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Retinal dysfunction parallels morphologic alterations and precede clinically detectable vascular alterations in *Meriones shawi*, a model of type 2 diabetes

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ABSTRACT

Diabetic retinopathy is a major cause of reduced visual acuity and acquired blindness. The aim of this work was to analyze functional and vascular changes in diabetic *Meriones shawi* (*M.sh*) an animal model of metabolic syndrome and type 2 diabetes. The animals were divided into four groups. Two groups were fed a high fat diet (HFD) for 3 and 7 months, two other groups served as age-matched controls. Retinal function was assessed using full field electroretinogram (Ff-ERG). Retinal thickness and vasculature were examined by optical coherence tomography, eye fundus and fluorescein angiography. Immunohistochemistry was used to examine key proteins of glutamate metabolism and synaptic transmission. Diabetic animals exhibited significantly delayed scotopic and photopic ERG responses and decreases in scotopic and photopic a- and b-wave amplitudes at both time points. Furthermore, a decrease of the amplitude of the flicker response and variable changes in the scotopic and photopic oscillatory potentials was reported. A significant decrease in retinal thickness was observed. No evident change in the visual streak area and no sign of vascular abnormality was present; however, some exudates in the periphery were visible in 7 months diabetic animals. Imunohistochemistry detected a decrease in the expression of glutamate synthetase, vesicular glutamate transporter 1 and synaptophysin proteins. Results indicate that a significant retinal dysfunction was present in the HFD induced diabetes involving both rod and cone pathways and this dysfunction correlate well with the morphological abnormalities reported previously. Furthermore, neurodegeneration and abnormalities in retinal function occur before vascular alterations would be detectable in diabetic *M.sh*.

1. Introduction

Diabetic retinopathy (DR) is a leading cause of blindness in adults (Congdon et al., 2003; Klein, 2007). The pathophysiology behind DR is complex: retinal vasculature and retinal neurons are both altered by the disease (Barber et al., 1998, 2000; Nguyen et al., 2007).

Clinically DR can only be easily diagnosed at the advanced stages based on the vascular abnormalities including microaneurysms, hemorrhages, cotton wool spots, hard exudates, intraretinal microvascular abnormalities, venous beading, loop formation and neovascularization (Gardner et al., 2002; Lai and Lo., 2013). Several techniques have been used for the detection, diagnosis and evaluation including eye fundus, fluorescein angiography and OCT (Salz and Witkin, 2015). While clinical diagnosis of DR focus on the visualization of retinal vascular lesions in diabetic patients, the disease also induces retinal electrophysiologic modifications including decreased contrast sensitivity, impaired color vision and ERG abnormalities (Ghirlanda et al., 1997; Greenstein et al., 1990; Hardy et al., 1992; Jackson and Barber, 2010). Indeed, these functional changes have been shown to occur in the retina prior to clinically detectable vascular symptoms (Juen and Kieselbach, 1990; Vadala et al., 2002; Yoshida et al., 1991) and may be the consequence of either the clinically undetectable microvascular pathology or the

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degeneration of glial and neural elements.

Much of the early disease progression seems to be similar between human patients and animal models; however, they never mimic exactly the human pathology (Wang et al., 2014). Consequently, choosing the right kind of animal model that is most representative of the structural, functional and biochemical features of human DR is of critical importance for investigating pathogenesis and for developing new and better therapeutic strategies. While rats and mice are the most commonly used animal models of DR, they have serious limitations including the different vasculature, the low cone number and the lack of fovea.

Meriones shawi (*M.sh*), a new animal model in DR researches, has the particularity to possess a prominent visual streak with outstandingly high cone and ganglion cell densities and specialized vasculature with the complete lack of major vessels in this area (Hammoum et al., 2017b). This anatomical composition resembling what can be seen in the human fovea makes this species a potentially useful model to mimic foveal pathology of DR including diabetic macular edema.

Furthermore, it's has been shown in our previous reports that feeding a high-fat diet (HFD) to *M.sh* for up to 3 and 7 months (Hammoum et al., 2017a, 2017b) caused evident retinal histological changes. In fact, we detected a significant decrease of retinal thickness, Müller glia activation with a significant increase in the number of microglia cells. In addition, prominent outer segment degeneration for both rods and cones, with a significant decrease in the number of all cones was visible. There was a decrease both in the staining intensity and in the number of stained elements for both AII and calretinin positive amacrine cells; furthermore, a significant loss of ganglion cells was also confirmed after 7 months in diabetic specimens.

The purpose of this study was to examine the clinical effects of HFD–induced diabetic conditions on retinal function and vasculature in *M.sh*, to evaluate how these changes progress with increasing diabetes duration and to correlate these changes with the previous results of the detailed histological evaluation. Special care was taken to examine the visual streak area in search for edema formation or any other specific pathological alterations. Additionally, we also aimed to extend the study to the key proteins of glutamate metabolism – the major neurotransmitter system of the retina - and synaptic transmission in general; as they have also been proposed to play important roles in the development of functional alterations and have not been examined in any previous report.

2. Material and methods

2.1. Experimental procedure

Approximately 3 months old male *M.sh* rodents (n = 40) were trapped in the semiarid region of Sidi Bouzid in BOUHEDMA Park (South of Tunisia), with the authorization of Tunisian Agriculture Ministry (number of approval: 2012–2016/2214-1693) and bred in our animal facility. All protocols on *M.sh* were approved by the ethics committee of Pasteur Institute, Tunis, Tunisia (number of approval: 2016/11/E/ISBST/V0). Animals were used and handled according to the principles of the Association for Research and Vision Research (ARVO) Statement for the Use of Animals in Ophthalmology and Vision Research.

Adult rodents, weighing 70–90 g were selected for the experiments. The animals were maintained at 24 ± 1 °C with 12/12 h light/dark photoperiod and free access to water and laboratory rodent chow.

After two weeks of adaptation period, the animals were divided into four groups. Two groups were fed a custom made high fat diet (Aissaoui et al., 2011) containing 14% protein, 60% complex carbohydrates, 10% sugar, 16% fat, 4% salt and 1% vitamin mix for an additional period of 3 months (n = 10) and 7 months (n = 10). The two other groups served as age-matched control animals maintained on standard laboratory diet containing only 4% fat (C3FG, El Badr company, Tunis, Tunisia) for 3 months (n = 10) and 7 months (n = 10). Water was supplied *ad libitum* to all groups. Body weights and blood glucose levels were monitored every month.

2.2. Clinical examinations

The examinations were performed at three months and seven months after HFD administration. Full field electroretinography (Ff-ERG), optical coherence tomography (OCT), eye fundus and fluorescein angiography (FA) were performed during the examinations.

2.2.1. Full field-Electroretinography

Retinal function was examined with Ff-ERG. Six responses were recorded with a Metrovision system (MonColor, Paris, France) according to the standardized protocol for clinical electroretinography by the International Society for Clinical Electrophysiology of Vision (ISCEV) and as it was already described in detail in our previous study (Dellaa et al., 2016). After 12 h of dark adaptation, animals were anesthetized under dim red illumination with ketamin (120 mg/kg, Tunisia) by intraperitoneal injection. Pupils were fully dilated with drops of tropicamide (25mg/5 ml; UNIMED, Sousse, Tunisia) and the animals were laid on a homeothermic blanket sets at 38 °C. The retinal potential was captured at the cornea with a low-mass, silver-coated, conductive (Dawson, Trick and Litzkow) fiber electrode (Sauquoit industries, Scranton, PA) acting as the active electrode. It was maintained on the cornea with an ophthalmic liquid gel lacryvisc (Carbomer 974 P, Alcon), to keep a proper conduction and to prevent corneal dryness. The reference and ground electrodes were inserted subcutaneously on the forehead and tail, respectively. The scotopic ERG responses (Amplification: x 12500; 1–1200 Hz bandwidth) were evoked to flashes of white light of 0.01 cd s m^{-2} for the rod response (RR) and 3 cd s m^{-2} for the mixed response (MR) and oscillatory potentials (OPs). The OPs were extracted from ERG signal using a 80–200 Hz bandwidth. After 10 min of light adaptation to a background of 30 cd m^{-2}, two photopic ERG responses (average, 20 flashes, inter stimulus interval: 1 s) were taken at 3 cd s.$m^{-2}$for the cone response (CR) and 30 Hz flicker response (FR).

2.2.2. Analysis of ERG parameters

Retinal function in diabetic and control animals was assessed by analyzing the amplitude and the latency of each waveform according to ISCEV protocol (Marmor et al., 2004; Zhang et al., 2014). The a-wave amplitude of the MR and CR was measured from the baseline to the first trough. The amplitude of the b-wave of the RR, MR and CR was measured from the first trough to the first peak. The amplitudes of scotopic and photopic OPs (OP1; OP2; OP3; OP4) and 30 Hz-Flicker response were measured from the through to the peak of each response component. The scotopic and photopic sum of the amplitudes of the OPs (ΣOPs = OP1 + OP2 + OP3 + OP4) was measured by adding the amplitudes of each of the sub-waves. The latency for all response peaks was calculated from stimulus onset to peak amplitude.

2.2.3. Optical coherence tomography

The pupils of animals were dilated as described above. Retinal imaging was performed using 3D OCT-2000 series (Topcon, Tokyo, Japan) with the integrated fundus camera. The average of the total retinal thickness measured from the nerve fiber layer to the outer margin of photoreceptor outer segments was calculated in diabetic and control *M.sh* from OCT-images by the FastMap software (Tokyo, Japan).

2.2.4. Eye fundus and fluorescein angiography

Fundus photography was taken as described previously (Huber et al., 2010; Paques et al., 2007; Xu et al., 2008). FA was performed by an intraperitoneal injection of fluorescein dye. To record FA, the device (Topcon medical systems TRC-50DX, Oakland, NJ) was operated in the fluorescence mode with the excitation light provided by a 488-nm