**OBJECTIVES**

- Fructose consumption might be a contributing factor to the development of obesity and the accompanying cardiovascular disorders (hypertension, ...) seen in the metabolic syndrome
- Patients with metabolic syndrome exhibit generalized endothelial dysfunction
- Daily treatment with PDE-5 inhibitors has beneficial effects on endothelial function in diabetic men

We postulated that chronic sildenafil in an experimental model of metabolic syndrome, the fructose-fed rat, could ameliorate arterial pressure regulation and biological surrogate markers of endothelial function such as (1) urinary 8-isoprostanes (IPT), a direct marker of non-enzymatic in vivo lipid peroxidation and the most reliable and clinically relevant marker of oxidative stress available to date, (2) urinary levels of TxB2, the stable metabolite of TxA2, a very potent vasoconstrictor. Tissular content of (3) cGMP and (4) ET-1 in vascular segments were also evaluated.

**MATERIALS & METHODS**

**Experimental animals**
- Water controls (n=14/group) were fed a standard chow (CONT) or a 60% fructose/5% fat (% by weight)-enriched diet for 8 weeks (FFR). From week 5 through 8, sildenafil was administered twice a day (20 mg/FRF-SIL), thus reaching clinically relevant plasma concentrations circa 20 min unheard brown to give efficacy in man (Pfizer Inc., date of ref). Then a 1-week wash-out period from sildenafil was observed.

In vivo basal blood pressure/heart rate and pressor response to noradrenaline (NE) in conscious unrestrained NE: In conscious unrestrained FFR: Restoration of normal blood pressure control in animals treated with chronic sildenafil, even one week after cessation of the treatment.

**Monitoring of endothelial biomarkers**
- At the end of the treatment period (week 8) as well as after the one-week wash-out period from the treatment (week 9), blood samples were taken after a 5 hr fasting period from the tail vein, plasma was separated and stored at -80°C where applicable. Determination of plasma glucose was performed by using a portable blood glucose meter on whole blood (Accu-check active, Roche diagnostics, France).
- To perform oral glucose tolerance test (OGTT), rats were fasted overnight. The following morning, rats were gavaged with a solution of glucose 1 g/kg. Blood samples were taken at 15, 30, 60 and 90 minutes after the gavage and determination of plasma glucose was immediately performed. The results were expressed as the percentage of increase in glycemia compared to the basal level for each rat and the total area under the curve (AUC) was calculated.
- Thioglycolic acid concentration was measured using a commercialized enzymatic colorimetric assay kit on plasma samples (Sigma, St Louis, MO, USA).
- For the determination of urinary IPT and TxB2 content on 24-hour urine samples, rats were fasted overnight and placed in metabolic cages at the end of the overnight wash-out period. Hormonometric determination of IPT and TxB2 were performed according to the manufacturer’s instructions (Cayman chemical, MI, USA). Urinary IPT and TxB2 levels were corrected by the clearance of creatinine to limit variability in the assay due to changes in renal excretory function.
- After BP evaluation, rats were deeply anesthetized with an intraperitoneal injection of urethane 1.2 g/kg. Tissue samples (thoracic aorta and superior mesenteric arteries) were harvested, immediately frozen in liquid nitrogen, and stored at -80°C until assessment of cGMP and ET-1 tissular content.

**RESULTS**

- No influence of the fructose-enriched diet on the animal body weight or blood glucose levels
- Impaired glucose tolerance in FFR corrected by a chronic treatment with sildenafil
- Chronic sildenafil treatment significantly countered the pronounced hypertriglyceridemia secondary to the fructose feeding in FFR and this effect was maintained even after the one-week wash-out period
- Baseline BP and heart rate were unchanged by the fructose-enriched diet, and chronic sildenafil administration did not modify these parameters, during or after one-week washout from sildenafil therapy
- Chronic sildenafil administration corrected the exacerbated pressor response to NE caused by the fructose-enriched diet, even one week after treatment cessation
- Neither the fructose diet nor the sildenafil treatment modified significantly tissue basal cGMP and ET-1 content in homogenates of aortas and superior mesenteric arteries after one-week washout from daily sildenafil treatment
- Chronic treatment with sildenafil was able to restore normal levels of urinary IPT and TxB2, even one week after cessation of the treatment

**CONCLUSIONS**

- Effect of chronic sildenafil treatment on physiological parameters relevant to the metabolic syndrome: Correction of the enhanced response to glucose overload, as well as the hypertriglyceridemia induced by fructose feeding
- Effect of chronic sildenafil treatment on exacerbated in vivo pressor response to NE in conscious unrestrained FFR: Restoration of normal blood pressure control in response to NE infusion
- Effect of chronic sildenafil treatment on urinary 8-isoprostanes and TxB2 excretion: Normalization of the excretion of a biological marker for oxidative stress and a biological marker of a potent vasoconstrictor

**EXACERBATED PRESSOR RESPONSE REGULATED BY DAILY TREATMENT WITH SILDENAFIL IN ANAnimal MODEL OF METABOLIC SYNDROME**

Behr-Roussel D.1, Oudot A.1, Compagnie S.1, Gomy D.1, Le Coz Q.1, Bernabé J.1, Wayman C.2, Alexandre L.1, Giuliano F.1,3

1 PELPHARM, Domaine CNRS, 5, avenue de la terrasse, Bâlintier S, F-81190 GI-sur-Yvette, France – p.christine@pelpharm.com
2 PSL-HP Raymond Poincaré Hospital, Department of Neurological rehabilitation, 104 Bd R. Poincaré, F-92380 Garches, France - giuliano@cyber-sante.org

**Figure:** Baseline blood pressure and heart rate after one-week wash-out from daily sildenafil treatment

**Table:** Baseline and post-treatment concentrations of (pg/ml/24h) of urinary 8-isoprostanes (IPT) and TxB2 in conscious unrestrained rats

**Graph:** Pressor response to NE in conscious unrestrained rats

The beneficial effects of daily sildenafil on blood pressure regulation gives additional insight to the possible mechanism of action of sildenafil in cardiovascular disorders related to the metabolic syndrome that could be explored in future clinical trials. Moreover, this study provides preclinical support for the predictive value of biological markers such as urinary IPT and TxB2 as surrogate markers in future clinical trials addressing cardiovascular risks.