

## Impaired Autophagy Diurnal Rhythmicity in Rodent Diabetic Retinopathy

Xiaoping Qi<sup>1</sup>, Sayak Mitter<sup>1</sup>, Yuanqing Yan<sup>2A</sup>, William Dunn<sup>2B</sup>, Juliet Busik<sup>3</sup>, Maria Grant<sup>1</sup>, Michele Boulton<sup>1</sup>

**Purpose:** Retinal homeostasis is under both diurnal and circadian regulation. However, diurnal changes in retinal autophagy have not been hitherto explored. We sought to investigate the diurnal expression of autophagy proteins/genes in normal rodent retina to determine if this is impaired in diabetic retinopathy.

**Methods:** Eyes from C57BL/6 mice and BBZ rats maintained under a 12h/12h; 6am/6pm light/dark cycle were enucleated every 2 or 3 hours over a 24 hour period. Eyes were also collected from C57BL/6 induced STZ for 2 or 9 month as type 1 and BBZDR/wor type 2 diabetic rats for 4 months.

Immunohistochemistry, Western-blot and real-time PCR were performed for Atg7, Atg9, LC3 and Beclin. Retina vessel pathology and superoxide were assessed by enzyme digestion and a spectrofluorometer.

**Results:** Autophagy proteins (Atgs) were abundantly expressed in neural retina and endothelia cells in both mice and rats with differential staining pattern across the retinas and demonstrated a distinctive diurnal rhythmicity. All Atgs showed localization to retinal blood vessels with Atg7 being the most highly expressed. Analysis of the immunostaining demonstrated distinctive diurnal rhythmicity of which Atg9 and LC3 shared a biphasic expression cycle with the highest level at 8:15 am and 8:15 pm. By contrast, Beclin revealed a 24-hour cycle with the highest level observed at midnight. Atg7 was also on a 24-hour cycle with peak expression at 8:15am, coinciding with the first peak expression of Atg9 and LC3. In diabetic animals, immunohistochemistry showed dramatic reduction in all four Atgs and this was further confirmed by Western Blot, especially a decrease in LC3II/LC3I ratio (a measure of autophagy flux). Furthermore, the distinctive diurnal rhythmicity of these autophagy proteins was significantly impaired and phase shifted in diabetic animals.

**Conclusions:** Autophagy proteins show both spatial and diurnal-dependent expression in normal rodent retinas and this is severely impaired and phase shifted in both type 1 and type 2 diabetic animals.

Decreased autophagy in diabetic animals may in part explain the increased generation of reactive oxygen species in diabetic retinopathy. Therefore, restoration of diurnal rhythmicity and facilitating autophagy pathway expression may provide new treatment strategies for diabetic retinopathy.