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Volume 2011 | Number 5 Article 28

2011

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Collier, Miranda; Floyd, Kyle; Wilson, Landon; Smith, Daniel; Srivastava, Om; and Barnes, Stephen (2011) "The Effects of Diet on Crystallin Protein Levels in the Zebrafish Lens," *Inquiro, the UAB undergraduate science research journal*: Vol. 2011: No. 5, Article 28.
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research paper

The Effects of Diet on Crystallin Protein Levels in the Zebrafish Lens

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Abstract

Crystallins are the major class of proteins within the lens, constituting over 90% of the total soluble protein. There are three major classes of crystallins: α, β, and γ. Defects in crystallin levels and structures have been linked to lens cataract disease. The zebrafish (*Danio rerio*) is rapidly emerging as a model for the study of ocular conditions. However, there has been little research on the role diet may have upon lens composition and function in the zebrafish and other research organisms. The goal of this study was to identify and relatively quantify some of the most abundant, and perhaps most important, crystallin proteins from the lenses of zebrafish that had been maintained on five diets of different protein sources: wheat gluten, soy protein isolate, fish protein hydrolysate, casein vita-free, and a mixture of all four. The overall proteome of the zebrafish lens was analyzed using mass spectrometry to identify the proteins present in the lens. From these data, specific crystallins were selected for comparison across the diet groups. For each crystallin, a proteotypic peptide was chosen to use for relative quantitation. The lenses of five zebrafish from each of the five diet groups were then analyzed for crystallin levels using a multiple reaction monitoring (MRM) mass spectrometric method. It was found that the lenses of zebrafish on the diet containing soy protein had significantly greater levels of α- and β-crystallins as compared to fish on the wheat protein and casein diets, while γ-crystallin levels remained approximately the same across all groups. The implications of this study could lead to new research on the correlation between diet and lens composition as well as lens-related diseases such as cataracts.

Introduction

The lens is an avascular organ that focuses light coming into the eye onto the retina. As the lens develops, epithelial cells on the anterior lens surface differentiate into fiber cells that form tightly packed concentric layers.[1,2,3] In order for the lens to be transparent, mature fiber cells are programmed to eliminate all internal organelles, which would otherwise scatter light. Without organelles, mature lens cells lack the necessary machinery for protein synthesis or maintenance and are left only with the proteins synthesized when they were undifferentiated.[1] The zebrafish lens is fully functional by 72 hours post fertilization. Epithelial cell proliferation followed by fiber cell differentiation, as with most other vertebrates, continues throughout the life of the zebrafish, making it reasonable that diet could be a factor affecting the protein composition of the new cells that are forming.[2]

After the loss of its cellular organelles, the lens still maintains a high concentration of proteins in order to refract and focus incoming light onto the retina.[1,4] Over 90% of these proteins are crystallins, which are divided into three distinct classes (α, β, and γ).[5] Often comprising over 40% of total lens protein, the α-crystallins are small heat-shock proteins (sHSP) that act as chaperones and are important in maintaining the solubility of unfolded proteins within the lens.[1,5,6,7] By constraining proteins in large complexes that involve αA- and αB-crystallins (20 kDa subunits that are 57% homologous)[1,5], the crystallins maintain the structure and solubility of other proteins. When α-crystallin chaperone activity is compromised, a decrease in solubility causes aggregation of many proteins, which has been associated with cataract formation.[1,5,6,7] Post-translational modification of proteins can change the internal environment of the lens after fiber cells have differentiated and organelles are no longer present. In the mammalian lens, C-terminal truncation of αA-crystallin has led to a 50% decrease in the protein's activity as a chaperone.[6] In humans, levels of full-length αA-crystallin are reduced as age progresses.[8] In rodents, the 1-53 αA-crystallin truncation product has been found localized to the nuclear region of the lens, where the most opaque region of small heat-shock proteins (sHSP) that act as chaperones and are important in maintaining the solubility of unfolded proteins within the lens.[1,5,6,7] By constraining proteins in large complexes that involve αA- and αB-crystallins (20 kDa subunits that are 57% homologous)[1,5], the crystallins maintain the structure and solubility of other proteins. When α-crystallin chaperone activity is compromised, a decrease in solubility causes aggregation of many proteins, which has been associated with cataract formation.[1,5,6,7] Post-translational modification of proteins can change the internal environment of the lens after fiber cells have differentiated and organelles are no longer present. In the mammalian lens, C-terminal truncation of αA-crystallin has led to a 50% decrease in the protein's activity as a chaperone.[6] In humans, levels of full-length αA-crystallin are reduced as age progresses.[8] In rodents, the 1-53 αA-crystallin truncation product has been found localized to the nuclear region of the lens, where the most opaque region of many cataractous lenses is found.[1] In contrast, in zebrafish cloche mutants that express a cataract phenotype, overexpression of αA-crystallin has led to a 64% increase in lens transparency.[9] These data show that α-crystallins are clearly integral to lens structure and function, which implies that normal levels of unmodified α-crystallins are crucial for a properly functioning lens.

The β- and γ-crystallins serve primarily as structural proteins, contributing to the refractive properties of the lens. They share the same core tertiary structure and are often referred to as members of the βγ-family. It has been proposed that these crystallins protect lens epithelial cells from stress.[4] Insolubilization of β-crystallins after proteolysis by the protease calpain II has been associated with lens maturation and cataract formation in rodent lenses.[10] The chaperone properties of α-crystallins aid in retaining β- and γ-crystallins in solution and preventing aggregation.[4] Crystallins have important structural and functional roles in the lens, and crystallin deficiencies and patterns of degradation are linked to increased lens opacity and cataract disease.

The zebrafish is an increasingly popular model for the study of physiological pathways, including those related to lens biochemistry and ocular diseases.[11,12] They are small and more economically sustainable than larger animal species, and their generational span is short (3-4 months). Additionally, a single mating pair can produce 100+ offspring each week, making them an ideal organism for providing large amounts of data.[3] The formation of the zebrafish lens follows typical vertebrate morphology. Multiple zebrafish mutants have been identified that model human ocular disorders, including but not limited to glaucoma, coloboma, retinitis pigmentosa, and cataractogenesis. The physiology and developmental processes of the zebrafish eye, as well as the species' heavy reliance on visual orientation, make it an ideal model for the study of human ocular disorders.[11,12] Zebrafish eyes are regularly exposed to light, giving them a degree of similarity to human eyes that most rodent models, which are predominantly nocturnal, naturally lack. Regarding studies of the crystallins in particular, characteristics of mammalian and zebrafish α-crystallins are similar. Zebrafish αA-crystallin has 71.7% percent homology and 90.8% similarity to human αA-crystallin, although αB-crystallin has a lower homology (58%).[13,14] If the zebrafish is to continue to be used for ocular studies, factors that could affect lens composition and experimental outcome need to be taken into consideration. One of the most obvious but often least controlled of these is diet.

Besides its impact on the future of zebrafish eye research, the experiment was conducted for its potential contribution to the future of treatment and prevention of human ocular diseases, the most common of which is cataracts. Cataracts are the leading cause of human blindness worldwide. Surgical intervention is presently the only method of cataract treatment.[15] Lifestyle intervention such as dietary control may be an early step to reduce the incidence or slow the progression of cataracts at an early stage. In addition to the effects of this phenomenon on quality of life, the high incidence of cataract surgery represents a significant economic cost to the nation, especially to the Medicare program.[16] The Federal government spends an estimated $3.4 billion annually treating cataracts through Medicare. In America, cataracts affect nearly 20.5 million people in the over-40 population, representing 1 in 6 within this age group.[15] Even a small breakthrough in cataract treatment that presents an alternative to surgery would save hundreds of millions of dollars per year in healthcare costs. While cataract surgery is readily accessible and prevalent in the U.S., many countries around the world do not have access to the facilities and capabilities necessary for the operation. Thus, a better understanding of cataract disease and the potential alternatives to surgery could result in widespread economic and individual benefits. This study looks at the effects of diet on crystallin levels in the lens of the zebrafish, an area that could lead to new approaches in the development of a novel preventative method for cataract treatment.

Materials and Methods

Wild-type adult zebrafish on a standard lab diet of fish protein were provided by Stephen Watts, PhD, UAB Department of Biology. The zebrafish on specific diets used in this experiment were part of a study conducted by Daniel Smith, PhD, UAB Department of Nutrition Sciences. All fish were fed a standard background mixture supplemented with one of five protein sources: fish protein hydrolysate, casein vita-free, soy protein isolate, wheat gluten, or a mixture of these four. Protein sources were isonitrogenous and constituted approximately 48% of the overall diet by weight. Lipid content was 24-25%, and carbohydrates constituted roughly 19%. Inorganic compounds and fiber accounted for the remaining makeup of the diet. The fish began the diets at 4-6 weeks of age, and tissues were collected at 4 months of age. All animal research was performed at the University of Alabama at Birmingham under approved protocols by the Institutional Animal Care and Use Committee and within the guidelines set forth by UAB IACUC.

Part 1: Total Lens Proteomic Analysis

Lens Extraction and Processing

Fish were euthanized by immersion in ice-cold water. Eyes were removed and stored at -80°C until used. Frozen intact eyes were thawed, and lenses were dissected with the aid of a dissecting microscope. Lenses were washed with deionized water to remove any contaminants. Both lenses from each animal were combined and homogenized into the same fraction. Two water-soluble extractions were performed by homogenization in 50 µL of 50 mM Tris-HCl (pH 7.4) followed by centrifugation at 18,000 x g and 4°C for 15 minutes. For the third extraction, the pellet was homogenized in 50 µL of 50 mM Tris-HCl - 6 M urea and re-centrifuged. A BCA protein assay was used to determine the concentrations of the extracts. Water-soluble and water-insoluble/urea-soluble proteins were resolved by SDS-polyacramide gel electrophoresis (PAGE) on a 10.5-14% (w/v) acrylamide Criterion Tris-HCl gel (Bio-Rad). The gel was fractionated into 12 bands from the water-soluble lane and 13 bands from the water-insoluble/urea-soluble lane. The gel image and bands that were excised are shown in Figure 1.

Gel fractions were de-stained overnight in 50 mM ammonium bicarbonate (ABC)/50% acetonitrile (ACN). Samples were then reduced by treatment with 20 mM dithiothreitol (DTT) in 100 mM ABC for 1 hour at 37°C. The DTT was removed and the

samples were then alkylated by treatment with 55 mM iodoacetamide (IA) in a dark environment for 1 hour. The IA was removed, and samples were de-hydrated with 100% ACN. Trypsin was added to samples at a 1:50 trypsin to protein ratio. Samples were incubated at room temperature for 10 minutes and then covered with 100 mM ABC. The digestion was allowed to continue for 48 hours at 37°C. After this time, peptides were extracted from gel pieces at room temperature using 50% ACN-5% formic acid (FA) for 1 hour. The supernatant was removed and saved, and the extraction was repeated a second time. Again the supernatant was saved, and the gel pieces were then dehydrated with 100% ACN. The supernatants were combined and then dried down in a Speed-Vac™. Samples were de-salted twice using C_{18} ZipTips (Millipore, Inc.). After de-salting, samples were dried down using a Speed-Vac™ and resuspended in 0.1% w/v FA containing 1 femtomol of trypsin-digested bovine serum albumin (BSA) as an internal standard. An aliquot (2 μL) of each digest was loaded onto a 2 cm x 75 μm i.d.PepMap100 C_{18} reverse-phase trap cartridge (Dionex, Sunnyvale) at 2 μL/min using an Eksigent autosampler.

After washing the cartridge for 4 minutes with 0.1% formic acid in ddH_2O, the bound peptides were flushed onto a 15 cm x 75 μm i.d. PepMap100 C_{18} reverse-phase analytical column (Dionex) with a 40 min linear (5-50%) acetonitrile gradient in 0.1% formic acid at 300 nl/min using an Eksigent Nano1D+ LC. (Dublin, CA). The column was washed with 90% acetonitrile-0.1% formic acid for 15 minutes and then re-equilibrated with 5% acetonitrile-0.1% formic acid for 30 minutes. The Applied Biosystems 5600 TripleTof mass spectrometer (AB-Sciex, Toronto, Canada) was used to analyze the protein digest. The IonSpray voltage was 2300 V, and the declustering potential was 60 V. Ionspray and curtain gases were set at 10 psi and 20 psi, respectively. The interface heater temperature was 120°C. Eluted peptides were subjected to a time-of-flight survey scan from 400-1250 m/z to determine the top twenty most intense ions for MS/MS analysis. Product ion time-of-flight scans at 50 msec were carried out to obtain the tandem mass spectra of the selected parent ions over the range from m/z 400-2000. Spectra are centroided and de-isotoped by Analyst software, version TF (Applied Biosystems). A β-galactosidase trypsin digest was used to establish and confirm the mass accuracy of the mass spectrometer. In-house MASCOT database searches were carried out against the *Danio rerio* genome on the UniProt database. The mass tolerances for precursor scans and MS/MS scans were set at 0.05 Daltons. One missed cleavage for trypsin was allowed. A fixed modification of carbamidomethylation was set for cysteine residues, and a variable medication of oxidation was allowed for methionine residues. Proteins with at least one individual peptide MOWSE score of <40 were considered significant.

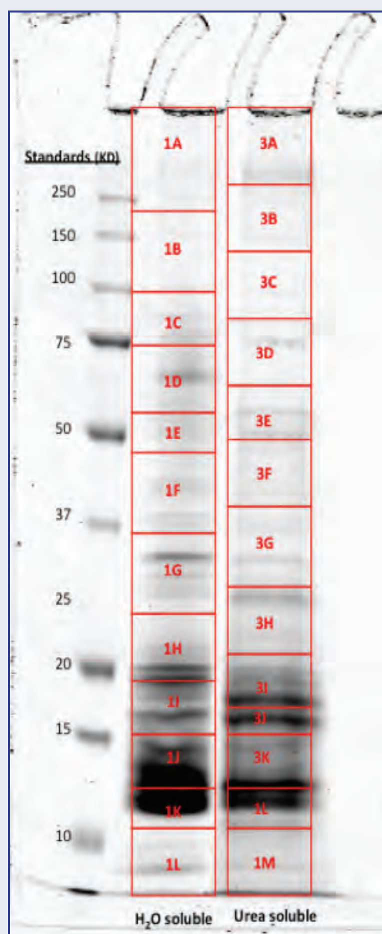

Figure 1: SDS-PAGE zebrafish lens homogenates. The first lane (Fractions 1A-1L) displays 7 μg of water-soluble proteins; the second lane (Fractions 3A-3M) displays 7 μg of urea-soluble proteins on a 10.5-14% Criterion Tris-HCl Gel. Darkest bands are in the 10-20 kDa range, corresponding with the masses of the major crystallins.

Peptide selection for LC-MRM-MS relative quantitation

The α-, β-, and γ-crystallins with the highest intensities from the overall proteome analysis were examined. Peptides were analyzed for relatively strong intensity, a comprehensive spectrum of y- and b- ions, and a mass range of 800-1600 Da (to have a 400-800 m/z $[M+H]^{+2}$ "doubly charged" mass). Selected peptides that did not contain missed cleavages from the enzymatic digest were subjected to BLAST (Basic Local Search Alignment Tool) searches to ensure that they were unique to the particular protein they were going to be used to quantify for the zebrafish. Peptides were also chosen on the basis of minimum post-translational modifications. A peptide meeting each of these criteria was found for 17 of the crystallins. These peptides were used as precursor ions, and for each crystallin, a fragment ion was chosen for use in relative quantitation. The crystallins, the peptide sequences and the ions used are shown in Table 1.

Part 2: Comparison of Crystallin Levels in Diet-Group Zebrafish

Lens Extraction and Processing

Lenses were dissected using the same methods described previously. Samples were randomized. Each pair of lenses was homogenized in 50 mM ABC with 8 M urea and centrifuged at 18,000 x g and 4°C for 15 minutes. A BCA protein assay was used to determine the concentration of the 25 extractions. A 20 μg portion of the total protein from each sample was loaded onto an 18-well criterion 12% Bis-Tris gel (Bio-Rad) for SDS-PAGE analysis. Instead of running the SDS-PAGE gel to completion, the samples were run only ~1 cm into the gel. The gel was then fixed in 30% methanol/10% acetic acid for 10 minutes, washed with water twice for 5 minutes each, and stained with Coomassie blue for 5 minutes. The bands were then excised and allowed to

Protein Name	Peptide Sequence	$[M+H]^{+1}$ Mass of parent ion	$[M+H]^{+2}$ Mass observed (m/z)	$[M+H]^{+1}$ Mass of ion used for relative quantitation
α-crystallin A chain	FTVYLDVK	985.53	492.77	736.424
α-crystallin B chain	FVINLDVK	946.55	474.28	701.4192
α-crystallin βB chain	ILFPIFFPR	1148.68	575.35	776.4454
βA1-2-crystallin	HSGDFQHWR	1168.52	585.26	1032.4646
βB3-crystallin	VSLYEFENFR	1302.62	652.32	1004.4472
β-crystallin A4	ENYLGR	750.37	376.19	232.1404
β-crystallin B2	LVIYEQENFQGR	1494.75	748.38	1170.5174
βA1c-crystallin	HSGDYQHWK	1156.51	386.5	688.2685
crystallin, βB1, like 2	HFNEYGAR	992.45	497.23	856.3948
crystallin, βB1, like 3	GYQYLFEFGSYK	1500.69	751.36	877.409
crystallin, γM2a	VVFYEDR	926.45	464.23	729.3202
crystallin, γM7	IIFYEDR	954.48	478.25	729.3202
crystallin, γMX	ITFYEEK	928.45	465.23	715.3297
crystallin, γS1	IIFFEDK	910.48	456.24	685.3192
γ-crystallin D	YSDWGALSSR	1140.52	571.27	590.3297
γ-crystallin N-A	IVFFEGK	838.46	420.23	627.3137
γ-crystallin N-B	GEYPEFQR	1024.46	513.24	676.3413

Table 1: Unique crystallin peptides and fragment ion masses. Zebrafish crystallins that were studied and the peptides and ions used for relative quantitation across the diet groups.

de-stain overnight in 50 mM ABC/50% ACN. Proteins were reduced and alkylated as described previously. Trypsin was added in a 1:50 trypsin to protein ratio, and samples incubated at room temperature for 10 minutes and then were covered with 100 mM ABC. The digest proceeded for 48 hours at 37°C. Peptides were extracted and dried down as described previously. Samples were then re-suspended in 0.1% FA mm. Samples were then de-salted twice using C_{18} ZipTips (Millipore, Inc.). After de-salting, samples were dried down using a Speed-Vac™ and resuspended in 0.1% w/v FA containing 1 femtomol BSA. Aliquots of the resuspended extract were resolved by nanoLC as described previously. Analysis was performed to relatively quantitate the peptides selected in Table 1, using a multiple reaction monitoring (nanoLC-MRM) mass spectrometric (MS/MS) method on a 5600 TripleTOF mass spectrometer (AB Sciex). Doubly charged ions corresponding to the m/z values of each of the selected peptides were filtered by the quadrupole for successive periods of 50 msec; these were subjected to collision-induced dissociation and the fragment ions analyzed by the TOF analyzer. The collected data were processed to obtain the total peak area of the most abundant fragment ion and compared to the BSA peptide for relative quantitation.

Results

From the analysis of the overall zebrafish lens proteome, a total of 306 different proteins were detected in the fractions. Of these, 120 proteins were detected in both fractions. There were a total of 73 crystallins identified, accounting for 24% of the total number of proteins. Of the 73 crystallins detected in the lens, 20 were chosen to compare levels across the diet groups using nanoLC-MRM-MS analysis – 17 of them were proteotypic (Table 1).

The experiment began with n=5 zebrafish for each diet group. Two samples from the casein diet group and one from the diet group containing all four protein sources were deleted from the final analysis due to technical errors in processing and handling. One sample from the soy diet group was omitted due to technical issues during mass spectral analysis, and another sample from the soy diet group was dropped during data processing due to adverse abnormalities that skewed the data to an unacceptable degree. After these adjustments, the number and sexes of the fish included in the data were as shown in Table 2.

Diet Group	Males	Females	Unknown Sex	Total Fish
Wheat	3	1	1	5
Soy	2	1	0	3
Fish	3	2	0	5
Casein	2	0	1	3
Mix	3	1	0	4

Table 2: Zebrafish numbers and sexes for each diet group. Characterization of fish included in the data.

Duncan's Multiple Range Test was used for statistical analysis.[24] Soy protein isolate increased levels of αA-, αB-, and αBb-crystallin in the zebrafish lens when compared to wheat protein and casein diets (Fig. 2). The soy diet also led to significantly increased levels of βB3- and βB2-crystallins in the zebrafish lens when compared to wheat protein and casein diets (Fig. 3). The diets containing fish protein hydrolysate and a mixture of all four protein sources followed a trend of similar, intermediate crystallin levels for the α-crystallins and βB3- and βB2-crystallins. Of the β-crystallins, βB3 and βB2 were the most abundant, followed by βB4 and βB1-like-2. There was no significant difference between levels of βA1-2, βA4, and βB1-like-2 crystallins across the diet groups. The β-crystallins βA1-2 and βB1-like-3 were not very abundant

within the lens (Fig. 3). The various diets did not significantly affect the amount of λ-crystallin in zebrafish lenses in any consistent manner. λM7-crystallin was the most abundant λ, followed by λM2a (Fig. 4).

Discussion

Soy protein had the most significant impact on the amount of crystallins in the lens. The lens homogenates from zebrafish that were fed the diet containing 48% soy protein displayed greater levels of αA, αB, αBb, βB2, and βB3 crystallins than those of fish that were fed wheat gluten and casein. In general, fish on the soy diet were larger in size than fish on the other four diets, and they displayed some malformations such as deformities of the operculum. Conversely, wheat protein diet fish were the smallest, likely due to lysine deficiency. However, because the same amount of protein from each lens was originally loaded onto the gel and because many of the γ-crystallin levels were similar across diets, the differences in α- and β-crystallins are believed to be significant.

Because the α-crystallins were affected by the diet differences, the overall chaperone function of the lens is likely to be compromised in diets for which α-crystallin levels were lower. Soy contains isoflavones such as genistein, which has been shown to prevent the formation of diabetic cataracts by inhibiting the enzyme aldose reductase.[17,18] The results of this experiment – that soy contributes to high levels of α-crystallins, which should keep proteins from aggregating – are consistent with some of the past research regarding soy and lens physiology.

Soy's contribution to the high levels of certain β-crystallins is also consistent with past findings. β-Crystallins are mainly structural and aid in refracting light, so high levels of β-crystallins should indicate a normal functioning lens, whereas low levels or modified insoluble forms of β-crystallins may be associated with aggregation and lens degeneration. It is reasonable that high levels of chaperone α-crystallins corresponded with high levels of certain β-crystallins. If there is credence to the proposal that β-crystallins function in protecting lens epithelial cells from stress, then decreased levels of these proteins may lead to ocular health issues relating to eye conditions that result from stress.

Interestingly, soy protein did not seem to have an effect on the levels of γ-crystallins, nor did any other diet. Whereas α- and β-crystallins showed distinct variations amongst the groups, no pattern was observed within the relative intensities of γ-crystallins. While γ-crystallins are primarily structural in purpose, they have been linked to cataracts. A mutant strain of zebrafish (cloche) is deficient in αA-crystallin, displays high levels of insoluble γ-crystallin, and exhibits cataracts. In these mutants overexpression of αA-crystallin decreased cataract opacity by keeping γ-crystallins soluble.[9] Further study is needed to determine if there is an unseen significant correlation between diet and γ-crystallin levels; however, this study would indicate that there is not.
These experiments are best viewed as preliminary work toward further research in this area. In the future, larger sample sizes will permit the data to be stratified on the basis of characteristics of the zebrafish such as gender, size, and other potentially relevant factors. A method of standardizing fish consumption may be developed, as it is difficult to determine how much a single fish eats and variability is observed within and across tanks for body size (length and weight). A protein from the lens homogenizations could be identified for use as a control for total amounts of protein identified by mass spectrometry: most likely a non-crystallin protein that should be the same across all lenses (e.g. α-tubulin). A Western blot could confirm that the control is present in equal amounts in different samples. Having such a control will allow normalization for slight variations in the samples that occur during mass spectrometry analyses; however, quantitation would still be relative. For absolute quantitation, a synthetic peptide could be generated that is identical to the proteotypic peptide used for a certain crystallin. This synthetic peptide would be analyzed at several known concentrations to generate a standard curve. Comparing intensities and areas under the peak of the peptides in lenses to this standard would allow for absolute quantitation of the crystallins.

Another issue to consider in future experiments is the forms of the proteins that are being quantified. With this method, C-truncations of the crystallins due to proteolysis were not identified. In the lens of ICR/f rats, 16 different αA-crystallin truncation products have been identified.[1] The peptide used for relative quantitation of αA-crystallin was FTVYLDVK, corresponding to amino acids 72-79 from a protein whose full-length form contains 173 amino acids. The full-length αA-crystallin (aa 1-173) would be detected with the same intensity as a C-terminally cleaved 1-100 truncation product. αA-crystallin loses 50% of its chaperone ability when just 16 amino acids are cleaved, so it is apparent that differences in crystallin form could play a prominent role in lens morphology.[6]

It would also be beneficial to look into the pathway by which these diets are influencing lens protein composition. Do the differences arise at the transcriptional level, the translational level, or post-synthesis? The soy diet may have contributed to higher α-crystallin levels by creating a more stable lens environment, preventing protein degradation, or increasing synthesis. One method that could look into this question would be to explore gene regulation techniques. It is suspected that proteolysis of α-crystallins is accomplished by means of calcium-dependent enzymes known as calpains, which are known to have proteolytic activity in rodent, human and bovine lenses.[18,19,20] If one or more calpains were identified and overexpressed or inhibited in fish on specific diets, the effects would give insight to the particular biochemical mechanisms affected by diet.

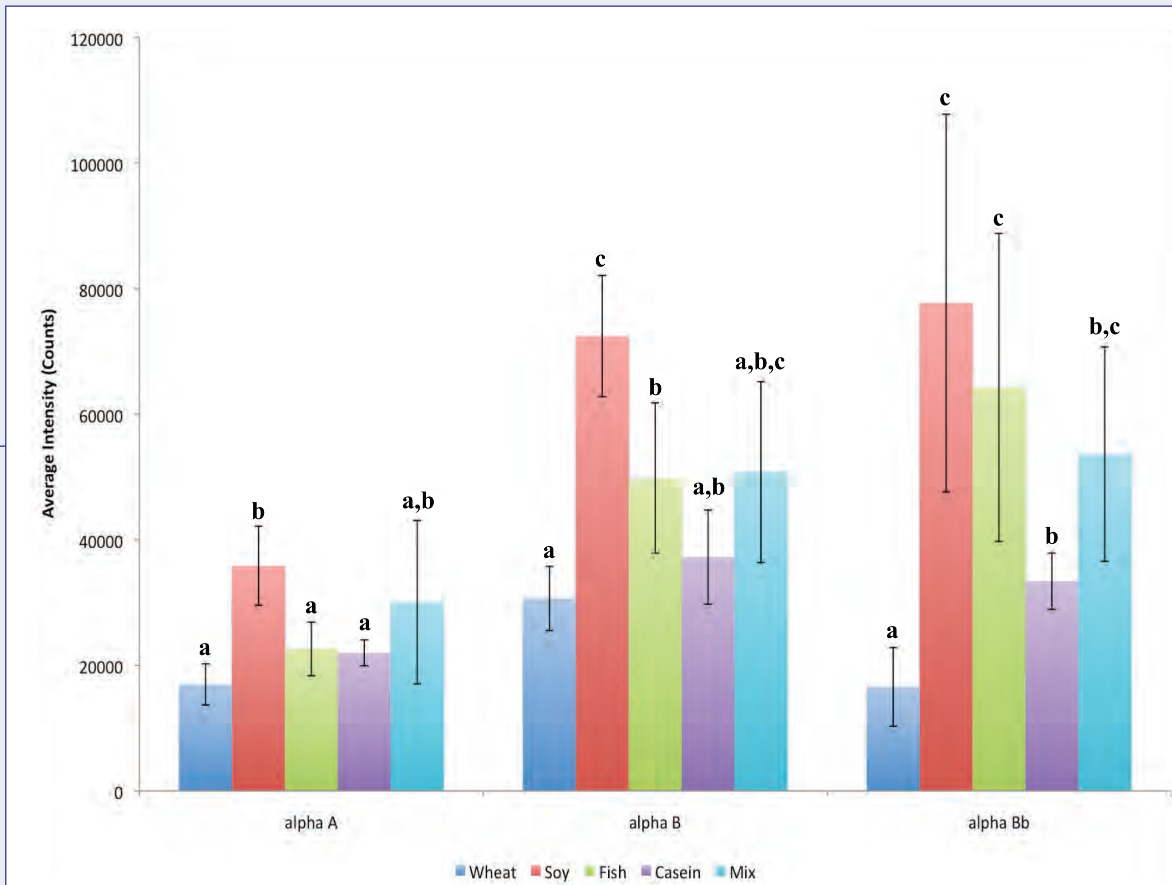

Figure 2: Relative quantitation of α-crystallin levels between diet groups, plotted with standard error of the mean and analyzed using Duncan's multiple range test. Differences between diet group intensities are compared within individual protein types, not across different proteins.

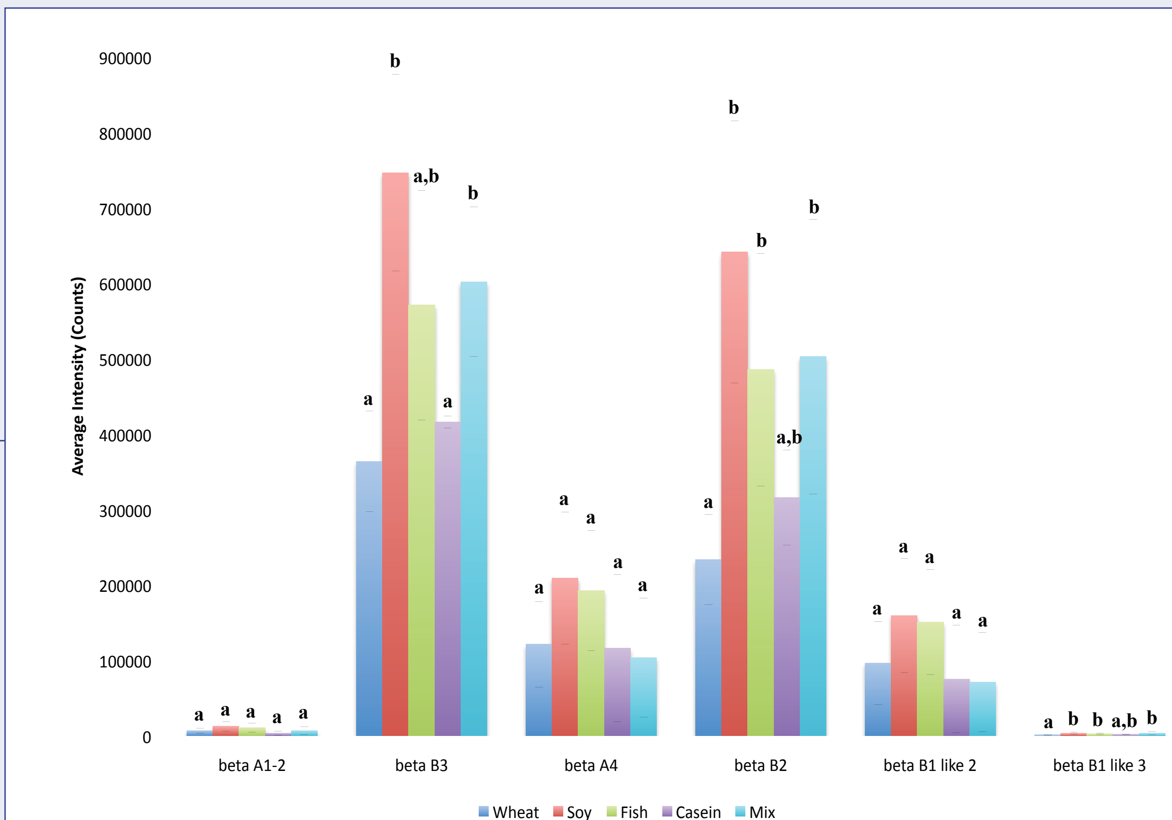

Figure 3: Relative quantitation of β-crystallin levels between diet groups, plotted with standard error of the mean and analyzed using Duncan's multiple range test. Differences between diet group intensities are compared within individual protein types, not across different proteins.

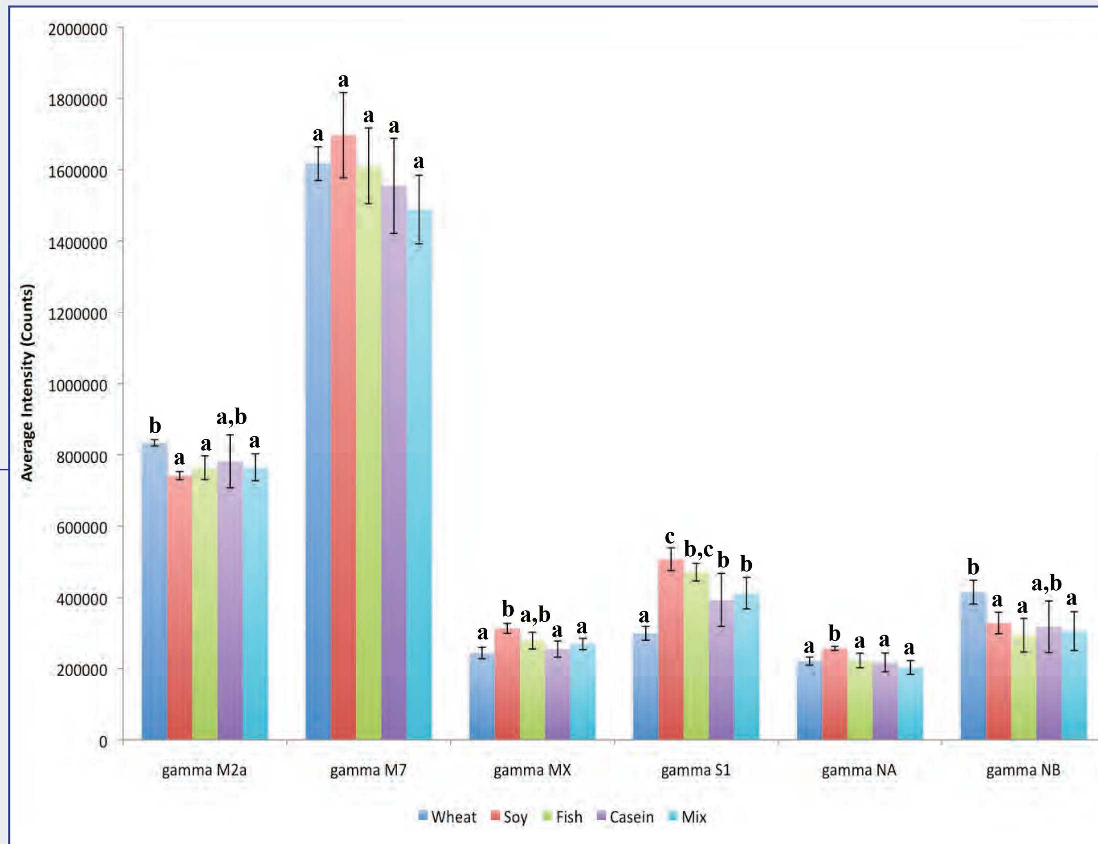

Figure 4: Relative quantitation of γ-crystallin levels between diet groups, plotted with standard error of the mean and analyzed using Duncan's multiple range test. Differences between diet group intensities are compared within individual protein types, not across different proteins.

The idea that diet and eye health are linked to one another is not new. Studies have suggested that women whose diets are in agreement with the diet recommended by the publication Dietary Guidelines for Americans have a smaller percentage of cataracts than those who have a lower Healthy Eating Index.[21] Another study saw a correlation between diet and vitamin intake and nuclear lens opacities in adult humans.[22] Animal studies have demonstrated that diets low in methionine and diets with restricted caloric intake lead to delays in cataract progression.[17,23] However, none of these examine how diet affects lens composition on the biochemical level.

Here it is demonstrated that different protein sources have direct impacts on the amount of major functional and structural proteins in the zebrafish lens. With further studies that examine the effect of diet on issues such as PTMs, truncated forms, and protein insolubilization in the lens, a more conclusive view of the extent to which diet regulates ocular health can be attained.

Acknowledgements

The authors would like to thank the labs of Dr. Stephen Watts and Dr. Daniel Smith for the zebrafish lenses. This work has been supported by the National Eye Institute (R21 EY020963, SB, PI) and from a pilot/feasibility grant (DS, PI) from the UAB Nutrition and Obesity Research Center from the National Institute for Diabetes, Digestive and Kidney Diseases (P30 DK56336, David Allison, PI). Support for the mass spectrometry experiments came from NCRR Shared Instrumentation grants S10 RR17261/RR19231/RR027822 (SB, PI), the UAB Center for Nutrient-gene Interaction (U54 CA100949, Connie Weaver, PI), the UAB O'Brien Acute Kidney Injury Center (P30 DK079337, Anupam Agarwal, PI), the UAB Skin Disease Research Center (P30 AR050948, Craig Elmets, PI), the UAB Diabetes Research and Training Center (P60 DK079626, Tim Garvey, PI), the UAB Center for Free Radical Biology, and the UAB Lung Health Center.

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