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# The Role of Antioxidants in Combating the Aging Process

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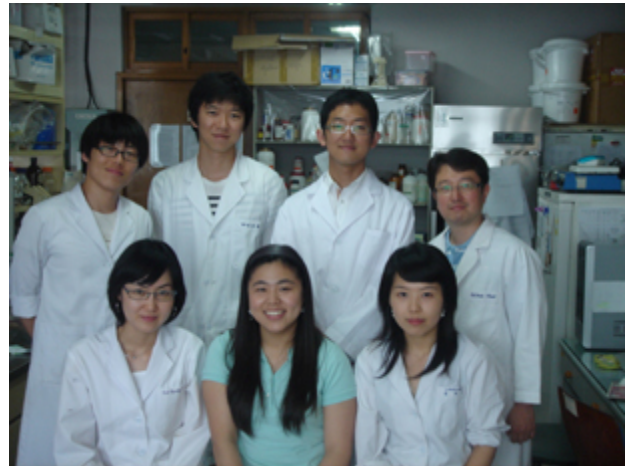
research article

## The Role of Antioxidants in Combating the Aging Process

—KwangLi Park (Edited by Kristina Griffin and Alex Miklos)

Aging and its undesirable effects is an inexorable process we all like to avoid. With greater technology, improved lifestyle and larger disposable incomes, much attention has been given to anti-aging supplements and procedures in the plastic surgery industry such as face lifts and Botox. One may hope to find a quick fix or a magic pill that will retain a youthful appearance. However, aging is a long, complex process, and miracle treatments are unrealistic.

Aging is controlled by several factors which occur at the cellular level, ultimately leading to organ deterioration and the onset of diseases such as cancer, arthritis, chronic inflammation, asthma, and heart disease. Lifestyle and environmental factors can greatly influence the aging process and may affect the vulnerability of an individual to age-related diseases. While an individual's genes associated with aging cannot be controlled, many studies suggest that modifications of exercise and nutrition, especially the use of antioxidants, can slow down the aging process. While society's current focus on anti-aging might be to preserve youthful beauty, I am more interested in preventing the diseases associated with aging.



*Members of the Molecular Gerontology/Biochemistry Laboratory. Author is front row center.*

This past summer (2008), with the help of a Summer Undergraduate Research Fellowship from the University of New Hampshire, I researched the effects of antioxidants on reducing pro-inflammatory proteins, which produce the physical deterioration connected with aging. I returned to my native country of South Korea to perform this research—a country I had left ten years ago.

During the flight from the United States in June, I wondered nervously what my Korean lab and colleagues would be like. When I arrived at the Kimhae International Airport in Pusan, the sound of people speaking Korean and the smell of *kimchi* welcomed me home. Despite the exciting and nervous beginning to my summer research experience, the ending in August was quite sad. Saying farewell to Korean families and friends was very difficult, but I was ready to come back to the U.S. and start my senior year at UNH. The U.S. and Korea—two countries apart in thousands of miles, two countries which I call home—are always closely connected in my heart. This summer's experience brought them even closer.

## An Unexpected and Unforgettable Experience

The nine weeks I spent in Dr. Hae–Young Chung’s Molecular Gerontology/Biochemistry Laboratory at the Longevity Life Science and Technology Institute of Pusan National University were not easy. They were full of new research experiences and of eventual success after initial failure.

First, however, I had the unexpected opportunity to help prepare body tissues at the Aging Tissue Bank, which is supported by the Korean National Research Resource Center. I assisted in caring for young and old rats that had been fed different chemical components found in natural products. I also was given the opportunity to dissect some of the rats. This enabled me to observe the deteriorating organs and tissues in old rats, which lead to increased vulnerability to diseases resulting from the aging process. I observed much healthier organs in rats that had been placed on special or restricted calorie diets when compared to their age–matched littermates.



*Organs of a young (left) and an old (right) rat. The organs of the old rat are enlarged with more fat around them.*

It was tiring work. I remember the day we had to dissect sixty–five rats, then separate and label the different organs; it was a twelve–hour process. I was inspired, however, by the passion of my fellow researchers. Working with them brought me a step closer to being a scientist.

Although this work was not officially part of my research project, it was an unforgettable experience. The comparison of color and size of the organs of old and young rats was very vivid. I could clearly see the tissue damage from the process of aging. Many of the older rats had even developed tumors.

## Understanding the Molecular Process of Aging

Aging can be defined as functional deterioration over time, accompanied by an increased vulnerability to disease. Even though the exact cause of aging is not fully understood, there are several hypotheses for the process of aging. Currently, much attention has been given to the oxidative stress hypothesis of aging, which proposes that an imbalance in a reduction–oxidation process in the body leads, through a complex genetic path, to vascular inflammation. Inflammation is our body’s essential defense for fighting off or repairing damage caused by foreign agents; however, many scientists believe that *chronic* inflammation may be the underlying cause of age–related deterioration and disease.

What is the reduction–oxidation, or redox, process? Cells in the body, while doing their jobs such as producing energy, also produce free radicals as by–products. These molecules lack an electron and are, therefore, unstable. They “attack” nearby molecules and “steal” electrons to become stable, thereby rendering the attacked molecules unstable free radicals. These in turn become electron “thieves,” and so the chain reaction continues. This chain reaction, if not shut down, activates certain genetic transcription factors which lead to the creation of pro–inflammatory proteins. (In technical terms, transcription factors allow the copying of RNA from a DNA pattern to create a new RNA molecule.)

My research focused on suppressing the activation of the transcription factor *Nuclear Factor kappa B*, or NF–kB, for short. NF–kB is involved in the regulation of a large number of genes that control various aspects of the immune and inflammatory responses. To suppress the activation of NF–kB, the redox chain reaction must be shut down. “Police” are needed to capture the free radical “thieves,” which have caused a redox imbalance.

These police are antioxidants, molecules which “scavenge” the free radicals and bind to them without becoming unstable themselves. The thieves are put in jail and the crime spree is ended! The result is less inflammation and thus less damage to the body.

Where do these “police” come from? Research has shown that certain foods are rich in antioxidants and that eating these foods can shut down the redox chain reaction and prevent the accumulation of free radicals in the body. This would result in better health and less age-related deterioration and disease. I carried out two experiments to find natural products that could modulate NF-kB activation. The first was not successful, the second was.

## Failure and Success of the Research

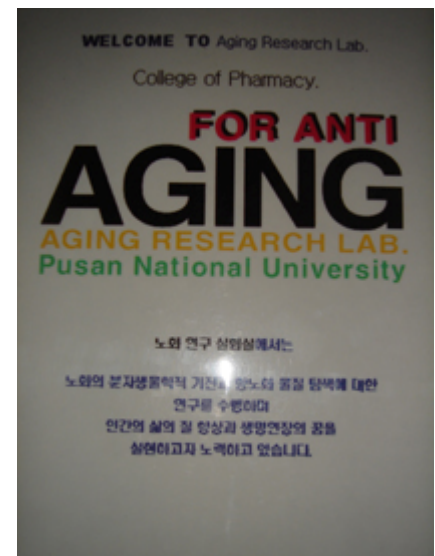
My first experiment focused on soy products, which contain the antioxidant isoflavone: Could age-related diseases be prevented or at least slowed down through the consumption of controlled amounts of isoflavone? Studies published by Dr. Chung’s lab had suggested that isoflavone has a role in reducing the amount of pro-inflammatory proteins in the aging process.

To investigate this question, I worked with kidney tissues from old rats which had been continually fed genistein, a type of isoflavone. The kidney tissues, from variously aged, old rats, were supplied by the Aging Tissue Bank. After preparing the tissues, I examined them for the presence of activated-NF-kB transcribed protein products. Results were then compared to those from our control sets, comprised of a young rat and an age-matched old rat that had not been fed genistein. Three methods were used to analyze the kidney tissues: total Reactive Species Assay, total Protein-SH level detection, and western blotting.

The extensive tissue preparation and testing took over a month and presented many problems and inconsistencies. Eventually, results showed that genistein did not appear to significantly inhibit the activation of NF-kB in the kidney tissues examined. The preparation problems coupled with the unexpected results of the experiment showed the project to be a failure. I was very disappointed, but then became involved in a second research project, which had much greater success.

In this second project, I tested the free radical scavenging activities of nine different antioxidant chemical compounds found in different natural products. The compounds tested were rutin, quercetin, morin, kaempferol, aloin, vanillin, salicin, linalool, and silymarin which are the main components of potato, grapes, mulberry leaves, green tea, aloe, vanilla beans, willow, bergamot, and thistle, respectively. As controls, I tested compounds with known, strong antioxidant activity. The tests were carried out by adding different doses of each chemical compound to samples containing known amounts of free radicals. By measuring the resulting, reduced number of free radicals in each sample, I was able to determine which chemicals had the greatest antioxidant activity and at what dosage. Morin and silymarin proved to be the most effective scavengers of free radicals.

A second test was carried out to determine if these two chemicals were effective scavengers at the cellular level. This test, the “intracellular reactive oxygen species test,” differs from the first only in that free radicals are induced, or stimulated, to produce more free radicals within cells, which in this case, were human embryonic kidney cells. These cells were first treated with morin and silymarin, and then a chemical agent was added to stimulate the production of free radicals in the cells. Treating the cells *before* inducing free radicals tested the preventive abilities of the two chemicals. This addressed one of my main research objectives, which was to investigate whether consuming the right amounts of antioxidants could *prevent* the damaging accumulation of



*Welcome sign on the lab bulletin board that says “We devote our biochemistry research to human longevity.”*

free radicals. Both morin and silymarin proved to be active free radical scavengers in this test also. Does that mean they can help reduce inflammation?

A third test addressed this question by investigating if the two chemicals could inhibit the activation of NF- $\kappa$ B, a key part of the process of producing pro-inflammatory proteins. For this test, samples were chemically treated to over express, that is, produce excess quantities of NF- $\kappa$ B. (The over expression would make measuring levels of NF- $\kappa$ B easier.) The samples of kidney cells were then treated with different doses of morin and silymarin. Finally, free radicals were added to the samples, which usually causes the concentration of NF- $\kappa$ B in the cells to rise. If the level of NF- $\kappa$ B remains the same or is reduced when the free radicals are added, this would indicate that the antioxidant present is successful at neutralizing the free radicals and thereby suppressing the activation of NF- $\kappa$ B. The results of this test revealed that morin was effective but silymarin was not.

### **Impacts the Research had on Me**

This summer experience taught me how advanced research is conducted. I learned many lab techniques, such as tissue homogenation, protein assays, and western blotting. Most importantly, though, I experienced the thrill of active research for the first time and learned that there is truly no limit to my ideas for scientific pursuits. Furthermore, working on these projects inspired me to pursue graduate studies and further research in the field of nutritional science. This summer experience led to my present interest in the relationship between nutrition and aging. My goal is to focus my efforts on the biochemical levels of nutrition and their effects on the body. In particular, I am interested in investigating the differences between Eastern and Western nutrition and the corresponding human susceptibility to different types of chronic diseases.

My long term goal is to work for a government agency, such as the United States Department of Agriculture, the National Institute on Aging, and the Centers for Disease Control, or for an academic research institute. Wherever I go, I want to make greater contributions to nutritional science and the welfare of society. I believe that balanced nutrition is critical in the prevention of chronic diseases and for slowing down the aging process. I hope that my studies and research on this topic will ultimately lead to a healthier world and a better understanding of nutrition and its relationship with aging.

*I would like to thank Dr. Laue, my UNH faculty mentor and academic advisor, for his help and guidance through this research project as well as my undergraduate years at UNH. I would also like to thank Dr. Chung, my foreign mentor, for hosting me in his lab for the summer and helping me build on my passion for the research in aging. Dr. Chung was named the "Best Researcher in Korea" in 2008, and I was honored to work with him. Special thanks goes to Jimin Kim, Ph.D student, who taught me all the lab techniques for the research I performed and mentored me throughout the entire project. I admire her knowledge and kindness. I thank Eun-kyu Choi for teaching me Western blot, and all the other lab members for the great experience which they were a part of. I could not have had this opportunity without a SURF Abroad grant, so I thank very much the Hamel Center for Undergraduate Research and their donors. This was one of the most rewarding experiences I had during my undergraduate years at UNH. Thank you.*

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### ***Author Bio***

*A native of Korea, KwangLi Park moved to Concord, New Hampshire, at the age of thirteen, and will graduate in May of 2009 with a Bachelor of Science from the University of New Hampshire. She is majoring in biochemistry with a minor in nutrition and is a member of the University Honors Program. KwangLi received a Summer Undergraduate Research Fellowship Abroad from UNH to pursue research at the Pusan National University in Pusan, Korea. KwangLi's research focused on natural substances that can slow down the aging process, such as foods rich in antioxidants. Her research project stemmed from an interest in Korean herbal medicine. Over the summer KwangLi was able to apply skills learned at UNH to lab work she did at the Aging Tissue Bank. She learned that even when research is well planned, it can fall through. KwangLi plans to pursue graduate studies in nutritional science, and would like to work for a public health agency which focuses on nutrition and its role in improving human susceptibility to disease.*

### ***Mentor Bio***

*A faculty member of twenty-four years at the University of New Hampshire, Professor Tom Laue teaches in the Molecular, Cellular, and Biomedical Sciences Department, where he specializes in physical biochemistry. Dr. Laue worked with KwangLi Park in the summer of 2008 as her UNH mentor during the research she performed in Korea. He has mentored other students at both the undergraduate and graduate levels, but this was his first experience mentoring a student abroad under a Summer Undergraduate Research Fellowship. Dr. Laue found KwangLi's project interesting, and was intrigued by how Korean biologists are working with botanical folk remedies to find their active compounds and their biological reactions in humans.*