

# Measurement of S-Nitrosothiols, Nitrate and Nitrite in the Obese and Lean Zucker Rat

Sophie A. Rocks<sup>1</sup>, Claire A Davies<sup>1</sup>, David Laight<sup>2</sup>, Martin Carrier<sup>2</sup>, Erik Anggard<sup>2</sup>, and Paul G Winyard<sup>1</sup>

<sup>1</sup>Bone and Joint Research Unit, <sup>2</sup>Experimental Therapeutics, Barts and The London Medical School, Charterhouse Square, London, EC1M 6BQ, UK

The “prediabetic” obese Zucker rat exhibits oxidative stress in response to a prooxidant challenge from hydroquinone and buthionine sulphoximine (BSO), previously shown by increased 8-epi-prostaglandin- $F_{2\alpha}$  synthesis. The measurement of nitric oxide (NO) metabolites (nitrosothiols (RSNOs), nitrate and nitrite) in this model could clarify whether NO production increases due to the onset of diabetes, or decreases due to impaired endothelial function.

Zucker rats (n=24) were split equally into 4 groups: control lean, control obese, stressed lean, and stressed obese. The stressed groups were injected intraperitoneally with 50mg/kg hydroquinone and BSO daily for 7 days, whilst the control groups were injected with saline over the same period. Plasma samples were obtained on day 8 and deproteinised with ethanol before addition of the spin trap complex, iron *N*-methyl *D*-glucamine dithiocarbamate ((MGD)<sub>2</sub>Fe<sup>2+</sup>) and measurement of RSNO concentrations by electron paramagnetic resonance (EPR) spectrometry. Plasma nitrate and nitrite were measured by capillary electrophoresis.

The mean RSNO concentration in the deproteinised plasma samples was significantly higher in the stressed obese rats (644 ± 321 (1SD) nM) when compared to each of the other groups (control obese 214 ± 198nM; control lean 220 ± 155nM; stressed lean 27 ± 17nM) (p<0.05). No significant differences were seen in the mean plasma nitrate, nitrite and NO<sub>x</sub> concentrations of the groups and these measurements did not correlate with the RSNO concentrations.

We conclude that, whilst endothelial dysfunction may occur in prediabetic (stressed, obese) Zucker rats, the over-riding influence on RSNO levels in these animals appears to be an increase, possibly due to the induction of nitric oxide synthase activity.

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