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Research article

A short term high-fat high-sucrose diet in mice impairs optic nerve recovery after injury and this is not reversed by exercise

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ABSTRACT

The aim of the current work was to test whether increased intake of dietary fat and sucrose in mice modifies the response of retinal ganglion cells (RGCs) of the optic nerve to injury, and whether any effects of diet are influenced by physical activity levels. C57BL/6J mice were given a high-fat high-sucrose (HFS) diet for 7 weeks, with or without exposure to regular exercise by swimming (60 min/day, 5 days/week). Injury to RGCs was subsequently induced by acute elevation of intraocular pressure (IOP) and retinas were assessed for function and structure. We report that mice on a HFS diet had similar body mass and blood glucose levels compared to mice on a control diet but suffered a 30% greater loss of RGC function following injury, as measured *in vivo* with the electroretinogram. RGC dysfunction in retinas from mice on the HFS diet was accompanied by activation of retinal macroglia but was not associated with neuronal cell loss. Exercising mice by swimming did not prevent HFS-induced RGC dysfunction in response to injury. This study shows for the first time that a short term increase in dietary fat and sucrose enhances the vulnerability of RGCs to dysfunction and cell stress after an acute injury, and that this is independent of obesity or hyperglycemia. Furthermore, our results suggest that detrimental effects of diet predominate over protective effects of exercise.

1. Introduction

Glaucoma is characterized by the accelerated loss of retinal ganglion cells (RGCs), the innermost neurons of the retina that project axons along the optic nerve to visual centres of the brain. Growing evidence suggests that the susceptibility of RGCs to injury and degeneration can be modified by lifestyle factors. We believe that identifying these protective and risk factors will inform new strategies to stop or slow down RGC loss in diseases like glaucoma.

Western pattern diets, which are characterized by a high intake of fat and sugar, have been putatively associated with higher rates of neurodegenerative brain diseases in humans (reviewed in Francis and Stevenson, 2013). Animal studies demonstrate that diets high in fat and sugar can influence neuronal structure and function in various regions of the brain (Cisternas et al., 2015; Molteni et al., 2002) and exacerbate neuronal loss after injury (Agrawal et al., 2015; Bousquet et al., 2012; Choi et al., 2005; Morrison et al., 2010; Wu et al., 2003). However, there is limited information with respect to how these same dietary patterns impact retinal neurons.

We have demonstrated that aerobic exercise protects RGCs of the mouse eye against dysfunction and cell stress after an insult induced by elevation of intraocular pressure (IOP) (Chrysostomou et al., 2014, 2016). Recently, it has become evident that exercise can also reverse some of the harmful effects associated with high fat consumption in the CNS. Animal studies found that physical activity protected mice against high-fat diet-induced microglial activation and inflammation in the hypothalamus (Yi et al., 2012); and prevented high-fat diet-induced oxidative stress in the hippocampus and deficits in spatial learning (Molteni, 2004). Whether

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exercise might be effective in reversing any detrimental effects of diet on retinal neurons has not been studied.

Accordingly, we hypothesized that increased intake of dietary fat and sucrose would exacerbate outcomes after RGC injury and that regular exercise may blunt (or prevent) these harmful effects. To explore this possibility, we tested the impact of feeding mice a modified diet with high levels of fat and sucrose, alone or in combination with increased levels of physical activity, on RGC function and structure in response to acute IOP elevation.

2. Methods

2.1. Animals and diet

All animal procedures conformed to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research, and with the requirements of the Royal Victorian Eye & Ear Hospital Animal Research and Ethics Committee. C57BL/6J mice were housed in a temperature- (22 ± 1 °C), light- (12 h light, 12 h dark) and humidity-controlled (30–40%) environment with free access to food and water. Male and female mice were used equally. At 8 weeks of age mice were either maintained on a control diet (AIN93G; Specialty Feeds, Australia) or switched to a modified version of the control diet in which total fat content was increased from 7% to 36% (SF03-002: Fat Modification of AIN93G; Specialty Feeds, Australia) for 7 weeks ($n = 12$ per dietary group). Details of diet compositions are shown in Table 1. Body weight of all mice was measured weekly. Non-fasting blood glucose measurements were taken weekly using a glucometer (Optium Xceed; Abbott Diabetes Care Inc).

2.2. Elevation of intraocular pressure

Retinal ganglion cell (RGC) injury was induced by short-term elevation of intraocular pressure (IOP), a well-characterized non-ischemic insult that has been described in detail (Crowston et al., 2015). Briefly, animals were anaesthetized with intraperitoneal injection of ketamine (60 mg/kg) and xylazine (10 mg/kg) before resting eye pressure was measured using a hand-held rebound tonometer (Icare TonoLab; Colonial Medical Supply). The anterior chamber of the eye was cannulated with a 50 μm borosilicate needle connected in series with a reservoir filled with sterile-filtered endotoxin-tested saline. IOP was raised and maintained at 50 ± 1.0 mmHg for 30 min using a feedback syringe driver (PHD Ultra; Harvard Apparatus) in line with a pressure transducer (Hugo Sachs Electronik; Harvard Apparatus).

Table 1
Composition of mouse diets.

	Control diet [*Standard AIN93G Rodent Diet*]	High-fat high-sucrose diet [*SF03-002 Modification of AIN93G*]
Nutritional parameters		
Protein	19.4%	19.4%
Fat	7.0%	36.0%
Carbohydrate (starch)	61.5%	0%
Sucrose	100 g/kg	346 g/kg
Digestible energy	16.1 MJ/kg	22.8 MJ/kg
Fatty acid composition (%)		
Total n-3 fatty acids	0.98	0.74
Total n-6 fatty acids	1.51	2.05
Total mono unsaturated fats	3.98	12.20
Total polyunsaturated fats	2.50	2.79
Total saturated fats	0.50	20.92

2.3. Exercise

Mice in the exercise group underwent 60 min of swimming five days a week according to published protocols (Chrysostomou et al., 2014; McMullen et al., 2003). Mice were exercised for 6 weeks prior to RGC injury and continued to exercise during the 7 day recovery period. To minimize potential confounding influences of stress or environmental enrichment, mice in sedentary control groups were placed in an empty swimming tank alongside exercising mice for a comparable period each day, and enrichment objects were excluded from all home cages.

2.4. Electroretinography

The full-field flash electroretinogram (ERG) was used to assess retinal function in dark-adapted anaesthetized mice according to published protocols (Chrysostomou and Crowston, 2013). In brief, responses to a series of stimulus intensities (−5.92 to 2.22 log cd.s/m^2) were recorded simultaneously from both eyes using a Ganzfeld system (Espion E2; Diagnosys LLC). Three components of the ERG waveform, representing the activity of different populations of retinal neurons, were analysed. The positive scotopic threshold response (pSTR) is elicited with low intensity illumination and is derived predominantly from RGCs in the rodent retina (Saszik et al., 2002). Amplitudes of the pSTR were measured at a fixed time of 110 ms after a flash stimulus of −4.54 log cd.s/m^2, which coincides with the pSTR peak. Maximum amplitudes of waveforms derived from photoreceptors (a-wave) and ON-bipolar cells (b-wave) were measured in response to a flash stimulus of 2.22 log cd.s/m^2. ERGs were recorded serially in animals, one day before (baseline) and 7 days after elevation of IOP.

2.5. Immunohistochemistry

Animals were euthanized by cervical dislocation before eyes were enucleated and immersion-fixed in 4% paraformaldehyde for 3 h, followed by overnight cryoprotection in 15% sucrose. Eyes were embedded in optical cutting temperature medium and 12 μm sections were cut through the pupillary-optic nerve axis. Cryosections were immunolabeled for glial fibrillary acidic protein (GFAP; 1:800, Dako, Campbellfield VIC, Australia) and nuclear-counterstained with Hoechst (1:10,000) using standard procedures (Kezic et al., 2013).

2.6. Quantification of retinal thickness and ganglion cells

The thicknesses of cellular and synaptic retinal layers were measured on digital images of Hoechst-stained cryosections as described (Chrysostomou et al., 2009). To quantify RGCs, sections cut through the optic nerve head and ora serrata were scanned from superior to inferior edge and the numbers of Hoechst-labelled nuclei in the ganglion cell layer were counted by a masked observer.

3. Results

3.1. A short term non-obesogenic and non-hyperglycaemic high-fat high-sucrose diet

The high-fat high-sucrose (HFS) diet used in this study had a total fat content of 36% fat by weight with carbohydrate content from sucrose only (346 g/kg; Table 1). The control diet was comprised of 7% fat content by weight and 100 g/kg sucrose, with fat substituted for carbohydrate (61% by weight). Mice that were fed a HFS diet for 7 weeks did not exhibit significant increases in body weight relative to mice that were fed the control diet (Fig. 1A).