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Effects of a Hypersaline Sodium-rich Carbonated Natural Mineral Water on Structure and Expression of VEGF, VEGFR1, VEGFR2, Ang1, Ang2 and Tie2 of Fructose-treated Rat Corpus Cavernosum

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Metabolic Syndrome (MS) definition is based on a cluster of metabolic risk factors that identifies subjects at high risk for forthcoming type 2 diabetes mellitus and atherosclerotic cardiovascular diseases (CVD). Although the exact aetiology of the MS still remains unclear, it is known to involve complex interactions between genetic, metabolic and environmental factors, with diet and oxidative stress playing important roles. Regular increased fructose consumption has been associated to some metabolic adverse changes observed in the MS and, thus, fructose-fed is considered a suitable animal model of diet-induced MS. On the other hand, calcium, magnesium and potassium, generally deficient in MS-inducing diets, and abundant in natural mineral-rich waters, have been proposed protective against the MS [1-4]. Although their exact effects are not yet fully clarified, natural mineral-rich waters present some antioxidant properties [5-8] that exert protection against reactive oxygen species that are chief contributors to the increase of CVD risk, considering their reactivity to nitric oxide (NO). Such waters also improve some MS metabolic risk factors [9, 10]. Degradation of NO seriously compromises the vasodilatation mechanism leading to endothelial dysfunction, which always precedes atherosclerosis — the main contributor to CVD and erectile dysfunction. Penis erection is a vascular process that strongly depends on NO-induced smooth muscle relaxation. Moreover, NO mediates indirectly the vascular endothelial growth factor (VEGF)-induced angiogenesis, which is fundamental to maintain endothelium integrity in the cavernous tissue. VEGF binds specifically to VEGF tyrosine kinase membrane receptors (VEGFR1 and VEGFR2), and crosstalk in vivo with other angiogenic factors such as angiopoietins that compete for binding to the endothelial-specific Tie2 receptor [11]. Previous work from our group demonstrated that long-term consumption of antioxidant-rich beverages modifies the expression of VEGF, Ang1, Ang2 and their receptors VEGFR1, VEGFR2 and Tie2 in the cavernous tissue of the rat, preventing atherosclerosis progression [12].

Thus, we aimed to characterize the effects of a fructose-rich diet on smooth muscle cells (SMC) content and on the expression of vascular growth factors and receptors in the corpus cavernosum (CC) of the rat, and if those effects are modulated by consumption of a hypersaline sodium-rich carbonated natural mineral water (Água das Pedras®).

Twenty one adult male Sprague Dawley rats were randomly divided into 3 groups (n=7) and maintained during 8 weeks with free access to standard laboratory chow diet and: a) tap water (C), b) 10% fructose in tap water (FRUCT) or c) 10% fructose in Água das Pedras® (FRUCTMIN). At the end of the treatment, all animals were sacrificed, the penises were excised and divided in two fragments: one being immediately fixed in 10% buffered formaldehyde and the other frozen at -80ºC (for molecular analysis). In the former case, penises were embedded in paraffin, oriented along its transversal axis, and 5 μm thick sections were placed onto 0.1% poly-L-lysine coated microscopy slides for immunodetection of α-actin, a specific marker of the SMC. Images were captured in an optical microscope connected to a digital camera (Carl Zeiss MicroImaging GmbH, Göttingen, Germany) and analysed by computer-assisted color histomorphometry by ImageJ® software (NIH, Maryland, USA) to assess the proportion of the smooth muscle to total erectile tissue area, by pixel classification. Immunofluorescence detection of α-actin/PECAM-1(endothelial cell marker), VEGF/VEGFR1, VEGF/VEGFR2, Ang1/Tie2 and Ang2/Tie2 was performed in CC, observed in an Apotome fluorescence microscope (Zeiss, Göttingen, Germany) and recorded with AxionVision 3.0

program (Zeiss System) (Figure 1). The semi-quantification of VEGF, VEGFR1, VEGFR2, Ang1, Ang2 and Tie2 was carried out by Western blotting (WB), using β-actin as loading control for normalization. The WB bands were quantified by densitometry using the ScionImage software (Scion Co., NIH, MN, USA). The morphometric study demonstrated that FRUCTMIN rats presented a higher proportion of SMC in CC (15.6±0.9) when compared to C (10.5±1.1, p=0.003) and FRUCT groups (9.3±.2, p=0.001). The immunofluorescent study revealed endothelial expression for VEGF, VEGFR2 and Tie2 and SMC expression for VEGFR1, Ang1 and Ang2. VEGF was also detected in SMC, co-localizing with VEGFR1 in all experimental groups. No significant differences were observed among the 3 groups for any of the studied protein in semi-quantitative WB.

The characterization of the CC of fructose-fed rats treated with a natural mineral-rich water is presented for the first time, however further molecular analysis will be necessary to clarify its exact contribution for vascular function.

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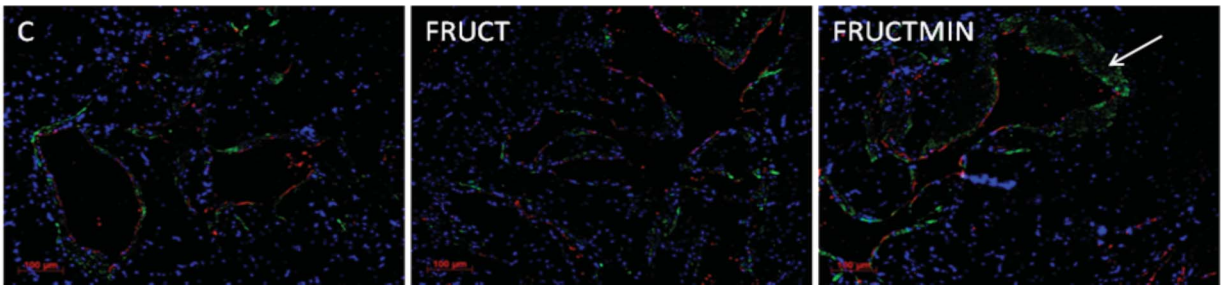

Figure 1. Dual immunolabelling of endothelium (PECAM-1, red) and smooth muscle cells (α-actin - green) in corpus cavernosum of rats from all experimental groups. Note the increase of smooth muscle layer thickness in FRUCTMIN group (white arrow).

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