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“Fluctuating Hyperglycemia Induces Severe Hypoxia in Human Retinal Müller Cells under Normoxic Conditions: Implications for Early Diabetic Retinopathy”

Objective: Fluctuating hyperglycemia (FHG) has been identified as an independent risk factor for diabetic retinopathy. However, the role of FHG in relation to hyperglycemia-induced hypoxia in human retinal Müller cells has not been well studied. Using immunohistochemistry and the Seahorse Extracellular Flux Analyzer, we conducted an analysis of human Müller glial cells isolated from healthy donor eyes to assess vulnerability to hyperglycemia-induced hypoxia and to characterize metabolic stress contributing to retinal hypoxia under normoxic conditions. We hypothesize that FHG induces severe hypoxia in human retinal Müller cells via dysregulation of mitochondrial metabolism.

Approach: We treated human Müller glia (HMG) in constant hyperglycemia (CHG) or FHG for a total of 6 hours before staining with pimonidazole hydrochloride to localize hypoxia intensity. In addition, we assessed mitochondrial metabolic function in Müller glia by measuring oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) using the Seahorse XFe24 analyzer.

Results: After normalization to normoglycemic (NG) controls, pimonidazole staining intensity significantly increased under both CHG (1.9-fold) and FHG (1.8-fold) in HMG (n=4). Bioenergetically, Müller glial cells exposed to CHG and FHG demonstrated reduced basal OCR (CHG: 89.74 ± 3.59 pmol/min, FHG: 91.39 ± 8.88 pmol/min) as compared to NG controls (97.25 ± 10.76 pmol/min) and reduced spare respiratory capacity, indicating diminished ability of human Müller glial mitochondria to respond to stress (n=4). Hyperglycemia also reduced mitochondrial function without a compensatory increase in ECAR, suggesting that acute hyperglycemic insult compromises mitochondrial oxidative respiration in human retinal Müller glia.

Conclusions: These preliminary data indicate that FHG induces severe hypoxia and oxidative stress in human retinal Müller cells via disruption of mitochondrial oxidative metabolism. Such findings suggest that hyperglycemia-induced hypoxia may arise due to an increase in glucose availability that leads to decreased mitochondrial oxygen consumption in human retinal Müller glia.