NUTRITIONAL GENOMICS

The Impact of Dietary Regulation of Gene Function on Human Disease
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Preface

Until recently, the etiology of chronic diseases had been described in terms of biochemical and physiological changes in tissue function. Unfortunately, by the time changes are diagnosed, metabolic dysfunctions already exist, and therapy is designed primarily to reduce the progression of the disease state. Selective dietary regimens and pharmacologic agents have been used to block or slow the progression and alleviate some of the side effects of these diseases. Population groups having similar chronic disease characteristics have consistently been noted to contain responders, nonresponders, and negative responders to each therapeutic regimen.

The future of health care may increasingly depend on identification of personalized nutrition targets based on nutritional genomic markers, enhancing intervention outcomes chosen to benefit the population as a whole. Nutritional products would be safe for all to consume but by characterization of individual genomic health needs, appropriate guidelines could enable consumers to better select nutrient sources to meet their individual genomic health needs. As such, it is imperative that scientists in agriculture, food science, nutrition, genomics, and clinical therapy all work together to assure safe and efficacious application of nutritional genomics to enhance health.

The goal of this book is to provide an opportunity for researchers to establish an integrated perspective for successful development of nutritional genomics. The content of the chapter material is not all encompassing, but in fact has targeted specific areas of interest within the field of nutritional genomics that will rely on further integration to complete the vision for utilization of nutritional genomics as an additional tool for improved health care.

Since the sequencing of the human genome, it has become more apparent that many gene variants exist, which may present a predisposition to a specific disease. Whether or not that genotypic potential will be expressed depends upon a complex interplay among external factors, diet, and environment, and the molecular components that regulate gene expression affecting the physiologic processes they control. The potential integration of dietary components on the phenotypic outcome has led to the development of the field of nutritional genomics.

Nutritional genomics is the study of the interactions between our genetic makeup and the foods we consume, and the health outcomes that may occur. The goal of nutritional genomics is to provide a platform from which a dietary regimen can be tailored to affect individual genetic profiles, potentially increasing the risk of developing a chronic disease or cancer, with the expectation that the onset of these conditions may be delayed or prevented if intervention occurs early enough. The role of bioactive food components, nutrient and non-nutrient, may be to affect multiple mechanisms related to cell signaling, transduction, and transcription factors that regulate gene expression. The regulatory efforts include the use of dietary components in altering chronic diseases and cancer development.

Advances in genomics have increased our understanding of the inherited basis of disease, including both genetic and epigenetic, as well as our understanding of how people differ in their response to their diet and its composition (Chapter 1). The role of epigenetic mechanisms in the etiology of cancer has been recognized in recent years, and more recently it has been associated with diabetes and other metabolic disorders (Chapters 1–3). Diet and other environmental factors may prove to be significant regulators of epigenetic mechanisms.

Different bioactive food components have been shown to exert protective effects through alterations in DNA methylation of CpG islands in promoter and other genomic regions, chromatin silencing, and post-translational modification of histone tail domains. Alteration of these epigenetic marks has been associated with modulation of numerous cellular processes associated with carcinogenesis, including differentiation, inflammation, apoptosis, cell cycle control/proliferation,
carcinogen metabolism, and angiogenesis, among others. Epigenetic silencing of tumor suppressor and proapoptosis genes in cancer cells, unlike genetic mutations, can be reversed by the use of DNA demethylating agents (to remove methylation marks) and HDAC inhibitors (to retain histone acetylation marks). Characterization of dietary effects depends on quantity, timing of exposure, chemical form, digestion, and bioavailability (Chapter 1).

Plant-based foods are a natural source of modifiers of the epigenetic machinery. For example, lunasin is a dietary peptide in soy foods, which survives digestion, undergoes absorption into the circulation, and becomes bioavailable to the cells. This peptide causes upregulation of chemopreventive gene expression by specific epigenetic modifications and provides another bioactive food component with the potential to reduce cancer risk (Chapter 2). Both clinical and in vitro studies have clearly demonstrated the importance of maintaining a good diet and glycemic control to prevent or slow the progression of diabetic complications. Elevated plasma glucose can induce epigenetic modifications in target cells, resulting in activation of various signaling pathways and genes associated with these complications (Chapter 3).

Type 2 diabetes is a complex disease involving several risk factors including insulin resistance (IR), obesity, dyslipidemia, and genetics. With elevated glucose, each of these risk factors has the potential to induce epigenetic changes in the chromatin structure affecting gene expression patterns in various target tissues. These risk factors participate in the epigenetic mechanisms responsible for metabolic memory as they relate to diabetic complications, when they might be expressed, and how they might be prevented.

Diet has profound effects on short-term and long-term changes in biologic systems. Recent advances in high throughput technologies, such as genomic, transcriptomic, proteomic, and metabolomic analysis, have made measurement of these effects possible. However, the volume of data generated requires mathematical models and use of large computers to pool the data into systems to clarify the molecular basis of human disease (Chapters 4–9). The multiple layers of information need to be integrated into networks to identify the common intersections (hubs) controlling the signaling pathways (Chapters 4 and 12).

N-3 polyunsaturated fatty acids (n-3 PUFA) decreased the risk factors for atherosclerosis in most human studies (Chapter 6). The n-3 PUFA diets containing both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), resulted in decreased fasting and postprandial plasma triglycerides, large VLDL particles, total LDL and small dense LDL particles, and RLP-C, and an increase in large LDL particles. Many studies also indicated anti-inflammatory and lowering of blood pressure (BP) effects. The reduction in BP and the number of small dense LDL particles appeared to respond best to DHA. These results suggest that conflicting data from published research may occur due to a need for optimizing individual doses for EPA and DHA and detailed evaluation of their use together.

Cellular communication is responsible for transduction of signals from external signals and transduction pathways, nuclear receptors (NRs) and coactivators to regulate transcription (Chapters 5, 7, 8, and 13). Fatty acids are believed to regulate gene expression through several transcription factors (TFs), including PPAR, HNF4α, and RXR, which act as fatty acid sensors, selectively binding specific fatty acids to targeted nuclear receptors. The lipid bound TF binds to a responsive element in the promoter region of the target genes and attracts protein coactivators to form the requisite complex for activation of transcription. Thus, NR-mediated transcription and the coactivators have been found to play important roles in many different human disease states related to inflammation, atherosclerosis and those involved in energy metabolism (Chapters 7 and 8).

Other nutritive and non-nutritive bioactive components found in foods have been shown to modify gene expression, affect the inherited genotype, and alter the expressed phenotype. Plant polyphenols, especially the flavonoids, have been most actively studied (Chapters 5 and 9). The green tea polyphenol epigallocatechin gallate has been effective against cancer in animal models. Curcumin polyphenols and cinnamon polyphenols provide anticancer, anti-inflammatory properties, blocking of angiogenesis, and induction of apoptosis. Resveratrol alters global patterns of gene regulation in animal models, perhaps acting through SIRT1, which slows aging and associated physiologic
decline, decreases oxidative stress and cancer, and alters the effects of obesity on insulin sensitivity. Quercetin provides a strong antioxidant effect and acts synergistically with resveratrol.

Enormous challenges remain to clarify the beneficial effects of polyphenols. More than 8000 distinct polyphenolic molecules have been identified, yet little understanding exists of their functional similarities and differences, or their cumulative effects over time. For example, the original study that identified resveratrol as a SIRT1 activator also identified 16 other structurally related polyphenols possessing similar activity (Chapter 9). Among the few compounds that have been studied in relative detail, similar effects have been identified in vitro and in vivo. All have antioxidant capacity. Resveratrol and curcumin bear little structural resemblance to one another, yet both show potent anticancer and anti-inflammatory effects, block angiogenesis, activate glutathione-S-transferases, inhibit cyclooxygenase, induce apoptosis in tumor cells, and are rapidly metabolized. The nature by which the polyphenolic compounds participate in the complex cellular signaling system and in gene expression needs to be characterized in greater detail. Many of the polyphenolic compounds pass through the plasma membrane to bind with nuclear receptors and act as TFs to signal gene expression (Chapter 5). Indeed, if diet can be used to alter phenotypic expression, then nutrition and food scientists can work together to design food products and personalized nutrition guidelines to affect the onset of chronic diseases, such as obesity, hypertension, type 2 diabetes, and cardiovascular disease (Chapters 10, 11, and 16).

Elucidation of the mechanisms by which obesity leads to increased morbidity and mortality has been the focus of intense research. There appears to be a strong link between inflammation and these chronic diseases. In the adipose tissue, expansion of the fat mass with increasing obesity initiates the inflammation cascade, caused by poor delivery of nutrients and limited delivery of oxygen to the core of the expanding adipose mass, increased fatty acid flux, increased cytokine and chemokine secretion, and adipocyte death. All changes lead to recruitment of macrophages to the site of inflammation and contribute to insulin resistance (Chapter 10). Proinflammatory pathways are initiated in the neighboring insulin target cells, hepatocytes, adipocytes, and myocytes, which release additional cytokines and adipokines, exacerbating general inflammation and enhancing insulin resistance. When occurring together, these events contribute to IR, T2DM, and the metabolic syndrome.

Vitamin D metabolism is well established for regulation of calcium absorption and utilization. Recently, hypovitaminosis vitamin D (25-dihydroxy vitamin D) has been associated with increased risk of cardiovascular disease, elevated BP, and increased cardiohypertrophy, while the vitamin D receptor-agonist (VDR-A) participated in the inhibition of key steps in the inflammation process, angiogenesis and small muscle cell proliferation (Chapter 11). The recognition of genomic-mediated effects of 1,25-dihydroxy vitamin D$_3$ and VDR-A within the cardiovascular system has initiated new insight into strategic nutritional interventions to treat cardiovascular disease.

Vitamin D deficiency produced an increase in renin levels, which was reversed by an increase in vitamin D intake. Research, using vitamin D receptor knock-out (VDR KO) or 25-hydroxy vitamin D$_1$α-hydroxylase KO, indicated that the lack of 1,25-dihydroxy vitamin D$_3$ or the VDR, suppressed the renin gene expression independent of the calcium metabolic pathway. The resultant conclusion is that 1,25-dihydroxy vitamin D$_3$ acts as an independent endocrine regulator of the renin–angiotensin–aldosterone system allowing a decrease in blood pressure (Chapter 11).

Due to the multiple levels of cell signaling involved in the regulation of gene expression, a new methodology, network analysis, has evolved (Chapters 12–15). The analysis is based on comparison of normal cell metabolism to dysfunctional cell metabolism using the same probes (external perturbation) and measuring changes over time in the transcription profiles used to compute and identify hub genes that control the intersection of those pathways.

For example, use of normal breast cells (MCF10A) and inflammatory breast cancer cells (SUM 149) in a network assessment identified the cancer cell hub genes IL-1A and IL-1B, cytokine synthesis, and NFκB were activated. Using EGFR as the probe, these markers were upregulated in the SUM 149 cells but not affected in the normal MCF10A cells. The blockade of the EGFR effect
leads to growth arrest and loss of viability in the cancer cells, indicating a control site that could be targeted in functional nutritional genomics (Chapter 12).

Targeting of specific cancer genes, signaling proteins and transcription factors is now considered to have the most effective potential to prevent cancer. Nutrients and other dietary factors, such as, epigallocatechin gallate (EGCG), [6]-gingerol, resveratrol, and various flavonoids, including kaempferol, quercetin, and myricetin, are of interest because of their potential to affect protein kinases and/or transcription factors. Transcription factor proteins bind to specific DNA gene sequences to regulate transcription. Activator protein-1 (AP-1), nuclear factor-kappaB (NF-κB), p53, nuclear factor of activated T cells (NFAT), and cAMP response element binding (CREB) protein have been shown to play a critical role in carcinogenesis and are all regulated by the mitogen activated protein kinase (MAPK) cascades. Activation results in transcription of genes that encode proteins that regulate a multitude of cellular responses including apoptosis, differentiation, development, inflammation, and proliferation (Chapters 9 and 13). A large number of proteins have been screened to identify specific binding sites for the natural polyphenols. Importantly, those identified had binding constants at potential physiologic levels, and demonstrated numerous specific regulatory effects on protein targets. For example, EGCG binds to vimentin, which inhibits AP1 activation and inhibits the p65-CREB-NFκB pathway.

The components of green tea extracts have been shown to inhibit a variety of carcinogenic events in vitro cell culture and in vivo animal models. The polyphenols, including EGCG, have been examined. The green tea polyphenols were found to play an active preventive role in photoinduced carcinogenesis in animal models (Chapter 14). UVB causes skin damage, initiating DNA damage and stimulating an inflammation response. From green tea polyphenol intake, EGCG stimulates DNA repair and nucleotide excision repair of UVB skin damage. They also decrease production and release of inflammatory cytokines.

Certain bioactive nutrients produce a “U”-shaped health curve indicating an increased incidence of cancer at low and high concentrations (Chapter 15). A low intake of vitamin D is associated with an increased colorectal cancer risk while a similar population with recommended levels of vitamin D indicated a decreased risk. Using meta-analysis, low vitamin D status was also found to be related to a greater risk in breast, skin, and prostate cancer.

In addition to the kidney, multiple cells contain the mitochondrial 1α-hydroxylase enzyme. Active 1, 25-dihydroxy vitamin D stimulates cell differentiation and proliferation. The function of vitamin D is closely linked to the vitamin D receptor (VDR), which has a large number of polymorphisms. One of them, the Fok1 polymorphism, in conjunction with a low calcium level is related to an increased incidence of colorectal cancer (Chapter 15). Inclusion of more dietary vitamin D and calcium reversed the cancer risk of this group. On the other hand, elevated vitamin D has also been correlated with an increased risk of prostate cancer, pancreatic cancer, breast cancer, and esophageal cancer. This effect may be cell specific and/or dependent on precancerous development, such as initiated by smoking. The need then is to specifically quantify the dose, the cell types, and the regulation mechanisms by which vitamin D protects and may increase cancer development.

Similarly, low dietary intake of folate might increase the risk for colorectal cancer, as well as breast, ovary, pancreas, brain, lung, and cervical cancer. Animal studies indicate the regulatory pathways for cancer are expressed through the Wnt cascade and are p53 dependent. The result is a decrease in thymidylate synthesis and a decrease in methylation of cytosine in DNA. A protective effect, 40% reduction in cancer risk, was related to an elevated dietary folate intake and increased plasma levels. While evidence has indicated inadequate folate with cancer risk, increased folate levels in subjects with a history of neoplasms or existing polyps indicate an increase in cell growth and proliferation. Thus, elevated folate could also be a concern for individuals already predisposed to certain cancers (Chapter 15).

The gastrointestinal immune system reflects an array of intricate biological systems, including a highly regulated and carefully controlled inflammatory process and a diverse community of microbes entitled the human microbiome (Chapter 16). Individual inflammatory responses are genetically
diverse. The gastrointestinal tract and the microbiome reflect a significant barrier to protect the body from challenges entering the gastrointestinal system. Selected bacterial colonization can alter intestinal physiology by gene modulation that impacts nutrient absorption, mucosal defenses, and xenobiotic metabolism. Thus, dietary choices can affect the inflammatory defense system.

The ultimate goal of nutritional genomics is to use whole foods in our diet to delay or prevent many of the catastrophic health outcomes that are currently overtaking our children, and impact their future health and longevity throughout their life time. Enhancement of nutrient and/or bioactive content in agricultural crops by genomic alteration is one of the important means to achieve this goal. The breeding challenge is to prevent loss of existing positive parameters while making nutritional gains. Specific agricultural breeding efforts have been used to increase calcium uptake into crops (Chapter 17) and genomic efforts to improve the nutritional content of lettuce (Chapter 18). Targeted small gains are those consumed in large quantities, but are lacking essential nutrient value. Thus, small gains in content can provide significant impact on dietary intake status.

Another means to enhance nutritional value is to utilize food technology to enhance bioactive food ingredients in our food supply. Naturally occurring anthocyanins, in blue carrots and blueberries, and the carotenoids in other vegetables can contribute varying levels of bioactive components (Chapter 19). If used as an additive in processed food, the amount would contribute to the overall dietary intake of the bioactive profile.

Current manufacturing practices have continued to improve food safety and enhance preservation of nutritional quality and assure bioavailability of functional foods (Chapter 20). Novel processing techniques and product structuring at the nano-, micro-, and macrolevels include utilization of microwave, high hydrostatic pressure, high-intensity pulsed light, high-intensity pulsed electric fields, ultrasonics, and others. Discussion of their use indicates their successful retention of nutritional and bioactive ingredients.

New restructured whole foods have been designed to more effectively deliver bioactive components (Chapter 21). A processing method has been created to liquify whole grains in a manner to assure delivery of all nutrients, fiber, and bioactive components into a very palatable “grain milk” which can also be added to sauces to enhance delivery of needed dietary fiber to meals or ready-to-eat (RTE) food products. The successful use of these methods may lead to enhancing the delivery of bioactive components from foods designed by agricultural intervention or by the creative processing of our foods contributing to enhanced bioavailability.

The closing chapter assesses the future of food labeling in the United States that will impact nutritional genomic foods and consumer selection (Chapter 22). The discussion examines the existing regulations of labeling and suggests areas needing broader review. Efforts to make the label easier to use are examined, and evaluated in light of FDA responses and decisions. The use of the product label must be considered an essential communication tool to aid consumers in selection of foods to meet individualized nutritional and bioactive component needs.

With this understanding, opportunities will occur to develop personalized nutritional interventions, using evidence-based genomic diets that enable individuals to implement dietary recommendations very early in life and maintain them as a lifelong regimen designed to achieve optimal health. Nutritional genomics provides an opportunity to link future health status for each of us and our families by clear identification of the diet–gene paradigm, enhancing health by personal decisions and commitment. The focus on personalized nutrition will have important implications for the agricultural and food industries to deliver food products to meet individual genomic needs. The future application of nutritional genomics may well bring revolutionary changes to health care and nutrition, changing how we prevent and treat disease, and how food is selected, grown, processed, and provided to the discerning health conscious consumer.

Wayne R. Bidlack, PhD
Editor