Ultrastructural Retinal Neuronal Changes in Juvenile Miniature Ossabaw Pigs Fed a Western Diet

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Vision loss is one of the most debilitating conditions with an adverse impact on the quality of life. It is significantly associated with depression in adults [1], increases dependency of afflicted elders on their caregivers [2], and is a substantial economic burden in the United States [3]. According to a consensus from the National Eye Institute and Prevent Blindness America, the leading causes of age related eye diseases are cataract, glaucoma, age-related macular degeneration and diabetic retinopathy (DR) [4].

DR is the main cause of irreversible blindness associated with diabetes mellitus in young adults. More than 642 million people are estimated to be diabetic by year 2040, with 90% of them being type 2 diabetic (T2DM), and an increasing proportion of the T2DM reported in children younger than 14 years of age [5]. An average 10% of patients will be afflicted with some form of retinopathy within 10 years of having diabetes. As a result, one-third of the economic cost from eye disorders was predicted to be incurred by adults younger than 40 years of age [3]. Current DR treatment mostly in the late stages involve anti-angiogenic drug injections into the eye, and laser photocoagulation surgery. However, there is no treatment for the early non-proliferative stage of the disease, and early signs of DR are impossible to detect or visualize with modern advanced imaging techniques. One of the major limitations to characterize the early events in DR is the unavailability of a suitable animal model especially in the early stages of disease pathogenesis. Also, there are no translational large animal model currently available which represents the physiological and clinical development of juvenile diabetes expressing DR features. Since detection is key to prevention of this disease, there is an urgent need to develop new models targeted at the pre-/early diabetic stage to better understanding the progression of DR.

Ossabaw pigs are a breed of wild boar that adopted a “thrifty genotype” to ensure their survival on the harsh conditions of Ossabaw island. As a result, Ossabaw pigs are a good model for diet-induced obesity, and develop classical features of metabolic syndrome (MetS) when fed a high-fat diet [6]. Human patients diagnosed with MetS has been shown to be at greater risk of developing conditions such as cardiovascular diseases [7], diabetes [8] and DR [9]. While the juvenile Ossabaw pigs on western diet had been an animal model for the study of cardiomyopathic dysfunction, steatohepatitis and renal injury, retinal diseases in the Ossabaw has not yet been studied. Hence, this study aims to evaluate the Ossabaw pigs for early retinopathy symptoms associated with pre-/early diabetes.

Twelve 14 week old Ossabaw pigs divided into Lean and Obese groups. Lean animals were kept on normal chow until the end of study, while Obese pigs were fed a high fat/high fructose/high calorie diet for 10 weeks. Animals were euthanized at 6 months of age. Eyes were enucleated within 10 minutes of confirmed
death. The eyes were hemisected circumferentially at ora ciliaris, and 1ml of 2% paraformaldehyde, 2% glutaraldehyde in 100 mM sodium cacodylate buffer was injected into the vitreous of the eye. The entire posterior eyecup was then submerged in fixative and left overnight in 4°C fridge. Next, a sharp razor blade was used to segment selected portions of the eye into small 3mm cubes. Each cube was immobilized in histogel, and rinsed with 100 mM sodium cacodylate buffer, pH 7.35 containing 130 mM sucrose. 1% osmium tetroxide in cacodylate buffer was used for secondary fixation. After an hour in 4°C, en bloc staining was performed using 1% aqueous uranyle acetate. Following overnight incubation at 4°C, graded dehydration series (100 Watts for 40s, per exchange) was performed using ethanol. Tissues were then transitioned into acetone and infiltrated with Epon resin (250 Watt for 3 min) and polymerized at 60°C overnight. Retinal cubes were cut transversely with an ultramicrotome to obtain 2µm thick sections. Toluidine blue staining was performed for gross retinal histological examination under Leica light microscope at 400X magnification. Retina tissue block was further trimmed to 500µm length, and cut with diamond knife to yield 75nm sections for TEM. Images were acquired with a JEOL JEM 1400 transmission electron microscope at 80 kV on a Gatan Ultrascan 1000 CCD, at 1200X magnification.

Histological examination of the Obese Ossabaw pigs showed changes in neuronal architecture in all layers of the retina without any loss of thickness. Numerous disintegrated cell bodies were seen in the ganglion cell layer (GCL), and both the inner and outer nuclear layer (INL, ONL) showed disorganization of cells with uneven distribution of nuclear/cytoplasmic content. Ultrastructure examination under electron microscopy showed nuclear distortion and degenerative changes in the retina pigmented epithelial (RPE), INL and GCL of the Obese pigs. Increased gaps between photoreceptor (Prh) cell bodies were observed in ONL, with uneven nuclear sizes and shapes. Cone Prh in the Obese group had a smaller inner segment, along with loss and disarrangement of disc in the outer segments. Numerous large vacuoles were seen in the INL. Neuronal processes were bigger and less compact in the nerve fiber layer (NFL) of Obese animals. Capillaries in this group also had thicker endothelial basement membrane as compared to the Lean control.

This is the first study to examine retinal changes in a MetS large animal model. Juvenile Ossabaw pigs fed a western diet exhibit early signs of DR including neuronal defects and basement membrane thickening, hence making it a translational animal model for DR [10].

References:

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