Review
Phenolics, inflammation and nutrigenomics

David A Evans, Julie B Hirsch* and Slavik Dushenkov
WellGen, Inc., 63 Dudley Road, New Brunswick, NJ, USA 08901

This paper is dedicated to Dr David Evans who passed away after a brief illness on June 1, 2006. Dave was WellGen, Inc.'s chief executive officer from 1999 until his death and provided the vision for WellGen. More than a leader, Dave was a dear friend and a wonderful human being who will be sorely missed.

Abstract: Nutrigenomics is the study of the effects of bioactive compounds from food on gene expression. In the last several years, an increasing body of scientific evidence has demonstrated that individual compounds, as well as complex mixtures of chemicals, derived from food alter the expression of genes in the human body. By turning on or off genes, bioactives in food alter the concentration of specific proteins directly or indirectly associated with human diseases. Several human diseases result in multiple inflammatory responses which are associated with many diseases including arthritis, cancer, cardiovascular disease, dermatitis, asthma, obesity, and others. Detailed mechanisms of action as to how food derived components play an active role in prevention of inflammation have been elucidated. Such biologically active compounds include theaflavins and catechins from tea, curcumin from turmeric, resveratrol from grapes, and lactones from chicory. While chronic diseases are very complex, an opportunity exists to regulate genes involved in inflammation by enriching our diet with the specific foods inherently rich in such compounds, enriched foods containing standardized extracts of well studied sources, or dietary supplements.

Keywords: nutrigenomics; nutrigenetics; inflammation; gene expression; nutraceuticals; plant extracts

FOOD AND GENE EXPRESSION

Food is one of the most effective factors capable of multi-level regulation of expression of human genes. Nutrient/gene/disease interactions have been documented for several common food substances. Vitamins (tocopherol, folate, biotin), minerals (zinc), and phytochemicals (flavones, catechins) have all been demonstrated to alter gene expression in human cells in culture.

Several mechanisms have been identified whereby nutrients alter gene expression. These include (1) direct involvement in DNA synthesis (niacin), (2) DNA methylation (folic acid), (3) binding to transcription factors (vitamin A), (4) alteration of the turnover of regulatory proteins, and (5) alteration of mRNA stability (vitamin D). Examples of each of these types of gene regulation are numerous (see Table 1).

Niacin is a precursor of NAD^+, which is a precursor of a polymerase that is directly involved in the cell's repair of DNA damage.[1] Folic acid contributes methyl groups for the conversion of dUMP to dTMP, a key step during DNA and RNA synthesis.[2] Folic acid also plays a key role in DNA methylation, which involves the addition of a methyl group to the 5′ position of cytosine that precedes a guanosine within a DNA sequence. Methylation occurs in C–G rich regions of DNA; nearly 50% of our genes have such regions.[3] When C–G rich promoters of genes are methylated, the expression of those genes is inhibited. Methylation of DNA is a key mechanism for the silencing of many genes involved in cancer, including those for cell cycle regulation, receptors, DNA repair, and apoptosis. Epigallocatechin Gallate (EGCG), one of the major polyphenols in green tea, has been shown to inhibit DNA methylation and thereby activate some methylation-silenced genes in human colon, esophageal, and prostate cancer cells.[4] Hypomethylation of DNA, which can lead to expression of otherwise suppressed genes, is a prerequisite for proliferation of cancer cells. In individuals fed a folate deficient diet, hypomethylation of DNA was detected.[5] Other micronutrients and vitamins are known to alter DNA methylation.[6] Often genes are hidden in chromatin and thus unavailable for the transcription factors needed to activate expression of the genes. Recent results have now identified that addition of a single acetyl group to a specific lysine located in the tail histone 4 (H4) can prevent folding and keep chromatin opened up so that gene expression can take place.[7] Histone acetylation, along

* Correspondence to: Julie B Hirsch, WellGen, Inc., 63 Dudley Road, New Brunswick, NJ, USA 08901
E-mail: jhirsch@wellgen.com
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Table 1. Mode of action of nutrients on gene expression

Impact on gene expression	Example	Reference
DNA repair	Niacin, folate	Hageman and Stierum[1]
Bind to transcription factor	Vitamin A, theaflavins	Pégorier *et al*.[14]
DNA methylation	Folate	Davis and Uthus[6]
mRNA stability	Iron, glucose	Salati *et al*.[16]
Turnover of regulatory proteins	Catechins	Shay and Banz[67]

with DNA methylation, could result in significant difference accumulated through the lifetime even in monozygotic twins.[8] These epigenetic modulations could be targets for food bioactive intervention.

Another important nutrient–gene mechanism of action is the ability of some nutrients to bind with transcription factors, which are proteins that bind to the promoter region of specific genes and can either enhance or suppress gene expression. Binding of a nutrient to a transcription factor can impact the ability of a transcription factor to bind to DNA, either enabling or disabling transcription. For example, peroxisome proliferation-activated receptors (PPARs), which are transcription factors, impact the expression of genes associated with fatty acid metabolism[9] and inflammation. In a multistep process involving retinoid and other transcription factors, fatty acids bind to PPARs to create a complex that can bind to DNA. This results in reduction of fatty acid synthesis and increase in fatty acid oxidation. PPARs also control expression of genes directly linked to inflammation, including *NF-κB* and *AP-1*.[10] PPARs are implicated in inhibiting the induction of pro-inflammatory cytokines, adhesion molecules and extracellular matrix proteins. They also modulate the proliferation, differentiation and survival of immune cells including macrophages, B cells and T cells.[11,12] The list of genes regulated by fatty acids is extensive,[13,14] and includes genes involved in fatty acid transport, oxidation of fatty acids, desaturation of fatty acids, glycolysis, lipogenesis and lipoprotein metabolism.[15] Mice fed diets rich in ω-6 or ω-3 fatty acids which exceeded nutritional requirements, showed expression changes in over 300 genes.

Stability of mRNA can also be altered, thereby reducing protein levels produced by genes. Salati *et al*.[16] reviewed the involvement of nutrients in mRNA processing, including the impact of dietary components on mRNA stability, control of rate of mRNA translation, and processing of primary transcripts to mature mRNA. Effects of food ingredients on gene expression are a very complex multi-level processes. Study of the mechanism of those effects requires a system approach and coordinated efforts of the research community.[17]

INFLAMMATION

Inflammation is a complex process that involves cellular and molecular components that lead to widespread changes in physiological systems. The result of each inflammatory reaction may be beneficial (defense against agents interfering with homeostasis) or harmful (causing damage to cells and tissues). The molecular events occurring during inflammation have been characterized and are quite complex.[18] Using a human endotoxin challenge, induced inflammation model, it has been shown that the response of human blood leukocytes to such acute systemic inflammation includes widespread suppression and concerted dysregulation of leukocyte bioenergetics and transcriptional modulation.[19] The expression of several secreted pro-inflammatory cytokines such as tumor necrosis factors (TNF-SF2, TNF), interleukins 1 (IL-1A, IL-1B), and chemokines (CXCL1 (GROa), CXCL2 (GRO-), CCL2 (MCP-1), CXCL8 (IL-8) and CXCL10)) reached a maximum 2–4 h after endotoxin administration and followed by the expression of several members of the nuclear factor kappa/relA family of transcription factors (NF-κB1, NF-κB2, REL-A and REL-B).

While acute inflammation is critical to ward off infection, prolonged chronic inflammation is mostly detrimental. Chronic inflammation may occur as a result of prolonged infection, extended irritation or autoimmune diseases, including arthritis, multiple sclerosis, and type-1 diabetes. There is growing evidence that low level inflammation linked to the increased risk of developing cardiovascular disease and associated with obesity is mediated by pro-inflammatory adipokines like A-SAA.[20–23] In addition, such inflammation, primarily the result of overproduction of pro-inflammatory cytokines, is associated with cancer, arthritis, Alzheimer's disease,[24] psoriasis, atherosclerosis and several other human diseases. In many cases, human diseases are initiated or worsened by chronic inflammation.

In cancer, the links between cancer and inflammation are less direct, but have been enumerated[25–27] to include the detection of cytokines in many types of cancerous cells. Elevated levels of TNF, IL-1 and IL-6, and various chemokines have all been detected in a wide range of types of cancers. These proinflammatory cytokines are involved in every phase of cancer including activities such as DNA damage, regulation of tumor suppressor proteins, angiogenesis and metastasis. Atherosclerosis was initially thought to be a simple disease of lipid storage, but recent studies have documented a critical role of chronic inflammation in all phases of atherogenesis.[28] Brod[29] has suggested that systemic inflammation, marked by inadequately regulated pro-inflammatory cytokines, exacerbates several diseases including Alzheimer's,

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atherosclerosis, arthritis and autoimmune disorders. Other indirect involvement of inflammation in human disease has been proposed based on studies of humans with obesity. Human adipose tissue secretes increased amounts of inflammatory proteins that may account for syndrome-X and the prevalence of atherosclerosis in overweight individuals.[30]

SCREENING METHODS AND INFLAMMATION

Most food contains a large number of chemicals, many of which have specific biological activity. The chemicals also interact with each other, confounding any effort to identify bioactives. Once it has been demonstrated that a plant extract impacts gene expression, it is necessary to isolate and identify the bioactive compound(s). Bioassay-directed fractionation is such a method that can be used to test fractions of an extract for gene expression activity, ultimately identifying bioactives that are responsible for gene regulation.

The direct or indirect involvement of extracts and their bioactive components in chronic inflammation in several important human diseases and the dietary causes of inflammation have been reviewed.[31] Naturally occurring nutraceuticals, specifically antioxidant bioactives, such as plant phenols, vitamins, carotenoids and terpenoids, have been shown to have significant beneficial effects for health promotion by reducing the process of sustained inflammation that accompanies chronic disease. The data supporting such nutraceutical therapies have been recently reviewed.[32] Recently, Liu-Stratton, *et al*.[2] reviewed and compared historical methodologies, including northern blot and real-time PCR, with the most current DNA microarrays, for the analysis of gene expression for nutraceuticals and toxicological purposes.

Several genes have been identified that are directly involved in inflammation (Fig. 1). These include the cytokines (*TNF-α*, *IL-1*, *IL-6* and *ICAM*) which are among the genes regulated by NF-κB. Downregulation of cyclooxygenase-2 (*COX-2*) and 5-lipoxygenase (*5-LOX*) enables a decrease in the production of the arachidonic acid pathway products, prostaglandins or leukotrienes, respectively. Because oxidation can also trigger an anti-inflammatory response, inducible nitric oxide synthase (iNOS) is also important.

Researchers at Rutgers University and the University of Medicine and Dentistry of New Jersey (UMDNJ, NJ, USA) pioneered published techniques for screening plant extracts for expression of genes (mostly anti-inflammatory genes involved with cancer), using human cultured cells.[33] Suppression of the *COX-2* gene is an interesting example where there has been much research done. Dannenberg and colleagues[34] screened nearly 1000 plant extracts in an effort to identify bioactives capable of modulating expression of the *COX-2* gene. Using human cell cultures several food substances have been identified which are able to downregulate one or more of these genes, including curcumin from tumeric,[35] resveratrol from grapes,[36] catechins from green tea,[37] theaflavins from black tea,[38] and vitamin E.[39] Cavin *et al*.[40] showed that chicory root extract, due to presence of sesquiterpene lactones, suppresses TNF-α mediated *COX* induction in human colon cells.

Plant extracts and their purified compounds have excellent therapeutic effects due to the ability to selectively turn off *COX-2*, which is induced during inflammation and often causes pain, while preserving *COX-1*, a housekeeping gene that, among other things, protects the lining of the stomach. Resveratrol in mammary epithelial cells,[41] curcumin in gastrointestinal epithelial cells,[42] and ursolic acid from rosemary,[43] all show suppression of activation of *COX-2* gene expression (as well as inhibition of the enzyme itself). Interestingly, many of the plant compounds work via the same mechanism, through interference with the AP1 transcription factor. These compounds above act through inhibiting the protein

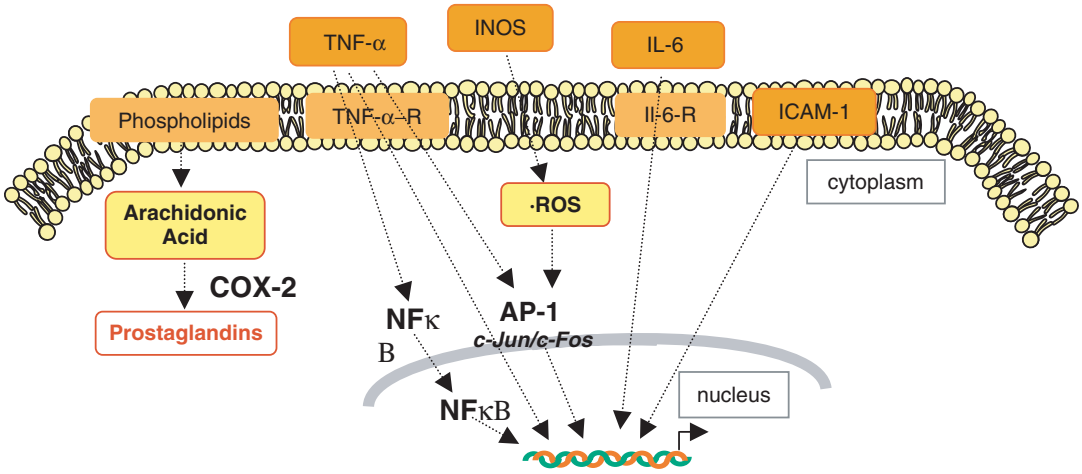

Figure 1. Inflammatory cytokines, such as TNF-α and IL-6, as well as iNOS and ICAM-1 can influence inflammation through gene expression and subsequent continuous transcription of additional cytokines, as well as prostaglandin generation through COX-2 activation.

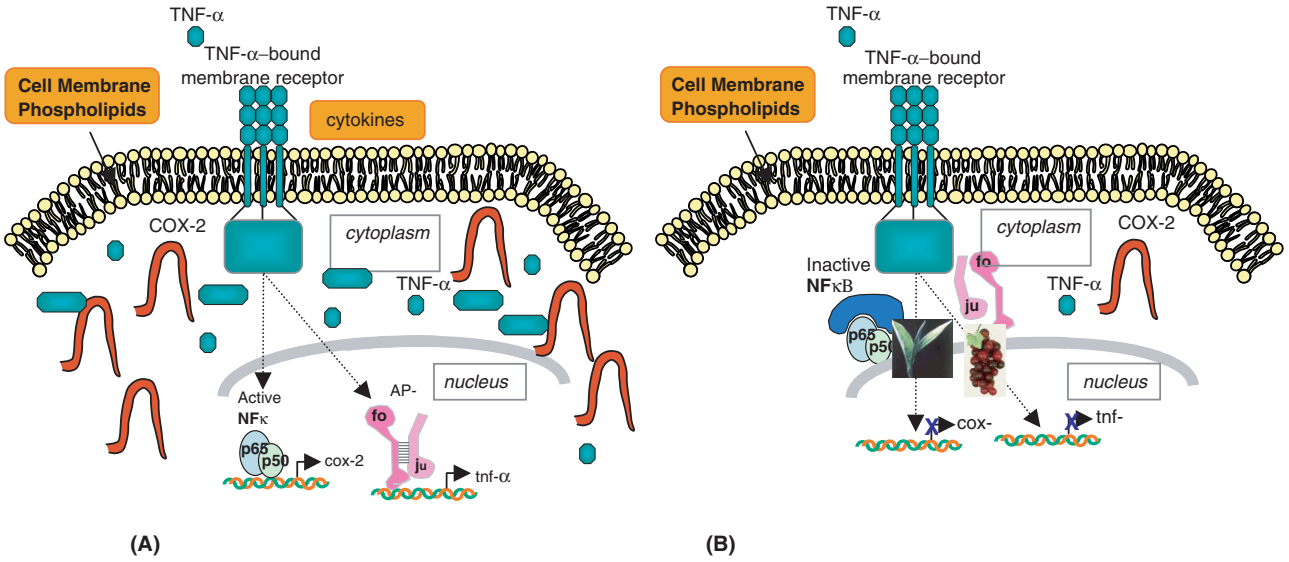

Figure 2. NF-κB and AP-1 are both transcription factors which are responsible for initiating transcription of numerous pro-inflammatory mediators, including COX-2, TNF-α, ICAM-1, and many others. NF-κB, a heterotetrameric protein, resides in the cytosol in an inactive form unless activated by cytokines or protein kinase activators. After activation, NF-κB translocates from the cytosol to the nucleus, and binds to a specific DNA sequence. AP-1 is a heterodimer composed of Jun and Fos proteins. AP-1 activity is induced by many stimuli, including cytokines and UV, which stimulate transcriptional activities. (A) Inflammation run amok. Inflammatory cytokines (TNF-α) and prostaglandins (generated by COX-2) are circulating in the cytoplasm and continually being churned out in the nucleus due to activation of transcription factors NF-κB and AP-1. (B) Inflammation in control with plant compounds. Shown are tea leaves (active compounds are theaflavins) and grapes (active compound is resveratrol). Concentration of inflammatory cytokines (TNF-α) and prostaglandins (generated by COX-2) circulating in the cytoplasm is much less due to prevention of activation of the transcription factors NF-κB and AP-1.

kinase c signal transduction pathway that, in turn, alters the DNA binding activity of the transcription factor AP1 to prevent transcription of *COX-2* (Fig. 2). Ultimately, it is the suppression or inhibition of prostaglandin synthesis which prevents the swelling, redness, lesions or pain associated with inflammation. In addition, high levels of prostaglandins are associated with multiple sclerosis, AIDS-associated dementia, and other neurological disorders.

Lu, *et al.*[44] compared the effects of theaflavin monogallates, derived from extracts of black tea, on the expression of the *COX-2* gene in normal (W138VA) versus cancer (caco-2) cells. Subsequent research in this laboratory demonstrated that the theaflavins downregulate *c-Jun* and *c-Fos*, the genes involved in the AP1 transcription pathway. Interestingly, they also found down regulation of cytokine-producing genes, including *NF-κB*, and downstream genes *TNF-α*, *IL-6* and *ICAM-1*, all involved in inflammation (Fig. 2). This reveals that compounds derived from plants work through multiple mechanisms to affect inflammation. Unlike molecular pharmaceutical targets which are synthesized to bind to specific targets, thereby shutting down pathways molecular pathways which may also have some beneficial metabolic effect, food derived compounds, while potentially not as potent, can work through several different means. This type of coordinated regulation lends itself to fewer side effects and an overall better safety profile for ingredients.

There are many examples of plant-derived compounds affecting multiple pathways. Using a microarray, Roy *et al.*[45] showed, using microvascular endothelial cells, the impact of *Boswellia* on expression of 113 of 522 genes implicated in induction by TNF-α.

All initial screening research to identify bioactives that regulate gene expression is completed with human or animal cell lines. Data from animal studies is encouraging. Modification of zinc deficiency by zinc supplementation has been demonstrated in rats to modify expression of many genes.[46] More recently, Fong *et al.*[47] demonstrated that zinc-deficient rats administered intra-gastric zinc had an 80% reduction in *COX-2* mRNA after only 8 h. Modulation of gene expression in rat livers has also been demonstrated by supplementation with vitamin E.[48] Caloric restriction has also been shown to result in increased gene expression of insulin related genes in rats.[49] Luceri *et al.*[50] demonstrated that polyphenols extracted from red wine inhibited gene expression of both *COX-2* and *iNOS* in rats. Schwerin *et al.*[51] observed differential gene expression in the livers of pigs fed casein or soy protein. Zeng *et al.*[52] observed increased levels of gene transcription for several apoptosis related in livers from mice fed selenium-enriched broccoli. With larger animals, Streltsova[53] demonstrated that black tea theaflavins inhibit gene expression of *TNF-α* in horses.

It is anticipated that alterations in gene expression should be associated with disease states. The studies by Zeng *et al.*[52] and Fong *et al.*[47] examined various molecular and cellular markers related to cancer. Treatment with selenium and zinc, respectively, suggested potent anti-cancer effects.

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Studies with humans are limited. Cao and Cousins[54] examined gene expression of a gene encoding a zinc binding protein (metallothionein) using blood samples from 16 men. Supplemented subjects were given the recommended daily amount (RDA) for zinc for 10 days. After 2 days significantly higher mRNA levels could be detected in blood cells, with elevated mRNA levels maintained throughout the 10 days of zinc supplementation.

BIOMARKERS

Nearly all evidence for the impact of nutritional factors on gene expression is derived from research using animal or human cells in culture with a paucity of direct human evidence. Human clinical studies are needed to establish a cause-and-effect relationship between the food affecting gene expression and the disease state. Carefully designed double-blind, placebo-controlled clinical studies are needed for any lasting credibility. Epidemiological studies analyzed to describe relationships among populations would also be extremely useful to support the science. These types of studies lay the foundation for exploring the multitude of reasons and factors why measurable benefits are achieved with the foods (or supplements) being studied.

Numerous biomarkers of inflammation have been directly associated with human disease. Gene product examples include NF-κB, TNF-α, C-reactive protein, IL-1 and several other cytokines.[18,55,56] In an effort to identify biomarkers associated with arthritis, Adarichev *et al*.[57] used high-density oligonucleotide arrays on the entire mouse genome. Thirty-seven early onset genes were identified which included genes related to chemokines, TNF-α signaling, and T-cell functions. Aigner and Dudhia[58] reviewed progress on functional genomics of osteoarthritis. From this type of analysis will arise new targets for control of inflammation will be identified.

NUTRIGENETICS

Nutrigenomics and nutrigenetics have been defined by several authors.[59] In essence, *nutrigenomics* studies the interaction of nutritional components (especially bioactives) with human genes. Nutrigenomics effects the change in expression of genes as a consequence of ingestion of a bioactive. *Nutrigenetics*, a subset of nutrigenomics, attempts to identify human allelic variations that respond differently to bioactives. Nutrigenetics recognizes an individual's hereditary predisposition to disease based on his or her genetic make-up. Ultimately, via a nutrigenetic approach, recommendations of dietary supplements and dietary recommendations, in the form of whole foods, will be made based on an individual's genetic background.

There are already fascinating, and hopefully prophetic, examples of nutrigenetics, in which genetic variation between individuals influences dietary responses. Ordovas *et al*.[60] demonstrated how a single-point mutation in the *APOA1* gene alters how an individual responds to the effect of polyunsaturated fatty acids on HDL cholesterol levels. Ordovas and Mooser[61] recently reviewed studies examining diet/genotype interactions involved in cardiovascular risk. Kornman *et al*.[62] have detailed a nutrigenetics approach to treatment of inflammation. Genetic polymorphisms are apparently common among cytokine genes. For example, *IL-1* genetic variations have been associated with inter-individual differences in IL-1 levels. Individuals have been shown to vary in the ability of ω-3 fatty acids to suppress TNF-α.[63]

Nonetheless, there are significant technical hurdles that must be overcome before we will see the benefits of nutrigenetics. Nutrigenetics is dependent on diagnostics. Several technical issues with diagnostics suggest that we are many years from development of meaningful low-cost diagnostic tests that can be used to predict personalized nutritional requirements. Marshall's report of inconsistent results from comparison among commercial DNA microarrays[64] underscores the need to validate any diagnostic test against biomarkers in a human clinical setting before commercial diagnostic tests are mass produced.

NUTRITIONAL INTERVENTION

Acute inflammation is essential for the protection of our bodies against infection. Chronic inflammation is involved in human diseases. Nutritional intervention to control inflammation needs to consider that inflammation is both critical to a human's survival and responsible for onset or proliferation of human diseases.

Treatment of pain and inflammation is primarily achieved with nonsteroidal anti-inflammatory drugs (NSAIDs). Evidence suggests an increase in cardiovascular risk with use of NSAIDs.[65] The opportunity exists to utilize nutrigenomics approaches to develop alternative treatments or dietary adjuncts that could reduce the use of NSAIDs for ongoing treatment of arthritis.

To this end Nakamura, *et al*.[66] investigated the impact of pre-op feeding of ω-3 fatty acids to patients undergoing surgery for cancer. Patients given ω-3 fatty acids had significant decreases in leukocyte elastase and IL-8, indicating reduced inflammation, on days 1 and 3 following surgery.

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