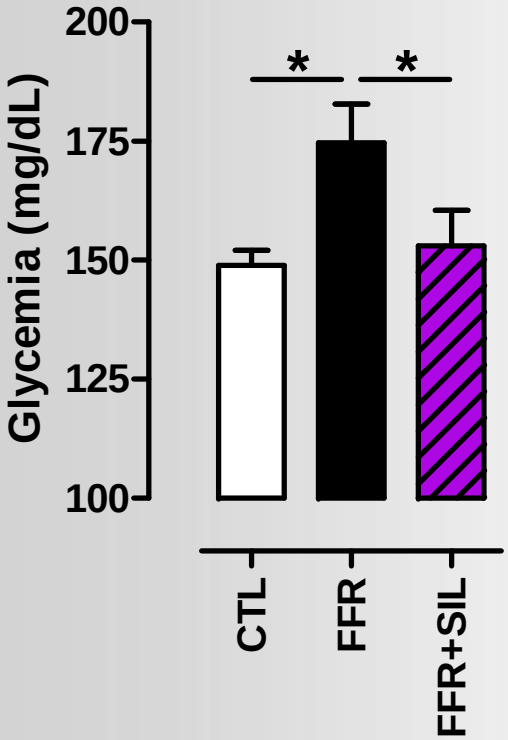
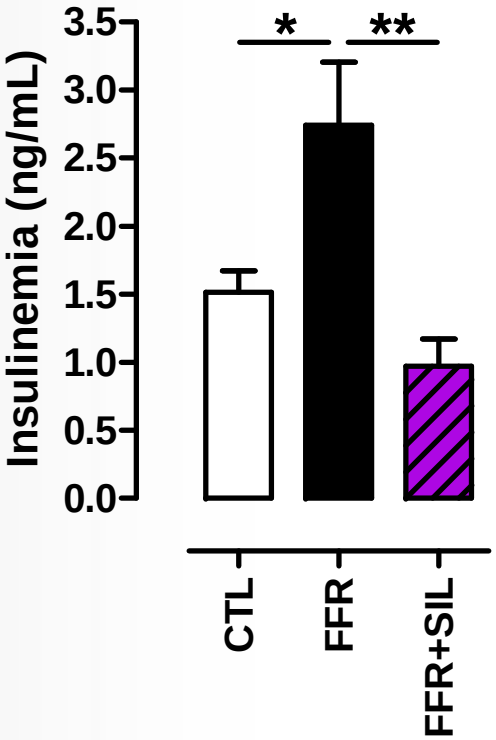
Results – Metabolic parameters

5h-fasting glycemia



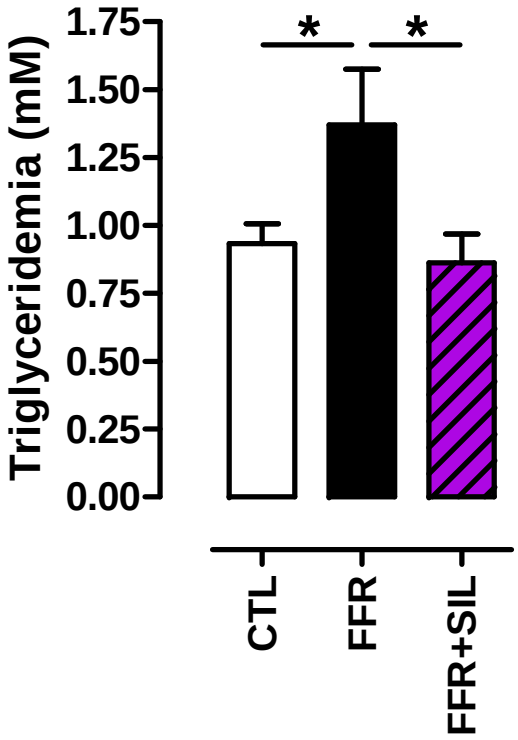
P<0.05, Newman-Keuls post-test following P<0.05 1-way ANOVA

5h-fasting insulinemia



*P<0.05, **P<0.01 Newman-Keuls post-test following P<0.01 1-way ANOVA*

Triglyceridemia



P<0.05, Newman-Keuls post-test following P<0.05 1-way ANOVA

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.

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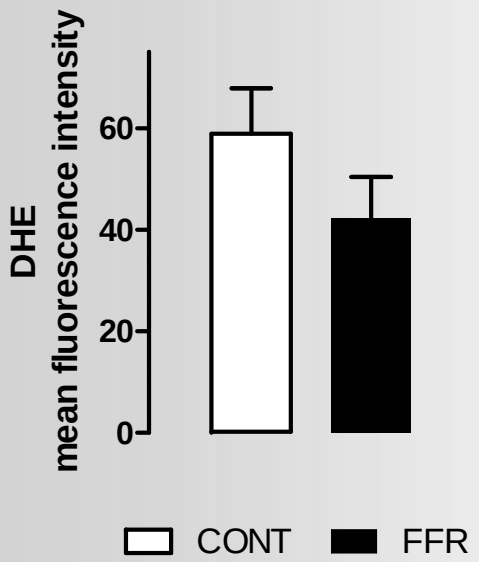
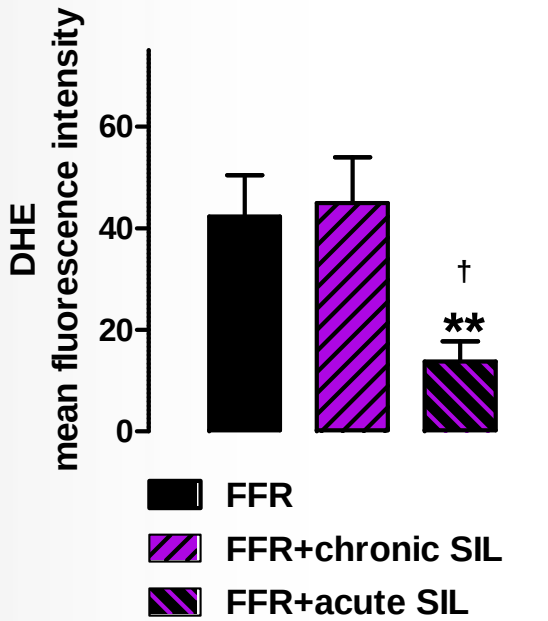
Results – effects on superoxide production (1)

Effect of fructose-enriched diet



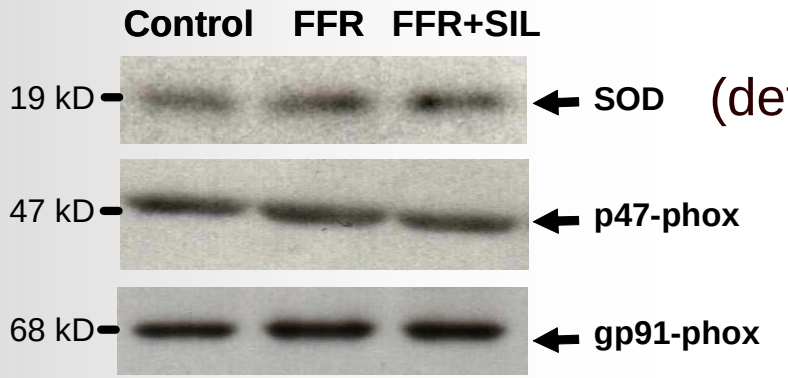
Effect of sildenafil



***P<0.01 vs FFR; †P<0.05 vs FFR+chronic SIL 1-way ANOVA*

In FFR rats, while 3 weeks of chronic sildenafil administration followed by a 1 week of washout had no effect either on $O_2^{\bullet-}$ production or SOD/NADPH oxidase expression, acute sildenafil significantly inhibited superoxide production.

Protein expression



(detoxifying $O_2^{\bullet-}$)

NADPH oxidase (producing $O_2^{\bullet-}$)

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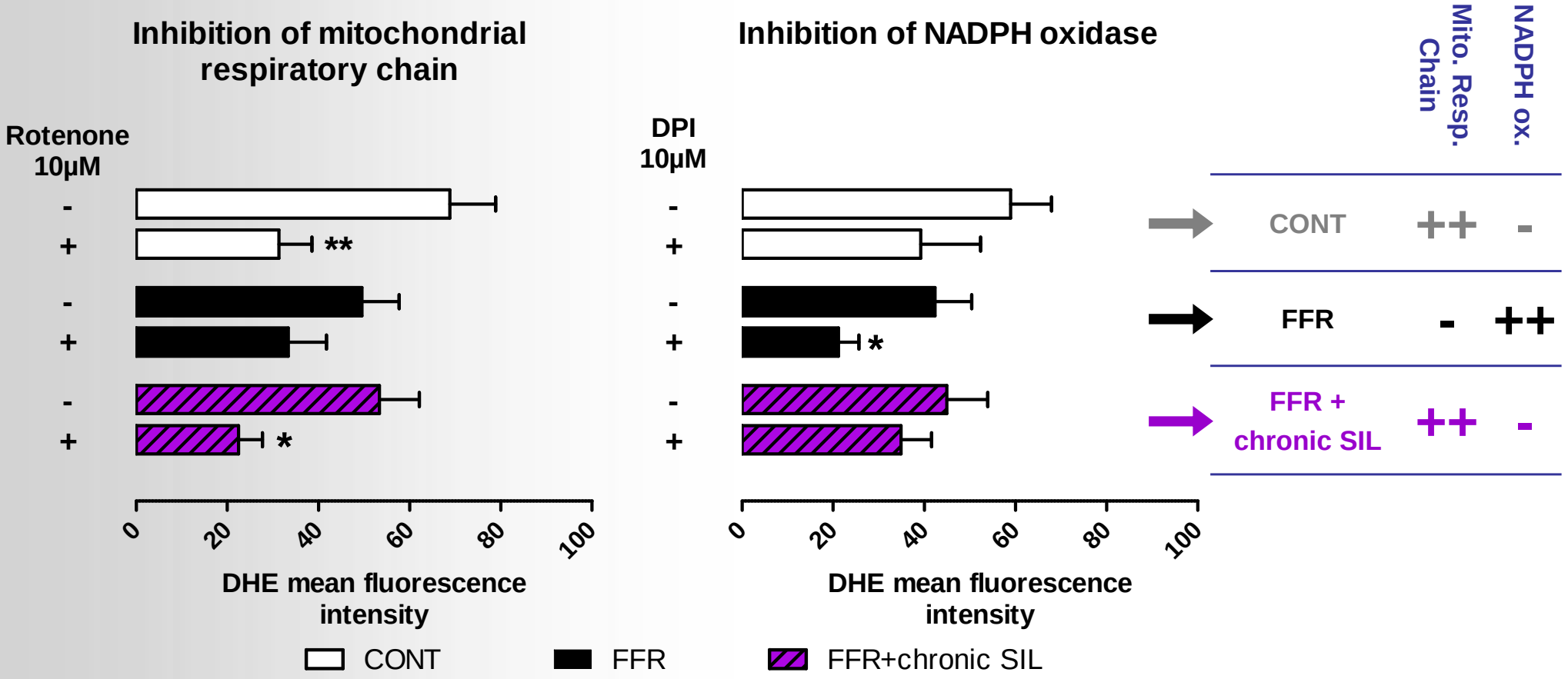
Results – effects on superoxide production (2)



*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 restored the contribution of mitochondrial respiratory chain and NADPH oxidase in global endothelial superoxide production

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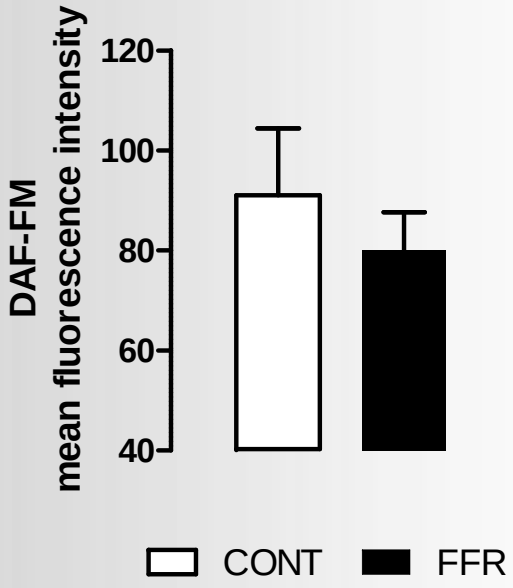
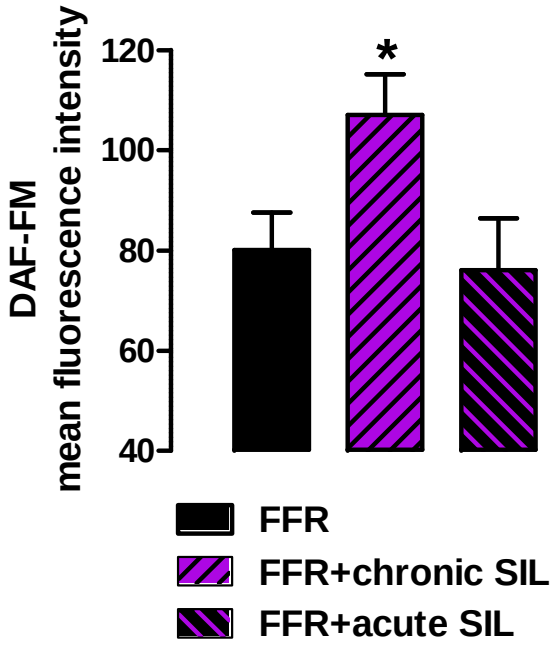
Results – effects on NO production (1)

Effect of fructose-enriched diet



Effect of sildenafil

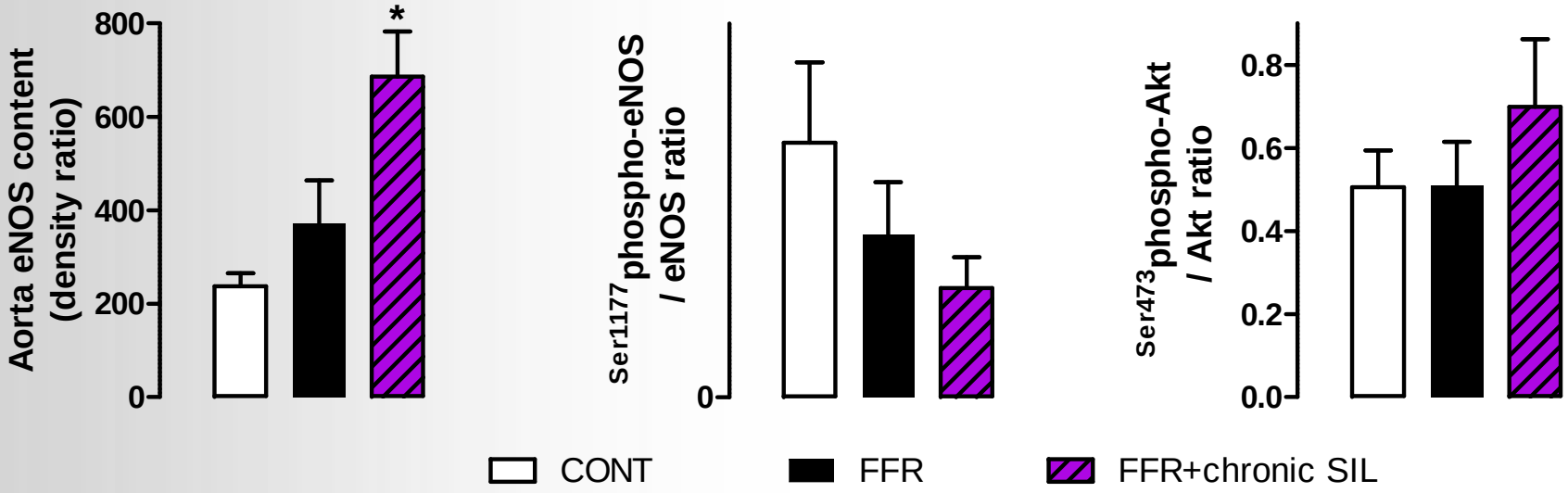


**P<0.05 vs FFR, Newman-Keuls post-test following P<0.05 1-way ANOVA*

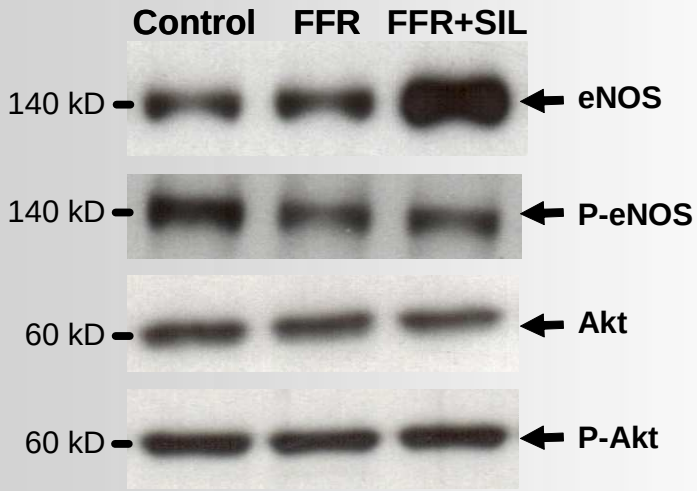
In FFR rats, 3 weeks of chronic sildenafil administration followed by 1 week of washout significantly increased endothelial NO production while acute sildenafil incubation did not.

Results – effects on NO production (2)

Protein expression



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



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.

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.

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