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Early adaptive response of the retina to a pro-diabetogenic diet: Impairment of cone response and gene expression changes in high-fructose fed rats

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ABSTRACT

The lack of plasticity of neurons to respond to dietary changes, such as high fat and high fructose diets, by modulating gene and protein expression has been associated with functional and behavioral impairments that can have detrimental consequences. The inhibition of high fat-induced rewiring of hypothalamic neurons induced obesity. Feeding rodents with high fructose is a recognized and widely used model to trigger obesity and metabolic syndrome. However the adaptive response of the retina to short term feeding with high fructose is poorly documented. We therefore aimed to characterize both the functional and gene expression changes in the neurosensory retina of Brown Norway rats fed during 3 and 8 days with a 60%-rich fructose diet ($n = 16$ per diet and per time point). Glucose, insulin, leptin, triacylglycerols, total cholesterol, HDL-cholesterol, LDL-cholesterol and fructosamine were quantified in plasma ($n = 8$ in each group). Functionality of the inner retina was studied using scotopic single flash electroretinography ($n = 8$ in each group) and the individual response of rod and cone photoreceptors was determined using 8.02 Hz Flicker electroretinography ($n = 8$ in each group). Analysis of gene expression in the neurosensory retina was performed by Affymetrix genechips, and confirmed by RT-qPCR ($n = 6$ in each group). Elevated glycemia (+13%), insulinemia (+83%), and leptinemia (+172%) was observed after 8 days of fructose feeding. The cone photoreceptor response was altered at day 8 in high fructose fed rats ($\Delta = 0.5$ log unit of light stimulus intensity). Affymetrix analysis of gene expression highlighted significant modulation of the pathways of eIF2 signaling and endoplasmic reticulum stress, regulation of eIF4 and p70S6K signaling, as well as mTOR signaling and mitochondrial dysfunction. RT-qPCR analysis confirmed the down regulation of *Crystallins*, *Npy*, *Nid1* and *Optc* genes after 3 days of fructose feeding, and up regulation of *End2*. Meanwhile, a trend towards an increased expression of αA- and αB-crystallin proteins was observed at day 8. Our results are consistent with early alterations of the functioning and gene expression in the retina in a pro diabetogenic environment.

1. Introduction

It is now considered as a postulate that long term adaptation to

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the environment is a key driver for the origin and evolution of living species. In *On the Origin of Species* published in 1859, Charles Darwin suggested that "It is not the strongest of the species that survive, nor the most intelligent, but the one most responsive to change" (Darwin, 1859). Among other powerful environmental factors, the ability to meet nutritional requirements by adapting to new foods plays a crucial role in the origin and evolution of species (Verginelli et al., 2009). More recently the early adaptation to dietary factors has emerged as an important contributor to behavioral and metabolic changes. It was shown that a high fat diet rewired hypothalamic neurons in the time frame of three days only. Moreover, the inhibition of this rewiring mechanism limited the metabolic adaptation of mice to high fat diet, leading to obesity (Benani et al., 2012). In conjunction with high fat, dietary high fructose induced short term insulin signaling and neuronal changes in the hippocampus after seven days of feeding (Calvo-Ochoa et al., 2014). In the retina, neuronal defects were the earliest detectable changes in diabetes, before the onset of vascular alterations. Functional changes have been suggested to be more sensitive indicators of retinal integrity than clinical ophthalmological endpoints (Antonetti et al., 2006). As recently reviewed by Abcouwer and Gardner, changes in the electroretinographic response including loss of photoreceptor light sensitivity have been reported in diabetic patients without diabetic retinopathy (Abcouwer and Gardner, 2014). Far less is known about the very early functional and gene expression changes in the retina in the context of pre diabetic states, such as obesity and metabolic syndrome that remain important risk factors for diabetes (Alberti et al., 2009). We therefore aimed to characterize the functional and gene expression changes in the retina of rats fed a high fructose diet after short term periods of feeding, accounting that feeding rodents with high fructose diets is a recognized and widely used model to trigger obesity and correlative metabolic syndrome (Dekker et al., 2010).

2. Materials and methods

2.1. Ethical concerns

All procedures were conducted in accordance with the ARVO Statements for the use of animals in ophthalmic and vision research and were approved by the local Animal Care and Use Committee (Comité d'Ethique de l'Expérimentation Animale C2EA nr 105, Dijon, France). Personal (nr 21CAE095) and institutional (nr B21231010EA) agreements were obtained according to French regulations.

2.2. Experimental diets

Standard and 60%-rich fructose diets (Table 1) were purchased from Ssniff Spezialdiäten Gmbh (Soest, Germany).

2.3. Animals

Male Brown Norway rats ($n = 64$, 6 weeks of age, Charles River, L'Arbresle, France) were housed in controlled temperature (22 ± 1 °C) and humidity (55–60%) conditions with a 12-h light/12-h dark cycle. After a 7-day-long quarantine, the animals were randomly allocated to the experimental groups corresponding to the feeding of either of the two experimental diets during 3 or 8 days ($n = 16$ per diet and per time point). Rats had unrestricted access to food and deionized tap water.

2.4. Electroretinography

The electroretinograms (ERG) were recorded after 3 and 8 days of feeding ($n = 16$ rats per group), according to previously described procedures and International Society for Clinical Electrophysiology of Vision (ISCEV) guidelines (Jaissle et al., 2001; Marmor et al., 2009). Rats were dark-adapted overnight before the experiments. All further procedures were carried out under dim red light ($\lambda < 650$ nm) at a constant temperature of 25 °C. Rats were anesthetized by intramuscular injection of ketamine (100 mg/kg, Imalgène 1000, Merial, Lyon, France) and xylazine (10 mg/kg, Rompun 2%, Bayer, Puteau, France). Pupils were dilated with 1% tropicamide (Mydriaticum, Laboratoires Thea, Clermont-Ferrand, France). After 10 min, rats were positioned on a warming plate, and the corneal electrodes were put in place. The ERG was recorded via corneal electrodes (thin gold wire with a 3-mm ring end) and reference and ground electrodes (silver needle) placed on the forehead and tail, respectively. The recording setup featured a Ganzfeld bowl, an amplifier, and a computer-based control and recording unit (RETI port/scan 21, Stasche & Finger GmbH, Roland Consult, Brandenburg, Germany). ERG responses were recorded from both eyes simultaneously after the rats were placed in the Ganzfeld bowl.

The first ERG examination consisted in light stimulations at growing intensities and at a fixed frequency of 8.02 Hz ($n = 8$ rats per group). The responses were recorded at ten intensities from 0.0003 cd s/m^2 to 10 cd s/m^2.

The second ERG examination corresponded to the scotopic single flash, scotopic threshold response (STR) and oscillatory potential (OP) recordings ($n = 8$ rats per group). The stimuli of the scotopic single-flash consisted in 10 increasing intensities from 0.0003 cd s/m^2 to 10 cds/m^2. At least ten responses were averaged with an inter-stimulus interval of 5 s (from 1 to 10 cd s/m^2) or 17 s (up to 0.3 cd s/m^2). The band-pass filter width was 1–300 Hz for the scotopic single-flash responses. The STR stimuli were recorded with three increasing intensities: −4.60 log, −4.32 log, and −4.02 log. A 2 s inter-stimulus interval was used between these stimuli. The band-pass filter width was 0.2–30 Hz for the STR recording procedure. After amplification, the signal was digitized and processed. For OPs recordings, two stimuli were applied at a frequency of 0.06 Hz. The band-pass filter width was 100–500 Hz. After amplification, the signal was digitized and processed on the basis of means of amplitude and time-latency measurements of each of the four typically characterized peaks (OP1–OP4) (Hancock and Kraft, 2004).

Table 1
Composition of the experimental diets.

	Standard diet	Fructose diet
	In g per kg of diet	
Casein	180	180
Cornstarch	460	90
Sucrose	230	0
Fructose	0	600
Cellulose	20	20
Mineral mix[a]	50	50
Vitamin mix[b]	10	10
Fat[c]	50	50

[a] Composition (g/kg): sucrose, 110.7; $CaCO_3$, 240; K_2HPO_4, 215; $CaHPO_4$, 215; $MgSO_4 \cdot 7H_2O$, 100; NaCl, 60; MgO, 40; $FeSO_4 \cdot 7H_2O$, 8; $ZnSO_4$, $7H_2O$. 7; $MnSO_4 \cdot H_2O$, 2; $CuSO_4 \cdot 5H_2O$, 1; $Na_2SiO_7 \cdot 3H_2O$, 0.5; $AlK(SO_4)_2 \cdot 12H_2O$, 0.2; K_2CrO_4, 0.15; NaF, 0.1; $NiSO_4 \cdot 6H_2O$, 0.1; H_2BO_3, 0.1; $CoSO_4 \cdot 7H_2O$, 0.05; KIO_3, 0.04; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.02; LiCl, 0.015; Na_2SeO_3, 0.015; NH_4VO_3, 0.01.

[b] Composition (g/kg): sucrose, 549.45; retinyl acetate, 1; cholecalciferol, 0.25; DL-tocopheryl acetate, 20; phylloquinone, 0.1; thiamine HCl, 1; riboflavin, 1; nicotinic acid, 5; calcium pantothenate, 2.5; pyridoxine HCl, 1; biotin, 1; folic acid, 0.2; cyanocobalamin, 2.5; choline HCl, 200; DL-methionine, 200; p-aminobenzoic acid, 5; inositol, 10.

[c] Composition of the oil mix (%): rapeseed oil, 18.7; oleic oil, 38.1; sunflower oil, 5; palm oil, 38.1. Omega 6 to omega 3 ratio = 7.5.