

Abstract #15-6311 Low Intensity Extracorporeal Shockwaves Therapy Improves Erectile Function in Diabetic Type II Rats Independently of NO/cGMP Pathway

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PELVI PHARM



OBJECTIVES

- Urological functional complications such as erectile dysfunction (ED) significantly impact the quality of life of diabetic patients who are poor responders to PDE5 inhibitors.
- Low intensity extracorporeal shock waves therapy (Li-ESWT) has been reported to significantly improve erectile function in ED patients
- The mechanism of action of Li-ESWT is still unknown

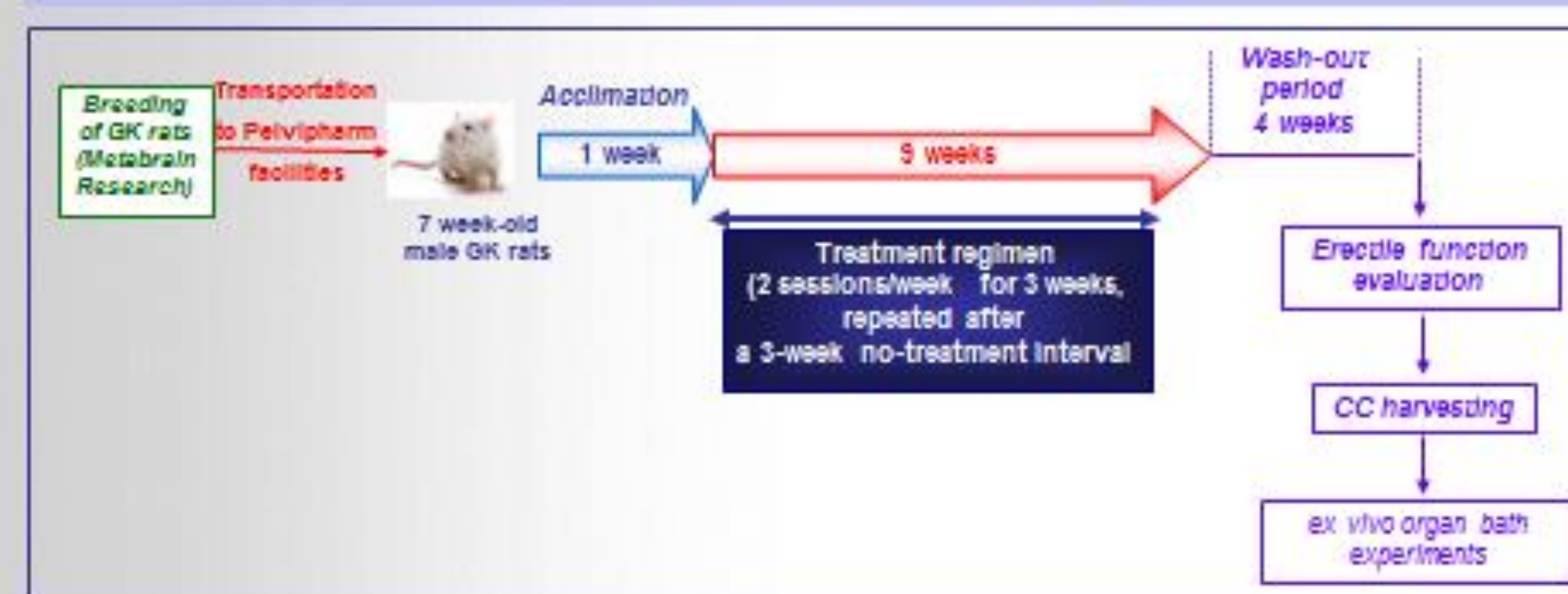
Aim of the study:

Assess the effects of Li-ESWT:

- In vivo on erectile function in the Goto-Kakizaki rats (GK), a validated model for type 2 diabetes-associated ED [1].

- Ex vivo on endothelium-dependent, -independent and nitrgic relaxations of cavernosal strips from GK rats

Summary of research design



Experimental groups

Groups	Strain	Li-ESWT treatment	N=
Control	Wistar	No	13
GK		No	12
GK_ESWT	Goto-Kakizaki	Yes	12
GK_Acute sil		No	12
GK_ESWT+Acute sil		Yes	12

- Male GK (n=12/group) and age-matched Wistar rats (n=13) were used at 7 weeks of age.

- ED function and ex-vivo experiments were performed at 21 weeks of age.

All procedures are performed in compliance with the legislation on the use of laboratory animals (NIH publication N°85-23, revised 1986) and Animal Care Regulations in force in France as of 1988 (authorization from competent French Ministry of Agriculture - Agreement No. A76-423-01, 2013).

Li-ESWT Treatment protocol (based on parameters described by Vardi et al.,[2;3] in clinical studies)

Male GK and age-matched Wistar rats received 2 sessions of Li-ESWT per week for 3 weeks, repeated after a 3-week no-treatment interval. Shockwaves were delivered by a calibrated probe yielding a controlled energy flux density of 0.09mJ/mm2 attached to a compact electrohydraulic unit with a focused shockwave source (Omnispec ED1000, Medispec Ltd, Germantown, MD, USA).

To facilitate coverage and transmission of the shockwaves, the penis of each anesthetized rat was manually stretched and dipped into a specifically designed water-filled tank. Following a 4-week wash-out period, erectile function was assessed by electrical stimulation of the cavernous nerve in rats under anesthesia.

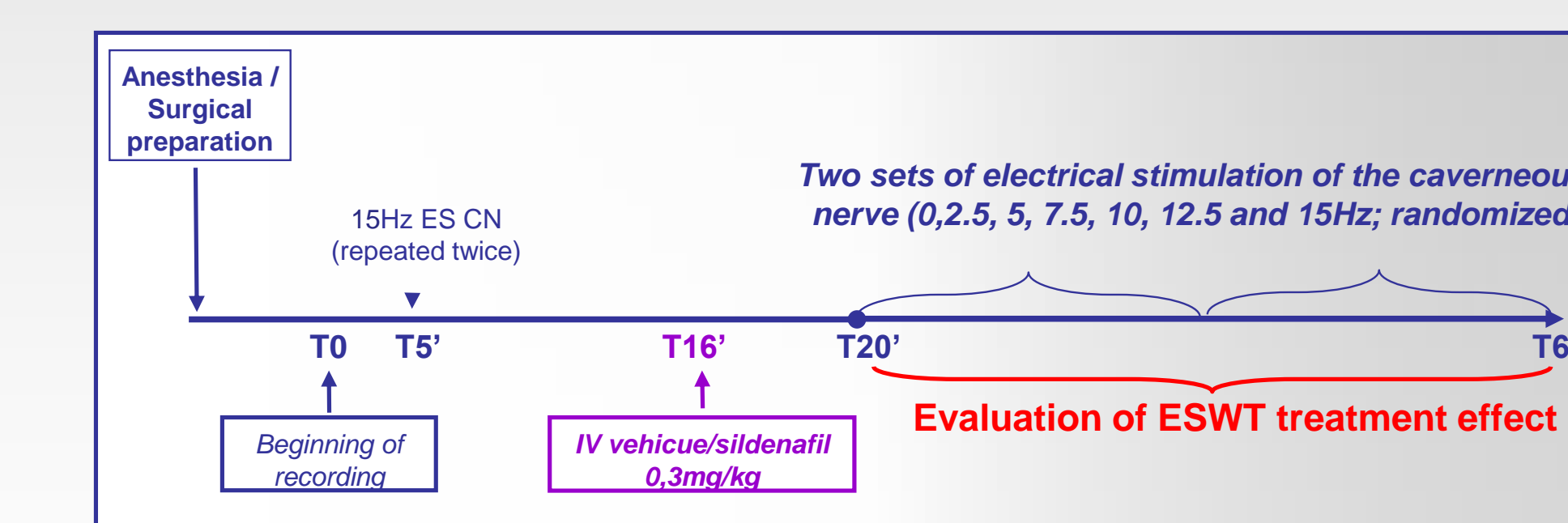


Li-ESWT treatments delivered using a special probe attached to a compact electrohydraulic unit with a focused shockwave source to the penis of anesthetized rat dipped into a salt water-filled tank

MATERIALS & METHODS

Erectile function evaluation: electrical stimulation of the cavernous nerve (ES CN) [4]

After 5 minutes of baseline recording of simultaneous computerized measure of mean arterial pressure (MAP) and intracavernous pressure (ICP), the CN was stimulated (6 V, 1 ms for 45 s) at different frequencies (0, 2.5, 5, 7.5, 10, 12.5 and 15Hz) at 3-minute intervals in a randomized manner in order to assess the erectile responses. Erectile responses to ES CN were expressed as a ratio of ICP (mmHg) / MAP (mmHg) x 100, ICP being the difference between ICP in the flaccid state, i.e. before stimulation and ICP during the plateau phase of the erectile response, and MAP, the mean arterial pressure during the plateau phase, and as the ratio of AUCtot / MAP and AUC45 / MAP, AUCtot and AUC45 being the area under the curve during the whole erectile response or the first 45 s during ES CN



Experimental design of ES CN investigation

Organ bath ex vivo experiments on isolated strips of corpus cavernosum At the end of erectile function evaluation, cavernosal strips were obtained and placed in organ chambers for isometric tension studies.

➤ Pharmacological stimulation of endothelium-dependent relaxations: The cavernosal strips were precontracted by phenylephrine (PHE 3.10⁻⁶ M for Wistar and 10⁻⁵ M for GK) in order to attain comparable levels of precontraction. Concentration-response curves (CRC) for acetylcholine, ACh, were performed by cumulative addition of increasing drug concentrations (ACh 10⁻⁹ to 10⁻⁴ M), to the baths in semi-log increments.

➤ In vitro nitrgic relaxation responses to electrical-field stimulation (EFS): Guanethidine (5 µmol/L) and atropine (1 µmol/L) were added to the organ chambers. The cavernosal strips were precontracted by PHE (10⁻⁶ M for Wistar and 10⁻⁵ M for GK) then transmural stimulations were performed using a stimulator delivering increasing single square-wave pulses. Frequency-response curves (FRC) to EFS were performed by successive stimulation of the cavernosal strips at different electrical parameters (1 ms - 10 s - 300 mA, 1, 2, 4, 8, 16 and 32 Hz).

➤ Pharmacological stimulation of endothelium-independent relaxations: The cavernosal strips were precontracted by PHE (10⁻⁶ M for Wistar and 10⁻⁵ M for GK) before inducing relaxations. CRCs for sodium nitroprusside, SNP, were performed by cumulative addition of increasing drug concentrations (SNP 10⁻⁹ to 10⁻⁵ M) to the baths in log increments.

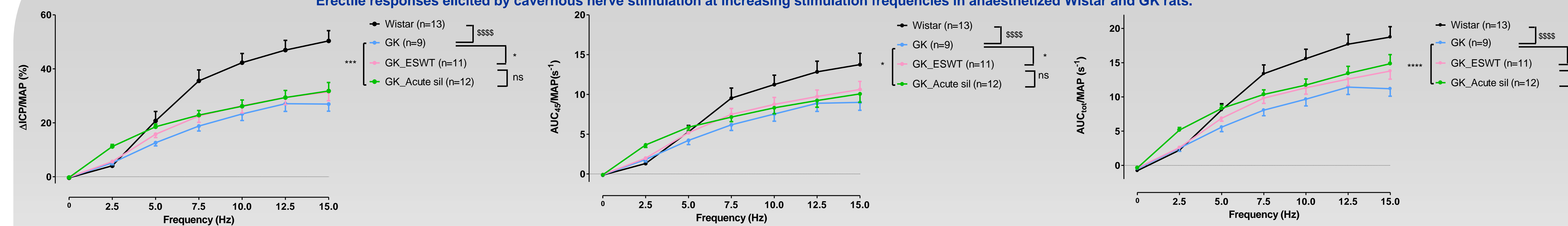
Statistical analysis

All results are presented as mean ± SEM. For erectile function evaluation, comparisons of frequency-response curves were performed with a two-way ANOVA statistical analysis test. For ex vivo experiments, statistical comparisons of the CRCs or FRCs were performed using a two-way ANOVA statistical analysis test. Statistical analysis was performed with GraphPad Prism® 5.04 software. P values < 0.05 were considered significant.

RESULTS

Effect of Li-ESWT on erectile function in GK rats

Erectile responses elicited by cavernous nerve stimulation at increasing stimulation frequencies in anaesthetized Wistar and GK rats.



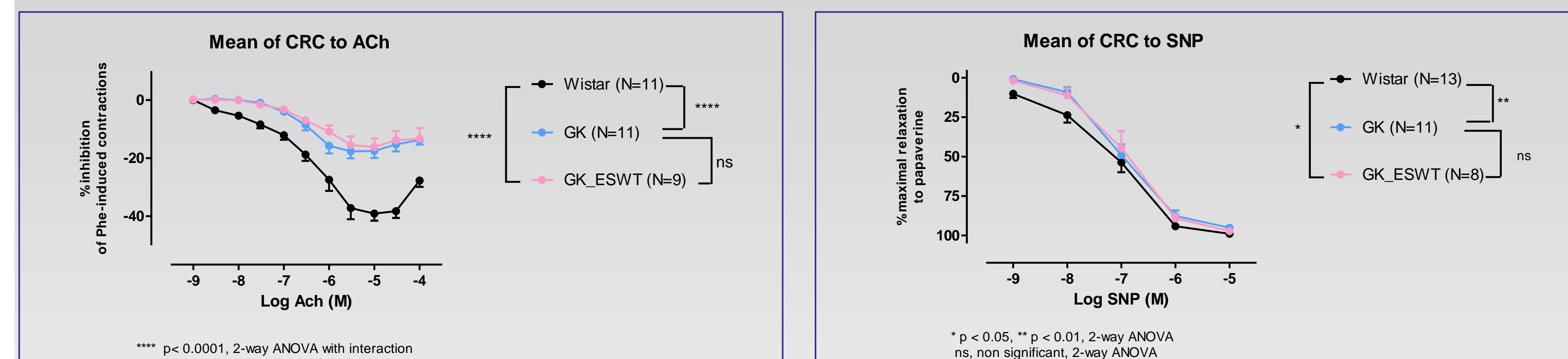
* p < 0.05, *** p < 0.001, 2-way ANOVA
§§§§ p < 0.001, 2-way ANOVA with interaction
ns, non significant, 2-way ANOVA

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ns, non significant, 2-way ANOVA

* p < 0.05, ** p < 0.01, **** p < 0.001, 2-way ANOVA
§§§§ p < 0.001, 2-way ANOVA with interaction

- The erectile responses elicited by electrical stimulation of the cavernous nerve (6V, 1 ms for 45s) were considerably decreased in GK compared to Wistar rats
- Both acute sildenafil or Li-ESWT significantly improved erectile responses in GK rats
- Neither treatment restored normal erectile responses

Effect of Li-ESWT on endothelium-dependent,-independent and nitrgic relaxations of cavernosal strips of GK rats

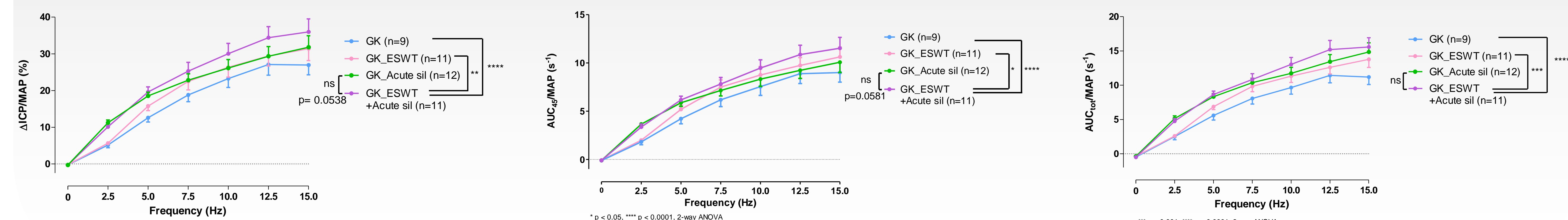


**** p < 0.0001, 2-way ANOVA with interaction
ns, non significant, 2-way ANOVA

* p < 0.05, ** p < 0.01, 2-way ANOVA
ns, non significant, 2-way ANOVA

The endothelium-dependent relaxations to ACh of cavernosal strips were impaired in diabetic GK rats compared to Wistar rats, mirroring endothelial dysfunction in cavernosal strips of diabetic GK rats. Li-ESWT did not improve endothelium-dependent relaxations to ACh of cavernosal strips in these diabetic GK rats.

The endothelium-independent relaxations to SNP of cavernosal strips were altered in diabetic GK rats compared to Wistar rats, indicating that the sensitivity of arterial smooth muscle cells to NO is altered in cavernosal strips of diabetic GK rats. Li-ESWT did not improve endothelium-independent relaxations to SNP of cavernosal strips from these diabetic GK rats.



* p < 0.05, ** p < 0.01, **** p < 0.0001, 2-way ANOVA
ns, non significant, 2-way ANOVA

* p < 0.05, **** p < 0.0001, 2-way ANOVA
ns, non significant, 2-way ANOVA

*** p < 0.001, **** p < 0.0001, 2-way ANOVA
ns, non significant, 2-way ANOVA

- The combination of Li-ESWT with sildenafil significantly potentiated the effect of Li-ESWT alone and tended to potentiate the effect of acute sildenafil

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- ✓ The present study describes a relevant preclinical paradigm to investigate Li-ESWT in the rat by adapting the machine and treatment modalities to adequately mimick what has been performed in men [2;3;5].
- ✓ Furthermore, this study reports, in an experimental model of ED associated to diabetes type II, :
 - the beneficial disease-modifying effect of Li-ESWT
 - the beneficial effect of Li-ESWT combined to an acute administration of PDE5-I compared to standard-of-care PDE5-I therapy alone
- ✓ To our knowledge, this is the first study to investigate the effect of Li-ESWT on endothelial and nitrgic relaxations of cavernosal strips from diabetic GK and age-matched Wistar rats. Interestingly, this study clearly demonstrates that the effect of Li-ESWT is not mediated via the NO/cGMP pathway.
- ✓ Thus, there is a need to search for alternative mechanisms of action to explain the beneficial effects of Li-ESWT i.e., possible angiogenesis and neovascularization to improve cavernosal blood supply ? Anti-inflammatory effect ? Anti-oxidative effect ?....

CONCLUSIONS