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An Experience in the Challenges of Research: Prevention of Age–Related Macular Degeneration

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research article

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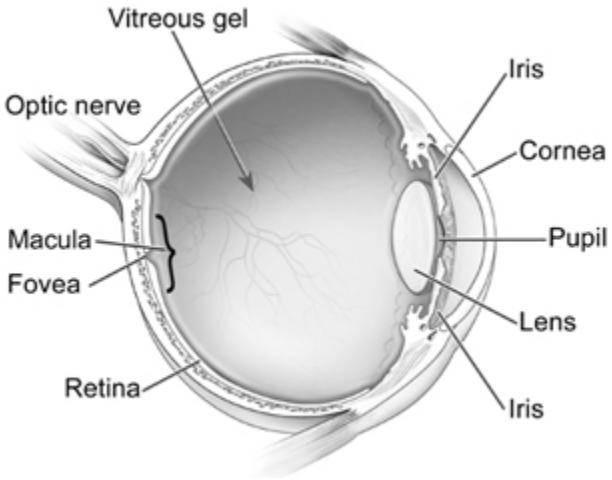
—**Hilary Snyder** (Edited by Sarah Matrumalo and Travis Taylor)

You are in your seventies and begin noticing changes in your vision. The center of your vision has become dark and hazy, making faces and objects difficult to discern. Your grandchild comes over to visit; and when you look down at him, you see a dark, cloudy void where his face would normally be. With great concern, you see your doctor and discover that you have age–related macular degeneration (AMD), the leading cause of blindness in people over the age of sixty–five (Johnson, 2005). You soon learn that there is no cure for AMD, but that researchers are trying to understand not only how to cure the disease but how to prevent it as well.

Age–related macular degeneration (AMD) is a disease that affects the central vision of more than six million Americans (Wang et al., 2007). Nearly 30% of Americans over the age of seventy–five will exhibit some signs of early AMD. Of that population, 7% will have advanced disease and suffer extreme loss of vision (Johnson, 2005). It is expected that the occurrence of AMD will triple in the next thirty years as the aging population increases (Johnson, 2005). The disease affects primarily the macula of the eye, the part of the retina responsible for central vision (Harvard, 2007).



The fovea centralis*, located in the center of the* macula*, is responsible for sharp central vision. AMD can cause a loss of clear central vision due to damage incurred by the retina. (Diagram courtesy of the National Institute of Health)*

In the summer of 2007, I joined the laboratory of Dr. Joanne Curran–Celentano at the University of New Hampshire in a project researching the role of lutein in preventing AMD. My part in this large project was to investigate how lutein is transported in the body to the eye. The challenges that I encountered in this research included working with human subjects, understanding lab procedures and learning how to use specialized equipment.

Tissue damage in the eye leading to AMD is primarily caused by an imbalance in a reduction–oxidation (redox) process in the cells. An excess of unstable molecules, known as free radicals, creates a chain reaction which, if not stopped or prevented, causes tissue damage or deterioration. Research has shown that antioxidants can potentially shut down the chain reaction by stabilizing the free radicals. Lutein, a type of carotenoid found in the macula, is an antioxidant. Lutein may also have a second, related and important role. A redox imbalance in the eye tissues can be triggered by such things as short–wave blue light. It has been hypothesized that the pigment in the macula filters out this light (Wenzel et al., 2007). Macular pigment is made up of two carotenoids: lutein

and zeaxanthin (L/Z). It is, therefore, important to know how lutein, once ingested, gets to the eye: the subject of my piece of the research project.

The Study and Challenges of Human Subjects

To determine the best way to deliver lutein and zeaxanthin into the macula of the eye, it must first be established exactly how L/Z reach the eye from our diets. L/Z are fat soluble molecules, and their absorption is affected by the same factors that affect fat absorption from food. They are absorbed from the intestines by means of large lipoproteins called chylomicrons. Chylomicrons carry lutein and other fat soluble dietary components from the intestine to the blood stream via the lymphatic system, which delivers them to the tissues (Johnson, 2000). Lutein is found principally in leafy green and yellow vegetables, but it is also available in a supplement form.

My study was a double–blind, placebo–controlled study where neither the participants nor any investigators knew who was receiving the L/Z supplementation and who was receiving the placebo. This study was done with eight healthy participants, ages eighteen to fifty–two. Four participants were randomly selected to receive the supplement containing 30 mg of lutein and 2.7 mg of zeaxanthin, while the remaining four received a placebo. The participants were instructed to take their supplement for 120 days. Blood was drawn by a phlebotomist at thirty–day intervals from 0–120 days. The samples would then be frozen until they could be tested for levels of lutein. My role was to prepare and process these blood samples.

I was very excited to have the opportunity to work with human subjects. While many students work with cell tissue or small laboratory animals, I was grateful to work with a species with whom I could directly identify. The subjects were asked to come to the lab in a fasted state (no food or drink except water since midnight the night before). As a result, they often arrived around 7:30 in the morning, an inopportune time for most people, including myself. Nonetheless, we all came together for the same purpose of working toward our research goal. Occasionally a subject would not show up, a phlebotomist would not show up or a subject's blood would be very difficult to draw. These situations were frustrating at times. However, the subjects were all volunteers, and many times there were extenuating circumstances which could not be avoided. While we encountered many challenges, the subjects were very dedicated to the study. I was extremely grateful for their participation, for without them, the study would never have been possible.

More Challenges: Procedures and Equipment

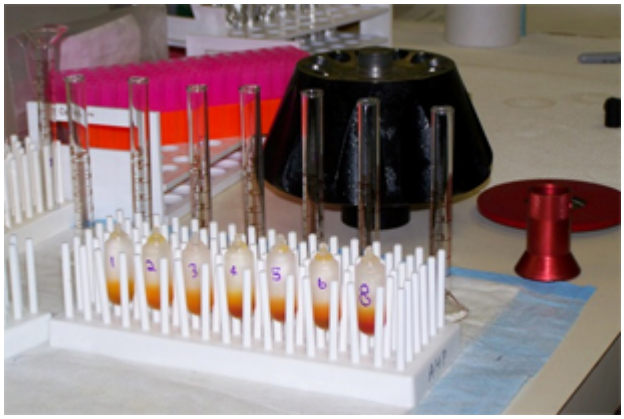
Prior to starting in the Celentano lab, my laboratory experience was limited to the microbiology and organic chemistry laboratories that accompanied my classes. I had only limited experience with pipettes, a critical tool used to measure small volumes of liquids; and I had never used a centrifuge or performed an assay to determine concentrations of sample contents.

I was very nervous about the new techniques and instruments that I would be using, but soon my anxieties were calmed when I met Sue Jalbert, the lab technician in the Celentano laboratory. She was instrumental in my becoming comfortable with the tests that I was performing. Although we had already begun receiving blood samples, I needed to practice many times in order to perfect my techniques. Our laboratory had standardized test blood on which I practiced prior to working with actual samples.

Author separating the lipoprotein layers in blood samples after an ultracentrifugation spin

I spent several days learning how to prepare the blood for density ultracentrifugation. Through a series of separate spins, the ultracentrifuge separated plasma into different densities, in this case very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL). Although I was very shaky and had difficulty controlling the pipette after each separation, I improved quickly with practice. It took quite a bit of trial and error in order to determine where the density layers should be cut after the ultracentrifugation, but with the assistance of Sue, I ultimately decided on the appropriate cut.

When it was determined that my performance was accurate and consistent, I progressed to processing the study samples. For several weeks I thawed our samples and separated the lipoproteins from each. While it may not seem like a lot of work, I had to separate nearly thirty five samples. This was extremely time consuming, for each sample required an eighteen–hour spin to separate VLDL, another eighteen hours to separate LDL, and a final forty–eight hours to separate HDL. It would take approximately a week of overnight spins in order to complete one visit's worth of samples. During this time we encountered problems with our ultracentrifuge; it was not spinning due to a damaged disk. We contacted the manufacturer to get instructions on how to replace the disk as soon as possible so that our work would not be delayed. Because the spins were happening overnight, we had to consider potential problems that could occur in my absence, such as a power outage. That summer there were many thunderstorms, so Sue and I developed a procedure to follow in the event of a power outage. Thankfully, we never lost power during a spin, but we were prepared in case one occurred.



Subjects' blood samples after an ultracentrifugation spin

In order to confirm that our separations were accurate, we performed a cholesterol assay that used certain wavelengths of light to determine the concentration of cholesterol in each sample. This was conducted at the very end of the summer and into the fall semester. Again we spent several weeks developing an appropriate protocol. The results of the assay confirmed that we had performed successful separations. It was great to know that our summer's work had been well done.

Challenges Met and Now the Future

This project has served as the foundation for my senior thesis research that will be completed in April 2009. The next step in this study will be to analyze the carotenoid content in the samples I separated. All of the samples from the ultracentrifugation will be extracted and analyzed using high–performance liquid chromatography to determine the lutein content of each separation. Through this process we will be able to see how the lutein supplementation affected our subjects. I am very excited to see all the research come together, and am anxious to present my final project at the Undergraduate Research Conference in April 2009.

I chose to pursue this research project because I find discovery exciting. I am passionate about medicine, and I believe that it is very important to try to prevent all possible diseases. Through preventive medicine, we will some day be able to assist the aging population in seeing their grandchildren's faces instead of dark, cloudy voids. Although my research will not find a cure for AMD or prevent it, it will add to the pool of knowledge and will help other researchers develop studies that may lead to answers. I am thrilled to have been a part of this research project and have learned skills that will be invaluable to me.

This project could not have been accomplished without the support of several people and organizations. First and foremost, I am so grateful for the constant guidance and support provided by Dr. Celentano, for without her I never would have begun this project. I must also thank Sue Jalbert, who was so patient with me as I learned all the laboratory techniques. She always was there to aid me in any way, especially when it came to troubleshooting. Special thanks to Dr. Sam Smith for his patience in teaching me how to use the ultracentrifuge. I thank the Hamel Center for Undergraduate Research and their donors for financial support in the forms of a Summer Undergraduate Research Fellowship in the summer of '08 and an Undergraduate Research Opportunities Program grant in the spring of '09.

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Author Bio

As a senior major in the Molecular, Cellular, and Biomedical Sciences Department at the University of New Hampshire, ***Hilary Snyder*** *has found research a satisfying experience. Coming from Seekonk, Massachusetts, Snyder is a member of the University Honors Program. She received from UNH a Summer Undergraduate Research Fellowship (SURF) in 2008 followed by an Undergraduate Research Award (URA) in the fall, and intends on using her research to complete her honors thesis. Her future plans include becoming a physician, and although she does not plan on continuing to work on lutein transport, her research experience has taught her a lot about the trial–and–error nature of experimentation. While Snyder was familiar with Inquiry early on in her undergraduate career, it was her mentor, Dr. Curran-Celentano, who encouraged her to write an article for the journal. According to Snyder, both conducting research and writing about it has been rewarding and useful for her future endeavors: "Medicine is so deeply reliant on research. I know that I will always be involved in research in some way, whether it is assisting in the writing of a grant, advising a committee, or helping to run a clinical trial."*

Mentor Bio

A frequent mentor to students doing research articles for Inquiry, Dr. ***Joanne Curran–Celentano*** *was well prepared to help Hilary Snyder with her research on age–related macular degeneration. Dr. Celentano has been a tenure track faculty member at the University of New Hampshire since 1986 and is a professor in the Department of Molecular, Cellular, and Biomedical Sciences. Her area of specialization is the nutritional sciences. As Dr. Celentano explained, "the research project that Hilary is completing is part of a larger effort to determine factors that influence the uptake and distribution of dietary carotenoids in tissue." In addition to helping Snyder in her research, Dr. Celentano has actively participated in the revision process for Snyder's Inquiry article. In Dr. Celentano's eyes, Snyder's research project "has been significant in her career progress and reflects very well on her commitment to studying medicine."*