

Affiliation	Professor
Name	Abdulrehman Al Sunaidy
Project's Title	Effect of glycation on the camel and mouse lens crystallins: Comparative analysis
Brief Introduction (max 300 words)	<p>Glycation is nonenzymatic reaction of reducing sugars with protein side chains. Glycated products derived from the reaction of sugars with proteins are formed during long-term incubation undergo complex multistep reactions and rearrangements to form advanced glycation end products (AGEs) [1].</p> <p>These AGEs are known to occur in vivo, in normal tissues, especially in long-living proteins such as collagen and eye lens proteins during normal aging and in people suffering from diabetes mellitus. These events can also be simulated in vitro by incubating sugars with proteins. In vivo, AGEs are found to contribute to the pathogenesis of diabetic complications, leading to renal insufficiency [2], cardiovascular and Alzheimer's diseases [3].</p> <p>The aim of the project is to find suitable biochemical characterization protocol, for this reason, camel and mouse lens homogenates are incubated with different concentrations of glucose, and its metabolites such as glyceraldehydes, glyoxal and methylglyoxal [4]. The isolated AGEs, crystallin peaks and lens homogenate are characterized and compared with respect to the extent of side chain modifications (lysine and arginine), the carbonyl content, free and total sulfhydryl contents, extent of glycation and the fibrillar state or amyloid formation using fluorescence and absorption spectroscopy [5].</p> <p>The purified AGEs peak is further characterized by incubating with cultured cells to observe the necrotic or apoptotic changes brought by AGEs to cell culture. The results will provide important aspects on the effect of AGEs on cell culture and allow comparing AGEs derived from modification of lens homogenates by glucose and its metabolites. The results will also suggest various conformational changes within the modified proteins, which may simulate the structural and functional change in proteins in diabetics [7].</p> <p>Antiglycating agents are currently under investigation for the prevention and treatment of AGEs accumulation and diabetic related complications. Effect of antiglycating agents (acetylcarnosine and carnosine) on isolated AGEs will be analysed [8]. The study of AGEs represents one of most promising areas of research today.</p>
Methodology (max 300 words)	<p>Preparation of AGEs</p> <p>AGEs were prepared by incubating of camel and mouse lens homogenate (25mg/ml) in 0.05M phosphate buffer, pH 8, 1mM NaN₃, 1mM EDTA using glucose (5 and 100mM) and glucose metabolites such as glyceraldehyde, methyl glyoxal and glyoxal (each 2 and 10mM) as modifiers for 2 weeks at 37 °C under sterile conditions. After the incubation, the homogenates containing AGEs and control are extensively dialyzed against PBS at 4 °C, aliquoted, and stored at -20 °C for further analysis. Protein concentrations are determined using the Bradford assay.</p>

	<p>Isolation of AGEs using Sephacryl S 1000 column Incubated camel and mouse lens homogenates (10mg/ml) are subjected to gel filtration chromatography on a Sephacryl S-1000 column (1x30 cm) (exclusion limit 108). AGEs peak is isolated in the first peak after void volume and fractionated α-,β-and γ - crystallin peaks were isolated and stored at -20oC for further analysis.</p> <p>Analysis mentioned below are performed either in AGEs, α-, β- and γ-crystallin peaks or in total homogenates of camel and mouse lenses.</p> <p>Determination of free amino groups using fluorescamine [9] and free arginine side chains using 9,10-phenanthrenequinone [10]. Concentrations of free- and total sulfhydryl groups in the AGEs, various peaks and total homogenates are assayed [11]. Determination of carbonyl content using 2,4-DNPH was assayed as described [3]. Fructosamine residues are quantified using nitro blue tetrazolium according to the method of Johnson et al. [12] and amyloid formation was quantified with thioflavin T method [13]. Extrinsic and intrinsic fluorescence and UV scans for adducts from with various sugars are recorded with spectrometers.</p>
<p>The project is applicable within (months)</p>	<p>8</p>
<p>References (max 300 words)</p>	<p>[1] VM Monnier, A Cerami, Nonenzymatic browning in vivo: possible process for aging of long-lived, Science, 211 (1981) 491-493.</p> <p>[2] R. Bucala, Z. Makita, G. Vega, S. Grundy, T. Koschinsky, A. Cerami, H. Vlassara, Modification of low density lipoprotein by advanced glycation end products contributes to the dyslipidemia of diabetes and renal insufficiency, Proc. Natl. Acad. Sci. USA 91 (1994) 9441–9445.</p> <p>[3] M.P. Vitek, K. Bhattacharya, J.M. Glendening, E. Stopa, H. Vlassara, R. Bucala, K. Manogue, A. Cerami, Advanced glycation end products contribute to amyloidosis in Alzheimer disease, Proc. Natl. Acad. Sci. USA 91 (1994) 4766–4770.</p> <p>[4] B.K. Kilhovd, I. Giardino, P.A. Torjesen, K.I. Birkeland, T.J. Berg, P.J. Thornalley, M. Brownlee, K.F. Hanssen, Increased serum levels of the specific AGE-compound methylglyoxal-derived hydroimidazolone in patients with type 2 diabetes, Metabolism 52 (2003) 163–167.</p> <p>[5] A.J. Furth, Methods for assaying nonenzymatic glycosylation, Anal. Biochem. 175 (1988) 347–360.</p> <p>[6] Nass N, Weissenberg K, Somoza V, Ruhs S, Silber RE, Simm A, Cell culture condition-dependent impact of AGE-rich food extracts on kinase activation and cell survival on human fibroblasts, Int J Food Sci Nutr. (2013) 14-18</p> <p>[7]] A. Lapolla, D. Fedele, L. Martano, N.C. Arico', M. Garbeglio, P. Traldi, R. Seraglia, D. Favretto, Advanced glycation end products: a</p>

highly complex set of biologically relevant compounds detected by mass spectrometry, *J. Mass Spectrom.* 36 (2001) 370–378.

[8] O.K. Argirov, and O. Argirov, Inhibition of ascorbic acid-induced modifications in lens proteins by peptides, *J. Pep. Res.* 9, (2003) 170-176

[9] De Bernardo, S., M. Weigele, V. Toome, K. Manhart, W. Leimgruber, P. Böhlen, S. Stein, and S. Udenfriend, Studies on the Reaction of Fluorescamine with Primary Amines, *Arch. Biochem. Biophys.* 163 (1974)390-399.

[10] R.E. Smith, R. MacQuarrie, A sensitive fluorimetric method for the determination of arginine using 9,10-phenanthrenequinone, *Anal. Biochem.* 90 (1978) 246–255.

[11] T. P. Hum and R. C. Augusteyn, The state of sulphhydryl groups in proteins isolated from normal and cataractous human lenses *Curr. Eye Res.* 6(9), (1987) 1091-1101.

[12] R.N. Johnson, P.A. Metcalf, J.R. Baker, Fructosamine: a new approach to the estimation of serum glycosylprotein. An index of diabetic control, *Clin. Chim. Acta* 127 (1983) 87–95.

[13] H. LeVine III, Thioflavine T interaction with synthetic Alzheimer's disease beta-amyloid peptides: detection of amyloid aggregation in solution, *Protein Sci.* 2 (1993) 404–410.

[1] VM Monnier, A Cerami, Nonenzymatic browning in vivo: possible process for aging of long-lived, *Science*, 211 (1981) 491-493.

[2] R. Bucala, Z. Makita, G. Vega, S. Grundy, T. Koschinsky, A. Cerami, H. Vlassara, Modification of low density lipoprotein by advanced glycation end products contributes to the dyslipidemia of diabetes and renal insufficiency, *Proc. Natl. Acad. Sci. USA* 91 (1994) 9441–9445.

[3] M.P. Vitek, K. Bhattacharya, J.M. Glendening, E. Stopa, H. Vlassara, R. Bucala, K. Manogue, A. Cerami, Advanced glycation end products contribute to amyloidosis in Alzheimer disease, *Proc. Natl. Acad. Sci. USA* 91 (1994) 4766–4770.

[4] B.K. Kilhovd, I. Giardino, P.A. Torjesen, K.I. Birkeland, T.J. Berg, P.J. Thornalley, M. Brownlee, K.F. Hanssen, Increased serum levels of the specific AGE-compound methylglyoxal-derived hydroimidazolone in patients with type 2 diabetes, *Metabolism* 52 (2003) 163–167.

[5] A.J. Furth, Methods for assaying nonenzymatic glycosylation, *Anal. Biochem.* 175 (1988) 347–360.

[6] Nass N, Weissenberg K, Somoza V, Ruhs S, Silber RE, Simm A, Cell culture condition-dependent impact of AGE-rich food extracts on

kinase activation and cell survival on human fibroblasts, *Int J Food Sci Nutr.* (2013) 14-18

[7]] A. Lapolla, D. Fedele, L. Martano, N.C. Arico', M. Garbeglio, P. Traldi, R. Seraglia, D. Favretto, Advanced glycation end products: a highly complex set of biologically relevant compounds detected by mass spectrometry, *J. Mass Spectrom.* 36 (2001) 370–378.

[8] O.K. Argirov, and O. Argirov, Inhibition of ascorbic acid-induced modifications in lens proteins by peptides, *J. Pep. Res.* 9, (2003) 170-176

[9] De Bernardo, S., M. Weigele, V. Toome, K. Manhart, W. Leimgruber, P. Böhlen, S. Stein, and S. Udenfriend, Studies on the Reaction of Fluorescamine with Primary Amines, *Arch. Biochem. Biophys.* 163 (1974)390-399.

[10] R.E. Smith, R. MacQuarrie, A sensitive fluorimetric method for the determination of arginine using 9,10-phenanthrenequinone, *Anal. Biochem.* 90 (1978) 246–255.

[11] T. P. Hum and R. C. Augusteyn, The state of sulphhydryl groups in proteins isolated from normal and cataractous human lenses *Curr. Eye Res.* 6(9), (1987) 1091-1101.

[12] R.N. Johnson, P.A. Metcalf, J.R. Baker, Fructosamine: a new approach to the estimation of serum glycosylprotein. An index of diabetic control, *Clin. Chim. Acta* 127 (1983) 87–95.

[13] H. LeVine III, Thioflavine T interaction with synthetic Alzheimer's disease beta-amyloid peptides: detection of amyloid aggregation in solution, *Protein Sci.* 2 (1993) 404–410.

[1] VM Monnier, A Cerami, *Science*, 211 (1981) 491-493.

[2] R. Bucala, Z. Makita, G. Vega, S. Grundy, T. Koschinsky, A. Cerami, H. Vlassara, *Proc. Natl. Acad. Sci. USA* 91 (1994) 9441–9445.

[3] M.P. Vitek, K. Bhattacharya, J.M. Glendening, E. Stopa, H. Vlassara, R. Bucala, K. Manogue, A. Cerami, *Proc. Natl. Acad. Sci. USA* 91 (1994) 4766–4770.

[4] B.K. Kilhovd, I. Giardino, P.A. Torjesen, K.I. Birkeland, T.J. Berg, P.J. Thornalley, M. Brownlee, K.F. Hanssen, *Metabolism* 52 (2003) 163–167.

[5] A.J. Furth, *Anal. Biochem.* 175 (1988) 347–360.

[6] Nass N, Weissenberg K, Somoza V, Ruhs S, Silber RE, Simm A, *Int J Food Sci Nutr.* (2013) 14-18

[7]] A. Lapolla, D. Fedele, L. Martano, N.C. Arico', M. Garbeglio, P. Traldi, R. Seraglia, D. Favretto, *J. Mass Spectrom.* 36 (2001) 370–378.

- [8] O.K. Argirov, and O. Argirov *J. Pep. Res.* 9, (2003) 170-176
- [9] De Bernardo, S., M. Weigele, V. Toome, K. Manhart, W. Leimgruber, P. Böhlen, S. Stein, and S. Udenfriend, *Arch. Biochem. Biophys.* 163 (1974)390-399.
- [10] R.E. Smith, R. MacQuarrie, *Anal. Biochem.* 90 (1978) 246–255.
- [11] T. P. Hum and R. C. Augusteyn, *Curr. Eye Res.* 6(9), (1987) 1091-1101.
- [12] R.N. Johnson, P.A. Metcalf, J.R. Baker, *Clin. Chim. Acta* 127 (1983) 87–95.
- [13] H. LeVine III, *Protein Sci.* 2 (1993) 404–410.
- [1] VM Monnier, A Cerami, *Science*, 211 (1981) 491-493.
- [2] R. Bucala, Z. Makita, G. Vega, S. Grundy, T. Koschinsky, A. Cerami, H. Vlassara, *Proc. Natl. Acad. Sci. USA* 91 (1994) 9441–9445.
- [3] M.P. Vitek, K. Bhattacharya, J.M. Glendening, E. Stopa, H. Vlassara, R. Bucala, K. Manogue, A. Cerami, *Proc. Natl. Acad. Sci. USA* 91 (1994) 4766–4770.
- [4] B.K. Kilhovd, I. Giardino, P.A. Torjesen, K.I. Birkeland, T.J. Berg, P.J. Thornalley, M. Brownlee, K.F. Hanssen, *Metabolism* 52 (2003) 163–167.
- [5] A.J. Furth, *Anal. Biochem.* 175 (1988) 347–360.
- [6] Nass N, Weissenberg K, Somoza V, Ruhs S, Silber RE, Simm A, *Int J Food Sci Nutr.* (2013) 14-18
- [7]] A. Lapolla, D. Fedele, L. Martano, N.C. Arico', M. Garbeglio, P. Traldi, R. Seraglia, D. Favretto, *J. Mass Spectrom.* 36 (2001) 370–378.
- [8] O.K. Argirov, and O. Argirov *J. Pep. Res.* 9, (2003) 170-176
- [9] De Bernardo, S., M. Weigele, V. Toome, K. Manhart, W. Leimgruber, P. Böhlen, S. Stein, and S. Udenfriend, *Arch. Biochem. Biophys.* 163 (1974)390-399.
- [10] R.E. Smith, R. MacQuarrie, *Anal. Biochem.* 90 (1978) 246–255.
- [11] T. P. Hum and R. C. Augusteyn, *Curr. Eye Res.* 6(9), (1987) 1091-1101.
- [12] R.N. Johnson, P.A. Metcalf, J.R. Baker, *Clin. Chim. Acta* 127 (1983) 87–95.
- [13] H. LeVine III, *Protein Sci.* 2 (1993) 404–410.

