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Emerging Genomics Technologies in Nutritional Sciences: Applications to obesity and hypertension research

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Summary

While the sequencing of several genomes was underway, several advanced techniques in genetics, molecular biology and protein chemistry emerged. Within the nutritional sciences, while the focus on nutrition education, epidemiology and public health aspects remains essential; it is crucial to incorporate the new advances in gene and protein discovery in nutritional studies. Nutrition is a discipline that has always integrated social, biochemical and physiological sciences from the studies at the molecule level to studies at the population level. For this reason, nutritionists are in a prime position to readily incorporate the current genomics approaches in nutrition research. All the available analytical techniques can and should be used in modern nutritional sciences. These include genetics, genomics, proteomics and metabolomics which also require integration and use of bioinformatics and computational methods for data analysis and management. These applications will be briefly reviewed with a primary focus on what the genomics and genetics approaches offer to nutritionists. We will use one of our research focus areas to illustrate uses of some of these applications in obesity-hypertension research. Our central hypothesis is that adipose tissue is an endocrine organ that plays a major role in obesity and related hypertension. We are primarily studying the renin angiotensin system (RAS). We provide evidence from our own studies and others for the paracrine as well as endocrine role of adipocyte-derived angiotensin II in adipocyte gene expression, adiposity and blood pressure regulation. Both cell culture studies as well as knockout and transgenic mice models are used to test our hypothesis. Genomics and proteomics technologies are currently

developed to complement our physiological and molecular studies on the RAS and for a fine analysis of this system and its function in health and disease.

Genes and Nutrition

Genetic research has and will continue to illuminate many disorders of single organs. Nutrition scientists use molecular biological approaches to find genes that are expressed in specific conditions and that are important for specific functions. Once such genes have been identified, several strategies exist for determining their functions and suggesting treatments.

While for example, enzyme deficiencies may lead to diseases due to abnormal substrate buildup such as ornithine, where over 30 different mutations in a single gene are able to produce the disease associated with ornithine buildup, in several tissues in the body - blood, urine, tears, spinal fluid, the most affected organ is the retina. Understanding the genetic cause of the disease has led to a medical treatment that seems effective: severely restricting the patient's diet to bring the ornithine levels down to nearly normal. But the diet is only a stopgap solution. Geneticists are searching for more effective remedies, including possible treatment for the gene defect itself. Other examples include the tentative use of gene therapy to treat cystic fibrosis (CFTR defects) and hyperlipidemia (LDL receptor defects). Ongoing research aims at optimizing and maximizing gene delivery into target tissues by developing more efficient transfections/transduction methods.

With the human genome sequencing nearing completion, the next challenge is to find functions for these new genes. Current knowledge indicates that mutations causing diseases are rare and future research may help identifying people with genetic predispositions to a specific major illness that can be modified with the lifestyle interventions. Some genes may be active or inactive depending on the environment or diet and thus the nutrient-gene interactions studies may provide useful information on how to modulate gene function just as one may use drug treatment for some of the important diseases that could be tailored to the genetically varied needs of patients.

Large scale analysis of gene expression:

The development of large scale and efficient gene analysis techniques such as real time polymerase chain reaction (RT-PCR) and microarray analysis (discussed later) has provided and will continue providing information on genes associated with a specific nutrient function or with a specific disease. Further analysis of these genes, including generation of animal models with inactivation or overactivation of these genes will provide key information of their function and response to nutrients and other environmental stimuli.

In addition to the transgenic and knockout models, targeted mutagenesis approaches such as large-scale chemically induced point mutations are the new emerging approaches in modern molecular genetics. These provide mice models for human diseases that can be readily analyzed. Such efforts are undergoing in Tennessee, where scientists at Oak Ridge National Laboratories use N-Ethyl-N-NitrosoUrea (ENU) as a chemical mutagene to randomly induce point mutations in the genome. From such an effort, we identified a new leptin receptor mutation that we are currently analyzing. The Tennessee Mouse Genome Consortium (www.tnmouse.org) was created in 1998 to take advantage of this unique mutagenesis program and provide mutant mice for virtually any non invasive phenotype screening for a disease of interest. Once a phenotype of interest is identified, the next step is to clone and characterize the gene responsible for the disorder.

Many aspects of human development and tissue functions will be clarified by work with mice, flies, and worms. Many of these lower species provide information relevant to humans and many of these genes have direct counterparts in mammals; some of which the function is not known yet.

Genomics and Proteomics

The drive to understand basic biological mechanisms has led to two distinct, yet related, approaches in the study of molecular biology: genomics and proteomics (Nakayama, 2002). Genomics is the study and identification of an organism's genetic makeup. Proteomics is the study of protein expression and protein interaction within cells.

There are several ongoing efforts in cutting-edge genomic research that are aimed at evaluating the "metabolic profile" of a given organism. That is, what are its gene functions, how do these functions distinguish this organism from any other and what metabolites are associated with a specific disease or nutritional status? Nutrition (as any other discipline) will be more effective by integrating multi-disciplinary teams of nutritionists, physiologists, chemists, biologists, engineers, computational scientists, and physicists who bring state-of-the-art technologies to the traditional nutritional biochemistry and molecular biology laboratory to address issues in establishing gene functions in health and disease (Ideker et al, 2001).

Intensive efforts and abundant resources have been expended over the last few years to determine the sequence of many genomes across the phylogeny, from microbes to mammals. The database of information is rich, and these genomic resources now open the way for many new approaches to biology in the "post-genomic" era. A variety of efforts across the scientific community are directed in the new area of functional genomics and functional proteomics, with experiments aimed at understanding biology in terms of networks of interacting genes and/or gene products and their regulation. New approaches that evaluate large sets of genes and/or gene products at a given time are especially important in unraveling the complex nature of human disease. We started developing methods to link genes to function using several animal models for human disease. These genomic tools will also be used to evaluate tissue-specific gene expression from cultured adipocytes from human patients and in mice models for obesity and cardiovascular disease. A long-term goal is the development of tools to use in our work with the mouse mutagenesis program at ORNL that will allow for high-throughput screening of mice using microarrays and proteomics as molecular phenotyping tools.

Microarray Technology:

Most of the instrumentation used in microarray analysis is now automated in large research centers. Microarray technology involves preparing DNA probes, preparing RNA samples, constructing microarrays and analyzing gene expression data. Probes, representing gene sequence, are spotted on nylon, plastic or more commonly on glass slides then hybridized to target RNAs (control vs treatment) labeled with Cy3 or Cy5. To provide labeling controls, the Cy3 or Cy5 labeling is

reversed on the targets in additional experiments. Both target RNA's are mixed prior to hybridization. Results are visualized using a fluorescence scanner and scatter plot are generated to display the expression data. The array includes probes whose expression patterns are well known and blank "spots" to check for autofluorescence. The results will identify several genes that are both over- and under-expressed in the test vs control RNA. Expression pattern must be subsequently confirmed by Northern blotting or RT-PCR experiments and must show consistency with the chip results.

2D-Gel Electrophoresis and Mass spectrometry:

The area of Proteomics is less advanced than genomics. Proteomics are important and complementary approaches to genomics and will be crucial in linking specific proteins to diseases. Many protein functions are unknown and this depends largely on lack of precise information on the structure of these proteins, and the need to identify other interactive proteins and molecules. Most proteins associated with specific diseases or nutritional response remain unknown. 2D gel electrophoresis is the main technonolgy used to identify proteins expressed in specific conditions, disease or treatment (Lei, 2001). This is followed by analysis of these spots by mass spectrometry and the available protein database. Additional non gel-based techniques are currently developed to help identify at a very sensitive scale proteins associated with nutritional and disease conditions.

Proteomic technologies offer significant opportunities to improve the health and find remedies for diseases. By focusing on protein activity levels, expression levels, and interactions, researchers are able to learn more about the role proteins play in causing and treating disease. This technology was used in several areas including characterization of adipose tissue secreted proteins (Halvorsen et al., 2000).

Data management and analysis: Bioinformatics.

The future of genomic and proteomic science lies in our ability to annotate sequence information so that researchers can understand the function of all the gene products encoded within a given genome, and furthermore, understand the interplay among all of the gene products and ultimately assign three-dimensional structures to the gene products.

With the technological advances in the post-genome era, the challenge for nutritional sciences is to develop into a multi and inter-disciplinary science that

include collaborative efforts with computer scientists, chemists, engineers, geneticists and biochemists, clinicians, epidemiologists and physiologists.

Applications to our ongoing obesity-hypertension research:

Obesity is a worldwide public health problem and has reached epidemic proportions, with 61% of adult Americans being overweight or obese (National Health And Nutrition Examination Survey, NHANES IV). Obesity is well recognized now as a significant risk factor for many disorders such as sleep apnea, respiratory problems, dyslipidemia, hypertension and coronary heart disease, the number one killer in the US. Higher BMIs are also associated with an increase in all-cause mortality (Flegal et al., 1998). In 1999, the National Heart, Lung, and Blood Institute (NHLBI) released the first Federal guidelines on the identification, evaluation, and treatment of obesity (http://www.nhlbi.nih.gov/guidelines/obesity/). More recently, the surgeon general issued a call to action to combat obesity (U.S. Department of Health and Human Services, 2001). Despite strong evidence linking obesity to several deadly diseases, the cellular and molecular mechanisms of this relationship are far from being understood.

Several obesity genes were cloned in the past decade where single gene mutation leads to obesity. Rare mutations in these genes were also identified in humans (Peruse et al., 2001, Chua et al., 1996, Zhang et al., 1994; Moustaid-Moussa and Claycombe, 1999) and these account only for a 5 to 7% of obesity prevalence. Human obesity is likely polygenic and results from gene-gene and gene-environment interactions (Perusee et al., 2001). High-energy, high-fat diets and low physical activity environments may modulate activity of permissive genes or gene products that control adiposity (Hill and Peters, 1998). High-fat diets, especially those rich in saturated fats, have been shown to promote several disease states such as obesity, cancer, hyperlipidemia, atherosclerosis, hypertension and cardiovascular disease (Hill and Peters, 1998). Research supported by NHLBI recently showed that subjects with high-normal blood pressure (systolic pressure of 130-139 mm Hg and/or a diastolic pressure of 85-89 mm Hg) had up to 2.5 times greater risk of suffering a heart attack, a stroke, or heart failure in 10 years than those with optimal blood pressure. The study used data from the NHLBI-supported Framingham Heart Study, a landmark epidemiological study that began in 1948

(Vasan et al., 2001). Current recommendations to decrease blood pressure include lifestyle changes such as following a healthy eating plan lower in saturated fat and cholesterol and losing extra weight; this emphasizes again the role of dietary fats and obesity in hypertension and CVD.

Because of the recently discovered endocrine function of adipose tissue that links adipocyte protein secretion to diseased states (Kim and Moustaid-Moussa, 2000), it is plausible that proteins secreted from adipose tissue may contribute to obesity, hypertension and cardiovascular disease. For example, a lack of leptin causes obesity in humans and rodents and a lack of angiotensin II (ang II) causes hypotension that can be reversed by the sole production of this hormone from adipocytes (Massiera et al, 2001a, b , Sone and Osamura, 2001). Some of these alterations are exacerbated by high-energy diets as specific genes cause obesity only in high fat but not in low fat diets.

To address the role of obesity in cardiovascular disease, we use both rodent and human models. These include genetic and diet-induced obesity models as well as engineered animal models for proteins secreted by adipose tissue (namely angiotensinogen (Massiera et al, 2001a, b). Most obese models exhibit altered angiotensinogen expression in adipose tissue, which may constitute a potentially important link of obesity to hypertension and cardiovascular disease (Jones et al., 1997b). We are characterizing these mice models using cutting edge genomics and proteomics technologies in collaboration with scientists at ORNL. We also use cultured murine preadipocytes, 3T3-L1 cells that convert into fat cells. A clinical application to this work is the use of cultured human adipose tissue derived from patients (Moustaid et al., 1996). We are interested in changes in protein and gene expression in adipose tissue of these models as well as in characterizing novel proteins secreted from human adipose tissue.

While abnormalities in adipose tissue grown and development are associated with several disorders including obesity, diabetes and cardiovascular disease, the exact function and role of the proteins secreted from this tissue are far from being understood. One of the proteins secreted form adipose tissue is angiotensin II, a hormone well known for its hypertensive effects. We propose that this hormone may constitute a potential link between obesity and hypertension. The following section discusses evidence supporting a role of angiotensin II in both obesity and hypertension.

a. Renin angiotensin system (RAS): Angiotensin II is a pressor endocrine hormone

known to regulate blood pressure. Classically, this hormone is generated by two enzymatic steps where renin and angiotensin converting enzyme (ACE) sequentially cleave the precursor angiotensinogen (agt), produced in the liver (Campbell, 1987). Several other peripheral tissues express their own RAS with potentially different tissue-specific functions (Cassis et al., 1988). We are primarily interested in the function of the adipocyte RAS in modulating not only adiposity but also blood pressure and cardiovascular function. Since agt is secreted from adipose tissue, and given the abundance of perivascular adipose tissue and its close proximity to vascular smooth muscle cells, it is possible that it contributes to controlling vascular tone and blood pressure (Soltis et al, 1991). Ang II effects are primarily mediated by an intracellular signal via AT1 or AT2 receptors, both of which are members of G-protein-linked receptors. Both receptors have been documented in adipose tissue from rodents and humans (Jones et al., 1997, Crandall et al., 1994) and have been linked to both adiposity and hypertension.

Adipose tissue expresses high levels of angiotensinogen (Frederick et al., 1992, Jones et al., 1997b). Expression of angiotensinogen is differentiation-dependent, nutritionally and hormonally regulated and differentially expressed in adipose tissue of lean vs. obese mice and rats (Saye et al, 1989, Frederick et al, 1992, Jones et al., 1997b). We were the first to report that angiotensinogen is highly expressed in human adipose tissue (Jones et al., 1997b). Taking into consideration the adipose tissue mass, adipocyte derived angiotensin II may be a key contributor to the hypertension of the obese patient. Ang II secreted by adipose tissue also acts in a paracrine way to regulate adipocyte metabolism and increase triglyceride stores (Jones et al., 1997a, Darimont et al., 1994). We have demonstrated that ang II increases the transcription of adipocyte lipogenic genes in a glucose-dependent manner (Jones et al., 1997b, Kim et al., 2001). We identified the Sterol Regulatory Element Binding Protein 1c/Adipocyte Differentiation and Determination factor 1 (SREBP1c/ADD1) as a potential transcription factor mediating this regulation (Kim et al., 2001).

Recently, we initiated characterization of angiotensinogen (agt) transgenic and knockout models, which were kindly provided to us by Dr. Gerard Ailhaud, University of Nice, France (Massiera et al., 2001 a,b). These studies indicate that agt expression in adipocytes alone (aP2-agt) reverses the kidney histological abnormalities seen in these knockouts; indicating that adipocyte-derived ang II reaches the circulation. Preliminary assays also indicate that lipogenesis and plasma insulin are significantly higher in aP2-agt transgenics compared to wild type

mice. Hyperinsulinemia/insulin resistance, combined with Ang II secretion from adipocytes may significantly increase obesity and the risk of CVD and merits further investigation.

Several epidemiological and genetic studies have shown a positive correlation between plasma agt levels, BMI and blood pressure in humans (Cooper et al., 1998, Hegel et al., 1995, Licata et al., 1994, Unger et al., 2002). However, the mechanisms by which adipocyte agt contributes to obesity-related hypertension have remained until recently largely unknown. Expression pattern of agt is linked to changes in blood pressure (Kim et al., 1995). Indeed, blood pressure of mice that express multiple copies of the agt gene increases in proportion to copy number (Smithies and Kim, 1994). Mice homozygous mice for the AT1 targeted mutation are viable and fertile and are hypotensive (Tanimoto et al., 1994). While the role of AT1 in hypertension is well documented, the role of AT2 is more controversial. AT2 knockout mice are normal and have moderately elevated blood pressure and increased pressor effects of Ang II (Hein, 1998), indicating that lack of AT2 may exacerbate the hypertensive effects of Ang II via AT1 receptors. AT2 receptor deficient mice exhibit cardiac abnormalities and left ventricular hypertrophy (Inagami and Senbonmatsu, 2001). Other mice models for the RAS and cardiovascular disease studies include ACE knockout mice (available at the Jackson Labs). ACE-/- are hypotensive and those with the ace gene duplication exhibit significantly decreased heart rates, heart weights and renal tubulointerstitial volumes with increasing copy number (Krege et al., 1995, 1996). We are developing genomics and proteomics approaches to study obesity and associated hypertension using large scale gene and protein analysis in mice models and cultured adipocytes. Identifying specific genes and proteins in the RAS transgenic and knockout models will provide crucial information towards understanding the role of this system in CVD and may provide key therapeutic targets.

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