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Dietary antioxidant intakes and neurologic disease risk

There is strong pathological and experimental evidence that oxidative stress has an important role in several neurodegenerative diseases. This evidence converges with results of large longitudinal epidemiological studies supporting a possible protective effect of antioxidant in foods, mostly fruits and vegetables, on cognitive decline and risk of Parkinson disease. On the other hand, results of randomized trials to assess the potential benefits of antioxidant supplements have been disappointing. Possible interpretations for this apparent discrepancy include: 1) a balanced combination of different antioxidants and consumption with foods rather than supplements is important to achieve the expected benefits; 2) the beneficial effects associated with dietary antioxidant reflect life long dietary habits that cannot be replicated with short-term supplementation; 3) the apparent benefits observed in observational studies are not due to antioxidants, but rather to other nutrients or other healthy habits (physical activity, etc.). Recent studies also suggest a protective effect of blood urate in Parkinson's disease. Urate, and the effects of diet on uricemia, have been largely ignored until recently in studies on oxidation and chronic diseases. Accounting for these effects may provide new clues to the importance of antioxidants in neurodegenerative diseases.

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Lipoic acid: a new therapy for multiple sclerosis?

Multiple sclerosis (MS) is a common disabling disease of adults in which a pathogenic immune response in the brain and spinal cord results in demyelination and axonal degeneration. We and others have demonstrated that lipoic acid is highly effective at suppressing and treating a murine model of MS, experimental autoimmune encephalomyelitis (EAE). Doses ranging between 15 and 100 mg/kg/day suppress EAE by 50-100%, reducing paralysis, demyelination, and axonal degeneration. When given after the onset of paralysis, lipoic acid results in significant clinical and histologic improvement. The mechanisms through which lipoic acid treats EAE are uncertain. Lipoic acid may inhibit T cell migration into the CNS by altering expression of adhesion molecules on brain endothelial cells and production of matrix metalloproteinase-9 (MMP-9) by T cells. Based on our EAE research, we have completed an early pilot trial of orally administered lipoic acid in MS subjects. This study indicates that with an oral dose of 1200 mg we can obtain serum levels of lipoic acid comparable to those required to obtain a therapeutic response in EAE. Moreover, lipoic acid appears to decrease serum MMP-9 production and sVCAM, which are associated with disease activity in MS. Lipoic acid, thus, is a promising new oral therapy for MS.

Raymond F. Burk

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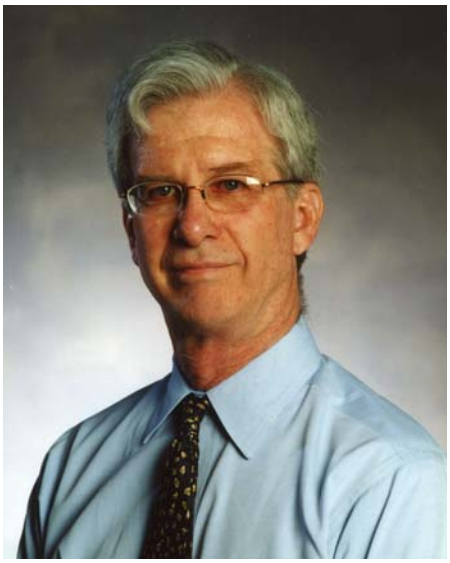

Dr. Burk received his B.A. in Mathematics from the University of Mississippi, in Oxford, Mississippi, and his M.D. from Vanderbilt University of Medicine in Nashville, Tennessee.

Models of antioxidant micronutrient deficiencies that cause central nervous system damage

The micronutrients vitamin E and vitamin C have antioxidant properties. We have examined their effects on the central nervous system of the guinea pig (GP). Weanling male GPs were fed a vitamin E-deficient diet for two weeks and then vitamin C was also removed from the diet, making it doubly deficient. Within a week, GPs began to suffer from neurological dysfunction. Paralysis began in the hind limbs and progressed to the front limbs and then to the respiratory muscles. Micronutrient-replete and singly deficient GPs developed no neurological signs. Histological examination of the brains and spinal cords of affected GPs revealed asymmetric infarct-like lesions in the spinal cords and brains. Staining for IgG to assess intactness of the blood-brain barrier showed extensive IgG around vessels and in brain tissue of deficient GPs. This indicates that the blood-brain barrier was not maintained in antioxidant micronutrient deficiency. These results are compatible with the hypothesis that vitamins E and C interact in vivo and suggest that combined deficiencies of them might have serious consequences in human beings. Selenium is also an antioxidant micronutrient. Deficiency of selenium in the mouse brain achieved by knockout of selenoprotein P or its receptor led to low brain selenium levels, and feeding a selenium-deficient diet led to neurological dysfunction and death. These studies demonstrate that antioxidant micronutrient delivery to the brain is essential for brain function and viability. *Supported by NIH grants AG16236 and ES02497.*

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Effects of dietary interventions on weight loss and lipoprotein metabolism

Despite the continuing effort to educate the public that excessive weight increases the risk of chronic disease, the prevalence of obesity continues to rise. The metabolic syndrome (MetSyn), associated with adult overweight represents a cluster of symptoms that are present just before the onset of type 2 diabetes. We have conducted weight loss interventions to evaluate whether macronutrient composition would reduce the risk for cardiovascular disease (CVD) and the MetSyn. In one study, 70 women (age 29.9 ± 5.1 y) who were classified as being overweight or obese (BMI 26-27 kg/m^2) followed a 10-wk intervention consisting of a low-calorie diet adapted to individual needs and level of activity. The diet had the following energy distribution: 30% protein, 30% fat, and 40% carbohydrates. Participants increased their level of activity by increasing the number of steps taken per day, which were assessed at baseline by use of a pedometer until they completed additional 4500 steps compared to their baseline. Women also took a supplement of either carnitine (3 g/d) or a placebo (cellulose) in a randomized double-blind design. All subjects received 90% of their food. At the end of the study participants had reductions in body weight, abdominal fat, waist circumference, LDL cholesterol, and triglycerides (TG) ($P < 0.001$), while the size of the LDL particle increased. Another key variable that was affected by the intervention was plasma insulin, which clearly indicates that insulin resistance decreases as body weight is reduced. There was also an 83% reduction in subjects who were initially classified as having the metabolic syndrome. In another study we evaluated the effect of adding soluble fiber to a carbohydrate-restricted diet (CRD) with a distribution of energy of ~60% fat, ~30% protein, and ~10% carbohydrate on plasma LDL cholesterol and other traditionally measured markers of CVD and the MetSyn. Using a parallel-arm, double-blind, placebo-controlled design, 30 overweight and obese men (BMI 25-35 kg/m^2) were randomly assigned to supplement a CRD with soluble fiber (Konjac-mannan, 3g/d) (n=15) or placebo (n=15). Plasma lipids, anthropometrics, body composition, blood pressure, and nutrient intake were evaluated at baseline, 6 and 12 wk. Compliance was excellent as assessed by 7-day weighed dietary records and ketonuria. The CRD resulted in significant reductions in body weight, trunk fat, plasma TG, and blood pressure, while there were increases in HDL cholesterol; thus these diets favorably affected all the symptoms associated with the MetSyn. CRD also had a huge impact on the number of lipoprotein particles. The total number of VLDL particles was reduced by 19.0% ($P < 0.001$) between baseline and 12

wk. Regarding LDL, particle size increased ($P < 0.001$) while particle number decreased ($P < 0.05$) from baseline to wk 12. The increase in particle size was due to a 35% increase in large LDL particles ($P < 0.001$) and a 25% reduction in very small LDL ($P < 0.001$). Consumption of a CRD for 12 wk also caused an increase in HDL particle size ($P < 0.01$). The duration of the intervention was relatively short but tightly controlled, thus clearly representing the true biological adaptations to a CRD. In summary, lifestyle modifications have a great influence on CVD risk factors and the MetSyn. However, weight reduction and diet modifications are the best alternatives. Among the dietary interventions, carbohydrate restriction appears to have the greatest impact.

Tory M. Hagen

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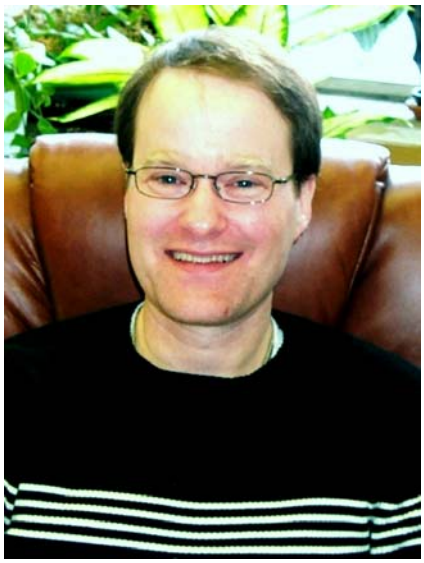

Dr. Hagen received his B.S. in Biochemistry from North Carolina State University in Raleigh, North Carolina, and his Ph.D. in Biochemistry from Emory University in Atlanta, Georgia.

Lipoic acid improves the age-related decline in cellular response to oxidative stress

One out of every five Americans will be over the age of 65 in just 25 years. Along with the "graying" of America are increased risks for chronic diseases that disproportionately afflict this age group. Geriatric medicine has primarily focused on "after-the-fact" treatments for specific diseases, which at best is palliative in nature. An alternative way for improving elder health would be to prevent development of debilitating disorders by targeting the causes that make age the leading risk factor for these insults. In this regard, it is notable that oxidative stress is a potent underlying factor for many age-associated diseases and may govern the rate of aging itself. Equally notable is that cellular stress defense mechanisms decline in the elderly and no longer adequately respond to oxidative and toxicological insults, elevating risk for acute and chronic pathologies. Thus, a practical approach to improving healthspan would be to simply maintain or reverse the loss in stress-resistance mechanisms.

In meeting our long-term goal to define the benefits of micronutrients in improving elder health, we observed that feeding old rats (*R*)-α-lipoic acid (R-LA) significantly increased hepatic antioxidant levels and markedly improved resistance to toxins. R-LA did not achieve this by merely acting as a dietary antioxidant; rather, it reversed the age-related loss of endogenous antioxidant capacity, especially glutathione (GSH). This led us to the discovery that nuclear levels of NF-E2 related-factor 2 (Nrf2), a transcription factor that regulates expression of GSH-synthesizing enzymes, markedly declines with age, but LA reverses this loss. These results, indicating that R-LA positively affects deficits in Nrf2-mediated defenses, have enormous implications for improving elder health because Nrf2 not only regulates GSH synthesis, but also the coordinate expression of over 100 genes containing the Antioxidant Response Element (ARE). These ARE-mediated genes are vital to cellular defenses against toxins, oxidants, and mutagens.

Ongoing work in the laboratory is now focused on understanding why Nrf2-mediated stress defenses decline with age and the precise mechanism(s) how R-LA acts to maintain this vital cellular defense system. Our focus is two-fold, namely to define age-related dysregulation of signaling pathways that may adversely affect Nrf2 nuclear translocation and tenure as well as to determine the extent that the aging

process alters the Nrf2 transcriptome at the gene level. Results to date indicate that a bimodal problem arises with age: nuclear Nrf2 levels significantly decline and a repressive transcriptional motif also develops that together, limits ARE-mediated gene expression. R-LA improves Nrf2 action by increasing nuclear tenure of Nrf2 and also promoting its binding to an imperfect, alternative ARE sequence. Thus, R-LA may be part of a unique class of dietary micronutrients that promote “healthspan” by maintaining vital cellular defenses, which otherwise decline with age.

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Dietary phytoestrogens & breast cancer: a complex safety issue involving dose & timing of exposure

Genistein, found in soy products, is a phytochemical with estrogenic activity. Our research has focused on the effects dietary genistein on growth of estrogen (E)-dependent mammary tumors both *in vitro* and *in vivo*. Genistein enhances the proliferation of E-dependent human breast cancer tumor growth. Genistin, the glycoside form of genistein, simulates growth similar to that of genistein, and withdrawal of either genistin results in tumor regression. We have also demonstrated that soy protein isolates processed to contain low, medium, and high amounts of isoflavones simulate tumor growth in a dose-dependent manner. Expression of the estrogen-responsive gene pS2 was also induced in response to treatment with dietary genistein. We also evaluated the effect of dietary genistein in the chemically induced (NMU) mammary cancer rodent model and have demonstrated that dietary genistein stimulates growth in this model. To evaluate whether dietary genistein interacts with current anti-estrogen breast cancer therapy such as tamoxifen (TAM), we implanted E-dependent tumors into ovariectomized athymic mice and administered estradiol, estradiol plus TAM, or estradiol, TAM plus dietary genistein. In these studies dietary genistein was able to negate the inhibitory effect of TAM on E-stimulated tumor growth. Genistein is present in soy as part of a complex mixture and the profile of these other bioactive compounds plays an important role; we will present data on how the profile of soy bioactive compounds can modulate genistein-stimulated, estrogen-dependent tumor growth. In summary, genistein can act as an estrogen agonist resulting in proliferation of E-dependent human breast cancer cells *in vivo* and can negate the inhibitory effects of TAM on E-stimulated growth of MCF-7 cell tumors *(in vivo)* implanted into ovariectomized athymic mice.

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Exercise training and the antioxidant α-lipoic acid in the treatment of insulin resistance and type 2 diabetes

An impaired ability of insulin to activate the glucose transport system in skeletal muscle is a critical defect that characterizes the insulin-resistant state of pre-diabetes and overt type 2 diabetes. While the etiology of this insulin resistance is multifactorial, accumulating evidence indicates that one major cause of insulin resistance is the defective expression and action of proximal aspects of the insulin signaling pathway, including the insulin receptor (IR) and insulin receptor substrate-1 (IRS-1). While several factors can lead to these defects in IR and IRS-1 functionality, one important condition associated with insulin resistance is oxidative stress, the imbalance between the production of damaging reactive oxygen species and the cellular and plasma antioxidant defenses. Exposure of skeletal muscle to an oxidant stress leads to impaired IRS-1-dependent insulin signaling and subsequently to reduced insulin-dependent glucose transport activity. Several recent studies have shown that the acute or chronic treatment of insulin-resistant animal models and type 2 diabetic human subjects with antioxidants, including α-lipoic acid (ALA), can induce enhancements of whole-body glucose tolerance and insulin action on the glucose transport system in skeletal muscle. In addition to this nutriceutical intervention, it is clear that endurance exercise training is very effective in ameliorating the insulin resistance of skeletal muscle in pre-diabetes and type 2 diabetes. Both exercise training and ALA can reduce indices of oxidative stress. Interestingly, we have demonstrated in a genetically obese animal model of pre-diabetes (the obese Zucker rat) that the combination of exercise training and the antioxidant R-ALA (the R-enantiomer of ALA) produces an interactive effect resulting in a greater improvement in insulin action on skeletal muscle glucose transport than either intervention individually. This interactive effect of exercise training and ALA is associated with an enhancement of the protein expression and functionality of IRS-1 in skeletal muscle of these obese animals. These results underscore the potential of combining endurance exercise training and the antioxidant R-ALA for optimally improving defective insulin action in insulin-resistant, pre-diabetic skeletal muscle.

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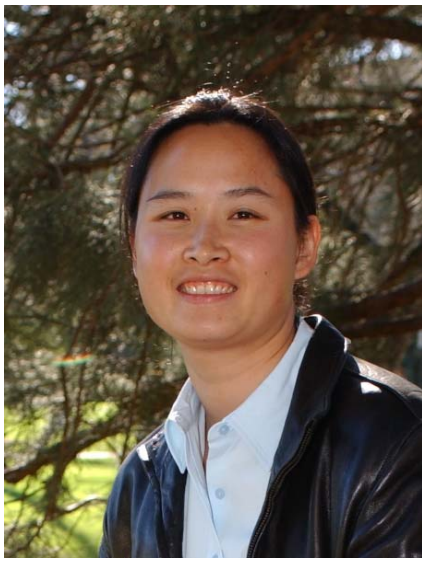

Dr. Ho received her B.S. in Nutritional Sciences from the University of Guelph, Guelph, Ontario, Canada, and her Ph.D. in Nutrition from The Ohio State University, in Columbus, Ohio.

Dietary histone deacetylase inhibitors for prostate cancer prevention

Prostate cancer is the most frequently diagnosed non-cutaneous cancer and is the second leading cause of cancer death in American men. The precise etiologic factors that initiate and enhance the progression of prostate cancer remain unknown, but epigenetic alterations and diet/lifestyle factors have come forth as significant contributing factors. During prostate cancer, alterations in acetylation patterns and increases in histone deacetylases are apparent. The use of pharmacological agents that inhibit HDACs for cancer prevention and therapy have gained significant interest. HDAC inhibitors cause increases in acetylated histones, selectively induce cell cycle arrest and apoptosis in cancer cells, and have shown promise in cancer clinical trials. Interestingly, several dietary components also appear to act as HDAC inhibitors. Sulforaphane (SFN) is an isothiocyanate found in cruciferous vegetables, such as broccoli. This anti-carcinogen was first identified as a potent inducer of Phase 2 enzymes, but evidence is mounting that SFN acts through other cancer chemopreventive mechanisms. We have recently reported that sulforaphane (SFN) suppresses tumor growth in animal models and inhibits HDAC activity in prostate both *in vitro* and *in vivo*. We have found SFN inhibits HDAC activity in prostate cancer cells, with a concomitant increase in accumulation of acetylated histones H3 and H4, increased associaton of acetylated histone with P21 and bax promoter regions and upregulation of p21 and Bax. In PC3 xenograft models, SFN consumed in the diet at an average daily dose of 7.5 μmol SFN per animal for 21 days suppressed the growth of human PC-3 prostate cancer cells by 40% in male nude mice. There was a significant decrease in HDAC activity in the xenografts, as well as in the prostates and peripheral mononuclear blood cells (PMBC) of mice treated with SFN, compared to controls. Finally, in human subjects, a single dose of 68 g broccoli sprouts inhibited HDAC activity significantly in PBMC, 3 and 6 h following consumption. These findings provide evidence that one mechanism through which SFN acts as a cancer chemopreventive agent *in vivo* is through the inhibition of HDAC activity. These studies are also significant because of the potential for the development of strategies for high-risk prostate cancer patients that decrease disease incidence and increase survival through targeting epigenetic events by incorporating easily accessible foods into a patient's diet.

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Calorie restriction and aging: effects on brain and behavior

Dietary caloric restriction (CR) has been demonstrated in many short-lived species to extend lifespan, reduce age-related disease, and maintain function later into life, including brain and behavioral function. CR in rodents attenuates age-related changes in many neurobiological parameters, including memory and motor impairments. Moreover, in rodent models of neurodegeneration, including stroke, Huntington's disease, Parkinson's disease, and Alzheimer's disease, CR attenuates damage and retards pathogenesis. Recent results from on-going CR studies in humans and nonhuman primates suggest that anti-disease and anti-aging benefits observed in rodents may apply to long-lived species. Studies in rhesus monkeys indicate that CR (30%) animals are healthier than fully fed counterparts based on reduced incidence of various diseases and specific risk factors and may be aging at a slower rate based on analysis of biomarkers. Recent behavioral studies also indicate enhanced performance in a motor task that is age sensitive, but no evidence has emerged showing attenuation of the age-related decline in neostriatal volume. In a related study it was observed that CR for 6 mo in adult rhesus monkeys protected against a neurotoxic insult. Increasing interest in the CR paradigm will expand knowledge about how nutrition modulates aging and the mechanisms responsible for this modulation.

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Cancer and imprinting: early nutritional influences

Human epidemiological and animal experimental data indicate that the risk of developing adult-onset diseases, such as asthma, diabetes, obesity, and cancer, are influenced by persistent adaptations to prenatal and early postnatal exposure to environmental factors. Two epigenomic targets that potentially link early environmental exposure to adult disease susceptibility are genes that are imprinted and those with metastable epialleles.

Genomic imprinting is an epigenetic form of gene regulation that results in monoallelic, parent-of-origin dependent gene expression. The functional haploid state of imprinted genes makes them susceptibility loci for diseases since a single genetic or epigenetic mutation can dysregulate their expression. For example, *IGF2* loss of imprinting is associated with an increased incidence of cancer and Beckwith-Wiedemann syndrome in children conceived by *in vitro* fertilization. We have also demonstrated in mice that early postnatal dietary methyl deficiency, and even exposure to a nutritionally complete synthetic diet, high in fat but low in fiber, increases biallelic expression of the oncogene, *IGF2*.

Genes with metastable epialleles have highly variable functions because of stochastic allelic changes in the epigenome rather than mutations in the genome. The viable yellow agouti (*Avy*) mouse harbors a metastable *Agouti* gene because of an insertion of a transposable element. We have used the *Avy* mouse to investigate the importance of maternal nutrition in determining the susceptibility of offspring to adult diseases. We have shown that maternal dietary supplementation during pregnancy with either methyl donating substances (i.e., folic acid, vitamin B12, choline, and betaine) or genistein, a phytoestrogen present in soy products, alters the coat color of the offspring and decreases their susceptibility to developing obesity, diabetes, and cancer by increasing CpG methylation at the *Avy* locus. Moreover, we now have evidence that these nutritional supplements can also block the deleterious DNA hypomethylation caused by the plasticizer and environmental toxicant, bisphenol A. *Supported by DOE grant DE-FG02-05ER64101 and NIH grants ES13053, ES08823, ES015165, and T32-ES07031.*

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Dietary modulation of UDP-glucuronosyltransferases in humans

UDP-glucuronosyltransferases (UGT) catalyze the conjugation of the glucuronyl group from uridine 5′-diphosphoglucuronic acid with endogenous and exogenous compounds, producing glucuronide products that are more polar, less toxic, and more easily excreted. Because UGT maintain sex steroid hormone balance and facilitate excretion of carcinogenic compounds such as PhIP, regulation of their expression and activity is hypothesized to affect cancer risk. Many phytochemicals have been shown to induce UGT in humans, rodents, and cell culture systems. Phytochemicals regulate transcription factors, such as the nuclear factor-erythroid 2-related factor 2 (Nrf2), aryl hydrocarbon (AhR) and pregnane X receptors (PXR), as well as proteins in several signal transduction cascades that converge on Nrf2 to stimulate UGT expression. AhR-binding sites have been identified in all *UGT1A* genes, as well as *UGT2B4, UGT2B15,* and *UGT2B17*, and *UGT1A* family members have been shown to be functionally inducible via AhR-ligands. This induction can be modified by several factors, including phytochemical dose and bioavailability and interindividual variation in enzyme expression. In humans, diets high in cruciferous vegetables, as well as a mix of crucifers, soy foods, and citrus fruits, increase glucuronidation. The *UGT1A1*28* polymorphism, characterized by the presence of 7, rather than 6, TA repeats in the promoter region of the gene, alters response to dietary exposures. Thus, on the basis of genotype, some individuals may derive greater benefit than others in terms of increased glucuronidation in response to diet. *Supported in part by NIH grant R01CA92288.*

vector was introduced (and expressed) into the arcuate nucleus (Arc) of the hypothalamus by stereotaxic injection. Expression of MCD, which should lower [malonyl-CoA] in the hypothalamus, reversed the suppressive effect of C75 on food intake, thus rendering the mice resistant to C75.

Taken together these findings provide compelling evidence that level of hypothalamic malonyl-CoA, which depends on the relative activities of ACC and FAS, is an indicator of energy status and mediates feeding behavior.

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Pharmacologic ascorbate concentrations selectively kill cancer cells: ascorbic acid as a pro-drug for ascorbate radical and/or H_2O_2 delivery to tissues

Pharmacokinetics data in humans indicate that intravenous (IV), but not oral ascorbate, could have an unanticipated role in cancer treatment. We hypothesized that millimolar ascorbate extracellular concentrations achieved only by intravenous administration could serve as a pro-drug for ascorbate radical and/or H_2O_2 delivery to the extracellular space, but with minimal formation in blood. Under *in vitro* conditions mimicking clinical IV use, we found that some cancer cells were killed by millimolar ascorbate, while normal cells were unaffected. Apoptosis and pyknosis/necrosis were caused by extracellular ascorbate, independent of intracellular concentrations, leading to H_2O_2 formation. H_2O_2 generation was dependent on ascorbate concentration and ascorbate radical formation, with 0.5-10% serum required. In contrast, millimolar ascorbate additions to hemolyzed and non-hemolyzed blood generated no detectable H_2O_2 and only trace ascorbate radical. To further test these concepts *in vivo*, rats were given intravenous (IV), oral, or intraperitoneal (IP) ascorbic acid using typical human pharmacologic doses (0.25~0.5 mg/g body weight). Extracellular fluid (ECF) was collected by microdialysis from muscles. After IV injection, ascorbic acid concentrations in blood and in extracellular fluid (ECF) increased from 50 μM baseline to peaks of approximately 8 mM. Upon IP injection, peaks approached 3 mM in both fluids. With the same doses given by gavage, ascorbic acid concentrations were always less than 150 μM in both blood and extracellular fluid. In blood, ascorbate radical concentrations were undetectable with oral administration and did not exceed 50 nM with IV and IP administration, even when ascorbic acid concentrations were as high as 8 mM. In extracellular fluid, ascorbate radical concentrations were as high as 250 nM and were an exponential function of ascorbic acid concentrations. At all doses and by all routes of administration, concentrations of ascorbate radical in extracellular fluid were approximately 4-12 fold higher than those in blood, as a function of ascorbic acid concentrations. Using a new assay, H_2O_2 in extracellular fluid was detected when ascorbate radical concentrations in this fluid exceeded 100 nM, which occurred only with parenteral administration. Taken together, these data validate the hypothesis that pharmacologic ascorbic acid is a pro-drug for preferential steady state formation of ascorbate radical and hydrogen peroxide in the extravascular space but not in blood. These data provide the fundamental rationale for pursuing pharmacologic doses of ascorbic acid as a drug for possible use in cancer treatment.

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Ascorbic acid transporters in health and disease

Neurons in the central nervous system (CNS) contain some of the highest vitamin C (ascorbic acid) concentrations of mammalian tissues. These high ascorbate concentrations are thought to be generated and maintained by the SVCT2 (*Slc23a2*), a specific transporter for ascorbate. Intracellular ascorbate serves several functions in the CNS, including antioxidant protection, peptide amidation, myelin formation, synaptic activity, and protection against glutamate toxicity. Recent results from this laboratory suggest that ascorbate is necessary for neuronal maturation, function, and antioxidant defense. The importance of the SVCT2 for CNS function is also highlighted by the finding that its targeted deletion in mice causes widespread cerebral hemorrhage and death on post-natal day one. The cause of death in these animals is not clear, but our data suggests it relates to neuronal damage and apoptosis. Neuronal ascorbate content as maintained by this protein also has relevance for human disease, since ascorbate supplements decrease infarct size in ischemia-reperfusion injury models of stroke, and since ascorbate may protect neurons from the oxidant damage associated with neurodegenerative diseases, such as Alzheimer's, Parkinson's, and Huntington's.

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Dietary polyphenols and health: defining the nutritional context

Polyphenols are the most abundant antioxidants in our diet. The large number of studies already published illustrates the wide diversity of their biological effects and suggests a protective role against a variety of diseases. However, few of the documented effects have been validated in a nutritional context. Most authors have used high polyphenol doses which could only be reached through drug administration. The biological effects associated to food consumption could be very different from those observed in these studies. It is therefore essential to characterize precisely dietary intake, metabolism, and tissue exposure to polyphenols. These different questions have been addressed in our laboratory. We are currently constructing a comprehensive database and food composition table for polyphenols, based on the analysis of the scientific literature. More than 500 phenolic compounds are already included with over 40,000 content values. Such a database will allow estimating the dietary intake of all these compounds. Beyond intake, the bioavailability of several polyphenols representative of different classes found in food has been compared in animal and human experiments. The nature and concentrations of the main metabolites have been determined in plasma and tissues. Altogether, these studies have provided the range of polyphenol concentrations and intake levels which should not be exceeded in *in vitro* or *in vivo* studies to be nutritionally relevant. We also showed that 15 phenolic metabolites, estimated by tandem mass spectrometry in urine samples from 154 human subjects, can be used as biomarkers of polyphenol intake, useful for epidemiological studies. This approach is now extended to a wider variety of phytochemicals using a metabolomic approach and a QT of mass spectrometer. Such a metabolomic approach should also lead to the discovery of the still missing robust markers of effects for polyphenols.

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The natural vitamin E α-tocotrienol and stroke-related neuroprotection

Seven years ago we noted that nanomolar α-tocotrienol (T3), but not α-tocopherol, blocked glutamate-induced neuronal death by suppressing early activation of c-Src kinase. A later independent study reporting that Src blockade provides cerebral protection following stroke enhanced the significance of our finding that α-T3 possesses c-Src regulatory effects. Our efforts to understand the mechanistic basis of neuroprotection by α-T3 have led to the observation that glutamate-induced neurodegeneration hinges on two key molecular checkpoints: (i) c-Src activation, and (ii) 12-lipoxygenase (Lox) activation (*JBC* 278:43508, 2003). Recently we noted that *in vivo*, α-T3 supplementation decreased stroke-induced damage to the brain of spontaneously hypertensive rats. α-T3 seems to protect neurons both by antioxidant-independent and –dependent mechanisms. Taken together, orally supplemented α-T3 is potently neuroprotective and should be considered as a nutritional countermeasure to contain stroke-related injury to the brain. Current developments in the laboratory will be reported. *Supported by NIH grant NS42617. Vitamin E is supplied by Carotech Inc.*

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Secrets of the Lean Plate Club: Tales from the Waistline Wars

Choose low-fat. Cut carbs. Eat more protein. Sip coffee. Skip coffee. Have more fiber. Eat fewer calories. Move more. Get five servings of fruit and vegetables. No make that nine servings daily. And what's a serving anyway? Consumers are confused, time-crunched and frustrated. No wonder they often head for the nearest golden arches when they're hungry. Cutting through the nutritional fog is the Lean Plate Club, founded by the Washington Post's nationally syndicated columnist Sally Squires, a veteran health journalist, who has a master's degree in nutrition. This virtual club now reaches more than six million members each week from coast to coast through newspapers, a free e-mail newsletter, a popular weekly Web chat at Washingtonpost.com as well as radio and television. Learn what Lean Plate Club members prove week after week: that small steps really can add up to big rewards in healthier eating and physical activity. Sally will also sign copies of the newly published *Secrets of the Lean Plate Club* (St. Martin's Press.)

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Ascorbylation reactions

Oxidation of lipids leads to the formation of reactive aldehydes and epoxides, which are potentially cytotoxic and genotoxic due to their ability to modify proteins and nucleic acids. The role of vitamin C (ascorbic acid) in the process of lipid peroxidation is not well understood: while ascorbic acid is best known as an antioxidant, experimental data from our laboratory and from other research groups suggest that the role of vitamin C is more complicated. We have shown that ascorbate acts as a mediator of lipid peroxidation (LPO) and that it has the ability to form conjugates with LPO products. The biological relevance of vitamin C conjugation (ascorbylation) of reactive aldehydes and epoxides is not clear at present. Cell culture experiments with vitamin C-adequate monocytes indicate that nonenzymatic ascorbylation of 4-hydroxy-2-nonenal (HNE), a known LPO product, is a minor pathway compared to enzyme-mediated glutathionylation of HNE. Feeding of ascorbylated HNE (AscHNE) to cultured monocytes showed efficient uptake of AscHNE, suggesting active transport, and subsequent conversion of AscHNE into glutathionylated HNE. Computational studies revealed that ascorbylation of acrolein (propenal), a more reactive LPO product compared to HNE, is slightly favored over glutathionylation. Using liquid chromatography coupled to tandem mass spectrometry and labeled standards, ascorbylated acrolein (AscACR) was detected in human urine at low nanomolar concentrations. While many ascorbylated natural products from plants have been reported in the literature, our data suggest that ascorbylation of reactive intermediates can also occur in mammalian systems.

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Redox-sensitive transcription factors and their regulators as prime targets for chemoprevention with anti-inflammatory and antioxidative phytochemicals

There are multiple lines of compelling evidence supporting the association between inflammatory tissue damage and cancer. A new horizon in chemoprevention research is the recent discovery of molecular links between inflammation and cancer. Components of the cell-signaling network, especially those converging on the ubiquitous eukaryotic redox-sensitive transcription factor, nuclear factor-κB (NF-κB), have been implicated in pathogenesis of many inflammation-associated disorders. A wide variety of chemopreventive and chemoprotective phytochemicals can alter or correct undesired cellular functions caused by abnormal pro-inflammatory signal transmission mediated by NF-κB and its upstream regulators, including IκB kinase. Modulation of cellular signaling involved in chronic inflammatory response by anti-inflammatory agents hence provides a rational and pragmatic strategy in molecular target-based chemoprevention and cytoprotection. Induction of phase-2 detoxifying or antioxidant genes represents an important cellular defensive in response to oxidative and electrophilic insults. Nuclear transcription factor erythroid 2p45 (NF-E2)-related factor 2 (Nrf2) plays a crucial role in regulating phase-2 detoxifying/antioxidant gene induction. Many antioxidants derived from dietary and medicinal plants have been found to activate this particular redox-sensitive transcription factor, thereby potentiating cellular antioxidant or detoxification capacity.

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Exercise neuroscience

Acute and chronic physical activity influence brain function, resulting in alterations in neuronal parameters, such as brain morphology, neuronal firing rates, cellular metabolism, neurotransmitter concentrations, and release, as well as influencing the number and sensitivity of receptors and the level of gene transcription and protein production. Investigations are now under way to determine how these changes in the structure and function of the brain after physical activity translate to alterations in behavior. These changes may help to elucidate the biologically plausible mechanism that explains the improvements in mental health observed after exercise.

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Indole-3-carbinol (and other phytochemicals) in the maternal diet of mice acts as a transplacental cancer chemoprotection agent

Indole-3-carbinol (I3C) is a major component of cruciferous vegetables and is also available as a dietary supplement. I3C provides chemoprotection against cancer in a number of animal models and has been utilized in human clinical trials, primarily for the prevention of breast cancer.

We have developed a mouse model of transplacental carcinogenesis in which pregnant animals administered dibenzo[*a,l*]pyrene (DBP, a polycyclic aromatic hydrocarbon produced by the burning of coal, diesel, cigarettes, and other carbon sources) a few days before giving birth produce offspring that exhibit high mortality due to a very aggressive lymphoma. One hundred percent of the offspring that survive to ten months of age have multiple lung tumors.

Incorporation of I3C into the maternal diet during the second and third trimesters and lactation provide significant protection for the offspring with respect to both the lymphoma and the lung cancer. These results are significant given that cancer is the second leading cause of death in children and leukemias/lymphomas are the most prevalent childhood cancers. Lung cancer is the number one cause of cancer mortality in both sexes in the U.S. We find it remarkable that a brief exposure early in development (fetal and infant) to the phytochemical I3C could provide this degree of chemoprotection, not just against cancer that impacts young adults (three to six months in a mouse), but also out to middle age (ten months).

Recently, we have expanded this model to test other mechanisms of cancer initiation and other phytochemicals. I3C in the maternal diet significantly protects offspring from lymphoma-dependent mortality due to loss of the tumor suppressor gene, p53, demonstrating that this strategy of chemoprotection can protect against genetically caused (in addition to chemical carcinogens) cancer.

Finally, we have demonstrated that, in the DBP-transplacental cancer model, caffeinated green tea or caffeine alone, administered to the pregnant mother, provided significant protection against lymphoma-dependent mortality in her offspring. Decaffeinated green tea and EGCG alone had no effect. However, all four treatments delayed the appearance of lung tumors in the ten-month-old mice. The most recent study documents the greatest chemoprotection effect yet. If the mother is given the

water-soluble derivative of chlorophyll, chlorophyllin, at the same time as the carcinogen, an almost complete elimination of lymphoma in the offspring is evident.

These results open a new avenue for cancer chemoprevention. We have demonstrated that it is possible to significantly reduce the risk of cancer in individuals if their mother is provided potent cancer chemoprotection phytochemicals in her diet, even if the individual never consumes those same phytochemicals. *Supported by NIH grants CA90890, ES07060, ES00210, and The Linus Pauling Institute.*

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Overview of flavonoids in human health

Flavonoids are dietary compounds which have some health implications. All are antioxidant *in vitro*, but their action *in vivo* is more complex. Although there are many flavonoids in nature, only a few are important in Western diets, including some catechins, procyanidins, flavonols (e.g. quercetin), flavanones (e.g. hesperidin, naringenin), and anthocyanins. In addition, the non-flavonoid but related phenolic acids (e.g. caffeic and ferulic acids) and isoflavones are also important. This paper will develop the hypothesis that dietary flavonoids and phenolics are “lifespan essential” since they have multiple actions *in vivo* which combine to reduce the risk of chronic disease (especially cardiovascular and other inflammatory diseases).

Different classes of flavonoids may have different effects and mechanisms, but human studies are generally not done on pure compounds, so it can be difficult to ascribe an effect *in vivo* to an individual flavonoid. They are not needed for growth and development, but are needed for stress protection, which includes returning or partially returning a biomarker back to optimal. For this reason, it is not always possible to observe an effect in young, healthy volunteers. In addition, a flavonoid must be bioavailable in order to exert an effect *in vivo*, and this aspect is also discussed in relation to the bioefficacy.

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Choline: critical role during fetal development

Choline is used to make a nerve messenger chemical (acetylcholine), cell membranes, and other important chemicals that are essential to the body's functioning. It's also the major source of methyl-groups in the diet, and affects very low-density lipoprotein transport from liver. Although our bodies are able to synthesize choline, most people need choline sources in the diet. Common genetic variations that occur in approximately half of the population (called single nucleotide polymorphisms) contribute to variation in human choline dietary requirements, and though the recommended intake is about half a gram a day, some individuals require significantly more.

Choline is critical during fetal development, when it influences stem cell proliferation and apoptosis (cell suicide) in the developing brain, thereby altering structure and function. Choline availability to the fetus also permanently influences memory function. At least in part, this effect of choline on brain development is mediated by altering the on-off switches that control genes (epigenetic changes involving DNA methylation). In addition, adults deprived of dietary choline develop fatty liver, liver cell death, and muscle cell death (rhabdomyolysis). Therefore, everyone should aim for a diet with great variety that includes foods rich in choline.

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