You Scream, We Scream for the Study of Proteins

THE PARADIGM THROUGH which health care is researched and applied continues to grow in scope as it scales down to mere nanometers. Still in its infancy, the field of proteomics offers registered dietitian nutritionists (RDNs) a glimpse of a future focused on wellness, prevention, and individualized medicine. As part of a larger field of study encompassing genomics, metabolomics, and lipidomics, proteomics promises to ultimately expand health care access to a world of underserved patients, while providing researchers with new tools to fight disease.

Those in the field observe that not enough evidence-based research yet exists for RDNs to fully use some of these concepts in practice, but the principles at hand are guiding present study, making for ample research opportunity. An outgrowth of genomics, proteomics requires a basic understanding of genetics and involves fields from cancer treatment to hunger. The Academy of Nutrition and Dietetics' (Academy's) position on nutritional genomics suggests that RDNs interested in this area of science and practice should acquire a "basic competency in genetics as a foundation for understanding nutritional genomics; proficiency requires advanced knowledge and skills."[1] Staying abreast of new technologies, such as those used in proteomics, will be crucial for RDNs seeking to optimize their patients' health through nutrition science. A basic overview of the field quickly opens one's mind to the seemingly limitless possibilities, particularly those involving cancer research, such as are described in the present article. But given that the technologies enabling these studies can be used across the scope of health care, it's reasonable to expect great change in all areas of nutrition.

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THE STUDY OF PROTEINS

Proteomics is the study of proteins, in terms of expression, structure, and function.[1,2] The term proteome is derived from the word *protein*, which is expressed by a *genome*, and it refers to the sum total of proteins produced by an organism, similar to the way a genome refers to an entire set of genes.[2] Any given human might have more than 2 million proteins within its proteome, with each individual protein carrying out a different function, from catalyzing biochemical reactions and enzymes to defending the body against disease.[2] One goal within the field of proteomics is to develop a map of the human proteome—much like work underway as part of the Human Genome Project—that identifies novel protein families, interactions, and signaling pathways.[2] But whereas the human genome remains fairly constant throughout one's life, the proteome is in a constant state of dynamic flux, as individual proteins interact not only with one another, but with the environment and an individual's diet. Whereas the genome serves as a metaphorical blueprint for the body, the involved proteins are the building blocks and, as such, are decidedly impacted by environmental conditions. As Colleen Spees, PhD, RDN, LD, assistant professor of medical dietetics and health sciences in the College of Medicine at The Ohio State University, Columbus, and author of that school's first graduate course on nutritional genomics, observed, the diet is the most intimate and continuous interaction between a host and its environment during a lifetime.

Spees points out that the proteome changes constantly in response to thousands of intracellular and extracellular environmental signals, varying widely from times of good health to those of disease, and also in response to drug treatments. In this sense, proteomics runs parallel to genomics in that the latter begins with the gene and makes inferences about the gene's products, many of which are proteins. Conversely, proteomics begins with the functionally modified protein and works backward to find the gene responsible for its modification.[2]

Through rapidly developing technologies, researchers are thus able to target individual proteins and deduce their role within a given system, as well as determine what interactions caused them to behave in a particular manner. This paves the way for individualized, point-of-care screening processes. Recent work conducted by researchers at Johns Hopkins Medicine took this approach to Nepal and, while working with children there, demonstrated that levels of certain proteins in the bloodstream can be used to estimate levels of essential vitamins and minerals without direct testing for each individual nutritional factor.[3,4] This indirect method allowed researchers to simultaneously analyze the levels of five vitamins and minerals—vitamins A (retinol), D (25-hydroxyvitamin D), E (α-tocopherol), copper, and selenium—in 500 Nepalese children aged 6 to 8 years.[3,4] The ability to test for such nutrients en mass, with relatively small samples of serum, represents a breakthrough in terms of lowering cost and increasing access to health care screenings.

Micronutrient deficiencies are common in undernourished societies, yet remain inadequately assessed due to the complexity and cost of the existing assays. A plasma proteomics-based approach holds promise in quantifying multiple nutrient–protein associations that reflect biological function and nutritional status.[3,4]

Spees says the growing field of "-omics"—these fields were born in the late 1980s as outgrowths of the International Human Genome Project[5]—is still relatively young, but quickly gaining global attention. The applications for such testing strategies are

easily seen here in the United States as well, where one in six citizens lives in a state of poverty, Spees said, noting that this economic status is often characterized by hard choices such as having to choose between purchasing food and paying rent, utilities, and health care bills. These struggles alone can develop into, or worsen existing, mental and physical health problems, she pointed out. Given that food insecurity is often correlated with poor dietary habits, it stands to reason that these individuals would benefit from more-frequent health care assessments with which RDNs could more quickly identify problems. Cost of health care services remains a barrier; thus, the potential value in lowering cost and increasing access. In food-insecure populations, testing procedures, such as those conducted by the Johns Hopkins team, would allow for the detection of mild and moderate nutritional deficiencies at earlier stages and potentially lead to treatment before the problem becomes more severe.

"Extensive applications of these tools could equate to more accessible health screenings for the underserved, as well as earlier diagnosis of diseases for the public," Spees said. "Basically, the concepts are there and scientists are eager. What is lacking is the necessary funding to develop, implement, and adequately evaluate these tools for clinical use."

MATHEMATICAL MODELS AND MICROSCOPIC MANIPULATION

The technology enabling these kinds of tests is aimed at the subatomic level, and the field of proteomics has attracted more than nutrition experts. Engineers, chemists, and mathematicians are likewise taking work in their fields to new levels by examining proteins. Daniel Morris, Jr, PhD, professor of chemistry at Rose-Hulman Institute of Technology, explained that new devices are being created across the country for just this purpose. Morris' work in microfluidic devices, high-performance liquid chromotography, capillary electrophoresis, and the role of metal ion binding in oxidative DNA damage, led him to a sabbatical at West Virginia University in connection with Protea Biosciences, where he worked on a new tool with which to conduct multidimensional proteome analysis.[6,7] That work on microfluidic and

Know Your —Omics

- Proteomics: The study of protein expression and function.
- Nutrigenomics: The interactions between dietary components and the genome, and the resulting changes in proteins and other metabolites that affect gene expression.
- Metabolomics: Research concerned with the comprehensive characterization of the small molecule metabolites in biological systems.
- Lipidomics: The study of large-scale pathways and networks of cellular lipids in biological systems.
- Allergenomics: The comprehensive analysis of putative proteinous allergens by way of a proteomic strategy.
- Secretomics: A subset of proteomics, the study of all secreted proteins within a cell, tissue, or organism.

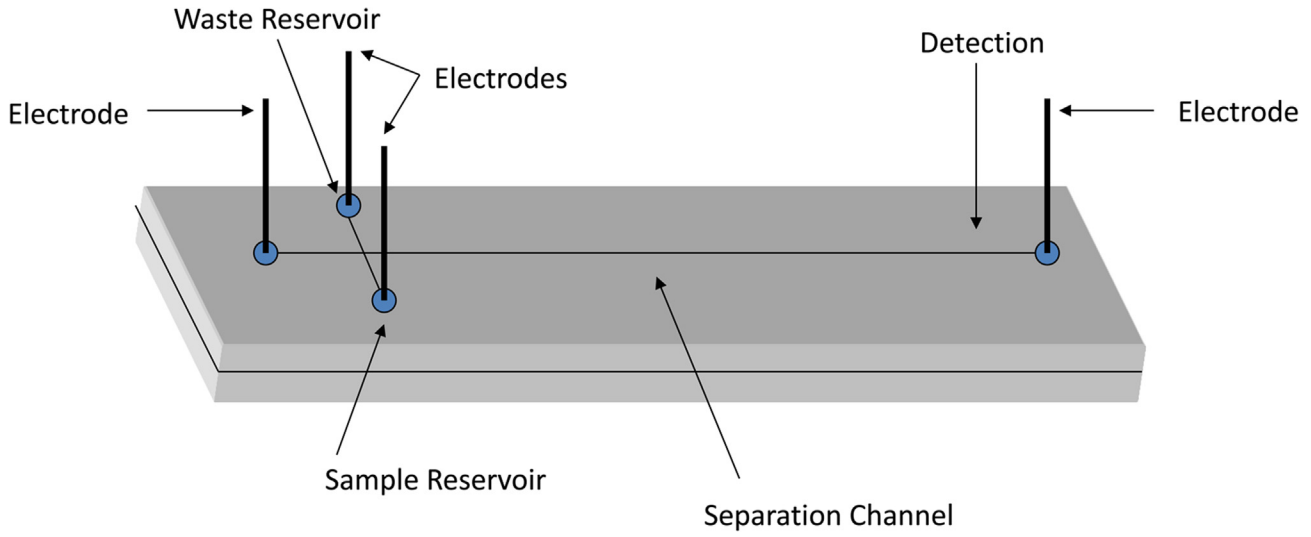

Figure 1. Microfluidic device for electrophoretic separations. Figure provided through the courtesy of Rose-Hulman Institute of Technology.

nanofluidic proteomic devices is in line with other projects of his, such as the "Lab-on-a-Chip" technology, whereby engineers design chips the size of microscope slides into which are etched micrometer-sized channels (Figure 1). Chemical mixtures are injected into those channels for analysis and could become an important tool for research, such as that conducted by Johns Hopkins in Nepal.

Likening the proteins within a proteome to letters in a bag such as those used in the game Scrabble (Hasbro), Morris explained that for scientists to determine whether various proteins are over-represented or under-represented and, thus, potentially correlated with disease or dysfunction, one must first "shatter" the proteome, or spill out the letters within the bag. A typical sample of human serum might contain 10,000 different proteins, he said, and just one might be the marker for disease. The protein albumin typically constitutes about 50% of the total proteins within one sample, and only 22 proteins make up roughly 99% within the blood count, he noted. Thus, identifying the one protein responsible for an interaction is exponentially tougher than attempting to randomly draw out any given letter from the metaphorical Scrabble bag. By shattering the proteome, one can take a "top-down," or deductive approach, to determine where each protein was, and thus deduce the problem at hand, he said, noting that the proteins should shatter the same way each time given their relationship to the overall genome.

The big value to proteomic strategies using high-performance liquid chromotography is that serum samples can be sized in the range of microliters, meaning less waste, less cost, and, thus, more testing opportunities.

And as challenging as the breakdown of the proteome is, distinguishing individual proteins that might be sized between 6 and 10 nm requires mathematical modeling much like that used in astronomy to locate distant stars and planets. The ability to deduce these interactions is presenting challenges to computer engineers and mathematicians alike. Yosi Shibberu, PhD, a professor of mathematics and colleague of Morris' at Rose-Hulman Institute of Technology, is among those researchers working to complete an "Atlas for Proteins," comparable to those established as part of the Human Genome Project. As with stars relative to galaxies, proteins within the human body are tiny but complex objects (for example, see Figure 2), and devising an atlas to map them requires extensive research.

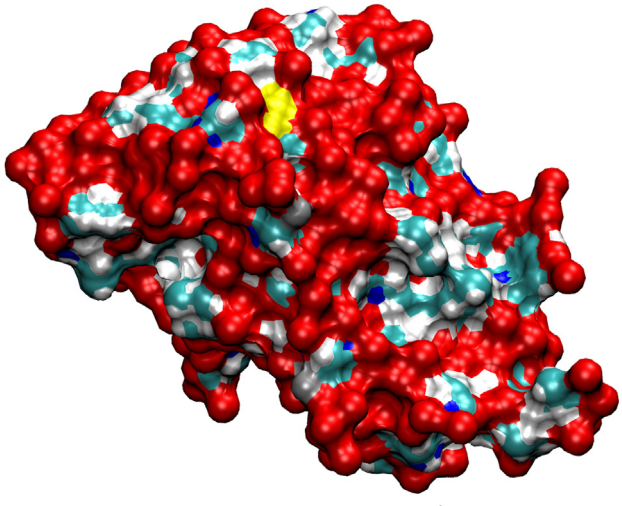

Figure 2. Human insulin protein. The 1,715 atoms of insulin are drawn using coordinates provided by Visual Molecular Dynamics. Figure provided by and printed with permission from Yosi Shibberu, PhD.

Shibberu said one could dedicate an entire career to studying just one protein.

"The better math will win out," Shibberu said, observing that in earlier centuries, European maps of Earth had large gaps where North America is now known to be. "Over time, and with the aid of satellite technology, humans have been able to account for much of the planet. The long-term goal of developing such an atlas of the human proteome is to eventually make it possible to design medicine with atomic resolution, or accuracy at the atomic level," he said, adding he's been working on this project since collaborating with colleagues at Washington University Medical School in 2005.

Shibberu said the ability to catalogue novel protein families and, thus, identify them within each individual will enable medicine to be practiced in a manner not unlike the advertising strategy of Internet search engine provider Google, where each individual user is targeted with advertising tailored to them. Sadly, the mathematical methods used in medicine lag far behind the methods used to target consumers by the advertising industry. "Historically, vitamins and minerals have been dosed in relatively gross terms, with one amount for adults and another for children, or perhaps by approximate body weight," he said.

"These numbers don't make any sense because they're not targeted to the individual," Shibberu said, explaining that the body changes dynamically in response to medication, stress, food, and other elements within the environment, at present making truly individualized medicine nearly impossible. But, as is the case in emerging cancer research, the ability to break down proteins and match up the reality next to the genetic blueprint will make all the difference, particularly if an entire proteome is accessible with one small blood sample.

"The whole idea of personalized medicine is going to be very big," Shibberu said. "Technology-wise, the ability to analyze human samples at this level is already in place, but costs are still high," he said. Those costs will come down as time passes and more people are tested, thus enlarging the database of proteins and genes, and ultimately an atlas of the proteome.

AN EXPANSIVE ROLE IN NUTRITION THERAPY

Spees, a member of The Ohio State University's Comprehensive Cancer Center and member of the Academy's Oncology dietetic practice group (DPG), remains an integral part of the first Nutritional Genomics graduate course offered at The Ohio State University's College of Medicine, which she helped design and launch. With a history of cancer in her family, the link between genetics and cancer led her and other members of her family to undergo testing using the Clinical Cancer Genetics Program at The Ohio State University, where it was determined that many of them were carriers of the autosomal dominant mutation known as Li-Fraumeni syndrome. A particularly severe cancer syndrome that increases cancer risk, it means that patients have a mutated or inactive p53

protein, which can promote carcinogenesis. In normal cell-cycle regulation, the p53 protein serves as a tumor suppressor and is known as a "guardian of the genome," she explained.

The relationship between disease, genetics, and proteins has inspired a good deal of her research, and Spees said the work crosses many fields of study.

"Nutritional genomics is an emerging area of nutrition science that offers the unique opportunity to conduct applied nutrition-related research to improve our ability to provide personalized nutritional prescriptions for optimizing health and treating disease," she stated. "As this field continues to advance, it is essential that RDNs are adequately trained and positioned to serve as the nutrition experts in nutritional genomic research, education, and training. The Academy's Oncology DPG is taking steps in exposing its members to nutritional genomics research and training opportunities, as most of the early nutrigenomic research was based on cancer models and bioactive food components impacting carcinogenesis. In addition, the Oncology DPG has several members that are national experts in this field."

Spees said one example of this tie to cancer oncology concerns the methylenetetrahydrofolate reductase (MTHFR) gene. MTHFR encodes for 5,10-MTHFR, an enzyme required for the successful conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. This critical chemical reaction is necessary for folate metabolism and the successful conversion of homocysteine to methionine. Two common MTHFR gene variants result in lower bioavailable folate and, thus, higher homocysteine levels. Medical nutrition therapy specific to mild-to-severe MTHFR deficiency consists of dietary modifications and folate supplementation in an attempt to restore enzymatic function. "RDNs," she said, "can be key members of their respective health care teams in identifying and correcting gene–diet and gene–environment interactions."

Spees' own laboratory research focuses on the evaluation of biomarkers for nutritional and dietary interventions in experimental and human carcinogenesis, and as a translational scientist she engages community research aimed at optimal

nutrition for disease prevention and health in disparate populations, such as those with whom she works as part of the Hunger.FOOD.Health Initiative. "RDNs have good reason to explore developments within the field of proteomics, regardless of their own individual specializations," she said.

"As an [Academy] strategic focus area, I do believe the Academy has made great strides in educating our members and students about nutritional genomics. Yet unlike other areas of expertise in dietetics, nutritional genomics spans the entire spectrum of health and disease. For our profession to truly thrive in this arena and become known as the experts in nutritional genomics, we must reevaluate core curriculum and research efforts," she said. "I envision a future in which each and every DPG will have a few nutritional genomics experts representing their area of expertise. We must expose all students to a working knowledge of the nutritional genomics field. Next, we must encourage and support interested students in pursuing focused areas of nutritional genomics research, such as diabetes, cancer, sports nutrition, and cardiovascular disease. Until we have RDNs engaging in nutritional genomics research and disseminating key findings in reputable scientific journals, we will not be distinguished as the true experts in the field."

And more research is needed. Spees said the field currently lacks enough evidence to support clinical recommendations, meaning RDNs should continue providing high-risk clients—those predisposed to disease by virtue of genetics—with evidence-based recommendations for modifiable lifestyle behaviors known to delay the onset of the disease, slow its progression, and improve quality of life. In the meantime, opportunities abound for those interested in adding to the growing body of research documenting the tiniest particles within each human being.

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