Angiogenesis Abstracts

**Se-[(Methyl)selenocysteine Causes Tumor Vessel Maturation Leading to Synergistic Antitumor Effect in Human Xenografts.** Arup Bhattacharya,1 Károly Tóth,1 Shousong Cao,1 Rami G. Azrak,1 Farukh A. Durrani,1 Yourcef M. Rustum,1 and Lakshmi Pendyala.2 Departments of 1Cancer Biology and 2Medicine, Roswell Park Cancer Institute, Buffalo, NY.

Pretreatment with 5-methylselenocysteine (MSC) before chemotherapy leads to synergistic chemomodulation with a wide range of anticancer drugs including taxanes, taxols, fluoropyrimidines, irinotecan (CPT-11), platinum compounds, and cyclophosphamide. This synergy is neither drug, tumor, nor host specific and was not seen in vitro. MSC down-regulates vascular endothelial growth factor, cyclooxygenase-2, inducible nitric oxide synthase, hypoxia-inducible factor-1α, and prostaglandin E2 in tumors, leading to an antiangiogenic effect. The hallmarks of tumor vasculature lacking smooth muscle and pericytes are that they are leaky, are chaotic, and shut off and on intermittently, imparting a tortuous tumor blood flow and distribution. The resultant adverse intratumoral interstitial fluid pressure retards intratumoral drug delivery and distribution. Studies were performed using female athymic nude mice bearing different xenografts that were treated daily for up to 14 d with MSC at 0.2 mg/d p.o. to determine whether antiangiogenic effects of MSC involve tumor vessel maturation and whether vessel maturation leads to higher intratumoral drug delivery, leading in turn to better therapeutic efficacy. Immunohistochemical study for vessel maturation used CD31 and α-smooth muscle actin double staining on frozen tumor sections. Immunofluorescence quantification. Within 24 h, 15 μmol/L of SUL clearly induced G2/M accumulation and premetaphase arrest in BAE cells. Moreover, immunofluorescence tubulin staining indicated that this same SUL concentration not only disrupted mitotic progression but also perturbed normal polymerization of mitotic (and cytoplasmic) microtubules. Furthermore, daily administration of SUL (100 nmol/d, i.v. for 7 d) to female BALB/c mice bearing VEGF-impregnated Matrigel plugs strongly and significantly (P < 0.05) suppressed angiogenesis progression as measured by hemoglobin concentration. Taken together, these findings suggest that the endothelial cell population is a novel target of sulforaphane action both in vitro and in vivo. This mechanism of SUL-induced endothelial microtubule disruption and early mitotic arrest may further discern a potential role of SUL as a chemopreventive agent.

**Sulforaphane Suppresses Angiogenesis and Disrupts Endothelial Mitotic Progression and Microtubule Polymerization.** Steven J.T. Jackson,1 Keith W. Singletary,2 and Richard C. Venema.1 1Vascular Biology Center, Medical College of Georgia, Augusta, GA; and 2Department of Food Science and Human Nutrition, University of Illinois, Urbana, IL.

Sulforaphane (SUL), an isothiocyanate derived from broccoli and other cruciferous vegetables, induces phase II detoxification enzymes, disrupts cancer cell microtubule polymerization, and triggers cell cycle arrest in breast and colon cancer cells. Here, we provide the first evidence that SUL also inhibits angiogenesis via suppression of endothelial cell proliferation. Bovine aortic endothelial (BAE) cells were exposed to concentrations of SUL up to 15 μmol/L before cell cycle analysis and mitotic index qualification. Pretreatment with 5-methylselenocysteine (MSC) before chemotherapy leads to tumor vessel maturation and whether vessel maturation leads to better therapeutic synergy. Immunohistochemical study for vessel maturation used CD31 and α-smooth muscle actin double staining on frozen tumor sections. Immunofluorescence quantification. Within 24 h, 15 μmol/L of SUL clearly induced G2/M accumulation and premetaphase arrest in BAE cells. Moreover, immunofluorescence tubulin staining indicated that this same SUL concentration not only disrupted mitotic progression but also perturbed normal polymerization of mitotic (and cytoplasmic) microtubules. Furthermore, daily administration of SUL (100 nmol/d, i.v. for 7 d) to female BALB/c mice bearing VEGF-impregnated Matrigel plugs strongly and significantly (P < 0.05) suppressed angiogenesis progression as measured by hemoglobin concentration. Taken together, these findings suggest that the endothelial cell population is a novel target of sulforaphane action both in vitro and in vivo. This mechanism of SUL-induced endothelial microtubule disruption and early mitotic arrest may further discern a potential role of SUL as a chemopreventive agent.

**In Vivo Imaging and Characterization of Hypoxia-Induced Neovascularization and Tumor Invasion.** Gina F. Lungu,1 Meng-Lin Li,2 Xueyi Xie,1 Lihong V. Wang,2 and George Stoica.1 1Department of Pathobiology, College of Veterinary Medicine, and 2Optical Imaging Laboratory, Department of Biomedical Engineering, Texas A&M University, College Station, TX.

A diet rich in fruits, vegetables, and other plant foods may reduce the risk of cancer, but few links to cancer have been established. Finding a method to determine the effect of dietary compounds on cancer and to monitor the disease progression will be of interest. This study provides an integrated approach to define oxygen status (hypoxia) of intracranial tumor xenograft using a novel spectroscopic photoacoustic tomography technology (SPAT). This approach is supported by our molecular biology investigation. Brain tumors can be identified by their distorted vascular architecture and oxygen saturation without any risk of additive host tissue toxicity. Better drug delivery leads to better therapeutic efficacy.

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images. Noninvasive in vivo tumor oxygenation imaging using SPAT is based on the spectroscopic absorption differences between oxyhemoglobin and deoxyhemoglobin. Sprague-Dawley rats inoculated intracranially with ENU1564, a carcinogen-induced rat mammary adenocarcinoma cell line, were imaged with SPAT 3 wk postinoculation. Proteins important in tumor hypoxia and invasion were detected in hypoxic brain foci identified by SPAT and were elevated compared with normal brain. Immunohistochemistry, Western blotting, and semiquantitative RT-PCR showed that hypoxia-inducible factor-α, vascular endothelial growth factor-A, and vascular endothelial growth factor-R2 protein and mRNA expression levels were significantly higher (P < 0.05) in brain tumor tissues than in normal brain. Gelatin zymography and RT-PCR demonstrated the up-regulation of matrix metalloproteinase-9 in tumor foci compared with normal brain. Together these results suggest the critical role of hypoxia in driving tumor angiogenesis and invasion through up-regulation of target genes important for these functions. Moreover, this report validates our hypothesis that SPAT is suitable for detecting tumors, hypoxia, and angiogenesis and for monitoring a cell-targeted therapeutic approach to brain malignancies. We hope that SPAT will be used in the near future to monitor the effect of different dietary compounds on brain malignancy.

**Apoptosis**

**Inhibition of Na+/K+-ATPase by Ouabain Potentiates Apoptosis by Inducing Perturbations in Cell Calcium Homeostasis: A Protective Role Selective for Bcl-2.** Michail Panagiotidis and John A. Cidlowski. Laboratory of Signal Transduction, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Apoptosis is a well-defined programmed process with distinctive characteristics including cellular shrinking, chromatin condensation, and formation of membrane-bound apoptotic bodies. Cell shrinkage occurs early in apoptosis and is accompanied by changes in the activity of ion channels and plasma membrane transporters. Na+/K+-ATPase couples the energy released from the hydrolysis of ATP to the transport of Na+ and K+ ions, thus controlling cell volume and plasma membrane potential. Additionally, the Na+/K+-ATPase is the only known receptor for cardiac glycosides including ouabain. We hypothesize that Na+ / K+ -ATPase is essential in modulating apoptosis, and thus, its inhibition with ouabain should potentiate the apoptotic process. We report that ouabain potentiated apoptosis in wild-type Jurkat cells exposed to Fas ligand (FasL) by increasing the percentage of shrunken cells, percentage of degraded DNA content, and caspase 3/7-like activity. When cells were treated with ouabain and then exposed to other apoptotic agents [H2O2, staurosporine, TNF-related apoptosis-inducing ligand (TRAIL), thapsigargin], only exposure to TRAIL showed potentiation of apoptosis, indicating that such potentiation is restricted to the death receptor pathway (FasL and TRAIL). In addition, we observed that ouabain induced perturbations in cell calcium homeostasis on both FasL and TRAIL treatments. Ouabain-induced potentiation of apoptosis was abolished in Bcl-2–overexpressing cells by inhibiting perturbations in cell calcium homeostasis, whereas Bcl-XL overexpression had no effect. To conclude, our data indicate a novel role for calcium in modulating ouabain-induced potentiation of apoptosis and that Bcl-2 selectively modulates calcium homeostasis.

**Biomarkers**

**Association of Hydroxynonenal- 1, N2-Propanodeoxyguanosine Adduct Levels in Human Lymphocytes with DNA Repair Gene XPA and Nutritional Markers in the Bavarian Nutrition Survey.** Erwin Eder, Odilia Popanda, Paul Wanek, Ellen Biskup, and Jakob Linseisen.

1Department of Toxicology, University of Würzburg, Würzburg, Germany; 2Toxicology and Cancer Risk Factors, German Cancer Research Center (DKFZ), Heidelberg, Germany; 3Clinical Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany. 1, N2-Propanodeoxyguanosine adducts of 4-hydroxynonenal are appropriate biomarkers for cancer risk from lipid peroxidation. Adduct levels were measured in human lymphocytes in the Bavarian Nutrition Survey. The study investigated dietary behavior and lifestyle in a representative population sample in Bavaria: 1050 participants aged 13–80 y. In a substudy, 568 adult participants of both genders aged 18–80 y with complete dietary information were invited for blood sampling. Here we present the results of 278 samples evaluated to date. A significant inverse correlation of adduct levels was observed.
with eicosapentaenoic acid [20:5(n-3)] concentration in red blood cell membranes. No clear correlations were found between adduct levels and other fatty acids. Surprisingly, a positive correlation was seen between plasma vitamin C concentrations and adduct levels. No significant correlations were observed between adduct levels and plasma concentrations of other antioxidants. Socioeconomic status significantly correlated with adduct levels: the higher the status, the higher the adduct levels. The individual 1,N2-propanodeoxyguanosine adduct level can be modulated not only by lifestyle and nutrition factors but also by the individual DNA repair capacity. We also studied the influence of repair polymorphisms. With the XPD Asp312Asn variants, no changes in adduct levels were seen. Adduct levels increased in the XPD Lys248Gln variants from 54.3 mean adducts/10^9 nucleotides in Lys/Lys (n = 126) to 60.6 in the Lys/Gln (n = 126) and to 63.2 in Gln/Gln (n = 40). In the XPA (-4G/A) variants, the adduct levels increased from 56.4 in variant G/G (n = 112) to 58.3 in G/A (n = 126) and to 64.4 in A/A (n = 40). After adjustment for nutrition factors, a significantly increased odds ratio of 2.5 (CI 1.05, 5.8) was determined for having high adduct levels in XPA A/A carriers, suggesting a higher cancer risk in these individuals.

DNA from Food as a Biomarker for Human Diet. Douglas Spangler,1 Richard Rivlin,2 and David S. Thaler.1

There is a recognized need to develop new, sensitive, quantitative, and objective biomarkers of human dietary intake (1). Species- and group (e.g., vegetable)-specific DNA sequences are potential biomarkers. We have developed a methodology of food-specific DNA analysis by Q(quantitative)PCR that can be applied to a wide range of clinical samples including blood, urine, salvia, and stool. Analyzing DNA in human biological fluids has the potential to provide novel biomarkers of dietary intake. Use of these biomarkers in clinical contexts will require and inform the understanding of individual variation in rates of digestion, intestinal transit, absorption, microbial processes, and excretion that are likely to be under genetic, physiological, and microecolological controls. A clinical study is ongoing at the Rockefeller University Hospital. Our first analyses were consistent with the hypothesis that DNA sequences unique to vegetables and to fish were present in the blood of normal humans who ingest these foods. However, DNA from these blood samples was isolated with commercial kits and columns manufactured by Qiagen. Our subsequent sequencing of the PCR amplicons indicates that at least some of the “food DNA” signals derived from purification reagents, most likely the silica-containing spin columns. Others have reported finding related contaminants in commercial DNA purification reagents (2,3). Contamination of silica columns may be difficult to avoid because of the way they are currently manufactured. Taken together, we believe that these findings call into question the use of currently available commercial silica-based purification columns for certain contamination-sensitive DNA purifications for research, medical-diagnostic, and forensic applications. [Supported by NCI and by The Sloan Foundation.]

Dietary Carbohydrate as a Biomarker of Red Meat and Dairy Intake. Eric I. Park. Department of Nutrition, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC.

High consumption of red meat has been associated with increased risk of colorectal cancer. However, the precise dietary component or the mechanism that accounts for this increased risk is unknown. One abundant component of red meat that is gaining interest is a type of carbohydrate moiety known as N-glycolyneraminic acid (NGNA). NGNA accumulates on the cell surfaces of colon cancer, breast cancer, and hepatocellular carcinoma cells, whereas the surrounding normal cells have only trace amounts. Even more intriguing is how this accumulation occurs, because normal and cancerous human cells cannot synthesize NGNA. One likely source of NGNA is the consumption of red meat and dairy products, which are abundant in NGNA, and the subsequent metabolism of the NGNA by the same intracellular enzymes and transporters that metabolize endogenous sialic acids. Despite the possibility of NGNA serving as a biomarker of red meat and dairy intake and for tagging and identifying cancer cells, little is known about the metabolism of dietary NGNA. Toward this goal, pilot studies were performed to determine the feasibility of using NGNA as a marker of red meat and dairy intake. First, a human cell line experiment showed that the amount of NGNA accumulation on the surface of cells depends on the NGNA concentration in the cell medium. Second, HPLC analyses showed that concentrations of NGNA were the same in raw and cooked meats. Third, the amounts of NGNA in human serum and urine samples were detected by HPLC and NMR methods, respectively. These studies suggest that the NGNA concentration in human samples may be useful as a marker of red meat and dairy consumption and a measurement to validate the real consumption levels of self-reported red meat and dairy intakes.

Food Composition

Sources of Flavonoids in the U.S. Diet Using USDA’s Updated Database on the Flavonoid Content of Selected Foods. D.B. Haytowitz, S. Bhagwat, J.M. Holden, S.E. Gebhardt, and J. Harnly. USDA-ARS, Nutrient Data Laboratory and Food Composition Laboratory, Beltsville, MD.

Flavonoids are biologically active polyphenolic compounds widely distributed in plants and have been linked to various chemoprotective effects (1). USDA first released a database on the flavonoid content of foods in 2003. The database was recently updated using data from USDA analysis of 20 different flavonoid monomers from a nationwide sampling of 59 fruits, nuts, and vegetables, providing high-quality U.S. data not available in the earlier database. Data from 102 scientific papers were also added. The new database contains flavonoid data for 395 food items and is available on Nutrient Data Laboratory’s (NDL’s) Web site (2). These data were combined with data from

NDL's Key Foods list derived from consumption data from the National Health and Nutrition Examination Survey to ascertain the intake of 5 classes of flavonoids—anthocyanidins, flavanones, flavonols, flavones, and flavan-3-ols—on a population basis. Black tea provided the largest amount of flavonols to the diet (32%), followed by onions (25%). Parsley was the largest contributor of flavones. Dried parsley contains a large amount—13.53 mg/100 g—though rarely is 100 g consumed at one time. Oranges (53%) and grapefruit juice (16%) provide significant amounts of the flavanones. Brewed tea provides the largest quantities of flavan-3-ols to the diet. Blueberries contributed the largest amount of anthocyanidins (31%), followed by bananas (21%) and strawberries (14%). Even though bananas contain considerably less anthocyanidins than any of the berries, U.S. consumption of bananas is much higher than that of individual berries. Daily per capita intake of flavonoids in the United States using these data were as follows: anthocyanidins, 5 mg; flavanones, 4 mg; flavones, 1 mg; flavonols, 10 mg; and flavan-3-ols, 112 mg. This expanded database provides researchers with new values on the flavonoid content of many more foods and should enable better assessment of the effect of flavonoid consumption on various chronic diseases.


Nuts and Seeds as Sources of α- and γ-Tocopherol.
Robin G. Thomas and Susan E. Gebhardt. USDA-ARS Nutrient Data Laboratory, Beltsville, MD.
Some nuts and seeds are among the highest natural sources of vitamin E in the U.S. food supply. In its chief function as an antioxidant, vitamin E prevents free radical reactions, which is important in protecting cells from oxidative damage. Vitamin E has been associated with reduced risk of certain cancers such as colon, bladder, and prostate cancers. Recent studies have focused on effects of γ-tocopherol as well as α-tocopherol. Of the 4 tocopherols (α, β, γ, and δ), α-tocopherol is the only one used to estimate the current Recommended Dietary Allowance (RDA) for vitamin E. The other tocopherols are absorbed and may have other functions but are not converted to α-tocopherol in the body. The RDA for vitamin E is 15 mg/d of α-tocopherol for adults. According to the National Health and Nutrition Examination Survey 2001–2002, >90% of adults do not meet the Estimated Average Requirement of 12 mg/d. Nuts and seeds are often cited as good sources of vitamin E. USDA recently updated tocopherol values in several nuts and seeds in the USDA National Nutrient Database for Standard Reference (SR). One-ounce portions of almonds, hazelnuts, and sunflower seeds provide >20% of the RDA for vitamin E, and Brazil nuts and pine nuts provide 10–20% of the RDA. One-ounce portions of cashews, macadamias, pecans, pistachios, black and English walnuts, flaxseed, and sesame seeds all provide 1–4% of the RDA. The highest nut and seed sources of γ-tocopherol are black walnuts and sesame seeds (28 mg/100 g), pecans (24 mg/100 g), pistachios (22 mg/100 g), and English walnuts and flaxseed (20 mg/100 g). These tocopherol values are derived from data from USDA studies, the food industry, and the scientific literature. Keeping SR up to date allows researchers to more accurately estimate nutrient intake, thus enabling them to study the relationships between diet and disease more effectively.

Disparities in Environmental Risk Factors for Cancer Prevention in 2 Rural Cities. Chellani S. Hathorn, Peter N. Gichuhi, Elaine Bromfield, Samia Ibrahim, and Adelia C. Bovell-Benjamin. Department of Food and Nutritional Sciences, Tuskegee University, Tuskegee, AL.

In the United States, African Americans (AAs) have the highest cancer incidence and lowest survival rate. The reasons for this disparity have not been elucidated, but dietary factors, which are largely modifiable, have been implicated. Potentially adverse dietary practices implicated in cancer risk have been associated with AA diets. This study examined nutrition knowledge, dietary beliefs, and perceived barriers related to cancer that prevent AA college students from adopting eating behaviors consistent with National Cancer Institute (NCI) dietary guidelines. A cross-sectional, in-depth structured interview was administered to students attending two historically black colleges and universities. Four hundred fifty-six students (238 male; 216 female) aged 18–30 y participated. A little more than half of the students (56.4%) correctly identified the food lowest in fiber from a given list, and the majority (84.7%) knew that lower fat intake reduces cancer risk. Although 38% of the students believed that cancer may be related to what people eat, 34.6% did not believe that cancer was related to diet, and 35% did not know that consumption of salt-cured, salt-pickled, and smoked foods is associated with cancer. Most students (83%) were unaware of NCI dietary guidelines. However, 82% of the students indicated that nutrition counseling and specific instructions on how to make dietary changes to prevent cancer could help them to modify their eating behaviors. Students believed that fatty and fast foods increased cancer risk. Availability, food preferences, convenience, cost, lack of support from family members, and little knowledge of healthy foods and their preparation were identified as barriers to healthy eating.

Dietary Pattern and Cancer Risk
Diet and Cancer Prevention Knowledge, Beliefs, and Barriers among Students in Historically Black Colleges and Universities. Adelia C. Bovell-Benjamin, Peter N. Gichuhi, Chellani S. Hathorn, and Elaine Bromfield. Food and Nutritional Sciences, Tuskegee University, Tuskegee, AL.

The burden of cancer is unequally distributed among populations and geographic locations. Minority populations, especially low-income African Americans, have higher mortality rates from cancer than other ethnic groups. Environmental factors explain some of the disparity in cancers among low-income minority groups; therefore, assessment of environmental risk factors (such as diet and physical activity) in communities may be important in cancer-prevention efforts. This study compared the availability of healthy food and physical activity choices that encourage cancer risk reduction in 2 rural cities: 1 high income and 1 low income. An unobtrusive, observational cross-sectional study was conducted using the United States Department of Agriculture Thrifty Food Basket Checklist. Four convenience stores, 9 fast-food restaurants, 7 restaurants, 7 large supermarkets, and 3 mass merchandisers located within a 4-mile radius in both cities were inventoried. In the low-income city, none of the 3 large supermarkets carried low-sodium vegetables. The fast-food restaurant inventoried offered no fruit items in the low-income city, whereas 2 in the high-income city did. Low-fat milk was available in all the large supermarkets. Low-sodium, low-fat cheese was available in only 2 supermarkets in the low-income city.
Meat Consumption, N-Acetyl Transferase 1 and 2 Polymorphism, and Risk of Breast Cancer in Danish Postmenopausal Women. Rikke Egeberg,1 Anja Olsen,1 Herman Autrup,2 Jane Christensen,3 Connie Stripp,3 Kim Overvad,3 and Anne Tjønneland.1 (1) Institute of Cancer Epidemiology, The Danish Cancer Society, Copenhagen, Denmark; 2Department of Environmental and Epidemiology Research Unit, RC, CHUM, University of Montreal, Montreal, Canada; 3Department of Environmental and Occupational Medicine, Institute of Public Health, University of Aarhus, Denmark; 4Department of Clinical Epidemiology, Aalborg Hospital, Aarhus University Hospital, Aalborg, Denmark.

In 1997 the World Cancer Research Fund concluded that meat intake might have a possible association with the risk of breast cancer. However, recent epidemiologic evidence concerning this relationship is controversial. We conducted a nested case-control study with 24,697 postmenopausal women included in the Diet, Cancer, and Health cohort study (1993–2000) to investigate the association between meat intake and breast cancer risk and whether polymorphisms in N-acetyltransferase 1 (NAT1) and 2 (NAT2) modified this association. A total of 378 breast cancer cases were identified and matched to 378 control subjects. The analyses were based on a conditional logistic regression analysis corresponding to a Cox proportional hazard model because of the study design used. All models were adjusted for baseline values of established risk factors for breast cancer. The incidence rate ratio (IRR) (95% CI) for breast cancer was 1.09 (1.02, 1.17) for total meat, 1.15 (1.01, 1.31) for red meat, and 1.23 (1.04, 1.45) for processed meat per 25 g/day increment in intake. The IRR (95% CI) in fast NAT1 acetylators compared with NAT1 slow acetylators was 1.43 (1.03, 1.99) and in intermediate to fast NAT2 acetylators compared with slow NAT2 acetylators was 1.13 (0.83, 1.54). Interaction analyses revealed that the positive associations between total meat intake and red meat intake and breast cancer risk were confined to intermediate-to-fast NAT2 acetylators (Pinteraction = 0.03 and 0.04), whereas no significant interaction was shown for NAT1 acylator phenotypes. In conclusion, our findings support an association between meat consumption and breast cancer risk and a modifying effect of NAT2 polymorphism on the association.

Diet Quality Predicts BRCA-Associated Breast Cancer Risk. André Nkondjock and Parviz Ghadirian. Epidemiology Research Unit, RC, CHUM, Montreal, Canada.

Although it has been suggested that dietary energy intake restriction may be related to reduced BRCA-associated breast cancer risk, it is currently not known whether overall diet quality could predict the risk among women with deleterious mutations in BRCA1 and BRCA2 genes who already have an elevated breast cancer risk. To assess possible relationships between diet quality—reflected by the Alternate Healthy Eating Index (AHEI), the Diet Quality Index-Revised (DQI-R), and the Canadian Healthy Eating Index (CHEI)—and BRCA-associated breast cancer risk, a case-control study was carried out within a cohort of 80 French-Canadian families involving 89 carriers of BRCA genes affected by breast cancer, 48 nonaffected carriers, and 46 nonaffected noncarriers. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated in unconditional logistic regression models. After adjustment for age, physical activity, and total energy intake, we did not detect any association between AHEI or aMED and breast cancer. However, a strong and significant inverse relationship was apparent between DQI-R and CHEI and BRCA-associated breast cancer risk. ORs comparing the highest and lowest tertiles of diet quality scores were 0.35 (95% CI 0.12 to 1.02; P< 0.034) for DQI-R and 0.18 (95% CI 0.05 to 0.68; P< 0.006) for CHEI, respectively. These inverse associations were not the result of a link with any specific component of the diet quality indexes. Findings from this study suggest that dietary guidelines reflected by DQI-R and CHEI may constitute preventive strategies for reducing BRCA-associated breast cancer risk.
Calorie Restriction

Glucose Restriction Prevents Epigenetic Changes Associated with Carcinogenesis. Joel B. Berletch, Liang Liu, Lucy G. Andrews, and Trygve O. Tollefsbol. Department of Biology and Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, AL.

It is well documented that calorie restriction (CR) is an effective experimental means for decreasing cancer risk in various model organisms. It is unlikely, however, that most humans would be willing to maintain a 30–50% reduction in calorie intake for the bulk of their adult life span, even if it means reduced cancer risk. A better option is to identify critical CR mimetics, agents that can induce the same beneficial effects as CR but without dieting, that can be used in dietary intervention. Dietary glucose may be the key factor determining the beneficial effects of CR in reducing the risk of cancer because reduced glucose metabolism is thought to have health- and longevity-promoting effects without actually decreasing food intake in rats. In fact, our preliminary studies indicated for the first time that glucose-restricted human fibroblasts undergo senescence at a slower rate than cells receiving normal levels of glucose. Early studies indicated that CR can change the level of gene products that are involved in epigenetic modifications of important cellular processes such as acetylation of histone proteins. DNA methyl- ation is another important epigenetic process that plays a critical role in gene regulation. Genome-wide hypomethylation occurs in aging cells and is associated with cancer. We have found that the induction of the DNA methyltransferase 1 gene, which is associated with cancer, is markedly decreased in glucose-restricted MCF-7 breast cancer cells. Taken together, these studies indicate that dietary glucose restriction may have important cancer-preventive implications.

The Effect of Intermittent Versus Chronic Energy Restriction on Breast Cancer Risk Biomarkers in Premenopausal Women: A Randomized Pilot Trial. Michelle Harvie,1 Mary Chapman,1 Jack Cuzick,2 Alan Flyvbjerg,2 Penny Hopwood,4 Susan Jebb,6 Gaenor Parfitt,6 and Anthony Howell.4 1School of Medicine, Withington Hospital, The University of Manchester, Manchester, UK; 2CRUK Department of Epidemiology and Statistics, Wolfson Institute, London, UK; 3Medical Research Laboratories, Aarhus University, Denmark; 4CRUK Department of Medical Oncology, Christie Hospital, Manchester, UK; 5MRC Human Nutrition Research Group, Cambridge, UK; 6Scholl School of Sport and Health Science, University of Exeter, UK.

Postmenopausal breast cancer risk increases 2-fold in women who gain significant amounts of weight (1), and there is evidence that energy restriction may reduce risk (2). Animal studies indicate that intermittent energy restriction (IER) reduces risk and may be superior to continuous energy restriction (CER) (3). We showed that chronic energy restriction reduces biomarkers of breast cancer in women at risk but is hard to maintain. We hypothesize that IER may be superior to CER in reducing bio- markers of breast cancer risk and may also be more acceptable to women. We are undertaking a 6-mo randomized trial to compare the 2 approaches in 104 premenopausal women aged 30–45 y, at high risk for breast cancer because of family history and adult weight gain of >7 kg. Women will be randomly assigned to either CER (75% estimated energy requirements: ~1500 kcal 7 d/wk) or IER (75% estimated energy requirements: 650 kcal for 2 d and ~1800 kcal 5 d/wk) over 6 mo. Study endpoints will be measures of insulin sensitivity (homeo- stasis model assessment, sex hormone binding globulin, testos- terone), potential breast cancer growth factors (insulin-like growth factor axis, leptin, adiponectin), inflammatory markers (C-reactive protein, sialic acid), oxidative stress marker (urinary F2 isoprostane), weight and body composition (waist-hip circumference, fat-free and total fat mass). The relative acceptability of IER and CER will be assessed using quality of life questionnaire (RAND SF-36) and scales of behavior change and adherence. The relative efficacy and acceptance of IER and CER will inform future weight loss programs to prevent breast cancer. Currently 14 women have been recruited to the study (5 CER and 9 IER). This pilot study will be completed by December 2007.


Obesity

Dietary Fat Predicts Biochemical Failure Only among Prostate Cancer Patients with Lower Gleason Scores. Yuko Yamamura,1 Patricia Troncoso,2 and Sara S. Strom.1 Departments of 1Epidemiology and 2Pathology, University of Texas M.D. Anderson Cancer Center, Houston, TX.

We demonstrated that obesity at diagnosis (BMI > 30) is a predictor of biochemical failure, measured by rising prostate-specific antigen, in prostate cancer (PCa) patients treated with radical prostatectomy. Given that Gleason score is the best
predictor of biochemical failure, we compared patients with less aggressive PCa [LAPCa; Gleason score < 7(3+4)] vs. more aggressive PCa [MAPCa; Gleason score > 7(4+3)] with respect to factors associated with obesity, such as dietary fat intake and weight gain, along with clinicopathologic and demographic variables. We hypothesize that risk of biochemical failure in LAPCa versus MAPCa is modulated by different factors. In a well-characterized cohort of 390 Caucasian men with PCa treated initially with only radical prostatectomy, we used multivariable Cox proportional hazards models to estimate risk of progression. As expected, in both groups pathologic stage and surgical margin involvement remained independent predictors of biochemical failure. Of 213 patients with LAPCa, energy-adjusted high-fat intake [hazard ratio (HR) = 3.59, \( P = 0.04 \)] was a significant independent predictor of biochemical failure; however, no association was found with weight change. In MAPCa patients, annual weight change between age 25 and diagnosis (continuous) but not dietary fat intake was associated with increased risk of biochemical failure (HR = 1.82, \( P = 0.03 \)). These data suggest that different combinations of clinicopathological and environmental factors, such as diet and weight gain, play different roles in LAPCa versus MAPCa in predicting biochemical failure after radical prostatectomy. To further our understanding of these findings, we plan to identify and explore possible mechanisms underlying our results. [Supported by NCI CA84964, CA90270, NIEHS ES07784.]

**Survivorship**

**The Effect of a Lifestyle Intervention on Body Weight, Psychological Health Status, and Risk Factors Associated with Disease Recurrence in Women Recovering from Breast Cancer Treatment: Study Protocol and Interim Findings.** Emma Scott,1 Amanda Daley,1 Nicola Woodroofe,2 Robert Coleman,3 Hilary Powers,4 Nanette Mutrie,1 Vanessa Siddall,1 Helen Crank,1 and John M. Saxton.1 1Centre for Sport and Exercise Science, 2Division of Genomic Medicine, 3Cancer Research Centre, and 4Human Nutrition Unit, Sheffield Hallam University, Sheffield, UK.

A large proportion of women gain weight after breast cancer diagnosis, and there is evidence that heavier women have an increased risk of disease recurrence and death compared with normal-weight women. After diagnosis, women can also experience high levels of emotional distress, which might affect stress hormone levels and impair immune function, thereby affecting cancer outcome. The aims of this research are to investigate the effects of a lifestyle intervention on body weight and psychological well-being in postmenopausal women recovering from breast cancer treatment and to determine the relation between the changes in these variables and circulating biomarkers associated with disease recurrence and survival. Patients are randomly assigned to 2 groups: a 6-mo lifestyle intervention or a normal-care control group. The lifestyle intervention combines dietary energy restriction and nutrition education with supervised aerobic exercise training. It was hypothesized that the lifestyle intervention would evoke a reduction in body weight and an improvement in psychological well-being. Furthermore, a decrease in body weight is expected to be associated with reductions in the circulating levels of estrogens and inflammatory mediators. Associations among changes in psychological well-being, stress hormone levels, and immune function are also being studied. Body weight and body composition, anthropometric measures, and psychological well-being are assessed at the start, midpoint, and end of the study, whereas circulating biomarkers are measured pre- and postintervention. Preliminary 12-wk findings for 8 women show greater reductions in body weight for the intervention versus control group (−1.58 ± 3.00 kg vs. −0.83 ± 2.11 kg, mean ± SD, respectively). Compliance to the intervention has been excellent, with women attending at least 87.5% of the dietary counseling and exercise sessions.

**Strategies for Health: Follow-Up Care Practices and Beliefs among African American Breast Cancer Survivors.** Renee Royak-Schaler,1 Susan Racine Passmore,1 Katherine Tkaczuk,1 Joseph Finkelstein,1 Jimmie Drummond,1 Peggy D. Nicholson,2 Alva P. Hutchison,1 and Shahnaz M. Gadalla.1 1University of Maryland School of Medicine, Baltimore, MD; 2Sisters Network Baltimore Chapter, Baltimore, MD; 3American Cancer Society, South Atlantic Division Inc., Atlanta, GA.

There is a gap in scientific understanding regarding the psycho-social and health concerns and follow-up care practices of African American breast cancer survivors after primary treatment is complete. Evidence-based guidelines for survivorship care are not readily available to clinicians (1). To address this gap we investigated 1) patient perceptions of patient-physician communication and follow-up care practices and 2) strategies for developing follow-up care plans that address the special needs of African American breast cancer survivors. Four focus groups were conducted with 39 African American women, mean age 55 y, to investigate the following themes: patient-physician communication and decisionmaking about follow-up care; other sources of information used in developing follow-up care plans (e.g., Internet or print material); and preferences and avenues for information delivery to survivors. Group members also completed survey questionnaires designed to support the focus group discussions. Participants identified no clear plan valued over others as a means to reduce their risk of breast cancer recurrence. They reported receiving minimal information from their physicians about state-of-the-art strategies for reducing their risk of recurrence, for example, weight management by low-fat diet and physical activity. Although 87% said they were satisfied with the information received from medical professionals, they acknowledged that it did not specifically target the health concerns of African American women (e.g., healthy eating in the context of traditional high-fat diets). African American survivors had a critical need for guidance from healthcare professionals in developing feasible plans of self-care that target the context of their lifestyles for improving breast cancer outcomes. [Supported by grants from the Lance Armstrong Foundation and the Susan G. Komen Breast Cancer Foundation, Maryland Affiliate.]


**Colorectal Cancer**

**Novel Combinations of Chemopreventive Agents Demonstrate a Synergistic Effect against Cell Proliferation in Colon Cancer Cell Lines.** Naveen Kantamneni and Sunil Prabhu. Department of Pharmaceutical Sciences, College of Pharmacy, Western University of Health Sciences, Pomona, CA.

We studied synergistic chemopreventive effects of a dual combination of agents (aspirin with folic acid; aspirin with...
Dietary Flax Products Suppress the Formation of Azoxymethane-Induced Colon Cancer in Fisher 344 Male Rats. Darlene S. Williams,1 Martha Verghese,1 Judith Boateng,1 Lloyd T. Walker,1 Louis Shackelford,1 Janak Khatiwada,1 David Asiamah,1 and Chandramohan B. Chawan.2 1Nutrition and Carcinogenesis Laboratory, Department of Food and Animal Sciences, Alabama A&M University, Normal, AL; 2USDA/AMS/PSY, Washington, DC. Flax is a rich source of bioactive components such as PUFA (fat); dietary fiber; α-linolenic acid (ALA), the essential (n-3) fatty acid; and lignans, which are phytoestrogens and antioxidants. This study was designed to determine the anticarcinogenic effect of flaxseed meal (FSM; 10% and 20%) and flaxseed oil (FSO; 7% and 14%) on azoxymethane (AOM)-induced colon cancer in Fisher 344 male rats at the initiation (I), promotion (P), and initiation plus promotion (I + P) stages of carcinogenesis. After an acclimatization period of 1 wk, 14 groups of Fisher 344 male weanling rats (15 per group) were created; 2 control groups were fed AIN 93G diet and AIN 93G + 14% soybean oil. The remaining 12 groups were assigned to 10% and 20% FSM (I, P, and I + P) and 7% and 14% FSO (I, P, and I + P). All rats received 16 mg/kg body weight of AOM at ages 7 and 8 wk. At age 45 wk rats were killed by CO2 asphyxiation. Tumor incidence and size, respectively, were significantly (P < 0.05) lower in groups fed FSM (10% and 20%) and FSO (7% and 14%) compared with controls. The ratio of tumors to tumor-bearing rats for groups fed the control diet, 10% FSM, and 20% FSM (I, P, and I + P) were 3.80; 1.60, 1.10, 1.0; and 1.66, 1.1, 1.0, respectively. In rats fed the 7% FSO, and 14% FSO (I, P, and I + P), the ratios were 1.40, 1.25, 1.42 and 1.90, 1.40, 1.42, respectively. The activity of glutathione-S-transferase (a phase II detoxification enzyme) was significantly (P < 0.05) higher in rats fed 10% and 20% FSM and 7% and 14% FSO compared with controls. The results of this study indicated that dietary flaxseed products may suppress colon tumors, particularly at the promotion stage, and may therefore be a potential chemopreventive agent.

Antitumor and Cytotoxic Properties of Red Kidney Beans (Phaseolus vulgaris): An In Vitro and In Vivo Model. Judith Boateng,1 Martha Verghese,1 Lloyd T. Walker,1 Louis Shackelford,1 Janak Khatiwada,1 Chandramohan B. Chawan,2 Darlene S. Williams,1 and David Asiamah.1 1Nutrition and Carcinogenesis Laboratory, Department of Food and Animal Sciences, Alabama A&M University, Normal, AL; 2USDA/AMS/PSY, Washington, DC. Dry beans are a good source of fiber and phytochemicals such as flavonoids and phenolic compounds and may be responsible for preventing the onset of chronic diseases. We examined the antitumor effects of red kidney beans (RKB) on azoxymethane (AOM)-induced colon tumors [aberrant crypt foci (ACF) and endpoint tumor model (EPTM)] and the cytotoxic effects of aqueous RKB extracts on colon cancer cell line CaCo2. For the ACF study, 12 Fisher 344 male rats were fed AIN-93G control diet containing fish oil and pectin diet induces apoptosis involves the prostaglandin and Wnt signaling pathways. [Funded by AICR 05B094, NIH CA61750, CA82907, NSBRI NASA NCC 9–58, CA59034, and NIEHS P30-ES09106.]
Breast Cancer

In Utero and Prepubertal Exposures to Indole-3-Carbinol Have Opposing Effects on Mammary Tumorigenesis in Rats. Leena Hilakivi-Clarke,1 Bin Yu,1 Moira B. Hilsher,1 and William Helferich.1,2 LCCC, Department of Oncology, Georgetown University, Washington, DC; and 2Department of Food Science and Human Nutrition, University of Illinois, Urbana, IL.

The age when an individual is exposed to various hormones or dietary compounds has a profound effect on how these compounds alter susceptibility to developing breast cancer. We used an animal model to investigate whether a maternal dietary exposure during pregnancy or prepubertally during postnatal weeks 2 and 3 to indole-3-carbinol (I3C), present in cruciferous vegetables, affects DMBA-induced mammary tumorigenesis in rats. The dose of I3C used, 2000 ppm, was previously used in several cancer prevention studies. Results indicated that offspring of dams fed I3C during pregnancy developed more mammary tumors than control rats fed AIN93 diet (increase of the Wnt signaling pathway in the I3C offspring may explain why in utero exposure increased later susceptibility to developing mammary tumors. We are now investigating whether prepubertal I3C exposure down-regulated the Wnt pathway.


Phytoestrogens are chemicals commonly found in foods of plant origin, and they can mimic the female sex hormone estrogen. The effects can be dramatic, such as the infertility in sheep from eating clover plants. Breast cancers are generally driven by estrogen, and effective treatments are based on removing or blocking this hormone. Can phytoestrogens in the diet or in dietary supplements act like estrogen and stimulate breast cancer growth, or can they block estrogen receptors like treatment with tamoxifen and slow down cancer growth? We are studying phytoestrogen levels in women with breast cancer to help answer this. In our study design, 2000 women in the United Kingdom with invasive breast cancer are recruited at diagnosis at 40 regional cancer centers, and their diet, phytoestrogen excretion, and clinical progress are monitored annually for 5 yr. The aim is to seek any association between phytoestrogen intake and time to death or time to first recurrence. Urine is collected to measure 9 phytoestrogens, and blood is collected to measure biomarkers and polymorphisms. Each year patients complete dietary questionnaires—the food frequency and 7-d food diary questionnaires developed for the European Prospective Investigation into Cancer and Nutrition—plus lifestyle, quality-of-life, and complementary therapies questionnaires. Phytoestrogen intake is measured from 24-h and random urine samples with mass spectrometry and an immune assay. The phytoestrogens currently measured are enterodiol, enterolactone, secoisolariciresinol, equol, daidzein, genistein, naringenin, glycitein, and O-desmethylangolensin. We have recruited 950 patients so far.


Previous studies from our laboratory reported the presence of several pathways of metabolic activation of ethanol to acetaldehyde and hydroxyl free radicals as well as the promotion of oxidative stress. In the present studies, we tested the possibility that after alcohol drinking, acetaldehyde accumulates in mammary tissue to reach concentrations higher than in blood. Three different doses of alcohol (low, medium, and high) were tested, and acetaldehyde concentrations in breast, liver, and blood were measured at times ranging from 1 to 24 h. We also measured alcohol dehydrogenase, aldehyde dehydrogenase, and CYP2E1-mediated p-nitrophenol hydroxylase activities. Oxidative stress induced hydroperoxide formation as determined with the xylenol orange procedure; α-tocopherol and glutathione content determinations are in course for each dose at different times of exposure. Hydroperoxide levels were increased at 6 h for the highest dose tested. Acetaldehyde concentrations at the 3 alcohol doses tested were always higher than those in blood. Peak concentrations of acetaldehyde in liver, although higher than those in breast, appeared to decrease to blood levels following a similar time sequence. Limited activities of alcohol dehydrogenase and aldehyde dehydrogenase in mammary tissue were observed. The microsomal CYP2E1-mediated p-nitrophenol hydroxylase in mammary tissue was several times smaller than that in liver. In summary, results suggest that the
mutagen acetaldehyde, either formed in situ or in small amounts continuously arriving via blood, tends to accumulate in mammary tissue as a consequence of a limited capacity of mammary tissue for detoxification. [Supported by grants from ANPCyT-SECyT (PICT 05–6045) and from UNSAM (PIB S05/03), Argentina.]

Prostate Cancer


The potential correlation between alcohol consumption and prostate cancer led to controversial findings. To shed some light on the problem, it is critical to learn whether interaction with ethanol leads to alterations in prostate tissue similar to those attributed to alcohol-promoted liver cell injury or cancer. To that end, previous studies from our laboratory showed that cytosolic and microsomal fractions from the rat ventral prostate are able to biotransform ethanol to acetaldehyde and 1-hydroxymethyl free radicals. Present biochemical and ultrastructural studies were performed in Sprague Dawley male rats fed with a nutritionally adequate liquid diet (1) containing ethanol for 28 d and compared with adequately pair-fed controls. Prostate microsomal fractions were found to exhibit a CYP2E1-mediated p-nitrophenol hydroxylase metabolism, and that activity was induced by repetitive ethanol drinking. Cytosolic activation of acetaldehyde led to acetyl radical, as detected by spin trapping with PBN and GC-MS analysis. Low activities of alcohol dehydrogenase and aldehyde dehydrogenase were observed in prostate tissue, and acetaldehyde accumulation occurred after ethanol administration. An increased oxidizability of prostatic lipids was detected by the t-butylhydroperoxide–promoted chemiluminescence emission test and by the increased levels of lipid hydroperoxides (shown by the xylene orange method). Ultrastructural alterations in the epithelial cells were observed. They consisted of marked condensation of chromatin around the perinuclear membrane, moderate dilatation of their endoplasmic reticulum, and some epithelial cells undergoing apoptosis. In summary, alcohol drinking leads to the formation of mutagenic acetaldehyde and to tumor-promoting oxidative stress. However, it exerts direct and indirect proapoptotic effects in the prostate epithelial cells. In the balance between these 2 actions might explain, at least in part, many controversial results observed in epidemiologic studies. [Supported by grants from ANPCyT-SECyT (PICT 05–6045) and from UNSAM (PIB S05/03), Argentina.]

Insulin-like Growth Factor-I Stimulates Lipid Metabolism in Prostate Cancer Cells. Shihua Wang,1 Lei Shen,2 Valerie L. DeGroff,1 and Steven K. Clinton.1 1Division of Hematology and Oncology, Department of Internal Medicine, and 2Division of Epidemiology and Biometrics, The Ohio State University College of Medicine and Public Health, Columbus, OH.

The hormone and growth factor insulin-like growth factor-I (IGF-I) has emerged as a potential promoter of prostate carcinogenesis. IGF-I exhibits pleiotropic activities including the stimulation of prostate tumor cell proliferation, survival, and resistance to apoptosis. To better understand how IGF-I modulates these processes and to identify novel biomarkers for future rodent and human studies, we examined global gene expression profiles (Affymetrix GeneChip - Rat Genome U34A) in AT6.3 rat prostate cancer cells after treatment for 24 h in serum-free medium (SFM), SFM with 10% fetal bovine serum, or SFM with 50 μg/L IGF-I (n = 6 each). The microarray data were analyzed by dChip and SAM software. Lipids are important structural components for cells and serve as signaling mediators associated with many critical cellular functions. This study reports our findings regarding IGF-I–regulated pathways involving lipid metabolism. Our results showed that the majority of genes involved in de novo cholesterol synthesis from mevalonate were significantly up-regulated by IGF-I and frequently down-regulated by serum. These genes included HMG-CoA synthase 1 (q = 1.2), HMG CoA reductase (q = 1.1), mevalonate kinase (q = 0), farnesyl-PP synthase (q = 0), farnesyl diphosphate farnesyl transferase 1 (q = 0.4), squalene epoxidase (q = 0), and 7-dehydrocholesterol reductase (q = 0). In addition, several enzymes related to fatty acid metabolism, such as acyl-CoA synthetase 3 (q = 0.9), and stearoyl CoA desaturase 1 (q = 0), were also up-regulated by IGF-I. These microarray observations were validated by subsequent RT-PCR and Western blots. Mevinolin (lovastatin), an inhibitor of HMG-CoA reductase, inhibited IGF-I–stimulated AT6.3 cell proliferation with an IC50 < 10 μmol/L. We previously observed that IGF-I increased anaerobic glycolysis, which is known as the Warburg effect, and that glucose uptake and metabolism were essential for IGF-I stimulation of prostate cancer cell proliferation and survival. Our findings suggest that IGF-I stimulation of lipid and glucose metabolism may be a critical mechanism whereby this hormone may contribute to prostate carcinogenesis. [Supported by AICR #05A131.]

Effects of Dietary Tomato, Broccoli, or Lycopene or Androgen Ablation on Proliferation and Apoptosis Biomarkers in Dunning R3327-H Prostate Adenocarcinomas. Kirstie Canene-Adams,1 Shihua Wang,2 Steven K. Clinton,2 and John W. Erdman, Jr.1 1Division of Nutritional Sciences, University of Illinois, Urbana, IL; and 2Department of Internal Medicine, Division of Hematology and Medical Oncology, The Ohio State University, Columbus, OH.

The androgen-dependent Dunning R3327-H transplantable rat prostate adenocarcinoma model was used to examine dietary interventions on tumor growth. Diets were initiated 1 mo before tumor inoculation (20 mg fresh tumor in Matrigel) and continued for ~18 wk in 6 cohorts of animals. Diets containing 10% tomato (P < 0.05), 10% broccoli (P < 0.01), or 10% tomato plus 10% broccoli (P < 0.001) or castration (P < 0.001) significantly reduced tumor weights compared with control tumors. Reduction in tumor weights were ~34% in rats fed 10% tomato, 40% in rats fed 10% broccoli, 52% in rats fed broccoli with tomato, and 62% in castrated rats. Finasteride and the low- and high-lycopenic diets (0.025 or 0.25 g/kg diet, respectively) did not significantly reduce tumor weights. Tumor tissue proliferation and apoptosis rates were estimated using proliferating cell nuclear antigen (PCNA) staining and ApopTag peroxidase in situ kits, respectively. Tumor sizes were substantially larger in 3 of the 6 cohorts. In these 3 cohorts lycopene, tomato, or broccoli (P = 0.01) caused a decrease in PCNA staining.

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compared with controls. PCNA staining was most suppressed with the tomato plus broccoli diet ($P = 0.001$), suggesting that one of the mechanisms behind the $>50\%$ decreased tumor growth from consuming both tomato with broccoli was a decreased rate of proliferation. Castration, tomato, broccoli, and the combination of tomato and broccoli all showed significant increases in apoptosis rates. We can conclude that diets containing tomato and broccoli significantly decrease prostate tumor growth by slowing the rate of proliferation. [Supported by AICR 01B061/ IFAFS 00–52101–9695.]

**Se-Methylselenocysteine, but Not Lycopene or γ-Tocopherol, Decreases Growth of Transplantable Dunning R3327-H Prostate Tumors.** Brian L. Lindshield, Nikki A. Ford, Kirstie Canene-Adams, Matthew A. Wallig, and John W. Erdman, Jr. Division of Nutritional Sciences, University of Illinois, Urbana, IL.

Lycopene, selenium, and vitamin E are 3 commonly consumed and supplemented micronutrients that have been associated with a decreased risk of prostate cancer. The potential protective effect of selenium (selenomethionine) and vitamin E (all-rac-α-tocopherol acetate) against prostate cancer is currently being evaluated in the Selenium and Vitamin E Cancer Prevention Trial (SELECT). However, there are few data on the effectiveness of these compounds or lycopene in vivo. Therefore, we evaluated the effects of lycopene, selenium, and vitamin E alone and in combination on the growth of Dunning R3327-H tumors.

The dietary sources of the supplemented compounds were selenium as Se-methylselenocysteine (2 mg/kg diet), vitamin E as γ-tocopherol (200 mg/kg diet), and lycopene (0.025 mg/kg diet) in the form of water-soluble beadlets. Isoenergetic and isonitrogenous AIN-93G–based diets were prefed for 4–7 wk to Copenhagen rats before tumor implantation by subcutaneous injection. Tumors were allowed to grow for 18 wk before study termination. Multiple linear regression analysis showed that Se-methylselenocysteine consumption resulted in highly significant decreases in tumor weight and tumor areas. Lycopene intake resulted in minor, nonsignificant reductions in tumor weights and tumor areas, whereas γ-tocopherol consumption led to nonsignificant increases in tumor weights and tumor areas. There were no significant interactions among nutrient combinations. In conclusion, among the 3 micronutrients studied, only Se-methylselenocysteine consumption reduced growth of transplantable Dunning R3327-H prostate tumors. [Supported by AICR grant 05A021.]

**Soy**

Radiosensitization of Prostate Cancer by Soy Isoflavones Targets Survival Pathways In Vitro and In Vivo. Gilda G. Hillman,1 Julian J. Raffoul,1 Sanjeev Banerjee,2 and Fazlul H. Sarkar.2 Departments of 1Radiation Oncology and 2Pathology, Barbara Ann Karmanos Cancer Institute at Wayne State University School of Medicine, Detroit, MI.

Epidemiologic studies showed that men who consume diets rich in soy isoflavones have lower incidence of prostate cancer. We previously demonstrated that genistein, the major bioactive component of soy isoflavones, acts as a radiosensitizer and potentiates prostate tumor cell killing by radiation in vitro and in animal orthotopic metastatic tumor models in vivo. However, when administered alone in vivo, pure genistein promoted increased lymph node metastasis. Recently, we found that a mixture of soy isoflavones, consisting of genistein, daidzein, and glycitein, was safer because it did not promote increased metastasis to lymph nodes in vivo. Furthermore, the combination of soy isoflavones with prostate tumor irradiation potentiated inhibition of primary tumor growth and metastasis, as observed with pure genistein and radiation. Soy isoflavones also increased radiation-induced tumor cell killing in vitro like pure genistein. Investigation of molecular mechanisms by which soy isoflavones potentiate radiotherapy revealed that soy isoflavones inhibited activation of nuclear factor (NF)-κB, a transcription factor critically involved in cancer cell survival. Like pure genistein, soy isoflavones also abrogated radiation-induced activation of NF-κB, probably driving the cells to apoptotic pathways as confirmed by induction of cleaved poly(ADP-ribose) polymerase in these cells. APE1/Ref-1, a DNA repair-redox protein implicated in tumor cell survival and radioresistance, functioned as a redox activator of transcription factors, including NF-κB. We demonstrated that soy isoflavones decreased APE1/Ref-1 expression in vitro, whereas radiation up-regulated it. Pretreatment with soy isoflavones followed by radiation inhibited APE1/Ref-1 expression in correlation with the decreased activation of NF-κB. The down-regulation of APE1/Ref-1 and
NF-κB by isoflavones in vitro could be recapitulated in vivo, supporting our hypothesis that these markers represent biological targets of isoflavones. Their down-regulation by isoflavones could potentially radiosensitize prostate tumor cells. Our studies demonstrate the safety and potential for the use of soy isoflavones to improve clinical strategies for prostate cancer radiotherapy.

**Genetic Influences on Interindividual Variability in Soybean Isoflavone Absorption and Metabolism.** Luisa Wakeling and Dianne Ford. Institute for Cell and Molecular Biosciences, University of Newcastle upon Tyne, Newcastle, UK.

Consumption of soybean isoflavones may have a number of health benefits, including reduced risk of cardiovascular disease and cancer. In unfermented plant sources, isoflavones occur as β-glucosides. Absorption requires cleavage by intestinal β-glucosidase enzymes lactase phlorizin hydrolase (LPH) and cytosolic β-glucosidase (CBG). Intestinal and hepatic UDP-glucuronosyl transferases (UGTs) including UGT1A1 are involved in phase II conjugation. We hypothesize that non-synonymous coding single nucleotide polymorphisms (SNPs) in LPH and CBG may contribute to variability in isoflavone absorption and that an insertion polymorphism [(TA)7] in the promoter region of UGT1A1 may alter isoflavone bioavailability. The Soy Isoflavone Metabolism Study (SIMS) is designed to establish whether genotype with respect to these polymorphisms influences the absorption and metabolism of a single oral dose of isoflavones. Healthy premenopausal women (n = 100) were genotyped for polymorphisms in LPH (G666A, A1095G, of isoflavones. Healthy premenopausal women (n = 100) were genotyped for polymorphisms in LPH (G666A, A1095G, Brijesh Patel, Kirk Pabon, and Kathleen W. Scotto. The Cancer Institute of New Jersey, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, New Brunswick, NJ.

Caffeine is the most widely consumed psychoactive substance in the world. It elicits multiple cellular functions including the inhibition of the adenosine receptor, induction of calcium release through the ryanodine receptor, and inhibition of the ATM/ATR protein kinases. Surprisingly, despite the seemingly pancellular cascades that caffeine induces, the effect of caffeine on gene expression has attracted little attention. We now show that caffeine, in doses equivalent to those contained in everyday consumables, is a potent transcriptional activator of the MDR1 gene. There is a dose-dependent activation of an MDR1 promoter-driven luciferase reporter construct in the presence of increasing concentrations of caffeine, concomitant with an increase in the expression of endogenous MDR1 mRNA and the MDR1 gene product, P-glycoprotein (P-gp). The overexpressed P-gp is properly translocated to the plasma membrane and is functional as evidenced by an increase in doxorubicin efflux in cells pretreated with caffeine. This increased doxorubicin efflux is correlated with an increase in cellular resistance to doxorubicin in SW620 cells. Furthermore, we show that the induction of MDR1 gene expression by caffeine is p53-independent and occurs in various cell types derived from different tissues. These findings suggest that caffeine consumption could play a role in the development of an MDR phenotype in vivo and could thus affect the outcome of chemotherapeutic regimens in the treatment of cancer.

**Sulforaphane and Erucin Increase the Expression of Multidrug Resistance Protein 1 in A549 Human Lung Carcinoma Cells.** Kristin E. Harris and Elizabeth H. Jeffery. Division of Nutritional Sciences, University of Illinois, Urbana, IL.

Multidrug resistance proteins (MRPs) have been termed the phase III detoxification system because they protect against xenobiotic insult by actively secreting foreign compounds from tissues such as liver and colon. However, MRP overexpression in tumors can lead to drug resistance, a major obstacle in the treatment of many cancers, including lung cancer. Isothiocyanates, such as sulforaphane (SF) and erucin (ER), which are derived from cruciferous vegetables, enhance the expression of phase II detoxification enzymes. Initial studies suggest that SF and ER may alter MRP transporter expression as well. The ability of SF and ER to modulate MRP expression is evaluated in this study. The expression of P-glycoprotein (P-gp), MRP1, and MRP2 in liver, colon, and lung cancer cell lines treated with ER or SF was analyzed by Western blotting. Sulforaphane and ER increased the level of MRP1 in HepG2 liver and A549 lung cells as well MRP2 in HepG2 and Colo-205 colon cells in a dose-dependent manner. Neither SF nor ER affected P-gp expression in any of the cell lines tested. Alterations in mRNA expression of MRP1 and MRP2 were evaluated by real-time PCR in SF-treated A549 cells. Treatment with SF led to a significant dose-dependent increase in MRP1 and MRP2 mRNA in A549 cells. These data suggest that SF and ER can increase MRP1 and MRP2 expression through a transcriptional mechanism. [Supported by USDA/IFAFS 2000–04766.]

**Phytochemicals**

**Caffeine Activates the Multidrug Resistance Gene Leading to Increased Drug Efflux and Chemoresistance.** Pellegrino G. Magro, Michael V. Mandola, Jia Shi, Zhen Hu, Brijesh Patel, Kirk Pabon, and Kathleen W. Scotto. The Cancer Institute of New Jersey, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, New Brunswick, NJ.

Caffeine is the most widely consumed psychoactive substance in the world. It elicits multiple cellular functions including the inhibition of the adenosine receptor, induction of calcium release through the ryanodine receptor, and inhibition of the ATM/ATR protein kinases. Surprisingly, despite the seemingly pancellular cascades that caffeine induces, the effect of caffeine on gene expression has attracted little attention. We now show that caffeine, in doses equivalent to those contained in everyday consumables, is a potent transcriptional activator of the MDR1 gene. There is a dose-dependent activation of an MDR1 promoter-driven luciferase reporter construct in the presence of increasing concentrations of caffeine, concomitant with an increase in the expression of endogenous MDR1 mRNA and the MDR1 gene product, P-glycoprotein (P-gp). The overexpressed P-gp is properly translocated to the plasma membrane and is functional as evidenced by an increase in doxorubicin efflux in cells pretreated with caffeine. This increased doxorubicin efflux is correlated with an increase in cellular resistance to doxorubicin in SW620 cells. Furthermore, we show that the induction of MDR1 gene expression by caffeine is p53-independent and occurs in various cell types derived from different tissues. These findings suggest that caffeine consumption could play a role in the development of an MDR phenotype in vivo and could thus affect the outcome of chemotherapeutic regimens in the treatment of cancer.

**Epigenetic Mechanisms Lead to Telomerase Inhibition in Breast Cancer Cells Treated with the Green Tea Component EGCG.** Joel B. Berlatch, Canhui Liu, Lucy G. Andrews, and Trygve O. Tollefsbol. Department of Biology and Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, AL.

Dietary polyphenols are common in the human diet and have chemopreventive and anticancer activities. Tea is one of the most...
consumed beverages worldwide, and a major constituent of green tea is the polyphenol (−)-epigallocatechin gallate (EGCG). Many studies have shown that EGCG has potent anticancer properties, but the mechanisms for these effects are unknown. Recent studies indicated that this dietary polyphenol alters the activity of DNA methyltransferase 1, leading to changes in the expression of methylation-controlled cancer genes. The gene for telomerase is important in oncogenesis that is controlled through DNA methylation, and we found that EGCG down-regulates transcription of hTERT (the catalytic subunit of telomerase) in cancer cells, leading to apoptosis. The hTERT gene is paradoxically hypermethylated in cancer cells that express this gene; we found that treating MCF-7 breast cancer cells with EGCG leads to hypomethylation of the hTERT promoter, presumably through DNMT1 inhibition and apoptosis of these cells. The hypomethylation of the hTERT promoter is especially pronounced in the E2F-1 methylation-sensitive binding sites. In rare cancer cells, such as multipotent HL60 cells, the hTERT promoter is hypomethylated but telomerase positive; we have found that EGCG has no effect on hTERT regulation in these cells. These studies collectively indicate that EGCG works through epigenetic mechanisms in most telomerase-positive cancer cells to lead to the down-regulation of telomerase and apoptosis of cancer cells. Ultimately, completion of this study may lead to elucidation of the mechanisms through which dietary polyphenols mediate their chemopreventive and anticarcinogenic effects, thereby facilitating more effective uses of green tea and polyphenols in cancer prevention and therapy.

Pharmacokinetics of Curcumin as Glucuronide and Sulfate Conjugates. Shaiju K. Vareed,1 Zora Djuric,2 Bill C. Frame,3 Daniel Normolle,2 and Dean E. Brenner.1 1Department of Internal Medicine, Hematology-Oncology Division, 2Department of Family Medicine, and 3Biostatistics Unit, and 4Department of Radiation Oncology, University of Michigan Comprehensive Cancer Center, Ann Arbor, MI.

Chemoprevention of cancer using natural products has the potential to be efficacious and yet nontoxic. Curcumin, the yellow pigment extracted from turmeric, has chemopreventive properties in vitro and in vivo. Curcumin has been used as a food additive and an herbal medicine for centuries because of its antioxidant, antimicrobial, and anticancer properties. We designed a clinical trial to 1) determine whether curcumin in a capsule formulation is absorbed and 2) assess the pharmacokinetics of curcumin at doses of 10 and 12 g. To assess bioavailability and pharmacokinetics, a trial was performed with 6 subjects per dose level using 10 and 12 g curcumin. After the single dose of curcumin was taken with a standardized fatty meal, blood specimens were collected at 0, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 10, 24, 36, 48, and 72 h. A reverse-phase gradient HPLC method was developed for identifying and quantifying curcumin in plasma. The assay was linear from 0.2 to 27 μmol/L. At doses of 10 and 12 g, no free curcumin was detected in human plasma. Essentially all the curcumin in plasma was conjugated, and enzymatic hydrolysis with β-glucuronidase and sulfatase was necessary to quantify curcumin. Our data indicate that the maximal concentration of the conjugated forms was achieved 2–10 h after oral administration with a concentration range of 4.1–10.1 μmol/L. Oral curcumin is absorbed in humans and circulates in the blood as conjugates; deconjugation at the target tissue may be responsible for its biological effects. [Supported by NCI-N01-CN-35160 and AICR#06A035.]


Breast tumors with overexpression of the oncogene erbB-2/neu (HER-2), a member of the epidermal growth factor receptor (EGFR) family, occur in ~25–30% of human breast cancers and are associated with chemoresistance resulting in poor clinical outcome. Although an antibody against the HER-2/neu receptor is available to treat breast tumors overexpressing HER-2/neu, many cardiotoxic effects have been noted, which complicates their use. Several studies documented that intake of indole-3-carbinol and 3,3’-diindolylmethane (DIM) present in the cruciferous family have antitumor effects against several types of cancer including breast cancer. This study investigated whether DIM has a growth-inhibitory effect on breast cancer cells with elevated expression of HER-2/neu by examining cell survival, apoptosis, and potential downstream EGFR-activated pathways. DIM inhibited growth of human MDA-MB-435eB and BT474 cells and MMTV-HER2/neu mammary tumor NMF11.2 cells in a concentration- and time-dependent manner. Results from the M3O CytoDeath and TUNEL assays showed that DIM induced apoptosis in NMF11.2 and 435eB cells in a concentration-dependent manner. The growth-inhibitory effects of DIM were associated with decreased EGF-induced phosphorylation of the HER-2/neu receptor, extracellular signal-regulated kinase 1/2, and AKT. Feeding MMTV-HER2/neu mice 550 mg/kg body weight of absorption-enhanced DIM for 12 mo reduced tumor incidence by 50% (P < 0.01, n = 6) and increased tumor latency with no toxic side effects. These findings suggest that DIM has potential benefits for use as adjuvant therapy for prevention and treatment of HER-2/neu–overexpressing breast cancer.

Proteomic and Genomic Analysis of Human Tumor Cells Treated with Diallyl Disulfide. Nagathihalli S. Nagaraj1 and Om V. Singh.2 1Department of Medicine, James Graham Brown Cancer Center, University of Louisville, Louisville, KY; and 2Department of Pediatrics, The Johns Hopkins School of Medicine, Baltimore, MD.

Diallyl disulfide (DADS), an important component of garlic, has a potential chemopreventive activity against human cancers. Evidence is also mounting to indicate that DADS can contribute to apoptosis in cancer cells, but the proteomic and genomic data leading to the proapoptotic effect of DADS are very poor. Using proteomic and genomic means, we systematically studied the responses of protein and gene expression simultaneously in breast cancer line MCF-7, prostate cancer cell line PC-3, and lung cancer cell line calu-1 induced by DADS. Protein profiles were analyzed by 2-dimensional gel electrophoresis and mass spectrometry. Differential gene expression patterns were determined in DADS-exposed versus nonexposed cells using high-density microarray RNA expression profiling. A total of 36 unique proteins and a set of 43 genes that were specifically up- or down-regulated in MCF-7, PC-3, and calu-1 cells were detected. Of these, 7 are typically expressed for apoptosis: S100A11, KRT8, FADD, CASP2, CASP3, CSTB, and APAF1. These were further examined by Western blotting and RT-PCR, resulting in data coincident with the proteomic and genomic evidence. Interestingly, 60–70% of proteins and genes in all the cell lines studied were highly associated with the apoptotic pathway. Furthermore, DADS induced a marked amount of Bax release,
mitochondrial membrane potential, cytochrome \(c\) release, caspase-3 activation, and poly(ADP-ribose) polymerase cleavage. DADS-treated cells triggered mitochondria-mediated signaling pathways that lead to apoptosis. The proteomic and genomic results presented support the hypothesis that garlic is a strong inducer of apoptosis in tumor cells.

Dietary Flavonoids Induce MLL Translocations in Human CD34+ Hematopoietic Stem Cells. Sahar Barjesteh van Waalwijk van Doorn-Khosrovani,1 Jannie Janssen,2 Lou Maas,3 Roger W.L. Godschalk,1 Jan G. Nijhuis,2 and Frederik-Jan van Schooten.1 1Department of Health Risk Analysis and Toxicology, The Nutrition and Toxicology Research Institute Maastricht, Maastricht University, the Netherlands; 2Department of Clinical Genetics and 3Department of Obstetrics and Gynecology, Academic Hospital, Maastricht, the Netherlands.

Genetic abnormalities leading to infant leukemias already occur during fetal development and often involve rearrangements of the mixed-lineage-leukemia (MLL) gene. These rearrangements resemble the aberrations observed in therapy-related leukemias following treatment with topoisomerase II (topoII)-inhibiting agents such as etoposide. Because flavonoids present in food (e.g., soy, fruits, vegetables, red wine, beer, and tea), herbal medicines, and dietary supplements are potent topoII inhibitors, we examined the effect of three widely consumed flavonoids (quercetin, genistein, and kaempferol) in primary hematopoietic CD34+ stem cells isolated from umbilical cord blood. Using neutral Comet assay, we demonstrated a dose-dependent double-strand-break formation after exposure to flavonoids, particularly genistein and quercetin. An incorrect repair of these double-strand breaks resulted in chromosomal abnormalities, as determined by fluorescence in situ hybridization (FISH) and an inverse PCR approach. Surprisingly, genistein and quercetin seemed to be more clastogenic than etoposide and kaempferol. A concentration of genistein as low as 1 \(\mu\)mol/L resulted in MLL translocations. The estimated frequency of MLL translocations after exposure to 50 \(\mu\)mol/L genistein or quercetin was 500 per 10^6 cells. Most of these translocations were formed by micro-homology-mediated nonhomologous end joining. Moreover, in all but one translocation, SINE/Alu or LINE/L1 repetitive elements were present in at least 1 side of the breakpoint junction. Beside MLL translocations, FISH analysis demonstrated monosomy or trisomy of MLL in 8–10% of the quercetin-exposed stem cells. Our study demonstrates that biologically relevant doses of flavonoids can induce MLL abnormalities in hematopoietic stem cells. This is particularly alarming because differences in metabolism and excretion rate between mother and fetus can lead to a higher flavonoid concentration in the fetus. Therefore, it is important to raise public awareness and set guidelines for marketing flavonoid supplements to reduce the risk of infant leukemias.

Nutritive Evaluation and Anticlastogenic Potential of Gracilaria sp. Kumvan Wiravan,1 Kupradinun Piengchai,2 Kangsadalampai Kaew,1 Teepsuwan Anong,2 Kusamran R. Wannee,2 and Buttyee Chaniphung.1 1Institute of Nutrition, Mahidol University, Nakhon Pathom, Thailand; and 2National Cancer Institute, Bangkok, Thailand.

A dietary red seaweed, Gracilaria sp., is consumed by Thai people as a food ingredient. The aims of this study were to determine the nutritive value and the clastogenic and anticlastogenic potentials of Gracilaria sp. induced by cyclophosphamide (CP), an indirect-acting carcinogen, and mitomycin C (MMC), a direct-acting carcinogen, by using the erythrocyte micronucleus assay in the mouse. The results, expressed per 100 g unprocessed and processed dry seaweed, showed that moisture content was 4.4–9.6 g; ashes, 4.0–37.9 g; protein, 9.0–9.4 g; fat, 0.6 g; total dietary fiber, 50.9–53.8 g; carbohydrate, 50.9–76.4 g; and iodine, 10.723–21.269 mg. The iodine content in Gracilaria sp. was very high when consumed in one serving, so high that consumption could lead to harm. Feeding diets containing 1% and 2% Gracilaria sp. for 2 wk had no significant effect in the erythrocyte micronucleus assay. For the anticlastogenic test, male mice were fed diets containing 1% and 2% powder of Gracilaria sp. for 2 wk. MMC and CP were given; blood was collected at 0, 24, and 48 h after administration; and the number of micronucleated peripheral reticulocytes (MNRETs) was counted. Both 1% and 2% Gracilaria sp. decreased the number of MNRETs in a dose-dependent manner. Significant inhibitory effects on carcinogens were shown by the diet containing 2% Gracilaria sp. at 48 h after carcinogen administration compared with the control group. In conclusion, this study demonstrated that Gracilaria sp. can be consumed as a source of protein, fiber, and iodine in a serving and it, interestingly, has anticlastogenic activity against MMC and CP in the erythrocyte micronucleus assay.

Lipids

Effect of Conjugated Linoleic Acid on Mammary Tumor Growth and Metastasis in a Transgenic Mouse Model of Breast Cancer. Margaret Flowers,1 Cynthia Thomson,1 Joyce Schroeder,2 and Patricia Thompson.3 1Departments of 1Nutritional Sciences, 2Molecular and Cellular Biology, and 3Pathology, University of Arizona, Tucson, A.

Conjugated linoleic acid (CLA) refers to a class of natural fatty acids derived from the biohydrogenation of linoleic acid by digestive bacteria in ruminant animals. CLA inhibits chemically induced mammary tumors and induces apoptosis in several cancer cell lines; limited evidence suggests isomer-specific properties. However, the mechanism of CLA action is still largely unknown. We are interested in the role of CLA as an anti-breast-tumor agent, which we hypothesize is primarily mediated through a dependent interaction with the peroxisome proliferator-activated receptors (PPARs), a class of nuclear receptors and transcription factors that perform a variety of cellular functions including fatty acid metabolism, energy homeostasis, and cell growth. Administration of the mixed isomer formulation of CLA commonly distributed over the counter as a weight-loss product showed weak antitumor activity in a genetic model of mammary tumorigenesis (\(n = 6\)). A preliminary analysis of selected tissue samples suggested an inhibitory effect on the protein kinase B/Akt cell survival pathway. Surprisingly, we found that dietary CLA was associated with higher cellular density and a distinctive tumor phenotype (i.e., densely packed tumor cells with an intact basement membrane and prolific) among tumors that arose in CLA mice compared with control animals. Our preliminary findings suggest a complex effect of CLA on mammary epithelial cell growth in animals genetically prone to breast tumors that warrants further characterization, particularly because of increased cellular density in the presence of an unexpected inhibitory action on the protein kinase B/Akt cell survival pathway. Future work will include molecular and pathogenic characterization of the tumor type arising in animals fed CLA compared with a control diet, including characterization
of their invasive potential, which we suspect to be low despite high cellularity.

**Eicosapentaenoic Acid and Docosahexaenoic Acid as Chemopreventive Agents against 17β-Estradiol Epoxide Formation and the Potential to Prevent Breast Cancer Carcinogenesis at Initiation.** Fu-Li Yu,1 Roger Greenlaw,2 Wanda Bender,1 and Katarzyna Berberka.1 1Department of Biomedical Sciences and 2Department of Medicine, University of Illinois College of Medicine at Rockford, Rockford, IL.

We found that 17β-estradiol (E₂) could be activated by the versatile epoxide-forming oxidant dimethylsulfoxide to inhibit nuclear RNA synthesis and to bind DNA, forming DNA adducts both in vitro and in vivo. Because DNA adducts can cause mutation, and mutation is the molecular basis for the initiation of carcinogenesis, we proposed that E₂ epoxidation is the underlying mechanism for initiation of breast cancer. A method to screen chemopreventive agents against breast cancer at initiation was developed. This report examines the transcriptional and DNA binding properties of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and their preventive effect on E₂ epoxidation. We found that both EPA and DHA strongly prevented the formation of E₂ epoxide, as reflected by the loss of the ability of E₂ to inhibit nuclear RNA synthesis. Additionally, we found that EPA and DHA prevented the binding of ³H-labeled E₂ to DNA. This preventive effect of EPA and DHA on the binding of E₂ to DNA was further confirmed when liver microsomes were used for the activation. We believe that this is the first report on the transcriptional and DNA binding properties of EPA and DHA and their inhibitory effect on the formation of E₂ epoxide. As dietary supplements, EPA and DHA may have the potential to prevent E₂-induced breast cancer carcinogenesis at initiation. [Partially supported by an Excel Academic Award from SwedishAmerican Hospital.]

**Effect of Walnut Consumption on Cancer Growth.** W. Elaine Hardman, Hyeong-Chan Jo, and Gabriela Ion. Department of Biochemistry and Microbiology, Marshall University School of Medicine, Huntington, WV.

Walnuts contain a high level of (n-3) fatty acids (18C, α-linolenic acid), phytosterols, vitamin E, and melatonin. Consumption of long-chain (n-3) fatty acids [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] and phytosterols and antioxidants (vitamin E and melatonin) has been shown to slow cancer progression. There is great interest in the role of polyunsaturated fatty acids (PUFAs) in either promoting [(n-6) class] or inhibiting [(n-3) class] inflammation and neoplasia. In contrast with plant and nematode cells, mammalian cells are devoid of a fatty acid (n–3) desaturase, an enzyme able to convert (n-6) PUFAs to (n-3) PUFAs, and consequently essential fatty acids must be supplied in the diet. In this study we have used transgenic mice engineered to carry the Caenorhabditis elegans fat-1 gene encoding a (n-3) desaturase 1 to assess PUFA production in colonic cells. Presence of (n-3) desaturase transcript was confirmed by RT-PCR analysis. To measure the conversion of dietary (n-6) PUFAs to (n-3) PUFAs, fat-1 mice and control littermates were fed for 7 wk AIN-76A standard diet supplemented with 10% (w/w) safflower oil rich (76%) in (n-6) polyunsaturated linoleic acid. The fatty acid profile in colons of control and transgenic mice was determined by lipid methylation and capillary gas chromatography. The results show that the (n-6)/(n-3) PUFA ratio in colonic cells of fat-1 mice is markedly lower (9.83 ± 2.62) than that of wild-type littermates (54.5 ± 9.24, P < 0.001); this process was associated with a significant decrease (P < 0.01) in (n-6) PUFA-derived prostaglandin E₂ production assayed by prostaglandin-E₂ monoclonal enzyme immunoassay. Cumulatively our results indicate that colonic cells of fat-1 mice have acquired the novel biochemical competence to convert (n-6) PUFAs to (n-3) PUFAs. These transgenic mice represent a unique model for studying dietary PUFA modulation of the inflammatory process and carcinogenesis. [Supported by AICR Grant 04B036.]


**Vitamins**


In preparation for the second report from WCRF/AICR, *Food, Nutrition, Physical Activity and the Prevention of Cancer: a Global Perspective*, a systematic review of the literature relating to...
stomach cancer has been undertaken. As part of this review, 1 objective was to assess the relation between vitamin C and risk of gastric cancer. All original, etiological, peer-reviewed studies published before January 2006 were considered with no exclusions on the basis of language or quality. Separate random-effects meta-analyses were performed for cohort and case-control studies based on derived linear dose-response curves. Data on dietary vitamin C intakes were extracted from 4 cohort and 28 case-control studies, with 2 and 17 of each study type providing sufficient information for meta-analysis. The pooled estimates of relative risk per 30 mg/d were 0.92 (95% CI 0.85, 1.00; P = 0.04) and 0.85 (95% CI 0.80, 0.90; P < 0.001) for cohort and case-control studies, respectively. Meta-regression suggested there was no excess heterogeneity within the cohorts, but there was a substantial amount of unexplained heterogeneity within the case-control studies (I² = 90%, Q = 184, df = 18, P < 0.001). Additionally, for these latter studies, Egger’s test suggested significant small-study bias (P = 0.002). Meta-analyses of data on blood vitamin C from 2 cohort and 3 case-control studies indicate consistency with the results on dietary vitamin C. The pooled estimates of risk from these studies were 0.98 (95% CI 0.95, 1.01, P = 0.3) and 0.85 (95% CI 0.80, 0.90; P < 0.001) per 1 μmol/L, respectively. These data indicate a statistically significant reduction in risk of stomach cancer associated with increasing levels of dietary and blood levels of vitamin C. However, the small size of the effect does not rule out the possibility that the association is caused by uncontrolled confounding or bias.

Supplementary and Dietary Vitamin D Intake and Renal Cell Cancer Risk. Robin Taylor Wilson,1 Jiangyue Wang,2 Vernon Chinchilli,3 John Richie,1 Lee E. Moore,3 and Demetrius Albanes.3 1Pennsylvania State College of Medicine, Penn State Cancer Institute, Hershey, PA; 2Pennsylvania State College of Medicine, Division of Biostatistics, Hershey, PA; 3National Cancer Institute, Division of Cancer Epidemiology and Genetics, Bethesda, MD.

Renal cell cancer (RCC) is the third most rapidly increasing cancer in the United States. Vitamin D has a suspected preventive role. We sought to determine the risk of RCC associated with supplemental and dietary vitamin D intake. Cases were identified through the Alpha Tocopherol Beta Carotene (ATBC) trial cohort (1985–2002). A detailed dietary history, fasting serum sample, and height and weight measurements were obtained at recruitment. Individuals diagnosed with prior cancer or using vitamin E, vitamin A, or RBP are also synthesized in extrahepatic tissues, where its

Pharmacologic Ascorbate Concentrations Selectively Kill Cancer Cells: Ascorbic Acid as a Prodrug for Ascorbate Radical or H2O2 Delivery to Tissues. Qi Chen,1 Je-Hyuk Lee,1 Liqun Zhang,1 Andrew Sun,1 Murali C. Krishna,2 Michael G. Espey,2 Chaya Pooput,1 Kenneth Kirk,1 Peter Choyke,2 Garry R. Buettner,3 Emily Shacter,4 and Mark Levine.1 1National Institute of Diabetes and Digestive and Kidney Diseases and 2National Cancer Institute, National Institutes of Health, Bethesda, MD; 3College of Medicine, University of Iowa, Iowa City, IA; and 4Center for Biologics Evaluation and Research, Food and Drug Administration, Rockville, MD.

Pharmacokinetics data in humans indicate that i.v. but not oral ascorbate could have an unanticipated role in cancer treatment. We hypothesized that millimolar ascorbate extracellular concentrations achieved only by i.v. administration would serve as a prodrug for ascorbate radical or H2O2 delivery to tissues with minimal formation of the prodrug in blood. Under in vitro conditions mimicking clinical i.v. use, some cancer cells were killed by millimolar ascorbate, whereas normal cells were unaffected. Apoptosis and pyknosis/necrosis were caused by extracellular ascorbate independent of intracellular concentrations and were critically dependent on H2O2 formation. H2O2 generation was dependent on ascorbate concentration and linearly related to ascorbate radical formation, with 0.5–10% serum required. In contrast, millimolar ascorbate additions to hemolyzed and non-hemolyzed blood generated no detectable H2O2 and only trace ascorbate radical. To test these concepts in vivo, i.v. ascorbate was administered to rats (0.25–0.5 mg/g), a dose on a weight basis that could readily be given to humans. Extracellular fluid (ECF) was collected by microdialysis from muscles. After i.v. administration, ascorbate reached 2–8 mmol/L for about 2 h in both blood and ECF. Ascorbate radical increased from below detectable to ~20 mmol/L in blood but >200 mmol/L in ECF and was dependent on ascorbate concentration. In ECF, H2O2 increased from <2 μmol/L before i.v. administration to about 20–30 μmol/L after i.v. administration. Similar data were obtained from urine. With oral rather than i.v. administration of the same dose, ascorbate concentrations in blood and ECF were <190 μmol/L and there was no ascorbate radical or H2O2 formation in either fluid. Taken together, these findings provide support for the hypothesis and plausibility for i.v. ascorbate use in cancer treatment and possibly in treatment of some infections in which H2O2 or reactive oxygen species may have therapeutic roles.

Vitamin A Signaling in Prostate Epithelium. Max E. Gottesman,1 Lesley Wassef,2 Silke Vogel,1 and Loredana Quadro.2 1Department of Medicine, Columbia University, New York, NY; 2Department of Food Science, Rutgers University, New Brunswick, NJ.

Alterations in vitamin A availability and metabolism are known to have profound effects on prostate growth and prostate cancer development. We used mice lacking retinol-binding protein (RBP) to investigate this issue. RBP is a specific serum transport protein that delivers retinol (vitamin A alcohol) from liver stores to target tissues. RBP−/− mice have low circulating retinol levels and rely predominantly on dietary vitamin A to maintain normal physiological functions. Although expressed mainly in the liver, RBP is also synthesized in extrahepatic tissues, where its

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function is largely unknown. In this context, we found that RBP is expressed in mouse prostate. Surprisingly, RBP-/- mice display elevated prostate retinol and retinyl ester levels at age 15 wk. This suggests that RBP synthesized in prostate epithelium may prevent accumulation of vitamin A by secreting excess retinol back into the bloodstream. In addition, the prostate epithelium of 15-wk-old RBP-/- mice showed increased cellular proliferation compared with wild-type age-matched controls, as assessed by proliferating cell nuclear antigen (PCNA) levels. However, total p27 levels were also increased. Phospho-Akt2 concentrations were elevated, whereas phospho-Akt1 levels remained unchanged. Androgen receptor and cyclin D1 levels were also unaffected. We also introduced the probasin-regulated transgene expressing the SV40 early genes (T/t antigens; TRAMP) into the RBP-/- background and generated a mouse strain with both increased prostate cancer incidence and altered retinoid metabolism (RBP-/- TRAMP). As expected, the transgene increased PCNA levels in prostate epithelium. However, PCNA levels were reduced in RBP-/- TRAMP mice compared with RBP-/- TRAMP controls. These data suggest that vitamin A may suppress proliferation in premalignant cells. In summary, alteration of vitamin A metabolism seems to have distinct effects on the prostate of wild-type mice and of mice prone to developing prostate cancer. Further analyses are ongoing to determine how vitamin A exerts these effects.

Use of Vesiculated α-Tocopheryl Succinate and Dendritic Cell Vaccination to Treat Metastatic Murine Breast Cancer. Emmanuel T. Akporiaye,1 Lalitha Ramanathapuram,1 Tobias Hahn,1 and Sharon Dial.2

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α-Tocopheryl succinate (α-TOS) is a semisynthetic analog of vitamin E that preferentially induces apoptotic death of cancer cells. The ability of α-TOS to suppress tumor growth in preclinical animal models has led to increased interest in its potential use in combination with immunotherapy for treating cancer. We combined vesiculated α-TOS (Vα-TOS) with non-matured dendritic cells (DCs) to treat metastatic murine breast cancer. Treatment with Vα-TOS plus nonmatured DCs inhibited the growth of established tumors and prolonged survival. The combination treatment dramatically reduced formation of lung metastases after resection of primary tumor. We also compared Vα-TOS with α-tocopherylxyacetic acid (α-TEA) which unlike α-TOS is resistant to intestinal esterases and can therefore be administered orally without concern for conversion to vitamin E. Comparison of intraperitoneal versus oral gavage administration of both agents revealed that only α-TEA suppressed tumor growth when administered by oral gavage. To make α-TEA treatment more clinically relevant, we administered α-TEA in mouse chow to prevent or treat established disease and metastases. Dietary α-TEA suppressed primary tumor growth and dramatically reduced lung metastases. In trying to understand the mechanism of action of the combination treatment, we showed that Vα-TOS– and Vα-TEA–treated tumor cells caused DC maturation in vitro evidenced by increased expression of costimulatory molecules, IL-12p70 secretion, and antigen presentation. These effects are mediated in part by heat shock proteins released from tumor cells after α-TOS or α-TEA treatment. These findings demonstrate enhancement of in vivo antitumor effects of α-TEA and α-TOS when combined with DC vaccination. The results also suggest that combining DC vaccination with dietary α-TEA may be a viable approach for treating metastatic breast cancer.

Evaluation of the Anticancer Actions of Natural Vitamin E Forms, RRR-α-Tocopherol and γ-Tocopherol. Weiping Yu. Molecular Genetics and Microbiology, College of Natural Sciences, University of Texas at Austin, Austin, TX.

Focus of previous studies of vitamin E compounds as antitumor agents has been on natural RRR-α-tocopherol (αT), the major vitamin E form in tissues, and synthetic vitamin E, all-rac-α-tocopherol, found in supplements. Data supporting these vitamin E forms as potent antitumor agents are inconclusive. Recent data suggest that γ-tocopherol (γT), the most abundant form of vitamin E in the American diet, exhibits antitumor properties. Studies reported here examined the proapoptotic properties of αT and γT in human estrogen-responsive MCF-7 and non-responsive MDA-MB-435 breast cancer cells, focusing on prodeath and prolife signaling pathways. γT but not αT induced both cell types to undergo apoptosis in a dose- and time-dependent manner while sparing human mammary epithelial cells from apoptosis. γT sensitized both cell lines to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)–induced apoptosis. γT increased death receptor DR5 mRNA and protein expression. siRNA knockdown of DR5 blocked both γT-induced apoptosis and sensitization of breast cancer cells to TRAIL-induced apoptosis. γT induced activation of JNK/c-Jun, activation of proapoptotic Bax, processing of Bid to its truncated form (tBid), and cleavage of caspase-8 and -9. Furthermore, γT down-regulated antiapoptotic phospho-Akt (pAkt) and survivin protein expression. Knockdown of pAkt as well as survivin enhanced γT-induced apoptosis. In summary, these studies showed that γT but not αT induced apoptosis and sensitized human breast cancer cells to TRAIL/DR5–induced apoptosis via JNK/c-Jun/Bax and caspase-8/tBid/Bax, leading to mitochondria-dependent caspase 9 activation as well as suppression of antiapoptotic pAkt and survivin. [Supported by AICR grant 26–7693 and The Foundation for Research.]

Minerals

The Inflammatory Response and Breast Cancer Metastasis to Bone: A Role for Selenium? Andrea M. Mastro,1 K. Sandeep Prabhu,2 Hema Vunta,2 Leah J. Novinger,1 and C. Channa Reddy.2 Departments of Biochemistry and Molecular Biology and Veterinary and Biomedical Sciences, The Pennsylvania State University, University Park, PA.

The skeletal system is a frequent target of metastatic breast cancer. Once in the bone, the cancer cells activate osteoclasts to degrade bone. Nonetheless, the osteoblasts appear unable to restore the bone even in the presence of drugs that inhibit osteoclasts. We propose that in the presence of metastatic breast cancer, the osteoblasts no longer function normally as bone-building cells. In support of this idea, we found that human metastatic breast cancer cells, MDA-MB-231 and MDA-MB-435, increase osteoblast apoptosis, suppress osteoblast differentiation, and affect osteoblast morphology. In addition, we found that in the presence of breast-cancer-cell-conditioned medium, osteoblasts undergo an inflammatory stress response exemplified by the production of IL-6, KC (IL-8), macrophage chemo-attractant protein-1, and cyclooxygenase-2, which attract and activate osteoclasts. Our studies are based on the premise that
the cancer cells bring about this inflammatory response through a change in the oxidative state of the osteoblasts that is controlled by cellular selenium status through the redox-regulated transcription factor, nuclear factor-κB. Nuclear factor-κB was activated in osteoblasts in breast-cancer-cell-conditioned medium. Exogenous addition of selenium or catalase inhibited this activation, suggesting a role for reactive oxygen species. Because dietary selenium has been linked to various stages of cancer progression including metastasis, it is important that the mechanisms be elucidated. This study may provide evidence to show how selenium status may affect metastatic bone loss.


Although iron is an essential micromineral, excess iron may promote oxidative stress and be a risk factor for chronic disease such as cancer. We investigated the long-term effects of maternal iron overload on oxidative stress in hepatic tissues in female rats and their offspring. Ten-week-old female Sprague-Dawley rats were mated with male rats. Pregnant dams were fed diets with normal iron (35 mg/kg diet), high iron (350 mg/kg diet), and excess iron (1050 mg/kg diet) during pregnancy and lactation. Dams and their pups were killed on postnatal day 16. There was no significant difference in weight gain and diet intake according to iron intake levels. However, the concentration of serum iron, hematocrit, and transferrin saturation were significantly increased in pups from dams fed the excess iron diet but not in their dams. With increasing maternal iron intake, the iron and ferritin content of the liver in both dams and their pups were significantly increased. Maternal excess iron intake significantly increased the content of malondialdehyde and protein carbonyl of the liver in both dams and their pups. Excess iron intake also decreased the activity of antioxidant enzymes glutathione peroxidase, glutathione reductase, and catalase in the liver of pups. Cell swelling, vacuolation, necrotic foci, and iron deposition showed cell damage in the liver of high-iron and excess-iron diet groups. In addition, the protein level of Bcl-2 as an antiapoptotic factor was remarkably decreased with increased maternal iron intake. Although iron overload during pregnancy and lactation had little effect on hematology measurements of dams, it led to the deposit of iron, the decline of activity of antioxidant enzymes, and cell death in the liver tissues in both dams and pups. These results suggest that hepatic iron accumulation as a result of excess iron intake may enhance susceptibility to cancer development related to oxidative stress.