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Lavoie DJ, Jacot JL, Knowles WJ, Carrington AL, Litchfield JE, Haigh B and Sredy J: (1999) **Characterization of Amadori-modified proteins in ocular tissues of experimental diabetic rats.** *IOVS* 40, 4, S607

Purpose: To determine by Western blot analysis the presence of Amadori-modified proteins in ocular tissue and whether they are increased in diabetic animals. **Materials & Methods:** Weight and aged-matched S-D rats were placed into a control (CON) or diabetic (DB) group (streptozotocin [STZ] 30 mg/kg i.p.-resultant blood glucose >345 mg/dl). After 4 wks of diabetes the rats were euthanized than enucleated. The eyes were placed in O.C.T. compound and stored frozen at -85oC until processing. Eyes were thawed in PBS, submerged in 150mM PMSF-PBS and dissected under a microscope. The following tissues were isolated and 4 eyes from different rates in each group were pooled: Cornea (CO), iris (IR), lens epithelium (LE), lens fiber mass (LF), vitreous (VT), retina (RT), and choroid (CH). Equal protein concentration so of the various tissue homogenates from CON and DB were loaded onto a SDS-PAGE 4-20% gradient gel and analyzed by Western blotting. A 1:5000 dilution of an unlabelled monoclonal mouse IgM antibody raised against glucose-derived Amadori-modified lysine residues (1-deoxyfructosyl lysine) with a secondary antibody at a concentration of 1:1250 was used. Detection was accomplished using alkaline phosphate-BCIP-NBT. **Results:** In both CON and DB rats multiple Amadori-modified proteins were detected in each tissue. Densitometry revealed increases in glycated proteins of low MW (in kD) from DB tissues in: LF (19-29), VT (29-43), RT (29-43). Higher MW glycated proteins were detected in: CO (43-200), IR (100-200), LE (100-200), and CH (68-200). **Conclusion:** Increased Amadori-modified proteins occur in ocular tissues of DB rats. This early protein modification is detectable in those ocular structures exhibiting diabetes-related complications, suggesting that it could contribute to the pathogenic mechanism (s) of diabetes related ocular disease.