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# Retinal changes in an experimental model of early type 2 diabetes in rats characterized by non-fasting hyperglycemia

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ABSTRACT

Diabetic retinopathy is a leading cause of acquired blindness in young, but also in elder adults, mostly affected by type 2 diabetes mellitus (T2DM). The aim of this work was to develop an experimental model of early human T2DM in adult rats, and to analyze retinal functional, morphological, and biochemical changes arising during the early stages of the moderate metabolic derangement. For this purpose, animals were divided in four groups: adult male *Wistar* rats receiving: tap water and citrate buffer i.p. (group 1), tap water with 30% sucrose and citrate buffer i.p. (group 2), tap water and 25 mg/kg i.p streptozotocin (STZ, group 3), or 30% sucrose and STZ (group 4). Fasting and postprandial glycemia, fructosamine and serum insulin levels were assessed. In addition, i.p. glucose and insulin tolerance tests were performed. Retinal function (electroretinogram, ERG) and morphology (optical microscopy), retinal nitric oxide synthase (NOS) activity (using $^{3}$H-arginine), lipid peroxidation (thiobarbituric acid reactive substances, TBARS), and TNFα levels (ELISA) were evaluated. At 6 and 12 weeks of treatment, animals which received a sucrose-enriched diet and STZ showed significant differences in most metabolic tests, as compared with the other groups. At 12 weeks of treatment, a significant decrease in the ERG a- and b- wave and oscillatory potential amplitudes, and a significant increase in retinal NOS activity, TBARS, TNFα, glial fibrillary acidic protein in Müller cells, and vascular endothelial growth factor levels were observed. These results indicate that the combination of diet-induced insulin resistance and a slight secretory impairment resulting from a low-dose STZ treatment mimics some features of human T2DM at its initial stages, and provokes significant retinal alterations.



## Introduction

About 350 million people across the globe are estimated to have diabetes (Danaei et al., 2011), and type 2 diabetes mellitus (T2DM) accounts for roughly 90% of all diagnosed cases (American Diabetes Association, 2008). Diabetic retinopathy (DR), one of the most feared complications of diabetes, is a leading cause of acquired blindness in young, as well as elder adults, mostly affected by T2DM (King et al., 1998; The Eye Diseases Research Prevalence Research Group-National Eye Institute, 2004). Experimental, epidemiological, and large scale intervention studies, such as the Diabetes Control and Complications Trial, and the UK Prospective Diabetes Study Group have demonstrated that hyperglycemia is a major trigger for its development, and lowering of blood glucose levels is essential for preventing or arresting DR development (Diabetes Control and Complications Trial Research Group, 1993; UK Prospective Diabetes Study (UKPDS) Group, 1998). In inadequately controlled patients with diabetes mellitus, the retinal microvasculature is constantly exposed to high glucose levels, and this insult results in many structural and functional alterations (UK Prospective Diabetes Study (UKPDS) Group, 1998). Current treatments for DR such as laser photocoagulation, corticosteroids,

*Abbreviations:* AUC, area under the curve; DR, diabetic retinopathy; ERG, electroretinogram; GCL, ganglion cell layer; GFAP, glial fibrillary acidic protein; HbA1c, glycosylated hemoglobin; IPGTT, intraperitoneal glucose tolerance; IPITT, intraperitoneal insulin tolerance test; MDA, malondialdehyde bis-dimethyl acetal; NOS, nitric oxide synthase; OP, oscillatory potential; SDT, spontaneously diabetic Torii; STZ, streptozotocin; TBARS, thiobarbituric acid reactive substances; T2DM, type 2 diabetes mellitus; TNF-α, tumor necrosis factor α; VEGF, vascular endothelial growth factor.

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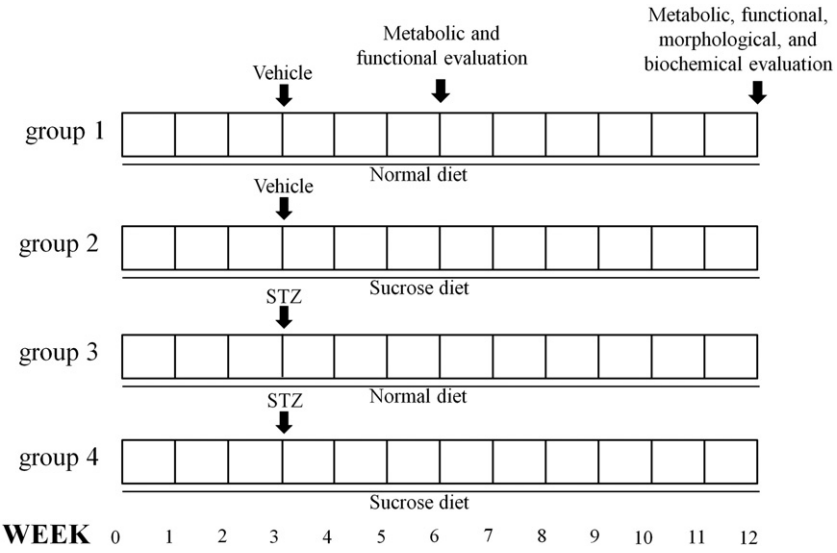


**Fig. 1.** Experimental groups. Animals were submitted to a normal diet (groups 1 and 3) or a sucrose enriched diet (groups 2 and 4) for 12 weeks. At the third week, animals were i.p. injected with vehicle (groups 1 and 2) or SZT (groups 3 and 4). Metabolic and retinal studies were performed at 6 and 12 weeks of treatment.

or anti-vascular endothelial growth factor agents are indicated for advanced DR, but have significant adverse effects. Therefore, new therapeutic treatments for the early stages of DR are needed. Understanding the molecular mechanisms of retinal damage associated with T2DM should help identify therapies to treat/postpone this sight-threatening complication of diabetes. For this purpose, a large number of genetically modified animal models including transgenic, generalized knockout, and tissue specific knockout mice have been employed. However, a typical diabetic profile is not always seen in these genetically induced models, nor do they absolutely mimic the pathogenesis of human T2DM (Matsumura et al., 2005; Movassat et al., 1995) since these gene mutations are extremely rare in human populations. Similarly, animal models of T2DM induced by removal of a portion of the pancreas (Portha et al., 1989) are not representative of the T2DM etiology in humans, which is typically preceded by obesity (Bray, 2004; Goralski and Sinal, 2007). In order to better understand the events which precede and precipitate the onset of T2DM, some nutritional animal models have been also developed (Mühlhausler, 2009; Shafrir et al., 2006; Srinivasan and Ramarao, 2007; Surwit et al., 1988).

Initially, the natural history of T2DM includes a period of normal or near-normal fasting plasma glucose levels and marked postprandial glycemic excursions (Monnier et al., 2007). However, the impact of these hyperglycemic spikes on retinal function is still unknown. Comprehension of the abnormalities that characterize the initial phases of DR may reveal therapeutic targets that could effectively block the progression of the disease. To our knowledge, there are no animal models that allow for the study of early retinal complications associated to mild fasting hyperglycemia and postprandial glycemic spikes. Thus, the present study was conceived in order to: a) develop an experimental model more closely mimicking the natural history of human T2DM, inducing insulin resistance in adult rats by means of a sucrose-rich diet, and then reducing pancreatic insulin release using low doses of streptozotocin (STZ), and b) analyze metabolic changes and functional, morphological, and biochemical alterations arising in the retina during the early stages of the moderate metabolic derangement.

## Materials and methods

### *Animals*

Male *Wistar* rats (400 ± 50 g) were housed in a standard animal room with food and water *ad libitum* under controlled conditions of humidity, temperature (21 ± 2 °C), and luminosity (200 lux), under a 12-hour light /12-hour dark lighting schedule (lights on at 7:00 AM). All animal procedures were in strict accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The ethics committee of the University of Buenos Aires School of Medicine, (Institutional Committee for the Care and Use of Laboratory Animals, (CICUAL)) approved this study.

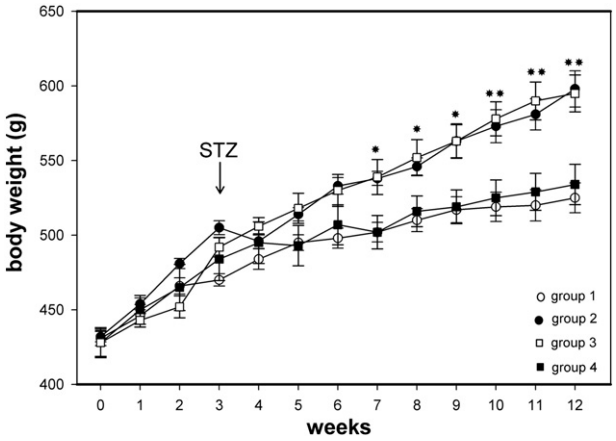


**Fig. 2.** Temporal course of body weight for all the experimental groups. Body weight significantly increased throughout the experiment in all groups, but from week 7 to 12, it was significantly higher in groups receiving sucrose (groups 2 and 4) than in those which did not (groups 1 and 3). Data are mean ± SEM ($n = 10$ animals/group). $^{*}p<0.05$, $^{**}p<0.01$ for groups 2 and 4 vs. group 1 and group 3, by Tukey's test.