Poster Abstracts


The World Cancer Research Fund International (WCRF) and American Institute for Cancer Research (AICR) are producing a second expert report to follow the landmark 1997 Food, Nutrition and the Prevention of Cancer: A Global Perspective. The report will comprise the judgments of a panel of 21 independent experts from around the world. The evidence linking food, nutrition, and physical activity to the risk of developing cancer will be obtained from 22 systematic literature reviews (SLRs) that cover >20 cancer sites, obesity, cancer survivors, and other reports. The SLRs will be conducted by independent academic institutions in the United States, United Kingdom, and continental Europe. These SLR centers will use a novel, standardized methodology designed, with advice from an expert methodology task force, specifically to address etiologic rather than therapeutic questions. This novel methodology has been tested by conducting SLRs on food, nutrition, physical activity, and risk of endometrial cancer at one U.S. and one UK institution to assess feasibility, utility, and reproducibility. The SLR process was demonstrated to be feasible, and the results of the test were used to streamline the process. The substantive SLRs began in January 2004. For each SLR, both the initial protocol and the final draft report are subject to peer review for nutrition and cancer content, method of review, and statistics. As of May 2005, 10 SLRs have been discussed at panel meetings. The volume of material and depth of process have led to a revised timeline. All SLRs will be updated in June 2006. Late in 2007, after the final panel meeting in March, the report will be published in printed as well as electronic format and is expected to be the most authoritative report of its type.

Biomarkers

Serum Levels of Insulin-Like Growth Factor-I in Premenopausal Women Participating in a Low-Fat High-Carbohydrate Dietary Intervention Trial. Norman F. Boyd,* Lisa J. Martin,* Limei Sun,* Feng Deng,* Jennifer Stone,* Philip Connelly,* Carolyn Greenberg,* and Salomon Minkin.* **Division of Epidemiology and Statistics, Ontario Cancer Institute, University Health Network, Toronto, Canada; and †J. Alick Little Lipid Research Laboratory, St. Michael’s Hospital, Toronto, Canada.

BACKGROUND: Blood levels of insulin-like growth factor-I (IGF-I) have been associated with risk of breast and other cancers. IGF-I levels are influenced by intake of total calories and protein, but little evidence is available about the role of dietary fat. We examined the effect of dietary fat reduction on blood levels of IGF-I and its principal binding protein IGFBP-3. METHODS: IGF-I and IGFBP-3 levels were measured at baseline and 2 y in blood samples from premenopausal subjects in the intervention (n = 370) and control groups (n = 365) of an ongoing dietary intervention trial that will examine the effects of a low-fat, high-carbohydrate diet on the incidence of breast cancer. RESULTS: Two years after randomization, intake of fat as a percentage of energy was 20% in the intervention group and 30% in controls, and blood levels of triglycerides and high-density lipoprotein cholesterol were significantly different between the groups. Mean IGF-I levels in the intervention and control groups were 22.9 and 23.4 nmol/L (175 and 179 µg/L), respectively, at baseline and 22.0 and 22.0 nmol/L (168 and 168 µg/L), respectively, at 2 y and did not differ significantly between the groups. IGFBP-3 levels were also similar in the 2 groups. Age at first pregnancy, parity, smoking, and change in energy intake were associated with changes in IGF-I levels over 2 y. CONCLUSIONS: Dietary fat reduction changed blood lipid levels but did not reduce circulating levels of IGF-I or IGFBP-3. Smoking cessation and a modest reduction in caloric intake may reduce IGF-I levels.

Is Cancer Risk as Biomonitored by Procarcinogenic DNA Adducts Increased at High Plasma Concentrations of Vitamin C and in People with High Socioeconomic Status? Decreased DNA Adduct Levels at High Eicosapentaenoic Acid in Red Blood Cell Membranes. Erwin Eder,* Paul Wanek,* and Jakob Linsein. †Department of Toxicology, University of Würzburg, Germany; and ‡Clinical Epidemiology of the German Cancer Research Center, Heidelberg, Germany.

Procarcinogenic 1,N2-propanodeoxyguanosine DNA adducts formed by the lipid peroxidation product 4-hydroxy-2-nonenal lead to mutations in hotspots of tumor suppressor genes and protooncogenes and can lead to cancer initiation and progression. Therefore, they are biomarkers for cancer risk arising from lipid peroxidation. In context with the Bavarian Nutri-
against lipid peroxidation–induced cancer. No clear correlations were seen between other fatty acids and DNA adduct levels. 2) We observed a significant positive correlation ($P < 0.001$) between plasma vitamin C concentration and DNA adduct levels, indicating an increased cancer risk from lipid peroxidation. In some investigations, ascorbic acid also showed oxidative effects. No significant correlations between DNA adduct levels and other antioxidant plasma concentrations were found. 3) There was a significant correlation ($P < 0.001$) between the socioeconomic status as defined by household net income, educational level, and career position and the white blood cell adduct levels: the higher the socioeconomic status, the higher the adduct levels. These results confirm our previous results from 200 of 300 evaluated samples, and the correlations are now much clearer than previously.

**Carcinogens**


Well-done meat cooked at high temperatures contains the carcinogens polycyclic aromatic hydrocarbons (PAHs) and heterocyclic amines (HCAs). We examined the association between breast cancer and lifetime intake of grilled, barbecued, and smoked meat intake as well as recent intake of cooked meat, PAHs, and HCAs for 1508 cases and 1556 controls in the Long Island Breast Cancer Project, a population-based, case-control study. Average lifetime intake of grilled, barbecued, and smoked meat intake as well as recent intake of cooked meat, PAH, and HCA intake for the year before the interview. Using unconditioned logistic regression with adjustment for age, intake of energy, fruits, and vegetables; and multivitamin supplement use, we observed increased breast cancer risk among postmenopausal women with high lifetime consumption of grilled, barbecued, and smoked meats [odds ratio (OR) = 1.47; 95% CI: 1.12, 1.92 for women in the highest versus lowest tertile of lifetime meat intake] but not among premenopausal women. The strongest effect was observed among postmenopausal women consuming low-fruit and -vegetable diets (OR = 1.74; 95% CI: 1.20, 2.50 for women in the highest versus lowest tertile of lifetime meat intake). No substantial associations were observed for any meat, PAH, or HCA intake measures in the year before the interview except for PAH intake from meat among postmenopausal women with tumors positive for estrogen and progesterone receptors (OR = 1.47; 95% CI: 0.99, 2.19). This large population-based case-control study lends modest support to the mounting evidence that consumption, particularly throughout the lifetime, of meats cooked by methods that promote carcinogenic formation may be important in the etiology of postmenopausal breast cancer. [Supported in part from grant nos. CA/ES66572, P30ES10126, P30ES09089, 1K07CA102640–01, AICR-03B091.]


The heterocyclic amine 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) is a carcinogen formed in meats during cooking. By binding to the DNA, PhIP forms DNA adducts, which may ultimately lead to cancer. PhIP targets the colon, prostate, and breast tissues. In this study we looked at several dietary compounds to determine their reductive, and therefore potentially chemopreventive, effects against PhIP DNA adduct formation. For 18 d, male F344 rats were given access to a nutritionally adequate diet that contained Metamucil®, phenethylisothiocyanate (PEITC), chlorophyllin, a combination of PEITC and chlorophyllin, or no additional additives. One group was given Flor-Essence®, an herbal tea, in its drinking water. The rats were dosed with [14C]PhIP (3 μg/kg body wt) once daily for 10 d during the study. The animals were then killed, and the blood plasma, liver, colon, and prostate were collected. On isolation of the albumin or DNA, the samples were analyzed by the highly sensitive technique of accelerator mass spectrometry. Preliminary results indicate that the combination of PEITC and chlorophyllin as well as chlorophyllin by itself significantly reduced the level of PhIP-induced DNA adducts in all tissues as well as in the plasma. Alone, PEITC and Flor-Essence had no significant effects. Metamucil was found to significantly increase the level of adducts in the liver. We conclude that a combination of PEITC and chlorophyllin as well as chlorophyllin by itself in the diet may act as potential chemopreventive compounds against the carcinogen PhIP. [Conducted under auspices of the U.S. Department of Energy(LLNL W-7405-ENG-48) and supported by NIH Grants AT 001730 and P41 RR13461.]

Transformation of Immortalized, Nontumorigenic Prostate Epithelial Cells by a Heterocyclic Amine Commonly Occurring in the Human Diet. Martin A. Whiteside and James M. Phang. Laboratory of Comparative Carcinogenesis, Metabolism & Cancer Susceptibility Section, National Cancer Institute, Frederick, MD; and Cancer Prevention Fellowship Program, National Cancer Institute, Rockville, MD.

Prostate cancer is believed to result from an accumulation of DNA mutations over time. 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) is a heterocyclic amine abundantly formed when meats are cooked well-done and is a suspected etiological factor in prostate cancer. Studies in rats and mice have confirmed that PhIP forms DNA adducts and causes DNA mutations in prostate tissue and, moreover, induces prostate cancer in rats, albeit at high doses not found in the human diet. Studies using C3H/M2 mouse fibroblasts treated with N-OH-PhIP, the activated form of PhIP, demonstrated loss of contact inhibition; however, other evidence of possible transformation was not provided. Similar studies have not
been performed in any epithelial cell type, from which most cancers develop. We therefore synchronously treated immortalized, nontumorigenic prostate epithelial cells, RWPE-1, with N-OH-PhIP at 0.4 μmol/L 4 times the 1st week and asynchronously treated the cells twice during the 2nd week. By the end of the 4th week, large foci of piled-up cells were observed (loss of contact inhibition), from which we established 5 separate cell lines (clones). Matrix metalloproteinase-9 (MMP-9) activity, which is often elevated in cancer cells, was significantly reduced in clone 4 compared with the untreated control. Previous studies with RWPE-1 cells demonstrated that cell transformation is accompanied by MMP-9 activity elevated at least 2-fold, and the cells formed tumors when injected into nude mice. Interestingly, in our study clone 4 invaded through a matrigel basement membrane similar to that of DU-145 prostate cancer cells and was the only clone that formed significant colonies in soft agar, which suggests that elevated MMP-9 activity, used as a biomarker of transformation, may not identify all transformed cell lines. This is the first report suggesting that diet-relevant doses of N-OH-PhIP can transform immortalized, nontumorigenic prostate epithelial cells in vitro.

**Bladder cancer**


**INTRODUCTION:** Isoflavones have anticancer activities but are poorly absorbed in their natural glycosylated state. Genistein combined polysaccharide (GCP[b]™) is a fermentation product of soy isoflavone extract and Basidiomycetes enriched in biologically active aglycone isoflavones. The purpose of these studies was to determine whether GCP is effective in vitro and in vivo against human bladder cancer cell lines. METHODS: Three human bladder cancer cell lines were used: RT-4 (wt p53), T24 (p53 partial function mutation), and HT-1376 (p53 loss of function mutation). Cell growth was monitored using the WST-1 assay. Apoptosis was analyzed by flow cytometry and detection of poly-(ADP-ribose)-polymerase cleavage. RT-4 and HT-1376 tumor xenografts were implanted into immunodeficient mice to study the effects of a 2% GCP-supplemented diet on tumor growth compared with a control diet for 5.5 wk. Expression of Akt, pAkt, p53, Mdm2, pMdm2, and p21 was analyzed via western blotting. RESULTS: Growth of all cell lines in vitro was significantly inhibited in a dose-dependent manner by 72 h of GCP treatment (P ≤ 0.005). GCP induced marked apoptosis in RT-4 cells but a lesser response in T24 cells. Apoptosis was not detected in HT-1376 cells. The 2% GCP diet suppressed RT-4 tumor xenograft growth (P < 0.0001) but not HT-1376 xenograft growth. GCP induced expression of p53 and p21 in RT-4 cells and suppressed pMdm2 in RT-4 and HT-1376 cells. Phosphorylation of Akt was suppressed in both cell lines. CONCLUSIONS: Our data suggest bladder tumor cells with wt p53 will activate cell cycle suppressive and apoptotic signaling pathways when exposed to GCP, whereas tumor cells lacking functional p53 activate only cell cycle suppressive activities. This could have significant implications for the ability of dietary GCP to be an effective chemopreventive agent for bladder cancers.

**Effect of Low-Fat, High-Fiber Diet and Exercise Intervention on Breast Cancer Risk Factors and Tumor Cell Growth and Apoptosis.** R. James Barnard, Jenny Hong, Maud Liva, and Tung H. Ngo. Department of Physiological Science, University of California, Los Angeles, CA.

**OBJECTIVE:** The purpose of the present study was to investigate the effect of a low-fat, high-fiber diet and exercise intervention on serum risk factors and the effect of serum changes on serum-stimulated growth and apoptosis of 3 estrogen receptor–positive breast cancer cell lines. METHODS: Fasting serum was obtained from postmenopausal women participants at the Pritikin Longevity Center Residential Program where they were placed on a low-fat (10–15% energy), high-fiber (>40 g/d) diet and attended daily exercise classes for 2 wk. Serum samples were analyzed for estradiol, insulin, insulin-like growth factor-I (IGF-I) and insulin-like growth factor binding protein-1 (IGFBP-1). Serum was also used to stimulate the growth and assess apoptosis in MCF-7, T-47D, and ZR-75–1 breast cancer cell lines. RESULTS: Estradiol was reduced in the women on hormone replacement therapy (n = 16) as well as those not on hormone replacement therapy (n = 10). Insulin and IGF-I were reduced in all women, whereas IGFBP-1 was increased. In 12 other subjects (on HRT), serum estradiol was reduced and growth in serum-stimulated breast cancer cell lines was reduced from 6.6% for the MCF-7 cells to 18.5% for the T-47D cells, whereas apoptosis was increased from 20% in the ZR-75 to 30% in the T-47D cells. Analysis by terminal deoxyuridine nick end labeling confirmed apoptosis in T-47D cells in the postintervention serum samples. CONCLUSIONS: A very low fat, high-fiber diet combined with daily exercise results in major reductions in recognized risk factors for breast cancer. These in vivo changes in serum factors slowed the growth rate and induced apoptosis in breast cancer cell lines in vitro.

**Low Body Mass Index but High Fat and Energy Intake Are Associated with the Emergence of a Less Malignant Phenotype of Breast Cancer Defined by Tumor Grade, Proliferation, and Sets of Cell Cycle Regulators.** Signe Borgquist, Elisabeth Wirfält, Karin Jirstström, Lola Anagnostaki, Bo Gullberg, Göran Berglund, and Göran Landberg. Division of Pathology, Department of Laboratory Medicine, Malmö University Hospital, University of Lund, Sweden.

**BACKGROUND:** The exact link among dietary behavior, body constitution, and risk of breast cancer is still ambiguous, potentially influenced by the fact that breast cancer is a multitude of diseases with different bases for transformation and consequently etiology. METHODS: In a cohort of 17,035 women enrolled in the Malmö Diet and Cancer population study, 346 emerging breast cancers were subcategorized by conventional pathology parameters such as tumor type, grade, and proliferation as well as by expression of key suppressor genes and oncogenes involved in the cell cycle control. Tumors were studied on tissue microarrays. Subcategories were then related to diet history information and objective body measurements determined several years before the cancer diagnosis. RESULTS: Lobular breast cancers were, in comparison to ductal cancers, linked to a higher alcohol intake. Smaller hip size and lower BMI were associated with low-grade tumors, whereas total energy, total fat, and energy-adjusted fat intake were inversely associated with tumor proliferation. Sim-
Leptin—a nutritional regulator—also has immunomodulating and growth factor activity. In this way, hyperleptinemia frequently described in breast carcinoma patients may be implicated in both the proliferation of tumors and the immune disorders in developing cancer. The aim of this study was to detect the leptin expression variations in ductal and lobular breast carcinoma. Human biopsies were obtained from the tumor bank of the Anti-Cancer Center. Immunohistochemical assays of normal, ductal and lobular premalignant (hyperplasia) and tumoral (in situ or invasive) breast cells were performed using a leptin antibody. We reported that leptin was expressed both by ductal and lobular benign (hyperplasia) and malignant breast cells. Moreover, leptin was found in normal tissue in the vicinity of the ductal and lobular lesions but not in normal tissue of healthy breasts. These findings enabled us to hypothesize concerning the role of leptin in breast cancer progression, particularly in obesity, a major risk factor for breast cancer development.


Recent studies from our laboratory showed that carcinogenic effects of ethanol consumption might be related to its in situ metabolism at the cytosolic and microsomal levels to the mutagen acetaldehyde and to hydroxyl radicals. In this work we report that when Sprague-Dawley female rats were exposed to the standard Lieber & DeCarli diet for 28 d we observed 1) induction of the cytosolic and microsomal pathways of ethanol metabolism, and 2) promotion of oxidative stress as evidenced by increased formation of lipid hydroperoxides, delay in the t-butyldihydroperoxide–induced chemiluminescence, significant increases in protein oxidation (increased protein carbonyls and decreased protein sulfhydryls), and induction of xanthine oxidoreductase activity. An additional pathway for ethanol metabolism to acetaldehyde was observed in mitochondria via a rotenone-insensitive NADH-dependent bioactivation. The epithelial cells evidenced marked ultrastructural alterations, consisting of markedly irregular nuclei with frequent invaginations at the level of the nuclear envelope, condensation of chromatin around the inner nuclear membrane, and marked dilatation of the nuclear pores, showing filamentous material exiting to cytoplasm. In conclusion, the presence in mammary epithelial cells of cytosolic, microsomal, and the here-reported mitochondrial pathways of ethanol bioactivation to carcinogenic and tumor-promoting metabolites may play a role in alcohol promotion of breast cancer. [Supported by a grant from the University of San Martín (PIDA UF105).]
Dietary Intakes of (n-3) Polyunsaturated Fatty Acids and Their Interactions with Antioxidant Vitamin E in Relation to the Risk of Breast Cancer: the E3N-EPIC Cohort Study. Anne C.M. Thiebaut,*† Véronique Chajes,** Mariette Gerber,‡ Franco Berrino,†† Elio Riboli,‡‡ Jacques Bénichou,† and Françoise Clavel-Chapelon.* †INSERM EA E3N-EPIC, Institut Gustave Roussy, Villejuif, France; ‡Centre de Recherche en Cancérologie, Rouen, France; **UMR-CNRS 8125, Institut Gustave Roussy, Villejuif, France; ††Centre de Recherche en Cancérologie, INSERM-CRLC, Montpellier, France; ‡‡Unité Operativa di Epidemiologia, Istituto Nazionale per la Cura e lo Studio dei Tumori, Milan, Italy; and **International Agency for Research on Cancer, Lyon, France.

BACKGROUND: Experimental studies suggest beneficial effects on mammary carcinogenesis of (n-3) PUFAs, possibly in interaction with antioxidants. However, epidemiological studies have been inconsistent, and few have assessed combined effects of (n-3) PUFA and antioxidant intakes on breast cancer risk. OBJECTIVE: The objectives of this study were to examine the association between (n-3) PUFA intakes and breast cancer risk and to evaluate effect modifications by vitamin E intakes. DESIGN: The study population included 67,629 women from the French E3N-EPIC cohort, aged 41 to 71 y, who completed a diet history questionnaire in 1993. For an average follow-up of 8.0 y, 2054 incident cases of invasive breast cancer were recorded. RESULTS: Overall, breast cancer risk was not related to individual (n-3) PUFA intakes. However, breast cancer risk was decreased with increasing intakes of α-linolenic acid [18:3 (n-3)] originating from vegetable sources [highest versus lowest quintile, hazard ratio (HR) = 0.82; 95% CI: 0.71, 0.95; \( P_{trend} = 0.009 \)] and increased with increasing intakes of α-linolenic acid from nuts (\( P_{trend} = 0.031 \)). Long-chain (n-3) PUFA intakes were positively associated with breast cancer risk in the lowest quintile of dietary vitamin E intake (HR = 1.65; 95% CI: 1.18, 6.09; \( P_{trend} = 0.036 \)). Conversely, in the highest quintile of vitamin E intake, HRs related to increasing quintiles of long-chain (n-3) PUFA intake were all below unity but not significantly so. CONCLUSIONS: These data suggest that the effect of α-linolenic acid intake on breast cancer risk may differ according to food sources, either reflecting different food patterns or different α-linolenic acid isomers. They also suggest that supplementation with (n-3) PUFAs should be counter-balanced by appropriate dietary antioxidant intakes to avoid carcinogenic effects. However, this study provides no support that vitamin E as a supplement may reduce breast cancer risk.


Although isoflavones and lignans are dietary phytoestrogens hypothesized to protect against breast cancer, a hormone-dependent disease, large prospective studies are needed to clarify their role in breast cancer. In 1993, 208-item diet history questionnaires were collected for 62,523 female participants of the French E3N-EPIC cohort, aged 41–71 y and not consuming soy supplements. We estimated usual dietary intakes of 4 isoflavones, 2 lignan precursors, and 2 enterolignans using a comprehensive food database based on published data. Until 2002, 383 premenopausal and 1115 postmenopausal women were diagnosed with invasive breast cancer. We used Cox proportional hazards regression to estimate relative risk (RR [95% CI]) for breast cancer associated with phytoestrogen intakes; stratifying on menopausal status and estrogen receptor (ER) and progesterone receptor (PR) status of the tumor; and adjusting for energy intake, reproductive history, and use of hormonal treatments. Estimated median dietary intakes (range) were 0.66 (0.01–8.14) mg/d of isoflavones and 5.07 (0.05–131.18) mg/d of vegetable lignans. Using the lowest quintile as reference, we found no phytoestrogens associated with risk of premenopausal breast cancer. Among postmenopausal women, we found a significant inverse association with highest intakes of lignans and the isoflavone genistein; when we stratified on receptor status of the tumors, these inverse relations were limited to ER+ PR+ tumors: RR = 0.75 (95% CI: 0.58, 0.96) for the highest quintile of genistein (\( P_{trend} = 0.02 \)) and RR = 0.73 (95% CI: 0.57, 0.94) for the highest quintile of vegetal lignans (\( P_{trend} = 0.04 \)), with similar results for their metabolites and enterolignans. In conclusion, levels of phytoestrogen intake in the E3N-EPIC cohort are typical of Western diets. At these levels, lignans and genistein were associated with a reduced risk of postmenopausal breast cancer tumors positive for ER and PR receptors. The role of dietary lignans in the biology of postmenopausal breast cancer in Western countries particularly deserves further investigation.

Exposure to the Dutch Famine of 1944-1945 and Shifts in Hormonal Set Points: Parathyroid Hormone. Paulus A. H. van Noord,* ‡Cécile M. Ronckers,* Sjoerd G. Elias,* and Petra H. M. Peeters.* †Julius Center UMC Utrecht, the Netherlands; and ‡Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD.

BACKGROUND: Exposure to the 1944–1945 Dutch famine was shown to be associated with increased levels of androgens, estrogens, and insulin-like growth factor later in life, hormones under control of the pituitary luteinizing hormone/follicle stimulating hormone and growth hormone axes. Moreover, exposed women were at increased risk of breast cancer. A neurodevelopmental set-point-shift hypothesis, proposed to consolidate these findings, predicts a mechanism of shifting in hormonal set points. In the present study, we tested whether famine exposure affects parathyroid hormone (PTH). STUDY DESIGN: We used data from a study on osteoporosis nested within the DOM breast cancer screening cohorts including 212 women born in 1911 to 1925 whose hormones were measured in 1980. During a subsequent screening round 2 y later, 156 women provided information about exposure to the
Dutch Famine of 1944–1945, thus enabling secondary analyses. RESULTS: A clear univariate increase in postmenopausal PTH levels was found among women who indicated exposure to the Dutch famine. Odds ratio by extreme PTH tertiles was 1.5 (95% CI: 0.6, 3.4) for moderately exposed women and 2.3 (95% CI: 1.1, 5.2) for severely exposed women. In a regression analysis of famine exposure on PTH levels, after controlling for levels of phosphorus, calcium, calcium ion, alkaline phosphatase, and estrone; having been pregnant; parity; age at menarche; age at blood donation; smoking; hormone replacement therapy use at time of blood collection; height; weight; and socioeconomic status, the independent significant contribution of PTH remained. CONCLUSIONS: The results corroborate the hormonal set-point hypothesis and seem to extend effects of famine exposure to hormones such as PTH, which plays a role in early bone growth as well as in osteoporosis later in life. Though PTH is not under hypothalamic or pituitary control, it is partially under ganglion control. Embryologically, the parathyroids and the adenohypophysis share a common origin from pharyngeal endodermal pouches.

Cervical cancer

A Randomized Placebo-Controlled Trial to Evaluate Interactions between Riboflavin and Folate Intake and Genotype in Reducing Risk of Cervical Cancer. Emma L. Stuart,* John Tidy,† Margo Barker,* and Hilary J. Powers.*  *Human Nutrition Unit, University of Sheffield, Sheffield, UK; and †Gynaecological Oncology, Royal Hallamshire Hospital, Sheffield, UK.

INTRODUCTION: Diet can play an important role in protecting against cancers at various sites. Studies in humans have linked folate intake with the risk of cervical cancer. There is a plausible biochemical basis for this relation, because folate is linked to DNA synthesis, stability, and repair, which are important for cancer prevention. These effects of folate are modulated by the presence of the T allele for the C677T variant of MTHFR. Folate is also thought to protect against the potentially cancerous changes associated with human papillomavirus infection. Infection with human papillomavirus is a necessary causative factor for the development of cervical cancer. AIMS: By means of a randomized, placebo-controlled, double-blind intervention study, we wish to explore the protective effect against cervical cancer of folate and riboflavin supplementation in women who have biopsy-proven cervical intraepithelial neoplasia grade 1 (CIN1) and who are also positive for human papillomavirus infection. STUDY DESIGN: One hundred eighty women will receive 1.2 mg folic acid/5 mg riboflavin or placebo, every day for 12 mo. The two groups will be stratified for the C677T polymorphism. STUDY MEASURES: A cervical biopsy taken at 12 mo will enable assessment of the effectiveness of this strategy in causing regression of CIN1. Blood samples will be collected at baseline and at 12 mo for the measurement of folate and riboflavin status. Cervical cell samples collected at the same times will be used to measure DNA strand breakage, uracil misincorporation, and gene-specific hypermethylation. Results will provide an evidence base for dietary advice to women for the prevention of cervical cancer.

Colorectal cancer

Interaction of Diet and Colonic Flora on Colorectal Cancer Risk in African Americans. Daniel Y. Chung,* Nevine Mah-
were fed the assigned diets until they were killed by CO₂ at age 45 wk. Cecal pH, cecal weight, feed intake, weight gain, tumors in the colon and liver, and glutathione S-transferase activity were determined. There were no significant (P < 0.05) differences in cecal weight, although cecal pH differed. Tumor incidences (%) in the colon of rats in the control, I, P, and I + P groups at 10, 15, and 20% were 100, 75, 42, and 36; 100, 46, 41, and 41; and 100, 54, 36, and 23, respectively. Colon tumors per tumor-bearing rat were 3.22, 1.7, 2.0, and 1.0; 3.22, 3.1, 2.5, and 2.1; and 3.22, 2.2, 1.1, and 0.6, respectively. Glutathione S-transferase values were 12.5, 29.3, 30.4, and 31.7; and 12.5, 29.5, 30.5, and 31.2, respectively. The results indicate that dietary peanuts suppress colon tumors, particularly at the promotion stages in AOM-induced colon tumors. Peanuts may therefore play a role in chemoprevention if incorporated in the daily diet.

Wheat Class but Not Processing Affects Regression of Colonic Precancerous Lesions (Aberrant Crypts) in Rats. Ajmila Islam and Daniel D. Gallaher. Department of Food Science and Nutrition, University of Minnesota, St. Paul, MN.

Previously, our laboratory found that hard red wheat flour decreased colonic precancerous lesions (aberrant crypt foci, ACF) relative to soft white flour, regardless of processing (whole vs. refined). Here we report the effects of wheat class and processing on the regression of established ACF and sialomucin (SIM)-producing ACF in the promotion stage of colon cancer. Rats were adapted to an AIN-93G (control) diet for 10 d and administered dimethylhydrazine twice, 1 wk apart. Rats continued to be fed the control diet throughout the carcinogen treatment and for an additional 6 wk. One group (n = 15) then was killed, and the remaining rats were divided into 5 groups (n = 15). One group was kept on the control diet and the rest were fed diets containing 61.5% of either whole hard red (WHR), refined hard red (RHR), whole soft white (WSW), and refined soft white (RSW) for an additional 8 wk. ACF were significantly lower in the WHR and RHR groups, compared with the control and RSW groups. Rats fed diets containing hard red wheat flour, either whole or refined, had significantly fewer aberrant crypts, ACF, large ACF, and SIM-producing ACF and a lower multiplicity than those fed diets containing soft white wheat flour. However SIM-producing ACF, suggested to have greater tumorigenic potential, were reduced in both hard red and soft white wheat diets, compared with the final control diet group. There was a significant effect of wheat class (red vs. white) but no effect of processing (whole vs. refined) in reducing ACF numbers. Thus, hard red wheat flour, whether whole or refined, caused regression of ACF in rats, thereby reducing colon cancer risk. [Supported by the American Institute for Cancer Research.]

Effect of Wheat Bran on Azoxymethane (AOM)-Induced Aberrant Crypt Foci (ACF) in Fisher 344 Male Rats. Adrienne Johnson, Martha Verghese, Judith Boateng, Louis Sinha, and V. Mai. Food Science and Technology, University of Maryland Eastern Shore, Princess Anne, MD; Department of Epidemiology and Preventive Medicine, School of Medicine, University of Maryland, Baltimore, MD; Food Sciences, Purdue University, West Lafayette, IN; and Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD.

African Americans suffer from an increased incidence and mortality of colorectal cancer. The underlying causes for these differences are not well established. Diet has long been thought to play an important role in the development of cancer, and intestinal microflora have been suspected of mediating at least some effects of diet on colorectal cancer. We conducted a pilot study to compare dietary intake and intestinal microflora composition between African American (AA; n = 50) and Caucasian American (CA; n = 50) residents of the Eastern Shore of Maryland. Healthy participants aged 40 to 75 y completed an extensive nutritional assessment (Block 98 FFQ, National Cancer Institute meat consumption and preparation questionnaire, 4-d food records) and donated a stool sample for molecular microflora analysis. Preliminary analysis of the FFQ data indicated significant differences in macronutrient intake between the two groups. AAs consumed a diet higher in percentage of energy from carbohydrates than that of CAs. The AA diet was lower in supplemental vitamins A, C, D, and E; calcium; and magnesium. The CA diet was characterized by a higher percentage of energy from fats. Some of these dietary differences may contribute to the observed increased colorectal cancer risk in AAs. An initial analysis of fecal microflora composition did not reveal clear differences between groups. However, in this unique data set that combines comprehensive nutritional data with detailed molecular data on microflora composition, we detected a decrease in the numbers of bacteria belonging to clostridia cluster IVX, common members of the normal microflora, in subjects that consumed a high-fat diet. To our knowledge, this is the first time that a specific association between diet and microflora com-

Diet and Microflora Composition in African American and Caucasian American Residents of the Eastern Shore of Maryland. Q. McCrary,* Q. Lin, C. J. Boushey,** R. Sinha, and V. Mai. † Food Science and Technology, University of Maryland Eastern Shore, Princess Anne, MD; Department of Epidemiology and Preventive Medicine, School of Medicine, University of Maryland, Baltimore, MD; ‡ Food Sciences, Purdue University, West Lafayette, IN; and Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD.

Wheat bran contains most of the vitamins and minerals found in the seed of wheat. It also contains substantial amounts of protein and carbohydrates. Wheat bran also contains both soluble and insoluble fiber. Fiber has been known to reduce cholesterol and prevent cardiovascular diseases and cancer. The objective of this study was to examine the effects of feeding wheat bran on azoxymethane (AOM)-induced aberrant crypt foci (ACF) formation and liver glutathione S-transferase (GST) activity in Fisher 344 male rats. After a 1-wk period of acclimatization, 16 rats were assigned to 2 groups and fed AIN93G (C) and C + 5% toasted wheat bran. At ages 7 and 8 wk, all rats were treated with an s.c. injection of AOM (16 mg/kg body wt). Rats were killed at age 17 wk by CO₂ asphyxiation. Colonic ACF (preneoplastic lesions) were scored, and liver GST activity was analyzed. Results showed that there were no significant (P < 0.05) differences in body weight and feed intake between the control and test groups. Reductions in ACF formation in the proximal and distal colon were 28% and 64%, respectively, with an overall total reduction of 42%, compared with controls. There were significant (P < 0.05) increases in liver GST activity in rats fed wheat bran compared to the control. Our results indicate that wheat bran significantly (P < 0.05) reduced AOM-induced ACF and also increased the activity of hepatic glutathione S-transferase, a key phase II detoxification enzyme. The inclusion of wheat bran in daily diets may have significant health implications.

Previously, our laboratory found that hard red wheat flour and processing at 15% of the diet resulted in reducing ACF numbers. Thus, hard red wheat flour, whether whole or refined, caused regression of ACF in rats, thereby reducing colon cancer risk. [Supported by the American Institute for Cancer Research.]
position has been established using an FFQ dietary assessment method combined with molecular microflora analysis.

Genotype-Phenotype Relations in Glutathione-S-Transferases and the Role of Vegetable Consumption in a Dutch Sigmoidoscopy-Based Population. Mariken J. Tijhuis,* Marleen H.P.W. Visker,* Jac M. Aarts,† Wilbert Peters,** Frans Kok,* and Ellen Kampman.* *Division of Human Nutrition and †Division of Toxicology, Wageningen University, Wageningen, The Netherlands; and **Department of Gastroenterology, University Medical Centre St Radboud, Nijmegen, The Netherlands.

The large intestine has a relatively low glutathione-S-transferase (GST) expression and high occurrence of neoplasia. Both genetic and environmental factors are thought to influence the functionality of the GST system. GST genotype-phenotype relations were investigated in 94 Dutch patients scheduled for sigmoidoscopy. Subjects were free of bowel inflammation and colorectal cancer. They kept a 3-d dietary record, ending at the time of endoscopy, and filled out a general questionnaire on other lifestyle factors. Functional polymorphisms in 4 GST isoforms were assessed in DNA isolated from blood, among them single-nucleotide polymorphisms in GSTP1 (A313G, resulting in an amino acid substitution) and in GSTT1 (C-69T, part of a functional haplotype). GSTT1 and a 16-kb isoenzyme levels were measured in rectal tissue by Western blotting. Total GST activity was measured spectrophotometrically in rectal tissue and white blood cells using 1-chloro-2,4-dinitrobenzene as a substrate. Results from all laboratory assays were normalized to protein content. Statistical analyses were adjusted for age and sex. Rectal GST activity showed a significant correlation with GSTP1 level (partial $r^2 = 0.18$). Activity differed between GSTP1 A313G genotypes ($P < 0.001$), the AG and GG genotype showing a mean of $\approx 30$ and 60 nmol min$^{-1}$ mg protein$^{-1}$ lower GST activity, respectively. The same trend was seen for GST activity in lymphocytes but not in leukocytes. Consumption of allium vegetables was positively associated with rectal GST activity, while consumption of cucurbitaceae and brassica vegetables was negatively associated (AG and GG versus AA genotype, respectively).

Glutathione S-transferases (GSTs) metabolize cancer-protective compounds from cruciferous vegetables and may differentially do so depending on genetic variation. The possible interplay among cruciferous vegetable consumption; functional genetic variations in GST M1, T1, P1, and A1; and colorectal adenomas was investigated in a Dutch case-control study. The study included 746 subjects with at least 1 histologically confirmed colorectal adenomatous polyp ever in their lives and 698 controls without any type of colorectal polyp in their medical history and at index colonoscopy. Dietary habits were assessed by the Dutch version of the European Investment into Cancer food frequency questionnaire. Other lifestyle factors, such as smoking habits, were determined by a general questionnaire. Functional polymorphisms in 4 GST isoforms were assessed in DNA isolated from whole blood: the GSTM1 and GSTT1 deletion polymorphisms and single-nucleotide polymorphisms in GSTP1 (A313G) and in GSTT1 (C-69T, part of a functional haplotype). High- and low-consumption groups were formed based on a median split in the control group. Consumption of cruciferous vegetables was slightly positively associated with colorectal adenomas, odds ratio (OR) = 1.15 (95% CI: 0.92, 1.44). When genotype was taken into account, a positive association with high brassica intake was only apparent in individuals with the low-activity GSTP1 allele (GG-genotype, adjusted OR = 1.94; 95% CI: 1.02, 3.69), and this interaction was more pronounced in men, with higher age and with higher red- and processed-meat consumption. There may be a modifying role for the GSTT1 variant as well: the OR with higher intake compared with lower intake was 1.59 (95% CI: 0.94, 2.69) for individuals homozygous for the low expression variant (TT genotype). This was most apparent with younger age and higher meat intake. The GSTM1 and GSTT1 genotypes did not modify the association between cruciferous vegetable intake and colorectal adenomas.

**Endometrial cancer**

BMI in Patients with Postmenopausal Bleeding. Angela F. Logullo,* Luiz C. Albuquerque-Neto,† Wapnen J. Gonçalves,‡ Mona Gabriela Giusa-Chiferi, and Eduardo C. Baracat.* Pathology Department and †Obstetrics/Gynecology Department, Escola Paulista de Medicina—Universidade Federal de São Paulo, São Paulo, Brazil.

OBJECTIVES: The aim of this study was to evaluate BMI in patients with postmenopausal bleeding. METHODS: We studied 98 patients with postmenopausal bleeding treated at São Paulo Hospital (Escola Paulista de Medicina) at São Paulo Federal University in Brazil. All patients had their BMI calculated; they also had transvaginal ultrasonography, hysterectomy, and endometrial biopsy. When the results showed adenocarcinoma, the patient underwent surgery (abdominal exploration, total hysterectomy with bilateral salpingo-oophorectomy, omental biopsy, peritoneal washings, and pelvic lymph node biopsy). RESULTS: The average age was 63.64 y (range 46–88 y). We found 40 (40.81%) patients with endometrial adenocarcinoma, 38 (38.77%) with endometrial polyps, and 20 (20.41%) with atrophic endometrium. Only 21 (35.71%) had a healthy body weight (BMI < 25), and 63 (64.29%) were overweight or obese. BMI was associated with the presence of polyps but not with malignancy. Patients with endometrial polyps had higher BMIs than did patients with atrophic endometrium and endometrial adenocarcinoma (P = 0.0108). CONCLUSIONS: The highest BMIs were among patients with endometrial polyps, but in this study 64.29% of all patients were overweight or obese. The best risk factors for endometrial adenocarcinoma are chronic estrogen exposure, early menstruation, late menopause, and few or no children. Additional factors include morbid obesity, hypertension, and diabetes.

**Laryngeal cancer**

Peculiarities of the Effect of Main Risk Factors on Laryngeal Cancer in Lithuania. Lilija Jaseviciene* and Aurelija Novelskaite." Institute of Oncology of Vilnius University,
INTRODUCTION: In Lithuania, larynx cancer was responsible for 1.8% of all malignant diseases in a year. About 200 new cases are recorded per year; 90% occur in men. There is a distinct male predominance for cancer of the larynx, but recent data in Lithuania show that the ratio of affected males to females is decreasing as the result of increasing incidence among women. AIM: The aim of the study was to assess the relation between nutritional habits, smoking, and alcohol consumption and laryngeal cancer. METHODS: The role of laryngeal cancer risk factors was investigated in a retrospective case-control hospital-based study on 149 cases and 298 hospital controls. Information on smoking, alcohol consumption, and nutritional habits was obtained from questionnaires. RESULTS: We identified a relation between the illness and food preparation by frying (odds ratio = 2.12; 95% CI: 1.35, 3.33). However, no relation between nutritional habits and laryngeal cancer was established, and no significant association was found between daily consumption of vegetables and fatty foods and laryngeal cancer ($\chi^2 = 1.31$). Our analysis demonstrated that smoking is one of the main factors, increasing the risk of illness by 3.58 times (95% CI: 2.15, 5.95). The risk for laryngeal cancer also increased with increasing length of time of alcohol consumption ($\chi^2 = 18.36$) and alcohol strength ($\chi^2 = 29.92$). Consumption of alcohol increased the risk of illness 4.67 times (95% CI: 2.15, 10.15). The relation of smoking and drinking in the regression model increased the probability of illness by 3.71 times (95% CI: 2.34, 5.88). CONCLUSIONS: Despite Lithuanian eating traditions, it is obvious that the patients’ diet lacked plant-based foods and oils but contained too many fatty animal foods. The use of more and lower-quality alcohol and tobacco also increased the risk for laryngeal cancer.

Melanoma

The Protective Effect of Bluefish, Tea, and Fresh Herb Consumption on Cutaneous Melanoma. Cristina Fortes,*, Simona Mastroeni,*, Franco Melchi,*, Maria Antonietta Pilla,*, Massimo Alotto,*, Gianluca Antonelli,*, Elisabetta Luchetti,*, and Paolo Pasquini,*. *Clinical Epidemiology Unit and †Dermatology Unit, Il Comitato Etico dell’Istituto Dermopatico dell’Immacolata (ID-IRCCS), Rome, Italy.

BACKGROUND. Although exposure to sunlight can be considered the major etiologic factor for the development of melanoma, melanoma risk does not simply increase with an increasing amount of accumulated exposure to ultraviolet radiation, and exposure is not the only risk factor. The present study was designed to evaluate the role of dietary factors in malignant cutaneous melanoma. METHODS: During 2001–2003, a hospital-based case-control study of melanoma was conducted on 304 incident cases of cutaneous melanoma and 305 controls, frequency matched by sex and age ($\pm 5\,\text{y}$), and admitted to the reference hospital of skin disease for central-south Italy, IDI-IRCCS, in Rome. Exposure characteristics were obtained by interviewing study subjects. A food frequency questionnaire was used. Using logistic regression, odds ratios (ORs) for melanoma were computed for all dietary items. RESULTS: After careful control for sun exposure and other confounding variables, protective effects for cutaneous melanoma were found for high consumption of fruits and cooked vegetables in general (OR = 0.44, 95% CI: 0.26, 0.74 and OR = 0.44, 95% CI: 0.26, 0.75, respectively), cruciferous vegetables (OR = 0.38, 95% CI: 0.22, 0.64), leafy green vegetables (OR = 0.34, 95% CI: 0.20, 0.58), carrots (OR = 0.55, 95% CI: 0.32, 0.95), fish rich in (n-3) fatty acids (OR = 0.46, 95% CI: 0.29, 0.73), nuts (OR = 0.40, 95% CI: 0.21, 0.76), and tea drinking (OR = 0.27, 95% CI: 0.10, 0.77). Regular consumption of fresh herbs (sage and rosemary) was also associated with a decreased risk of melanoma (OR = 0.36, 95% CI: 0.17, 0.72). In a multivariate model, considering all food items simultaneously, the protective effect of high consumption of fruits, tea, and fish rich in (n-3) fatty acids and regular consumption of fresh herbs remained significant. CONCLUSIONS: Our results indicate that some food items typical of the Mediterranean diet are associated with decreased risk of melanoma.

Ovarian cancer

Effects of Sphingosine and Enigmol on Mouse Ovarian Surface Epithelial Cells Representing Early, Intermediate, and Late Stages of Ovarian Cancer. Eva M. Schmelz, Emilio P. Mottillo, Andrea C. Baxa, Nicole Doyon-Reale, and Paul C. Roberts. *Karmanos Cancer Institute and †Department of Immunology/Microbiology, Wayne State University School of Medicine, Detroit, MI.

Studies to identify early markers of ovarian cancer to support the development of chemopreventive regimens have been hindered by the lack of adequate cell models. Using the spontaneous transformation of mouse ovarian surface epithelium (MOSE) in vitro, we isolated MOSE cells from C57BL/6 mice and characterized distinct transitional states in their progression from a nontumorigenic to a highly aggressive malignant phenotype. During neoplastic progression, our ovarian cancer model undergoes distinct changes in proliferation, anchorage-independent growth, and tumor formation in vivo. Concomitant with these changes, we found the downregulation and/or aberrant subcellular localization of two tumor-suppressor proteins, E-cadherin and connexin-43. These changes were concurrent with a significant increase in growth rate and anchorage-independent growth capacity. Our novel MOSE model, which provides multiple stages and protein markers for treatment efficacy, was used for the initial in vitro evaluation of the chemopreventive and chemotherapeutic potential of sphingolipid metabolites and the novel sphingolipid analogue Enigmol. Treatment with either sphingolipid metabolites or Enigmol prevented or reversed the neoplastic progression of MOSE cells at distinct stages in vitro. We demonstrated that the use of low, nontoxic doses of sphingolipids and Enigmol appears to exert antiproliferative effects on MOSE cells; thus, they have chemopreventive properties. In contrast, high doses of sphingolipids and their derivatives have cytotoxic, apoptotic properties in ovarian cancer cells. The use of low doses of sphingolipids provides a promising strategy for both chemoprevention and chemotherapy of ovarian cancer, two strategies that are currently being investigated in our laboratories in greater detail in vivo using the immunocompetent C57BL/6 mouse model. [Supported by American Institute for Cancer Research Grant 04B071 (to EMS), and a grant from the Elsa U. Pardee Foundation for Cancer Research (to PCR).]

Pancreatic cancer

Characterization of Scutellaria baicalensis Root Extract: a Viable Plant-Based Adjunct Therapy against Pancreatic
Cancer. Melonni A. Dooley,* Pinku Mukherjee,† and W. Dennis Clark.* *Arizona State University, Tempe, AZ; and †Mayo Clinic, Scottsdale, AZ.

Single-modality treatments or even combinations of surgery and chemotherapeutic and radiation therapies have not been successful in the treatment of pancreatic cancer. To achieve more therapeutic efficiency, cancer strategies require multiple agents with different modes of action used in combination. One such strategy, which shows tremendous potential against other cancers, entails the use of low-toxicity, flavonoid-rich plant extracts in combination with current chemotherapeutic drugs. Flavonoids have been shown to have antioxidant, anti-inflammatory, antiviral, antibacterial, and antitumor properties. The goals of this study were to determine the flavonoid composition of hot-water extracts of Scutellaria baicalensis Georgii and to investigate its antitumor potential as a single agent or in combination with conventional chemotherapy. The root of S. baicalensis is rich in flavonoids, and it has been used in China to enhance liver function and treat inflammatory-related disorders for >2000 y. Analysis using reverse-phase high performance liquid chromatography and matrix-assisted laser desorption ionization–time of flight mass spectrometry showed that all hot-water extracts of S. baicalensis contained 4 flavonoids: 15.4% baicalein, 3.2% baicalin, 0.4% wogonin, and 2.6% wogonin-7-O-glucuronide. We further determined that the whole root extract had 2.25 times greater antioxidant efficiency than the standard antioxidant ascorbic acid or the individual flavonoids present in the extract. The antitumor activity of the extract was tested on 2 highly invasive human pancreatic cancer cell lines, BxPc-3 and MiaPaCa-2. Using the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction cell viability assay, we observed significant reduction in cell viability and proliferation of the pancreatic tumor cells treated with the extract. The LD50 value for both cell lines was determined to be 150 μg/mL of the extract. Future experiments will elucidate the antitumor synergy of the S. baicalensis extract in combination with standard chemotherapy (gemcitabine) for the treatment and prevention of pancreatic cancer.

Dietary Patterns and the Risk of Pancreatic Cancer. André Nkondjock,* Parviz Ghadirian,† Kenneth C. Johnson,** and Daniel Krewski.† *Epidemiology Research Unit, Research Centre, Centre Hospitalier de l’Université de Montréal (CHUM)-Hôpital-Dieu, Montreal, Canada; †McLaughlin Centre for Population Health Risk Assessment, Institute of Population Health, University of Ottawa, Ottawa, Canada; and **Surveillance and Risk Assessment Division, Centre for Chronic Disease Prevention and Control, Population and Public Health Branch, Health Canada, Ottawa, Canada.

Although individual foods and nutrients have been implicated in the development of pancreatic cancer, the effect of overall food consumption has received little attention to date. To investigate associations between broad dietary patterns and pancreatic cancer risk, the authors carried out a case-control study of 462 histologically confirmed pancreatic cancer cases and 4774 population-based controls in 8 Canadian provinces between 1994 and 1997. Dietary intake was assessed using a food frequency questionnaire. Major dietary patterns were identified by factor analysis. Unconditional logistic regression was used to describe associations between dietary pattern scores and the risk of pancreatic cancer. Three dietary patterns were identified. The Western pattern was characterized by a high intake of processed meats, sweets and desserts, refined grains, and potatoes. The fruits and vegetables pattern was characterized by a high intake of fresh fruits and cruciferous vegetables. The drinker pattern was characterized by higher consumption of liquor, wine, and beer. After adjustment for age, BMI, smoking, physical activity, province, and total energy intake, the fruits and vegetables pattern was associated with a 49% reduction in pancreatic risk among men (odds ratio = 0.51; 95% CI: 0.29, 0.90; P trend = 0.004) when comparing the highest and lowest quartiles of dietary pattern scores. No significant relation was observed with the Western and drinker patterns. These results suggest that the fruits and vegetables dietary pattern may reduce pancreatic cancer risk among male Canadians.

Prostate cancer

β-ionone Suppresses the Proliferation of Human DU145, PC-3, and LNCaP Prostate Tumor Cells. Sheila Jones and Huanbiao Mo. Department of Nutrition and Food Sciences, Texas Woman’s University, Denton, TX.

3-Hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase is the rate-limiting activity in the mevalonate pathway that provides essential intermediates for posttranslational modification and biological activity of growth-associated proteins. Assorted dietary isoprenoids found in plant foods suppress HMG CoA reductase and have chemopreventive activity. β-ionone, a cyclic sesquiterpene and an end-ring analogue of β-carotene, has been shown to suppress reductase activity and, consequently, tumor cell proliferation and the growth of implanted tumors. Growth-suppressive activity of β-ionone was evaluated in cultured human prostate tumor cells. The Guava ViaCount assay showed 9% (75 μmol/L), 21% (150 μmol/L), 59% (225 μmol/L), 71% (300 μmol/L), and 85% (375 μmol/L) inhibition of DU145 cell proliferation after a 72-h incubation, with concomitant cellular morphological changes suggestive of apoptosis. Human PC-3 prostate adenocarcinoma (IC50 = 130 μmol/L) and LNCaP prostate carcinoma cells (IC50 = 155 μmol/L) are more sensitive than DU145 cells (IC50 = 225 μmol/L) to β-ionone-mediated growth inhibition. Flow cytometric analyses revealed, in both DU145 and PC-3 cells, β-ionone–induced cell cycle arrest at G1 phase. A blend of β-ionone (225 μmol/L) and trans,trans-farnesol (50 μmol/L), an acyclic sesquiterpene shown to initiate the degradation of reductase, suppressed the net growth of DU145 cells by >100%, an effect equal to the sum of those of β-ionone (60%) and farnesol (47%), suggesting an additive effect. A cumulative effect was also observed with a blend of β-ionone and lovastatin, a competitive inhibitor of reductase. β-ionone, individually or in combination with other HMG CoA reductase suppressors, may have potential in prostate cancer chemoprevention.

Combined Dietary Intervention with Tomato-Based Foods and Soy in Men with Recurrent Asymptomatic Prostate Cancer. Elizabeth C. Miller, Thomas W.-M. Boileau, Valerie DeGroff, Tammy Bray, Nuray Unlu, Steven J. Schwartz, and Steven K. Clinton. The Ohio State University, Departments of Hematology and Oncology, Food Science and Technology and Human Nutrition, Columbus, OH.
Tomato products and soy foods are hypothesized to reduce the risk of prostate cancer or enhance treatment. We completed a study to determine whether men with prostate cancer will adhere to a diet rich in tomato products to achieve a lycopene intake of >25 mg/d and a soy supplement providing 40 g/d of soy protein (80 mg isoflavones). We enrolled 41 men (age 70 ± 7 y, mean ± SD) with recurrent but asymptomatic cancer characterized by a rising serum prostate specific antigen (PSA) level following primary therapy. After an initial 1-wk washout, the men were randomly assigned to 1 of 2 groups. Men in group A were advised to consume tomatoes and tomato products (no soy) for wk 0 to 4 and to combine tomato products with 40 g soy protein for wk 4 to 8. Group B consumed soy (no tomatoes) for wk 0 to 4 and the tomato-rich diet with soy for wk 4 to 8. Side effects were few (7%, grade 1 constipation).

Serum lycopene increased (P < 0.0001) and urinary isoflavone excretion increased (P < 0.05) in response to diet changes for weeks 0–4 and 4–8. During weeks 0 to 4, mean lycopene intake for group A was 43 ± 15 mg, and mean soy intake for group B was 39 ± 1 g. During weeks 4 to 8, compliance remained high (>97%). No changes occurred in blood counts, blood chemistry tests, or circulating hormones. Total cholesterol decreased 7% between wk 0 and 8 for both groups (P < 0.001). Serum PSA levels decreased at wk 8 vs. wk 0 for 14 (34%) of 41 men. Men with active prostate cancer are able to consume tomato products and soy together with excellent compliance and no toxicity. Further studies combining tomato and soy foods to determine clinical benefits should be undertaken.

Stomach cancer

**MTHFR Polymorphisms, Dietary Folate Intake, and Stomach Cancer Risk in a Polish Population. Fang Fang Zhang,** Mary Beth Terry,† Lifang Hou,* Jinbo Chen,* Jolanta Lisowska,** Meredith Yeager,* Witold Zatonski,* Stephen Chanock,* and Wong-Ho Chow.* Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD; Division of Epidemiology, Columbia University, New York, NY; and Division of Cancer Epidemiology and Prevention, Cancer Center, and M. Sklodowska-Curie Institute of Oncology, Warsaw, Poland.

Two common single-nucleotide polymorphisms in the MTHFR gene (677C→T; 1298A→C) have been implicated in carcinogenesis through abnormal DNA methylation and impaired DNA synthesis. Several case-control studies in the Chinese population reported an increased risk of stomach cancer associated with the variant allele in MTHFR C677T. However, no studies were conducted in other populations that may have variant genotype frequency and different levels of dietary folate intake that modify the genetic associations. Using data from the Polish Stomach Cancer Case-Control Study, we investigated whether polymorphisms in MTHFR C677T and A1298 C affected the risk of stomach cancer among 305 cases and 428 population-based controls. We used the Taqman assay for the genotyping and collected dietary folate information through a food frequency questionnaire. We modeled the associations using multivariate logistic regression. Our results did not show a significant association of the variant genotype in MTHFR C677T or A1298C with stomach cancer risk [ITT vs. CC in 677, odds ratio (OR) = 1.2, 95% CI: 0.7, 2.0; CC vs. AA in 1298, OR = 1.0, 95% CI: 0.6, 1.7]. The diplotype of CC-AT was related to a significant 30% reduction in stomach cancer risk as compared with the most common diplotype of CC-AC. Among individuals whose dietary folate intake was above the median (300 μg/d), carrying the variant allele in MTHFR was associated with a nonsignificant 60 to 70% increased risk of stomach cancer (OR = 1.7, 95% CI: 0.8, 3.8 for C677T; OR = 1.6, 95% CI: 0.8, 3.5 for A1298C). Among individuals who consumed a lower level of dietary folate, the variant allele was related to a nonsignificant reduced cancer risk. Our findings suggest that 2 common polymorphisms in MTHFR did not significantly affect stomach cancer risk in this population. Although the associations were not significant, our data suggest that the association between MTHFR polymorphisms and stomach cancer may be modified by dietary folate intake.

Mushrooms

**Ganoderma lucidum Inhibits Proliferation in Human Breast Cancer Cells by Estrogen Receptor–Dependent and Estrogen Receptor–independent Signaling Pathways. Jiahua Jiang,* Veronika Sliva,* Jan-Ake Gustafsson,† and Daniel Sliva.*** Cancer Research Laboratory, Methodist Research Institute, Indianapolis, IN; *Department of Medical Nutrition, Karolinska Institute, Huddinge, Sweden; and **Department of Medicine, School of Medicine, Indiana University, Indianapolis, IN.

Ganoderma lucidum, an oriental medical mushroom, has been widely used in Asian countries for centuries to prevent or treat a variety of diseases, including cancer. We previously demonstrated that G. lucidum inhibited growth and induced cell cycle arrest at G1/G0 phase through the inhibition of Akt/NF-κB signaling in estrogen-independent human breast cancer cells. However, the molecular mechanisms responsible for the inhibitory effects of G. lucidum on the proliferation of estrogen-dependent (MCF-7) and estrogen-independent (MDA-MB-231) breast cancer cells remain to be elucidated. In the present study, we showed that G. lucidum inhibits the proliferation of breast cancer in MCF-7 and MDA-MB-231 cells by the modulation of estrogen receptor (ER) signaling. Therefore, G. lucidum inhibits the ER activity, as assessed by the reporter gene assay and DNA-binding assay, in breast cancer MCF-7 (ERα and ERβ positive) and MDA-MB-231 cells (ERα positive and ERβ negative) by different mechanisms. G. lucidum suppresses the ER activity in MCF-7 cells by the downregulation of the expression of ERα, whereas the expression of ERβ in MCF-7 and MDA-MB-231 cells is not affected. The inhibition of ER activity also results in the downregulation of the expression of c-Myc. Collectively, these results suggest that G. lucidum inhibits cell proliferation of human breast cancer cells by ER-dependent as well as ER-independent mechanisms. These findings demonstrate that G. lucidum exerts its effect on estrogen-dependent and estrogen-independent breast cancer cells by multiple mechanisms and may have therapeutic potential for the treatment of human breast cancer.

Herbs and spices

**Zyflamend®, an Herbal Preparation with Nonselctive Cyclooxygenase Inhibitory Activity, Induces Apoptosis in LNCaP Cells Lacking COX-2 Expression. Jillian L. Capodice, Debra L. Bemis, Ralph Buttyan, and Aaron E. Katz. Department of Urology, Columbia University Medical Center, New York, NY.**
INTRODUCTION: Ongoing studies in our lab showed that Zyflamend® (New Chapter) has antiprostase cancer activities in vitro. We are testing this preparation of 10 herbal extracts (rosemary, turmeric, ginger, holy basil, green tea, hu zhang, Chinese goldthread, barberry, oregano, and Scutellaria baicalensis) to determine its mechanism of action in the human prostate cancer cell line LNCaP. METHODS: Cyclooxygenase (COX) inhibitory activity of the preparation was determined by a spectrophotometric-based assay using purified ovine COX-1 and -2. Effects on LNCaP cell growth and apoptosis in vitro were assessed by cell counting. Western blot detection of poly(ADP-ribose) polymerase (PARP) cleavage, and measurement of caspase-3 activity in treated and control cells. Western blots were also conducted to determine the effect on the expression of key cell signaling proteins p21, AR, p-PKCαβ, and p-Stat3. Phosphoprotein screens profiled its effect on 31 phosphorylation sites of 26 different cell signaling proteins.

RESULTS. The preparation dramatically decreased COX-1 (73.8 ± 1.83%) and COX-2 (85.7 ± 5.60%) enzymatic activity. Elevated p21 expression coincided with decreased cell growth after treatment of LNCaP cells. PARP cleavage fragments were evident, and caspase-3 activity was upregulated compared with controls, indicating the ability of the preparation to induce apoptosis in LNCaP cells. Androgen receptor expression levels declined by 40%, and decreases were observed in the active forms of Stat3 and PKCαβ in treated cells. COX-2 expression was not detected in LNCaP cells by PCR.

CONCLUSIONS: Zyflamend inhibited COX-1 and -2 enzymatic activities, suppressed cell growth, and induced apoptosis in LNCaP cells. However, our data suggest that the effects are likely due to COX-independent mechanisms potentially involving enhanced expression of p21 and reduced expression of AR, pStat3, and pPKCαβ. Our in vitro data support the potential benefit of this preparation as a chemopreventive agent. We are pursuing it in a Phase I clinical trial for patients with prostatic intraepithelial neoplasia.

Induction of Apoptosis and Gene Expression in Glioblastoma Cells by a Mixture of Standardized Extracts from Common Herbs and Spices. Mladen Golubiz*† Judy Bondar,* † Patrick Leahy,‡ Joan E. B. Fox,*** and Gene H. Barnett,*†

*Brain Tumor Institute, †Center for Integrative Medicine, and ‡Department of Molecular Cardiology, Cleveland Clinic Foundation, Cleveland, OH; and **Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH.

Modulation of inflammation by compounds found in many herbs and spices may be a mechanism by which diet influences cancer development and progression. A wide spectrum of human malignancies, including the most malignant brain tumor, glioblastoma multiforme, aberrantly overexpresses proinflammatory cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LO). Inhibitors of these molecules may be useful for cancer chemoprevention and treatment. We examined the effects of Zyflamend® herbal preparation (New Chapter) on apoptosis and global gene expression of an established human glioblastoma cell line, U87. The preparation consists of 10 standardized herbal extracts in olive oil (rosemary, turmeric, ginger, holy basil, green tea, hu zhang, Chinese goldthread, barberry, oregano, and Baikal skullcap) that are among the richest sources of naturally occurring and chemically diverse COX-2 and 5-LO inhibitors. The preparation, diluted in DMSO at 0.1 μL/mL, induced apoptosis of U87 cells compared with the olive oil–DMSO control at 48 h, as determined by the terminal deoxyuridine nick end labeling analysis. Global gene expression changes were determined by the GeneChip® Human Genome U133A (Affymetrix) oligonucleotide microarray in U87 cells at 2 and 24 h after treatment. In total, at 2 h after treatment we identified 7 genes that were upregulated (5 of them belonging to the heat shock protein family) and 2 that were downregulated >2-fold in both experiments, compared with the DMSO-treated controls. At 24 h, the expression of 84 genes was increased and that of 65 genes was decreased. The expression of 8 genes influenced by the Zyflamend treatment was confirmed by real-time PCR analysis. Our results suggest that phytonutrients that are present in the herbal preparation modulate the expression of genes involved in the regulation of apoptosis and inflammation that collectively induce apoptosis in the glioblastoma cell line.

Deleterious Side Effects of Tamoxifen May Be Ameliorated by Aqueous Vernonia amygdalina Leaf Extracts. Ernest B. Izevbige,* Michael Opata,* and Joseph L. Bryant.†

*The Laboratory of Phytoceuticals, Cancer Therapies and Prevention, Department of Biology and NIH Center for Environmental Health, Jackson State University, Jackson, MS; and †The Institute of Human Virology, University of Maryland Biotechnology Institute, Baltimore, MD.

INTRODUCTION: Breast cancer is the second leading cause of cancer-related deaths of women in the United States. One in every 8 women will be diagnosed with the disease in her lifetime. Tamoxifen (TAM), a commercially available drug, is one of the most common drugs used worldwide to treat breast cancer. TAM, although effective, is associated with many potential life-threatening side effects, such as stroke, thrombosis, uterine sarcoma, and endometrial cancer. Other less serious side effects include nausea, emesis, and more. Natural botanical products may be integrated with conventional cancer drugs to optimize treatment outcome, stimulate immune function, and minimize the side effects from conventional drugs. We recently discovered and reported antitumor activities of an aqueous fraction extract of edible Vernonia amygdalina (VA) leaves in preclinical studies in MCF-7 cells. The effects of treatment of cells with VA and conventional breast cancer drugs are unknown. OBJECTIVE: Our objective was to assess the effects of TAM, VA, and the combination of TAM and VA on cell number, DNA synthesis, and activation of extracellular signal–regulated kinases (ERKs) in MCF-7 cells. The combined effects of TAM and VA on apoptosis are being assessed in our laboratory. METHODS: Cell number was determined using a hemocytometer. DNA synthesis was determined by [3H]thymidine incorporation assays. ERK activities were determined using a commercially available kit. RESULTS: IC50 values for TAM, VA, and TAM-VA combination were 7.1 ± 0.2 μg/mL (P < 0.05), 10 ± 1.2 μg/mL (P < 0.05), and 1 ± 0.8 μg/mL (P < 0.05), respectively. TAM and VA alone markedly impeded ERK activation, but TAM-VA synergistically impeded ERK activation. CONCLUSIONS: 10-fold less concentration of TAM was required to inhibit cell growth by 50% when combined with VA (1 ± 0.08 vs.10 ± 1.2 μg/mL with and without VA, respectively). These results suggest that TAM-VA therapy may be beneficial to breast cancer patients.


LICR/UCL Breast Cancer Laboratory, Department of Surgery and Oncology, Royal Free and University College School of Medicine, London, UK.
The safety of various complementary medicines has been a subject of considerable debate. Many if not most cancer patients will try such remedies in the course of their disease, often without the knowledge of their surgeon or oncologist. Among the herbal preparations that are sometimes recommended by patient support or self-help groups is the “adaptogen” *Rhodiola rosea* or (golden or arctic root). This purports to generally enhance physical performance as well as improve cognitive function, claims that make it attractive to patients undergoing systemic chemo- or hormonal therapy. Following a report that *Rhodiola* extracts bind strongly to estrogen receptors (ERs) in an in vitro assay, we have investigated its biological effects on a number of ER-positive and ER-negative human breast cancer cell lines. Both aqueous and solvent (DMSO) extracts of a commercial *Rhodiola* preparation proved to be effective and potent stimulators of the growth of the receptor-positive cell lines, showing that its receptor-binding properties simulated the biological effects of estrogen. No stimulation of the ER-negative lines was observed. More significantly, and unlike estradiol itself and other phytoestrogens such as red clover (isoflavone) that were also tested, we found that the growth-stimulating effects of *Rhodiola* were not blocked by either tamoxifen or the pure antiestrogen Faslodex when these were added concurrently. Consumption of *Rhodiola* could, therefore, pose a significant hazard to patients with breast cancer who have ER-positive tumors and who are being treated with antiestrogens.

**Molecular Mechanism of Chemoprevention and Chemoprotection against Cancer and Inflammatory Diseases by African Edible Plants and Beverages.** Kensee S. Mossanda,* Joydeb K. Kundu,† Hye-Kyung Na,† An-Sik Chung,** and Young-Joon Surh.†

because inhibition of cyclooxygenase 2 (COX-2) is regarded as an effective and promising strategy for the prevention of digestive and liver cancers, we investigated the underlying mechanism of COX-2 inhibition in relation to some Southern African edible plants [Bambara groundnut (BG), Vigna subterranea] and beverages [cancer bush (CB), Sutherlandia frutescens; devil’s claw (DC), *Harpagophytum procumbens*; and Rooi bos tea (RT), *Aspalathus linearis*] in relation to their antitumor potential. We assessed the effect of their methanolic extracts on phorbol ester–induced activation of transcription factors and intracellular signaling kinases participating in COX-2 induction in mouse skin and in human breast epithelial (MCF-10A) cells. Western and Northern blotting analyses measured COX-2 mRNA expression after incubation of foodstuff extracts with the MCF-10A cells in vitro and in mice skin in vivo using tetradecano phorbol-3 acetate (TPA). Kinase assays for determining the catalytic activities of p38 and extracellular signal–regulated kinase (ERK) were carried out by using a nonradioactive mitogen-activated protein kinase assay kit. Phosphorylation of Elk-1 was selectively measured by immunoblotting. An electrophoretic mobility shift assay was performed using oligonucleotide harboring a consensus, either nuclear factor-κB (NF-κB) or activator protein 1 (AP-1) and cAMP responsive element-binding protein (CREB) binding sequence. Topical application in mouse skin and pretreatment of MCF-10A cells with foodstuff extracts inhibited TPA-induced COX-2 expression. As an underlying mechanism of COX-2 inhibition, these extracts diminished TPA-stimulated catalytic activity of extracellular signal–regulated protein kinase. TPA-induced activation of NF-κB was inhibited in MCF-10A cells, which is consistent with the observed attenuation of DNA binding activity of NF-κB.

Prostate cancer is the leading cancer diagnosis and the second most common cause of cancer-related death in U.S. men. One in 6 men will be diagnosed with prostate cancer during his lifetime. Therefore, discovering new and effective chemopreventive agents, including natural products, is urgently needed to reduce the incidence and mortality of this disease. *Ginkgo biloba* extract (ginkgo) is a popular, nontoxic, over-the-counter dietary supplement, mostly used by an aging population to improve cognitive function. Notably, ginkgo possesses certain properties such as antioxidative, anti-inflammatory, antiangiogenic, and gene-expression modulation activities, similar to known anticancer agents. Several lines of preclinical evidence support the antitumor effect of ginkgo. No data, however, exist regarding its role in prostate cancer. Based on its biological activities, we hypothesize that ginkgo may be a chemopreventive agent for prostate cancer. In the present study, we used in vitro cell models to determine the effect of ginkgo on prostate cancer growth. We found that 1) ginkgo treatment selectively inhibited the proliferation of both androgen-dependent (LNCaP) and androgen-independent (DU145) human prostate cancer cells, compared with its effect on immortalized normal prostate epithelial cells (RWPE-1); 2) ginkgo treatment induced a cell cycle arrest in G1 phase in LNCaP cells; and 3) ginkgo-induced growth inhibition was associated with a modulation of the expression of cell cycle regulatory proteins in LNCaP cells. These findings, for the first time, demonstrate the antiprostate cancer activity of ginkgo and potential mechanisms associated with its growth-inhibitory effect in prostate cancer cell lines. These results suggest that ginkgo may be a chemopreventive as well as chemotherapeutic agent for prostate cancer management and provide rationale to further test this innovative idea in animal models and clinical studies.

**Soy**

**The Growth and Lifestyle (GRLS) Study: Soy Intake and Puberty in Girls.** Pamela L. Horn-Ross,* Christine Collins,* and Stephen Barnes.†

Dietary intervention studies are usually conducted in adults, but earlier life exposures may be more important. Studies in rats suggest that puberty may be the critical period during which isoﬂavones have maximum impact in reducing mammary cancer risk. This risk reduction appears to be related to...
enhanced cell differentiation resulting in fewer terminal end buds, the structures most susceptible to carcinogenesis. In humans, adult soy consumption generally has been associated with breast cancer risk reduction in Asian women but not in non-Asian women. Recent epidemiological evidence suggests that adolescent exposure to soy may be critical for risk reduction to occur. Soy consumption during adolescence may explain the different results observed in epidemiological studies conducted in different populations. The Growth and Lifestyle (GRLS) study, a prospective cohort of 350 girls age 10 to 13 y, was recently funded to evaluate the translational potential of the experimental findings to humans by examining the effects of soy consumption on the onset of menarche, a strong risk factor for breast cancer. The study also will examine how polymorphic variation in genes in the steroid hormone pathway influences the onset of menarche. Girls who consume soy foods rarely (low-soy group) or ≥3 times/wk (high-soy group) are eligible. Usual dietary intake, physical activity, and physical maturation are assessed at baseline, 12 mo, and 24 mo through in-person interviews. Body composition is measured and blood and urine are collected. Validation studies of diet and activity are being conducted. Monthly follow-up will ascertain the onset of menarche and the establishment of regular cycles. Participants will be followed to determine whether time-dependent differences in physical maturation depend on level of soy consumption and, if so, whether these differences are consistent with reduced breast cancer risk in the high-soy group. [The GRLS study is part of the Center for Nutrient-Gene Interaction, funded under U54 CA110948, awarded to the University of Alabama, Birmingham.]

Lunasin, a Novel Cancer Preventive Soy Peptide: Bioavailability and Biokinetics in Animals. Ben O. de Lumen, Mark Fitch, Chang-su Lim, Iris Reys, Pia Vichayavilas, Hiu Tong Chu, Jennifer Lee, Helen Kim, Rachel Hurwitz, Yang Fang, and Hyung Jin Jeong. Department of Nutritional Sciences and Toxicology, University of California, Berkeley, CA.

Lunasin is a cancer-preventive soy peptide whose efficacy has been shown in mammalian cells and a skin cancer mouse model in our laboratory. Bioavailability and biokinetics studies were carried out in mice by oral administration of 3H-labeled synthetic lunasin with lunasin-enriched soy (LES) protein. Animals were killed and tissues were collected at 3, 6, 9, 12, and 24 h after oral administration. Scintillation counts confirmed a previous pilot study showing that lunasin ends up in all the tissues sampled, including stomach, small intestine, heart, liver, lung, kidney, prostate, breast, brain, skin, and blood, and urine and feces. At 3 h, 39% of the labeled oral dose is absorbed. Lunasin exists as a dimer in mouse blood. In rats fed lunasin-enriched soy, lunasin also exists as a dimer in liver and is bioactive in suppressing foci formation in mammalian cells induced by the chemical carcinogen 7,12-dimethylbenz[a]anthracene. Lunasin is found in Bowman-Birk inhibitor concentrate (BBIC), a well-known soy-derived cancer preventive agent. In vitro digestibility studies show that lunasin is protected from digestion by the soy protease inhibitors BBIC and Kunitz trypsin inhibitor. We conclude that lunasin is protected from digestion in soy by naturally occurring protease inhibitors.

**Phytochemicals**

Update of the USDA Database for the Flavonoid Content of Selected Foods. Seema A. Bhagwat,* David B. Haytowitz,* James Harney,* and Joanne M. Holden.* *Nutrient Data Laboratory and Food Composition Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD.

Evidence to suggest an association of dietary flavonoids and reduction in cancer risk is consistent. Flavonoids have also been associated with reduction in the risk of cardiovascular diseases. Reliable databases to estimate dietary flavonoid intakes are thus essential to study the health benefits and reduction in risks of chronic diseases. The USDA's Nutrient Data Laboratory (NDL) has developed databases for 3 different groups of flavonoids: monomeric flavonoids, polymeric proanthocyanidins, and isoflavones. A database for monomeric flavonoids for 26 selected compounds in 5 subclasses (flavonols, flavones, flavonanes, flavans, and anthocyanidins) was released in March 2003. The database contained values for 225 selected foods, most of which came from the analytical studies conducted in countries other than the United States. Therefore, NDL procured nationally representative samples of 59 fruits, vegetables, and nuts through the National Food and Nutrient Analysis Program (NFNAP). It was also observed that analysts frequently concentrated on quantifying 1 or 2 particular subclasses of flavonoids for the lack of a suitable analytical method to separate and quantify all 5 subclasses simultaneously. A kinetics method was developed to separate and quantify 26 compounds representing all 5 subclasses. Food samples collected by NDL through NFNAP were analyzed by the Food Composition Laboratory of the USDA using this method. The most significant finding from national sampling was the high degree of variability: an average relative standard deviation of 116% for composited samples and 197% for individual samples. These data will be incorporated into the revised database. In addition, published data from 2002 onward were collected and evaluated. Acceptable analytical data from ~90 studies will be combined with the previous data. The updated database now contains data on ~400 selected foods for flavonols, flavones, flavonanes, flavans, and anthocyanidins and is available on NDL’s website (http://www.nal.usda.gov/fnic/foodcomp).


Fruits and vegetables contain essential nonnutritive components called phytochemicals, which have exceptional antioxidative, anticarcinogenic, and antiatherogenic properties. These phytochemicals contribute to the vibrant color of fruits, and it has been reported that the darker the fruit, the higher the antioxidative or anticarcinogenic properties. Our objective was to investigate the effects of blueberries (BB), blackberries (bb), pomegranate juice (PJ), and cranberry juice (CJ) on azoxymethane (AOM)-induced aberrant crypt foci (ACF) in Fisher 344 male rats. After a 1-wk acclimatization, 40 rats were assigned to 5 groups and were fed AIN-93G control diet (C), C + 10% BB, C + 10% bb, C + 50% PJ, or C + 50% CJ. At ages 7 and 8 wk, all rats were treated with an s.c. injection of AOM (16 mg/kg body wt). Rats were killed at age 17 wk by CO2 asphyxiation, and colon and liver samples were collected.
The proximal colon of rats fed C, BB, bb, PJ, and CJ contained 78 ± 3, 9 ± 3, 32 ± 5, 19 ± 4, and 36 ± 7 ACF (mean ± SEM), respectively; the distal colon contained 135 ± 15, 31 ± 8, 54 ± 5, 38 ± 2, and 56 ± 10 ACF, respectively. The total ACF counts in rats fed C, BB, bb, PJ, and CJ were 217 ± 14, 41 ± 7, 86 ± 9, 57 ± 5, and 92 ± 8, respectively. Reductions in ACF formation compared with the controls were 79, 57, 70, and 57% for BB, bb, PJ, and CJ, respectively. There was a significant (P < 0.05) reduction in ACF induction in the groups fed BB, bb, PJ, and CJ. Glutathione S-transferase activity in the groups fed C, BB, bb, PJ, and CJ was 18 ± 1, 29 ± 5, 24 ± 1, 40 ± 5, and 33 ± 5 µmol/mg tissue, respectively. Results also showed that there were no significant (P > 0.05) differences in body weight and feed intake between control and treatment groups. Our findings suggest that BB, bb, PJ, and CJ significantly reduce the formation of AOM-induced ACF and may reduce the incidence of colon cancer.

Antiproliferative and Proapoptotic Effects of Green Tea Constituent Epigallocatechin Gallate in Human Burkitt’s Lymphoma. Gerren Ector,* Tara Humphrey,1 Marisela De Leon,* Mark Maloney,* and Kimberly M. Jackson.** Biology Department, Spelman College, Atlanta, GA; † Biology Department, Bennett College, Greensboro, NC; and **Chemistry Department, Spelman College, Atlanta, GA.

Green tea polyphenols are known to exhibit anticarcinogenic properties by suppressing growth and inducing apoptosis in a number of human cancer cell lines. Epigallocatechin gallate (EGCG), the major polyphenolic constituent in green tea, possesses a wide range of pharmacological properties and is a promising chemotherapeutic agent. In the present study, we examined the effect of EGCG on the proliferation and cell cycle progression of two human Burkitt’s lymphoma cell lines. Burkitt’s lymphoma is a malignant B-cell tumor most commonly found as a childhood cancer in certain parts of equatorial Africa and Papua New Guinea. In these B-cell lymphomas, the glycolipid globotriaosyl ceramide (Gb3) is highly expressed. Daudi cells (a Gb3-positive Burkitt’s lymphoma cell line) and VT300 cells (a Gb3-deficient Daudi mutant) were exposed to various concentrations (0, 10, 25, 50, 75, and 100 µmol/L) of EGCG for 72 h. Both cell lines exhibited growth inhibition in a dose-dependent manner, without any change in cell cycle progression. However, cell cycle data did reveal a sub-G1 peak in Daudi cells after EGCG treatment, indicative of apoptosis. To better understand the preventive effects of EGCG on human Burkitt’s lymphoma Daudi cells, apoptosis induction was further assessed by Annexin V-FITC and propidium iodide staining. Flow cytometric data revealed an increase in Annexin V-FITC binding in Daudi cells treated with EGCG at 50, 75, and 100 µmol/L. These data suggest that apoptosis induction should be considered when evaluating the antiproliferative activity of EGCG in Burkitt’s lymphoma cells.

Broccoli Sprout Modulation of the Urinary Excretion of Aflatoxin-DNA Adducts and Phenanthrene Tetraols in a Randomized Clinical Trial in Qidong, People’s Republic of China. Thomas W. Kensler,*** Jian-Guo Chen,† Patricia A. Egner,⁎ Jed W. Fahey,⁎ Lisa P. Jacobson,† Katherine K. Stephenson,**, Lingxiang Ye,**, Jin-Bing Wang,⁎ Yao Wu,⁎ Yan Sun,⁎ Qi-Nan Zhang,⁎ Bao-Chu Zhang,† Yuan-Rong Zhu,† Geng-Sun Qian,† Stephen G. Carmella,‡ Stephen S. Hecht,‡ Lorie Benning,⁎ Stephen J. Gange,† John D. Groopman,⁎ and Paul Talalay.** Departments of *Environmental Health Science and **Epidemiology, Bloomberg School of Public Health, and ***Pharmacology and Molecular Science, School of Medicine, Johns Hopkins University, Baltimore, MD; †Qidong Liver Cancer Institute, Qidong, People’s Republic of China; ‡Jiao Tong University, Shanghai, People’s Republic of China; and ‡‡University of Minnesota Cancer Center, Minneapolis, MN.

Residents of Qidong, People’s Republic of China, are at high risk for developing hepatocellular carcinoma in part because of consumption of foods contaminated with aflatoxin. Individuals in this region are also exposed to high levels of airborne phenanthrene, a polycyclic aromatic hydrocarbon formed from combustion. Epidemiological studies have demonstrated a strong link between the consumption of fruits and vegetables and a reduction of cancer. Specifically, cruciferous vegetables (e.g., broccoli, kale, brussels sprouts, cabbage) have been shown to contain high levels of protective phytochemicals that enhance detoxication pathways. The primary glucosinolate in broccoli sprouts is glucoraphanin, which can be converted by intestinal microflora to sulforaphane, a potent anticarcinogen. In a randomized, single-blind placebo-controlled trial in Qidong, we tested whether drinking hot-water extracts of 3-d-old broccoli sprouts nightly for 2 wk could modulate the excretion of aflatoxin-N7-guanine adduct (a biomarker of the effective dose of aflatoxin) and μ,μ,μ-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene, reflecting exposure to and biotransformation of polycyclic aromatic hydrocarbons. Urinary levels of the aflatoxin DNA biomarker and the phenanthrene metabolite did not differ between treatment and placebo groups (P = 0.68 and P = 0.29, respectively). However, despite the fixed dose of the glucoraphanin administered to each individual in the treatment group, the urinary excretion of sulforaphane metabolites (dithiocarbamates) varied dramatically. Strong inverse associations between dithiocarbamate excretion and aflatoxin-DNA adduct levels (P = 0.002) as well as the phenathrene metabolite (P = 0.0001) were observed, suggesting a protective pharmacodynamic action of the sprouts’ beverage on carcinogen disposition in humans.

Resveratrol Inhibits Phorbol Ester–Induced Expression of COX-2 through Inactivation of NF-κB in Mouse Skin by Blocking IκB Kinase-β Activity. Joydeeb Kumar Kundu, and Young-Joon Surh. National Research Laboratory of Molecular Carcinogenesis and Chemoprevention, College of Pharmacy, Seoul National University, Seoul, South Korea.

Inflammation is frequently linked to multistage carcinogenesis process. Aberrant expression of cyclooxygenase-2 (COX-2), a rate-limiting enzyme in the biosynthesis of inflammatory mediators, has been implicated in tumor promotion. Resveratrol, a dietary polyphenol with anti-inflammatory properties, was reported to inhibit chemically induced carcinogenesis in mouse skin. We investigated the effect of resveratrol on the expression of COX-2 and its underlying molecular mechanisms in 12-O-tetradecanoylphorbol-13-acetate (TPA)–treated mouse skin. Resveratrol (0, 0.25, and 1.0 µmol), applied topically to dorsal skin of female ICR mice 30 min before TPA, significantly inhibited TPA-induced COX-2 expression. The DNA binding of nuclear factor-κB (NF-κB), a redox-sensitive transcription factor known to regulate expression of COX-2, was attenuated by resveratrol in TPA-stimulated mouse skin. As underlying mechanisms, resveratrol attenuated TPA-induced phosphorylation and subsequent degradation of IκBα and the resultant nuclear translocation of p65. Resvera-
control also blunted TPA-induced phosphorylation of p65 and the interaction of phosphorylated p65 with a transcriptional coactivator cyclic AMP response element binding protein-binding protein, thereby blocking transcriptional activation of NF-κB. Pretreatment with resveratrol suppressed the phosphorylation and catalytic activity of extracellular signal-regulated protein kinase and p38 mitogen-activated protein kinase, which were reported to attenuate phosphorylation of p65 in TPA-treated mouse skin. We examined the role of IkB kinase (IKK)-β, an upstream kinase recently recognized as a molecular link between inflammation and cancer, in mediating TPA-induced activation of NF-κB and COX-2 expression in mouse skin in vivo. A kinetic study revealed that topical application of TPA caused rapid induction of IKK-β activity, which was suppressed by pretreatment with resveratrol or cotreatment with Bay 11–7082, an inhibitor of IKK. Topical Bay 11–7082 also abrogated TPA-induced NF-κB activation and COX-2 expression, suggesting a regulatory role of IKK-β in TPA-induced COX-2 expression in mouse skin. These findings suggest that resveratrol suppresses TPA-induced COX-2 expression in mouse skin by blocking activation of NF-κB via the IKK-β signaling.

Chemopreventive Effects of Curcumin and Celecoxib on Azoxymethane-Initiated and Dextran Sulfate Sodium–Promoted Mouse Colon Carcinogenesis. Jeong-Sang Lee,* Hyun Soo Kim,* Tae-Young Oh,† Marie Yeo,† Ki-Baik Hahn,† and Young-Joon Surh.* †National Research Laboratory of Molecular Carcinogenesis and Chemoprevention, College of Pharmacy, Seoul National University, Seoul, South Korea; and ‡Genomic Research Center for Gastroenterology, School of Medicine, Ajou University, Suwon, South Korea.

Multiple lines of compelling evidence support the causal relation between inflammation and cancer. In an attempt to clarify the contribution of ulcerative colitis to colon carcinogenesis, we treated male ICR mice with a single i.p. dose (10 mg/kg body wt) of azoxymethane (AOM) followed by 2% dextran sulfate sodium salt (DSS) in drinking water for 7 consecutive days. DSS administration caused chronic inflammation, which markedly enhanced the incidence and burden of AOM-initiated colon tumors. At 16 wk of AOM and DSS treatment, 83.33% of the mice developed colorectal carcinoma, mostly rectal and descending colon, whereas only 27.27% of AOM-treated and no DSS-treated mice developed tumors. These findings suggest that ulcerative colitis induced by DSS effectively promotes colon carcinogenesis in mice. Cyclooxygenase-2 (COX-2), inducible nitric oxide synthase, p16, and mutated p53 were markedly upregulated in the AOM-initiated and DSS-promoted colon tumors. The serum level of nitric oxide and proinflammatory cytokines, including interleukin (IL)-1β, IL-6, and IL-10, were also elevated in the colon tumors. In addition, the mRNA expression of matrix metalloproteinases (MMPs), such as MMP2, MMP9, MMP13, and MT2-MMP, was increased after AOM plus DSS treatment. On the contrary, the tissue inhibitor of MMP2 was downregulated by the same treatment. Using the same mouse model, we evaluated chemopreventive activities of a phytochemical (curcumin) and a selective inhibitor of COX-2 (celecoxib). Oral administration of curcumin and celecoxib (0.1 and 0.25 mmol/kg p.o., respectively) for 14 wk significantly lowered the AOM-plus-DSS-induced tumor incidence and multiplicity (83.33% and average 6.00 tumors/mouse in the AOM-plus-DSS group; 50.14% and 4.25 tumors/mouse in the low-dose curcumin group; 42.86% and 4.00 tumors/mouse in the high-dose curcumin group; 44.44% and 4.50 tumors/mouse in the low-dose celecoxib group; 40.0% and 1.75 tumors/mouse in the high-dose celecoxib group). The chemopreventive effects of combined treatment of curcumin and celecoxib on AOM-plus-DSS-induced mouse colon carcinogenesis are under investigation.


INTRODUCTION: HER-2/neu increases the aggressiveness of breast cancer and resistance to chemotherapy, 3′,3′-Diindolylmethane (DIM), a naturally occurring metabolite of indole-3-carbinol, a compound found in cruciferous vegetables, induces apoptosis in breast cancer. Because HER-2/neu confers resistance to paclitaxel and DIM has antitumor effects, we hypothesized that DIM would enhance the cytotoxic effects of paclitaxel on human Her2/neu breast cancer cells by stimulating apoptosis. METHODS: The MDA-MB-435B1 human Her2/neu breast cancer cells were treated with various concentrations of DIM (15 μmol/L) and/or paclitaxel (10 nmol/L). The cells were analyzed at 24, 48, and 72 h. Proliferation was measured by a cell proliferation assay. Cell cycle and apoptosis were determined by cytometry. Western blot was performed on cells treated with DIM and/or paclitaxel for 72 h or in serum-free medium for 24 h and then stimulated with endothelial growth factor for 15 min. RESULTS: Both DIM and paclitaxel exhibited time- and concentration-dependent inhibition of cell proliferation. This combination exhibited a significant decrease in proliferation compared with paclitaxel alone (P < 0.001) and increased apoptosis more than either agent alone. The presence of cleaved poly(ADP-ribose) polymerase (PARP), which measures apoptosis, was increased by the combination treatment. Bcl-2, an antiapoptosis protein, was downregulated by the combination. The activation of the Her2/neu receptor was decreased by DIM alone and the combination treatment, as was the activation of extracellular signal–regulated kinases 1 and 2 (ERK1/ERK2), a cell growth and differentiation protein mediated by Her2/neu activation. CONCLUSIONS: DIM and paclitaxel synergistically inhibit growth of Her2/neu human breast cancer cells through induction of apoptosis. The Her2/neu receptor pathway is involved in DIM’s effect on cell growth and differentiation (ERK1/ERK2), whereas apoptosis appears to be mediated through the mitochondrial pathway (Bcl-2/PARP). DIM enhances the cytotoxicity of paclitaxel on this aggressive breast cancer. Natural and nontoxic DIM may be a beneficial addition to traditional chemotherapy.

Acid Condensation and Degradation Products of N-Methoxyindole-3-Carbinol (N13C) and the Combined Inhibitory Effect of N13C and Indole-3-Carbinol on Colon Cancer Cell Lines. Hanne Mørkeberg, Thomas Mansoor, Fritz Dus, and Ole Vang. Department of Life Science and Chemistry, Roskilde University, Roskilde, Denmark.

Intake of fruit and vegetables is well known to reduce the risk of cancer. Some of the compounds responsible for this effect are indoles, which occur naturally in vegetables of the Cruciferae family (e.g., broccoli and cabbage). Recent studies in our laboratory showed that indole-3-carbinol (13C) and N-methoxyindole-3-carbinol (N13C) inhibit the growth of human cancer cell lines DLD-1 and HCT-116. N13C was shown to be
a more potent inhibitor of cell proliferation than I3C, and both indoles inhibit cell proliferation by different mechanisms. The aim of this study was to show the combined inhibitory effects of NI3C and I3C on cell proliferation and to determine the chemical stability of I3C and NI3C in an acidic solution. Our experiments clearly showed that I3C and NI3C synergistically inhibit cell proliferation. Furthermore, human colon cells treated with both compounds simultaneously showed a significant increase in the accumulation of cells in G0/G1 phase compared with cells treated with I3C or NI3C alone. When cells were treated with NI3C alone, a small amount of the cells arrested in S phase. These data showed for the first time that NI3C and I3C work synergistically, which explains why lower doses of the indoles have the same chemopreventive effect as the higher dose required for either indole alone. It is assumed that the acid-catalyzed conversion of I3C is compulsory for the biological effect in vivo and that the conversion primarily occurs in the acidic environment of the stomach. Whether this conversion occurs in cell culture is unclear. We presume that NI3C is more stable than I3C because of the methoxy group, and this difference in stability may explain the difference in their biological activity. Some preliminary results on the acid stability of NI3C will be presented.

Antiproliferative Effect of Scutellaria baicalensis Extract and Constituent Flavonoids in Colon Cancer Cell Lines. Yantao Niu, Mary L. Hardy, Qing-Yi Lu, Vay Liang W. Go, David Heber, and Diane M. Harris. UCLA Center for Human Nutrition and Department of Medicine, David Geffen School of Medicine, University of California, Los Angeles, CA.

The root of Scutellaria baicalensis (SB; Chinese skullcap or Huang Qin) has a history of traditional use as an anti-inflammatory agent, suggesting inhibition of arachidonic acid metabolism, which could also reduce colon carcinogenesis. Therefore we chose to study the effect of an extract of SB on 4 different human colon cancer cell lines that differ in phenotype: HCT116 (cyclooxygenase-2 (COX-2) negative), HT29 (COX-2 positive, 5-lipoxygenase positive), and SW480 and SW620 (2 lines from same patient, primary vs. metastasis; COX-2 negative). Ethanol (75%) extracts of SB (Botanica Biosciences) were assayed by HPLC for levels of a number of flavonoids. The principle component was found to be baicalin, at 224 mg/g SB. Concentrations of baicalin and skullcap flavone were some 10-fold less. However, a host of other flavonoids, including neobaicalein, wogonin, and rutin, were also present in small quantities. Colon cancer cell lines (initial 3000–5000 cells) were treated with increasing concentrations (0–200 μmol/L) of SB extract or selected flavonoids (baicalin, baicalein, wogonin) as well as pharmaceuticals sulindac sulfide and indomethacin for 48 h. Levels of SB were matched to baicalin content. Cell viability was assayed using an ATP-based cell proliferation assay (CellTiter-Glo; Promega). The SB whole extract was generally a more potent inhibitor of proliferation (IC50 = 24–60 μmol/L) than baicalin, baicalein, and wogonin individually. Indomethacin (IC50 > 188 μmol/L) and sulindac sulfide (IC50 > 119 μmol/L) were only mildly inhibitory in these lines. These results indicate that SB extract is a potent inhibitor of cellular proliferation in both COX-2-negative and –positive cell lines. The glucuronide baicalin and its aglycone baicalein accounted for a majority of this effect, but the other flavonoids may provide additional benefits. [Supported by NIH AT1535, CA4271.]
more, GTPs also suppressed cell adhesion, migration, and invasion of MDA-MB-231 cells. These anti-invasive effects of GTPs were the result of the inhibition of constitutively active transcription factors activator protein-1 and nuclear factor-κB, which further suppressed secretion of urokinase plasminogen activator (uPA) from breast cancer cells. From these results it can be hypothesized that GTP treatment resulted in the inhibition of the formation of signaling complexes responsible for cell adhesion and migration (uPA–uPA receptor—vitronectin—integrin receptor), and cell invasion (uPA-uPA receptor).

Our results indicate that GTPs may contribute to the anticancer effects of green tea by inhibiting the invasive behavior of cancer cells, resulting in the suppression of cancer metastasis.

Protection of Side Effects of 5-Fluorouracil by Anthocyanin-Rich Extracts. Ye Su,* Tao Yu,* Geeta Lala,* Sabine Francke-Carroll,* and Bernadne Magnuson.* *Department of Nutrition and Food Science, University of Maryland, College Park, MD; and †Pathology Branch, U.S. Food and Drug Administration, Washington, DC.

INTRODUCTION: 5-Fluorouracil (5-Fu) is a widely used cancer chemotherapy drug, especially for the advanced stages of colon, breast, and prostate cancers. However, side effects include gastrointestinal injury and bone marrow inhibition often limit the dose and duration of 5-Fu therapy. We reported that extracts rich in anthocyanins (naturally occurring pigments) have chemoprotective activity against colon cancer.

OBJECTIVE: Our objective was to determine whether an anthocyanin-rich extract (ARE) is beneficial in combination with 5-Fu chemotherapy.

METHODS: To assess the effect of ARE in combination with 5-Fu on cell growth in vitro, an untransformed colon cell line, NCM460, and 2 colon cancer cell lines, HT29 and SW620, were used. To assess the effect of dietary ARE on 5-Fu side effects in vivo, Sprague-Dawley rats were fed 1) AIN93 diet, 2) AIN93 diet plus 5-Fu (200 mg/kg), 3) ARE diet containing 0.5% anthocyanin, or 4) ARE diet plus 5-Fu. Rats were fed their assigned diet for 14 d. On d 11, groups 2 and 4 were injected with 3-Fu and gavaged 1 h later with sucrose solution; groups 1 and 3 were injected with control vehicle. Urine was collected and assayed for sucrose content as a measure of gastrointestinal damage. All rats were killed 3 d later, and total blood counts and pathological analysis of tissues were conducted.

RESULTS: ARE enhanced the growth inhibition by 5-Fu in colon cancer cell lines to a greater degree than in NCM460 cells. Rats treated with only 5-Fu exhibited increased sucrose excretion, inhibition of bone marrow function, and damage in the small intestine, compared with untreated rats. Rats fed ARE and given 5-Fu treatment had less small intestine damage compared with rats fed the control diet and given 5-Fu treatment. These data suggest that dietary ARE may reduce the adverse side effects of 5-Fu without impairing efficacy, but studies with advanced tumor development are needed.

Effect of Green Tea Extract on the Growth of Experimental Breast Tumors. Sophia Zaletok,* Levan Gulua,† Alexey Orlovsky,* Sergey Gogol,* Nino Omiadze,† Nani McHedlishvili,† and George Kvesitadze.* †R. E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine, Kiev, Ukraine; and ‡S. Durmishidze Institute of Biochemistry and Biotechnology, National Academy of Sciences of Georgia, Tbilisi, Georgia.

Numerous epidemiological studies support the link between green tea consumption and reduced cancer rates. The objective of this study was to investigate the effect of green tea extract consumption on the growth of experimental breast tumors.

To obtain green tea extract, fresh tea leaves (Camellia sinensis L., Georgian strain Kolkhida) were processed by special technology elaborated at the Durmishidze Institute of Biochemistry and Biotechnology. The effect of the green tea extract on the growth of Walker W-256 carcinoma was studied. Consumption of green tea extracts at 1 g/L in drinking water inhibited tumor growth depending on the number of transplanted tumor cells and the period between tumor cells transplantation and initiation of tea consumption. When 10^6 cells per rat were inoculated and tea drinking began 7 d after transplantation, tumors appeared only in 20% of rats (vs. 100% in the control group). When 4.5 × 10^6 cells per rat were inoculated and tea drinking began 3 d after transplantation, tumors appeared in 80% of the rats, and the tumor inhibition index was 55%. When 7.7 × 10^6 cells per rat were inoculated and tea drinking began when the first tumor appeared, tumors appeared in 80% of rats, and the tumor inhibition index was 28%. Tumor growth inhibition was accompanied by a reduction in the level of ornithine decarboxylase (the key enzyme of polyamines biosynthesis) and nuclear factor-κB–dependent oncogene proteins (bcl-xL, inducible nitric oxide synthase, cyclooxygenase-2). Thus, the data obtained showed that the antitumor effect of green tea extract may be mediated by polyamine–nuclear factor-κB–dependent signal pathways. [Supported by the Science and Technology Center, Ukraine, within the framework of Project G-122.]

Phytoestrogens

Phytoestrogen Intake and Plasma Concentrations in South Asian and British Women Resident in the United Kingdom. Dee Bhakta,* Isabel dos Santos Silva,* Craig D. Higgins,* Leena Sevak,* Herman P. Adlercreutz,† and Anthony J. McMichael.* †Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, UK; and *Institute for Preventative Medicine, Nutrition and Cancer, Folkhalsan Research Center and Division of Clinical Chemistry, University of Helsinki, Helsinki, Finland.

BACKGROUND: Phytoestrogens, naturally occurring hormones in plant food, may protect against hormone-related chronic diseases. South Asian migrants in the United Kingdom have a low incidence of hormone-related cancers, but the extent to which this may be due to phytoestrogen intake is not known.

OBJECTIVE: Our objective was to compare habitual phytoestrogen intake in migrant first-generation South Asian women and native British women using multiple 24-h recalls, multiple plasma samples, and an enhanced phytoestrogen database.

DESIGN: South Asian (n = 221) and native British women (n = 50) were recruited from general practitioners’ lists. Subjects were asked to provide monthly 24-h recalls for 1 y. A phytoestrogen database was compiled using data from a literature search and from previously unpublished data. A subsample of South Asian women (n = 100) and the native British women (n = 40) also provided blood samples every 3 mo concurrently during the 1-y period.

RESULTS: The median daily intakes (μg/d) of isoflavones [184.2, interquartile (IQ) range 121.1–277.6, vs. 333.9, IQ range 227.0–448.7] and lignans (110.8, IQ range 76.8–182.4, vs. 148.8, IQ range 87.4–228.6) were significantly lower in South Asians than in the native British (P < 0.001 and P = 0.04, respectively). There were no significant differences in plasma isoflavone...
levels, but plasma enterolactone was significantly higher in the native British than in South Asians \( (28.5 \pm 23.3 \text{ vs. } 13.9 \pm 17.5, P < 0.001) \). The main sources of phytoestrogens were bread and vegetables and vegetable dishes for both ethnic groups. CONCLUSIONS: Habitual phytoestrogen intake in South Asian and native British women was <1 mg/d and was higher in the native British diet. This was reflected in both dietary intake data and plasma levels. This study, therefore, does not lend support to the protective role, at a population level, of phytoestrogens in hormone-related cancers. [Supported by Cancer Research Campaign (now Cancer Research UK); Grant SP2315.]

**Antioxidants**

**Dietary Antioxidants and Cancer.** Harald Carlsen, Ingvild P. Knudsen, Siv K. Bohn, Monica H. Carlsen, and Rune Blomhoff. Department of Nutrition, Institute for Basic Medical Sciences, University of Oslo, Oslo, Norway.

A diet rich in fruits and vegetables reduces the risk of diseases related to oxidative stress, such as cancer. The mechanisms and the compounds involved in this protective effect have not been identified. Reduction of oxidative stress by antioxidants from fruits and vegetables has been regarded as the most likely antioxidant hypothesis. Although results from epidemiological studies and experimental model systems have demonstrated beneficial effects, the randomized intervention trials have not been supportive. Plants contain several thousand antioxidants. Our antioxidant hypothesis is that the beneficial health effect is contributed by antioxidants other than those tested so far (i.e., other than vitamin C, vitamin E, and \( \beta \)-carotene), and that the antioxidants work cooperatively in a network. Thus, a combination of different antioxidants may be optimal to protect cells against oxidative stress and inflammation. To test this hypothesis, we generated a table containing total antioxidant values for 1115 products from the USDA National Food and Nutrition Analysis Program and about 1500 other food items from different regions of the world. Food items containing high levels of total antioxidants include several berries, fruits, nuts, seeds, vegetables, drinks, and spices. Our hypothesis is now being tested in clinical intervention studies and epidemiological studies by assigning antioxidant values to existing databases. We also generated transgenic reporter mice for noninvasive monitoring of gene expression in vivo. Various oxidative stress- and inflammation-related gene promoters or specific response elements were fused to the luciferase reporter gene. Reporter activity is manifested by light, quantified, and visualized in living mice with a digital camera. This unique model allows the effects of a given diet or treatment to be monitored over time in the same experimental animal.

**The Diet, Supplements, and Health (DISH) Study: Rationale, Objectives, and Design.** Joanne Watters and Jessie Satta. Department of Nutrition, Schools of Public Health and Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Cancer incidence rates are generally higher for African Americans than for other racial/ethnic groups. High consumption of fruits and vegetables, which are antioxidant-rich foods, is associated with lower incidence of many cancers. Antioxidants decrease oxidative stress, the imbalance of free radicals over the body’s defenses, which can lead to carcinogenesis. However, little is known about relations among antioxidants, oxidative stress, and cancer risk in healthy (i.e., cancer-free) persons and potential racial differences; new methodologies are needed to assess antioxidant nutrient intakes. The hypothesis of the DISH study is that African Americans have lower antioxidant nutrient levels than whites (largely because of lower dietary intakes) and therefore have higher levels of oxidative stress, which may contribute to their higher cancer rates. Our objectives are to 1) determine whether antioxidant nutrients (carotenoids, vitamin C, and vitamin E), oxidative stress (oxidative DNA damage), and the association between antioxidants and oxidative DNA damage differ by race in healthy adults; 2) develop and test the relative validity of a new antioxidant nutrient questionnaire by comparison with 24-h dietary recalls and nutrient biomarkers; and 3) evaluate the degree of agreement between 2 measures of oxidative DNA damage (alkaline Comet assay and 7-hydroxy-8-oxo-2'-deoxyguanosine). To accomplish these aims, 150 healthy (i.e., nonsmoking, nonobese, chronic disease free) African Americans and whites, aged 20–45, are currently being recruited in the Research Triangle area of North Carolina. Participants complete a demographic and health questionnaire, four 24-h dietary recalls, and a dietary supplement inventory; have height and weight measured; and provide blood, urine, and toenail samples. To date, 83 participants have been enrolled. This study will fill important knowledge gaps by providing information about the relation between antioxidants and oxidative DNA damage in a sample of African American and white adults and optimal measures of oxidative DNA damage as well as, potentially, a validated instrument for assessing antioxidant nutrient exposure.

**Antioxidant Supplements Reported in the National Health and Nutrition Examination Survey (NHANES) 1999–2000.** Cuwei Zhao,* Karen Andrews,* Joanne Holden,* Amy Schweitzer,* Janet Roseland,* Jim Harnly,† Wayne Wolf,‡ Johanna Dwyer,** Mary Frances Picciano,** Joseph Betz,** Leila Saldanha,** Elizabeth Yetley,** Kenneth Fisher,** and Kathy Radimer.†* Nutrient Data Laboratory, †Food Composition Laboratory, U.S. Department of Agriculture, Beltsville, MD; ‡Office of Dietary Supplements, National Institutes of Health, Bethesda, MD; and **National Center for Health Statistics, Centers for Disease Control, Hyattsville, MD.

Oxidative injury is considered one of the initiators of cancer development. A large number of supplements containing antioxidant constituents are available in the marketplace to promote health and ostensibly to prevent the risk of various cancers. Analysis of U.S. consumption of antioxidant supplements is important in prioritizing ingredients for development of an analytically validated Dietary Supplement Ingredient Database (DSID). This study investigated dietary supplements containing antioxidants and their frequency of use in the National Health and Nutrition Examination Survey (NHANES) 1999–2000. Products were determined to be antioxidant-containing supplements if they contained at least 1 of the following ingredients: vitamins C and E, \( \beta \)-carotene, selenium, flavonoids, or isoflavones. NHANES data files were imported into Microsoft Access. Queries were constructed using structured query language (SQL) to search for dietary supplements containing these antioxidants. The number of respondents and weighted frequency of use were summed using SQL. Among the 1900 reported supplement products, >900 products (47%) contained at least 1 of the above antioxidants. A total of >3000 survey respondents, representing ~37% of
the U.S. population, reported taking at least 1 of these products. Vitamins C and E were the top 2 reported antioxidant ingredients. Approximately 680 products containing vitamin C were reported by >2900 respondents, and 560 products containing vitamin E were reported by ~2800 respondents. Other commonly reported antioxidants, in rank order, were selenium, β-carotene, and flavonoids, which were present in ~280, 260, and 150 of the reported products, respectively. Generally, >50% of multivitamin consumers took multitamins containing vitamin A, C, or E with 100% daily value. However, only about 30 products containing isoflavones were reported by 40 respondents. Vitamins C and E and β-carotene are the high-priority vitamins being evaluated in pilot studies for the development of the DSID.

Carbohydrates

Anticancer Function of IP6 through Modulation of Cell Signaling Pathways. Ivana Vucenik and Abul Kalam M. Shamsuddin. University of Maryland School of Medicine, Baltimore, MD.

Inositol hexaphosphate (IP6) is a naturally occurring polyphosphorylated carbohydrate, present in substantial amounts in almost all plant and mammalian cells, with a strong anticancer activity against numerous tumors. In vitro, IP6 affects signal transduction pathways, leading to inhibition of cell growth and transformation, reduced invasive behavior, enhanced apoptosis, and slowed angiogenesis. Uncontrolled proliferation is a hallmark of malignant cells, and IP6 can reduce the cell proliferation rate and cause G0/G1 cell cycle arrest of many cell lines, both human and rodent. Our studies using estrogen receptor–positive MCF-7 and estrogen receptor–negative MDA-MB-231 human breast cancer cells show that IP6 causes G0/G1 cell cycle arrest associated with inhibition of Akt and Erk1/2 and upregulation of PKC-θ and cyclin-dependent kinase inhibitor p27Kip1. As a result, phosphorylation of retinoblastoma protein was decreased, and cells were G0/G1 arrested. IP6 was selective and synergistic with standard chemotherapeutics. One important characteristic of malignancy is the ability of tumor cells to metastasize and invade normal tissue. A significant reduction in the number of lung metastatic colonies by IP6 was observed in a mouse metastatic tumor model using FSA-1 cells. In vitro, IP6 inhibits key steps of metastasis through effects on adhesion, migration, invasion, and inhibition of matrix metalloproteinases. Tumors depend on the formation of new blood vessels to support their growth and metastasis. IP6 inhibited the growth and differentiation of endothelial cells and inhibited the secretion of vascular endothelial growth factor from malignant cells. Apoptosis is a hallmark of action of many anticancer drugs. IP6 induces apoptosis involving cleavage of caspase 3, caspase 9, and poly(ADP-ribose) polymerase (an apoptotic substrate) in a time- and dose-dependent manner. Being a safe, selective, and potent anti-proliferative and pro-apoptotic agent, IP6 should be considered in our alternative or complementary strategies for cancer prevention and therapy.

Fatty acids

Conjugated Linoleic Acid Attenuates Cyclooxygenase-2 Transcriptional Activity via an Anti–AP-1 Mechanism in MCF-7 Breast Cancer Cells. Stephanie C. Degner, Michael Q. Kemp, and Donato F. Romagnolo. Department of Nutritional Sciences, The University of Arizona, Tucson, AZ.

Conjugated linoleic acid (CLA) refers to a mixture of isomers of linoleic acid. Previous studies demonstrated that CLA reduces the production of eicosanoids, which originate from the metabolism of arachidonic acid by cyclooxygenase-2 (COX-2). Overexpression of COX-2 has been regarded as a causative factor in the onset of tumorigenesis of the breast. In the present study, we investigated the effects of CLA on COX-2 transcription in breast cancer cells. Results of transient transfection studies revealed that treatment of breast cancer MCF-7 cells with CLA or selected isomers (t10,c12-CLA; c9,t11-CLA) at concentrations ranging from 20 to 160 μmol/L attenuated in a dose-dependent fashion COX-2 transcription induced by the proinflammatory factor 12-O-tetradecanoylphorbol-13-acetate (TPA). In addition, transient transfection studies showed that CLA inhibited TPA-induced activity of the human collagenase-1 promoter and an activator-protein-1 (AP-1) reporter– luciferase construct. Using electrophoretic mobility shift assays, we found that CLA reduced TPA-induced recruitment of nuclear proteins to a cAMP response element (CRE) (~59/~53) in the COX-2 promoter and a consensus TPA-responsive element (TRE), which are binding sites for members of the AP-1 transcription factor. Overexpression of the AP-1 member c-Jun reversed the inhibitory effects of CLA on COX-2 transcription and restored binding of nuclear proteins to CRE and TRE. These results suggest that CLA represses AP-1–mediated activation of COX-2 transcription. We conclude that the ability of CLA to inhibit AP-1 activity leading to inactivation of COX-2 transcription may contribute to its effectiveness as an anticarcinogenic and anti-inflammatory agent.

Regulation of Syndecan 1 in Human Breast Cancer Cells by (n-3) PUFAs: A Potential Mechanism for Apoptosis Induction. Iris J. Edwards, Isabelle M. Berquin, Hai Guo Sun, and Jamie L. Kistler. Wake Forest University School of Medicine, Winston-Salem, NC.

Epidemiological and animal model studies show an inverse relation between breast cancer incidence and fish oil consumption, and (n-3) PUFAs of fish oil have been proposed to have tumor-inhibitory properties. Our studies focus on a potential anticancer mechanism of (n-3) PUFAs. Syndecan-1 is a transmembrane heparan sulfate proteoglycan (PG) that is expressed on the surface of mammary epithelial cells and is known to regulate biological processes including cytoskeletal organization, growth factor signaling, and cell-cell adhesion. Our studies have shown that syndecan-1 production is significantly lower in MCF-7 and MDA-MB-435 human breast cancer cell lines than in the nontumorigenic MCF-10A cell line. We studied the effects of (n-3) PUFAs on syndecan-1 expression in these cells. PUFAs were delivered to the cells by LDL enriched in either (n-3) or (n-6) PUFAs. PG synthesis was measured by incorporation of [35S]sodium sulfate. No effect of LDL was observed in MCF-7 cells, whereas in MCF-7 cells, treatment with (n-3)- but not (n-6)-enriched LDL resulted in significantly greater synthesis of a PG identified as syndecan-1. Real-time RT-PCR was used to demonstrate that (n-3) LDL increased the expression of syndecan 1 mRNA. The effect was mimicked by an agonist for peroxisome proliferator activated receptor gamma (PPARγ) and eliminated by the presence of a PPARγ antagonist, suggesting a role for PPARγ in syndecan-1 enhancement. To demonstrate a potential functional consequence of the altered syndecan-1 expression, isolated syndecan-1 was shown to induce apoptosis in MCF-7 cells. These studies show that (n-3) LDL modifies the production of syn-
decan-1 in human breast cancer cells and suggest that a potential antitumor mechanism of (n-3) PUFAs involves the regulation of this apoptotic stimulus.

(n-3) Fatty Acids Prevent Prostate Cancer Progression to Hormone Independence. William E. Friedrichs,* Xiaonan Li,* Wen Wang,† Gabriel Fernandes,* and Linda A. de-Hormone Independence. William E. Friedrichs,* Xiaonan (n-3) Fatty Acids Prevent Prostate Cancer Progression to regulation of this apoptotic stimulus.

Androgen deprivation therapies for metastatic prostate cancer are useful initially, but progression to androgen independence usually results in relapse within 2 y and remains the primary obstacle to improved survival. To improve overall survival, novel treatment strategies based on specific molecular mechanisms that prolong the androgen-dependent state and that are useful for androgen-independent disease need to be identified. Both epidemiological as well as preclinical data suggest that (n-3) fatty acids are effective primary tumor prevention agents; however, their efficacy at preventing and treating refractory prostate cancer has not been as thoroughly investigated. Our overall hypothesis is that essential PUFAs can modulate genetic and epigenetic processes critical for prostate cancer progression to an androgen-independent state and that altered balance between (n-3) and (n-6) fatty acids results in changes to these processes that can be effectively exploited for preventing refractory prostate cancer. We used an in vitro model of androgen ablation to determine the effect of treatment with (n-3) fatty acids on the progression to androgen independence. Both (n-3) fatty acids, docosahexaenoic acid and eicosapentaenoic acid, prevented the progression of LNCaP cells to androgen independence, whereas the (n-6) fatty acid, arachidonic acid, actually promoted cell growth under conditions of hormone depletion. These results correlated with a decrease in the expression of the androgen receptor and a decrease in prosurvival proteins such as Bcl-2 as well as suppression of the Akt/mTOR signaling pathway, previously shown to be critical for progression to hormone independence. Connecting the mechanisms by which (n-3) fatty acids affect phenotypic outcome is important for effective exploitation of these nutrient agents as a therapeutic approach. The effect of specific dietary components on prostate cancer progression likely depends on a host of genetic and epigenetic processes. Understanding these processes is critical for the development of effective dietary intervention strategies that improve overall survival.

(n-3) Fatty Acids to Retard Breast Cancer Progression. W. Elaine Hardman. Pennington Biomedical Research Center, Baton Rouge, LA.

BACKGROUND: Canola oil contains a more favorable ratio of (n-3) to (n-6) fatty acids (~1:2) than corn oil and is readily available; is inexpensive; and can be easily used in cooking, baking, and salad dressings. If consumption of this oil could prevent or retard mammary gland cancer development, encouraging its use would be a cost-effective dietary change that could improve the future incidence of breast cancer. EXPERIMENTAL MODEL: The transgenic FVB-Tg (C3–1–TAg)cJeg mouse model was used for this study. On a standard diet, female hemizygotes develop hyperplasia in the mammary ducts by age 3 mo that progresses to mammary adenocarcinoma in 100% of mice by 6 mo. METHODS: Female SV 129 mice were divided into 2 groups and fed diets containing either 10% corn oil (control diet) or 10% canola oil (test diet); then were bred with male homozygous FVB-Tg (C3–1–TAg)cJeg mice. At weaning, female offspring were fed the corn oil or the canola oil diet. EARLY RESULTS: At age 3 wk (weaning), livers from female mice from mothers that consumed the canola oil diet had significantly lower levels of linoleic acid and arachidonic acid [(n-6) fatty acids] and significantly higher levels of eicosapentaenoic acid and docosahexaenoic acid [(n-3) fatty acids] than did the livers of female mice from mothers that consumed the corn oil diet. Mean plasma estrogen did not differ significantly by diet in nursing mothers or in their offspring at weaning. At 4 mo, the mammary glands of mice that consumed the canola oil diet were more differentiated than those of mice that consumed the corn oil diet. Because differentiated cells do not proliferate, this could be interpreted as reducing susceptibility of the mammary glands to carcinogenesis. DISCUSSION: Having canola oil instead of corn oil in the diet could reduce risk for developing mammary gland cancer. [Supported by a grant from the Department of Defense Breast Cancer Research Program.]

Neutral Sphingomyelinase Mediates Inhibitory Effects of (n-3) Polyunsaturated Fatty Acids on Breast Cancer Development. Rafat A. Siddiqui,e,*** Min Wu,* Nargiz Ruzmetov,* Kevin A. Harvey,* Zachary R. Welch,* Laura Sech,* Kim Jackson,† Gary P. Zaloga,** and William Stillwell.†

Background: Understanding these processes is critical for the development of effective dietary intervention strategies that improve overall survival. The effects of fish oils and their active (n-3) fatty acid constituents, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), on breast cancer growth were investigated. IN VITRO EXPERIMENTS: DHA and EPA inhibited the growth of cultured MDA-MB-231 cells in a dose-dependent manner (P < 0.05). Neutral sphingomyelinase (N-SMYase) activity was also increased 30–40% (P < 0.05) in the DHA or EPA-treated cells, where an increase in ceramide formation was observed. DHA and EPA both enhanced membrane bleb formation and also induced the expression of p21. Both bleb formation and p21 expression were inhibited by the N-SMYase inhibitor GW4869, which also inhibited apoptosis by ~40% (P < 0.05). IN VIVO EXPERIMENTS: Mice were fed diets that were rich in either (n-3) (fish oil) or (n-6) (corn oil) fatty acids. Three weeks after implantation of MDA-MB-231 breast cancer cells, tumor volume and weight were significantly lower (P < 0.05) for mice fed the (n-3) diet compared with those fed the (n-6) diet. Dietary fish oil also caused a 40% (P < 0.05) increase in N-SMYase activity in the tumors. The tumor tissues from fish oil–fed mice expressed elevated p21 mRNA, whereas tumor tissues from corn oil–fed mice did not. DHA and EPA also caused a translocation of N-SMYase from plasma membranes to intracellular sites. The results suggest that inhibition of breast cancer cell growth in culture by treatment with DHA or EPA and inhibition of breast cancer growth in nude mice by dietary fish oil is mediated by N-SMYase translocation from membranes to intracellular sites, with subsequent enhancement in N-SMYase activity.

Proteins and amino acids

Does a Dietary Methionine Restriction Alter the Polymorphonuclear Neutrophil Respiratory Burst in Advanced Can-
cer? Marie-Chantal Farges, Emilie Thivat, Xavier Durando, Philippe Chollet, and Marie-Paule Vasson.

Introduction: Preliminary clinical trials in patients with advanced cancer indicate that dietary methionine (Met) restriction has some antitumor activity and offers promise as a strategy, either alone or combined with chemotherapy. Because Met is a precursor of glutathione, a potent antioxidant, and polyamines and polymorphonuclear neutrophils (PMNs) are a key element of the innate immune system and form the first line of defense, the aim of this preliminary study was to verify that in vitro and in vivo Met restriction does not alter the microbicidal reactive oxygen species (ROS) generation in PMNs. Subjects and Methods: Ten patients with metastatic melanoma or glioma were treated every 2 wk with short cycles of Met-restricted diet (XMets Cys Maxamais®, 2400 kcal/d) concomitantly with chemotherapy (cyclophosphamide, 60 mg/m²). ROS production from phorbol myristate acetate-stimulated PMNs was quantified by flow cytometry before and after the Met-restricted diet on 1 to 4 consecutive regime days. Plasma Met concentration was quantified by ion exchange chromatography. In addition, for healthy subjects, the kinetics (0–6 h) of isolated PMN ROS production was determined for the medium Met concentration (0, 10, and 20 μmol/L). Two-way ANOVA and t tests on paired series (means ± SEM) were used. Results: The Met-restricted diet reduced the methioninemia by 42% (22 ± 7 vs. 12 ± 5 μmol/L, P < 0.05) and did not alter ROS production (107 ± 11 vs. 140 ± 17, P = 0.172). Whatever the medium Met concentration, ROS production by stimulated PMNs from healthy subjects was similar (P = 0.860), with a maximum at 2 and 3 h. Conclusions: In vivo, dietary Met restriction reduced plasma Met levels without affecting the intracellular oxidative stress. In vitro, Met depletion did not alter the stimulated PMN ROS production. Therefore, dietary Met restriction does not induce a decrease in the antioxidant capacity of the cell.

Vitamins

Soy Components Gender-Specifically Affect Apoptosis and Expression of the Vitamin D System in a Mouse Model for Human Colonic Premalignancy. Heide S. Cross, Giovanna Bises, Erika Bajna, and Enikő Kallay. Department of Pathophysiology, Medical University of Vienna, Vienna, Austria.

Colorectal cancer incidence is reduced in soy-consuming populations. Recent data suggest that the human colon is an estrogen-sensitive organ. Colonocytes express estrogen receptors, and certain phytoestrogens can reduce colonic proliferation in a mouse model. Recently we demonstrated that phytoestrogens regulate the colonic vitamin D system, which could lead to enhanced activation of this autocrine defense against tumor progression. Our model in this study was mice fed AIN76 with or without 20% soy meal. In other experimental groups, AIN76 was supplemented with 0.04% genistein and normal (0.5%) or low (0.04%) calcium. Our rationale was that positive effects of phytoestrogens on the vitamin D system become visible mainly when mucosal cells are induced to hyperproliferate by low calcium in the diet. Methods used were real time RT-PCR, semiquantitative PCR, and immunoblotting. Our data demonstrated gender- and segment-specific action of soy. The vitamin D receptor was modulated only in males and only in the right-side colon. This was paralleled by increased activation of caspase 3, a marker for enhanced apoptosis. Soy downregulated CYP24, the vitamin D–catabolizing hydroxylase, again mainly in the male and in the right-side colon. CYP24 was extremely low in the distal colon. There was no significant change in CYP27B1, the vitamin D–synthesizing hydroxylase, neither gender- nor segment-specific. Results from genistein-treated groups indicated that this substance was even more effective than soy: when hyperproliferation was induced in the mouse colon by feeding AIN76 containing 0.04% calcium, CYP24 increased dramatically, but genistein feeding reduced expression to control levels whereas CYP27B1 levels were increased. Our data showed that soy and genistein indeed regulate the colonic vitamin D system and, in parallel, also regulate proliferation and apoptosis in a direction that might be inhibitory for progression of colonic premalignancy. Interestingly, males appear to be affected more favorably than females. [Supported by a grant from the American Institute for Cancer Research, Washington, DC.]

Dietary Regulation of the Colonic Vitamin D System in Mice. Enikő Kallay, Kan Yang, Martin Lipkin, and Heide S. Cross. Department of Pathophysiology, University of Vienna Medical School, Vienna, Austria; and Strang Cancer Prevention Center, New York, NY.

Decreased dietary intakes of calcium, vitamin D, and folic acid and high fat consumption were suggested as major risk factors for colorectal cancer. The nutritional stress diet NWD used in this study is based on the AIN76A diet modified to contain 5 risk factors of the human diet: high fat and phosphate levels and low calcium, vitamin D, and folic acid. We examined the effect of NWD and some chemopreventive diets on expression of the colonic vitamin D system in mice. Mice were fed NWD for 3 (short exposure) or 18 mo (long exposure). Control groups were fed the AIN76 diet. During the short exposure, additional groups were fed supplemental calcium, vitamin D, or folic acid. The mRNA levels of the vitamin D receptor (VDR), 25-hydroxyvitamin D3 1α-hydroxylase (CYP27B1), and 24-hydroxylase (CYP24) were measured by real time RT-PCR. The VDR mRNA expression was not affected by the NWD diet, but VDR levels in older animals increased independent of diet. Feeding mice NWD for 18 mo enhanced CYP27B1 expression in the proximal colon only. Shorter exposure had no significant effect. In the proximal colon, NWD augmented CYP24 expression also. However, 18 mo exposure to NWD resulted in a higher increase of CYP24 levels than 3 mo exposure. CYP24 expression also rose significantly with age. In the distal colon, we observed no regulation of CYP24 expression. All 3 chemopreventive diets decreased CYP24 transcription; folic acid had the most significant effect. The supplements had no significant effect on VDR or CYP27B1 expression. This study confirms that colonic vitamin D hydroxylases in mice can be regulated by dietary means, but mainly in the proximal colon. Vitamin D, calcium, and folic acid are able to reduce expression of CYP24 in the ascending colon, which might lead to maintenance of levels of the vitamin D hormone sufficient to exert its antimitotic cancer preventive action. [Supported by the American Institute for Cancer Research, Washington DC (HSC).]
Minerals

Effect of Diet and Calcium on Intestinal Tumorigenesis. Jay Whelan,* Jong Sik Lee,* Shengli Ding,* Michael F. McEntee,* and Michael B. Zemel.* *Department of Nutrition and †Department of Pathobiology, University of Tennessee, Knoxville, TN.

OBJECTIVE: We previously reported that dietary PUFAs affect intestinal tumorigenesis by modifying PGE_2 levels in tumors and these effects were correlated with changes in intracellular calcium levels [Ca^{2+}]. To further investigate this link among diet, [Ca^{2+}], and early stages of colorectal cancer, we manipulated the [Ca^{2+}], of intestinal tumors through diet and pharmacologic intervention. METHODS: Apc^min^ mice, a mouse model that spontaneously develops intestinal polyps, were treated with agents that modify tumor [Ca^{2+}]. 3 levels of dietary calcium (0.2, 0.5, or 1.2%), 2 types of L-calcium channel blockers (verapamil or nifedipine), piroxican, and 2 types of prostaglandin E_2 (PGE_2) receptor agonists (16,16-dimethyl-PGE_2 or 17-phenyl trinor PGE_2). After treatment, the mice were killed and tumor [Ca^{2+}] levels and tumor load were determined. RESULTS: Treatment with the Ca^{2+}-channel blockers reduced tumor [Ca^{2+}], by >70% and resulted in nonsignificant reductions in tumor load (number and size). Both PGE_2 receptor agonists increased [Ca^{2+}], >2.8-fold; piroxican reduced [Ca^{2+}], and concomitant addition of the PGE_2 receptor agonists attenuated this response. However, tumor load did not change significantly under any of these conditions. When mice were fed diets with various levels of calcium (in the form of nonfat dry milk), [Ca^{2+}], in tumors was 61% lower in the high-versus low-calcium groups, yet the tumor number was paradoxically 67% higher in the high-calcium group (63 vs. 38 tumors/mouse), despite the literature showing antitumor effects with dietary calcium. Interestingly, dairy calcium showed antiobesity properties, and the mice on the high-calcium diets lacked any supportive adipose tissue. Follow-up experiments demonstrated that when the mice maintained their adipose stores, the high-calcium diets failed to significantly change tumor load. CONCLUSIONS: Although changes in [Ca^{2+}], may be working in concert with other mechanisms to modify tumorigenesis, as an isolated event, modifying [Ca^{2+}], was not correlated with changes in tumor load. [Supported by a grant from the American Institute for Cancer Research and support from the Tennessee Agricultural Experiment Station.]

Interaction of Dietary Calcium and Fat Stores in Modulating Intestinal Tumorigenesis. Shengli Ding,* Michael F. McEntee,* Jay Whelan,* and Michael B. Zemel.* *Department of Nutrition and †Department of Pathobiology, University of Tennessee, Knoxville, TN.

OBJECTIVE: Although high-calcium diets have been reported to reduce the risk of colorectal cancer, our preliminary data with the Apc^min^+ mouse strain show a paradoxic increase in intestinal tumor loads (>65%) with diets high in calcium. Because we previously demonstrated that increasing dietary calcium reduces adiposity, and Apc^min^+ mice fed high-calcium diets exhibited a profound loss of adipose tissue, we hypothesized that profound loss of adipose tissue explains in part the markedly increased tumor load in mice fed the high calcium diet. METHODS: We crossed tumor-prone Apc^min^+ mice with obesity-prone A^+/A^ mice to generate obese A^+/A^ mice. Diets low (0.2%), normal (0.5%), and high (1.2%) in calcium were fed to A^+/A^ mice and Apc^min^+ mice (n = 21/strain, n = 7/group) for 7 wk. Intestinal tumor size and number, fat depot mass, and plasma leptin and insulin levels were measured. RESULTS: The high-calcium diet reduced weight gain in both strains (A^+/A^: 13.38 vs. 4.89 g; Apc^min^+: 2.64 vs. 0.03 g) and reduced fat pad mass by 46 to 57% in A^+/A^ and by 65 to 71% in Apc^min^+. Feeding Apc^min^+ mice the high-calcium diet increased tumor number from 29 to 76 tumors/mouse but had no effect on tumor loads in the A^+/A^ mice. Serum leptin levels in A^+/A^ mice fed the high-calcium diet were significantly lower than in those fed the low-calcium diet (19.04 vs. 51.36 μg/L), whereas diet had no effect on leptin levels in Apc^min^+ mice. Diets higher in calcium had no effect on insulin levels in either genotype; however, plasma insulin was 2-fold higher in Apc^min^+ than in A^+/A^ (P < 0.001). CONCLUSION: The differential effect of dietary calcium on intestinal tumorigenesis may result from the loss of protective factors derived from adipose tissue because of the lack of body fat in Apc^min^+ mice fed a high-calcium diet, where substantial loss of adipose tissue could accelerate tumorigenesis. [Supported by a grant from the American Institute for Cancer Research and support from the Tennessee Agricultural Experiment Station.]

Dietary Selenium Homeostasis in Mice Expressing a Mutant Sec tRNA[Ser]Sec—a model for Colon Cancer Research. Robert Irons,* Bradley A. Carlson,† Dolph L. Hatfield,† and Cindy D. Davis,* ‡Nutritional Science Research Group, National Cancer Institute, Rockville, MD, and ‡Molecular Biology of Selenium Section, Laboratory of Cancer Prevention, National Cancer Institute, Bethesda, MD.

Dietary selenium intake is reported to be inversely associated with colon cancer risk in several epidemiological and preclinical studies. To determine the importance of selenoproteins in cancer protection, we conducted studies to characterize selenium homeostasis in transgenic mice that express a mutant Sec tRNA[Ser]Sec lacking the isopentenyladenosine (i^6A) base at position 37 and have selectively reduced selenoprotein synthesis. Wild-type (wt) and i^6A-deficient mice were fed diets containing 0, 0.1, or 2.0 μg selenium (as selenite)/g diet (deficient, adequate, and supplemental selenium, respectively; n = 5–6 mice/group). Compared with wt mice, liver and colon tissue selenium concentrations were lower in i^6A-deficient mice fed adequate selenium (1014 ± 67 vs. 252 ± 14 and 225 ± 11 vs. 63 ± 3 ng/g, respectively; P < 0.001). In contrast, when mice consumed supplemental selenium, there were no significant differences in liver selenium concentrations between the two strains. Western blot and enzymatic analysis revealed that i^6A-deficient mice had significantly reduced (P < 0.001) glutathione peroxidase 1 (GPX1) expression and activity in both liver and colon tissue, which was not corrected by supplemental selenium. However, neither mouse strain nor dietary selenium significantly affected protein levels of thioredoxin reductase (TR1), selenoprotein P, a 15-kDa selenoprotein, and GPX2. Compared with those fed the selenium-deficient diet, both wt and i^6A-deficient mice fed adequate and supplemental selenium diets had significantly increased (P < 0.05) TR1 activity in liver and colon tissue. i^6A-Deficient mice had significantly more (P < 0.005) azoxymethane-induced aberrant crypts and aberrant crypt foci than wt mice. Compared with adequate selenium, supplemental selenium decreased aberrant crypt formation in both wt and i^6A-deficient mice. In summary, supplemental selenium can increase tissue selenium concentrations and TR activity in i^6A-deficient mice but does not rescue GPX1 expression or activity.
These results also suggest that selenoproteins are important for the cancer-protective effects of selenium.

Boric Acid Inhibits Growth of Human Prostate Cancer Cell Line DU-145. Susan L. Meacham,‡‡ Kyler E. Elwell,† and Stephen W. Carper.** *Department of Nutrition Sciences, School of Health and Human Sciences, Division of Health Sciences; †Department of Chemistry, College of Sciences; and **UNLV Cancer Research Center, University of Nevada, Las Vegas, NV.

Epidemiological studies have suggested that dietary boron (high in foods and juices such as avocados, apples, grapes, pears, peanuts) may be inversely related to prostate cancer. To investigate a possible cellular mechanism we determined the effect of 1 mmol/L boric acid on the growth rate of cell lines: 3 human prostate and, for comparison, 4 human breast cell lines. Estrogen receptor–negative human breast cancer cell lines MDA-MB-231 and MBA-MB-435 cultured in MEM supplemented with 10% fetal bovine serum (FBS) and 25 mmol/L HEPES did not show any growth inhibition in the presence of boric acid. Estrogen receptor–positive cell lines MCF-7 and T47-D cultured in either MEM or RPMI1640 medium supplemented with 10% FBS and 25 mmol/L HEPES did not show any growth inhibition when exposed to boric acid. Androgen receptor–positive human prostate cancer cell line LNCaP and androgen receptor–negative human prostate cancer cell line PC3 cultured in RPMI1640 also did not show any response to boric acid. However, growth was completely inhibited by 1 mmol/L boric acid in the androgen receptor–negative human prostate cancer cell line DU-145 cultured in RPMI medium. This growth inhibition was reversible, because removing boric acid on d 3 allowed cell growth to resume by d 6. Flow cytometric analysis of DU-145 DNA indicated that boric acid did not block the cell cycle or induce apoptosis. Boric acid appeared to be acting as a cytostatic agent only in DU-145 cells. These results indicate that the inhibitory effects of boric acid are highly specific, affecting only one androgen receptor–negative prostate cancer cell line, DU-145, a line previously shown to be highly sensitive to nutrient therapies. Additional studies elucidating the selective mechanism of action of boron on this particular cell line will be needed before dietary recommendations are made.

BMI and exercise

Exercise and Bowel Cancer: Wheel Running versus Treadmill Running in Min Mice. Laura Basterfield and John C. Mathers. Human Nutrition Research Centre, School of Clinical Medical Sciences, University of Newcastle, Newcastle upon Tyne, UK.

We are using Min mice as models for studies of the effect of physical activity on development of intestinal neoplasia. These mice have a mutation at codon 850 in the Apc gene and develop multiple intestinal polyps spontaneously. The numbers, sizes, and anatomical distribution of these lesions can be altered by dietary and pharmacological agents. From age 5 wk, male and female Min mice were exercised by running on a treadmill at 18 to 21 m/min for 30 min on a 5% slope for 5 d/wk for 10 wk (treadmill mice). Additional groups of mice were provided with an exercise wheel (wheel mice) or with no exercise (control mice). Throughout the study, mice had free access to a Western-style high-fat diet. Nonexercise physical activity (NEPA) undertaken by the treadmill and control mice was quantified for 23 h/d (i.e., excluding the period associated with treadmill running) using an Infraomat device. On average, wheel mice ran 3.45 km/d (maximum 16.8 km/d), compared with 0.71 km/d for treadmill mice. Female mice were more willing treadmill runners and ran further in the wheels than did males. NEPA was also significantly higher (P = 0.032) for females and for treadmill compared with control mice (P = 0.042). Time asleep did not differ significantly between treadmill and control mice, but female mice slept for significantly (P = 0.004) less time than males (584 vs. 747 min). Although it is often supposed that experiments in rodents provide much greater control over nonimposed variables, these studies demonstrate that there is considerable interanimal variation in NEPA, which may modulate responses in tumorigenesis to any imposed exercise regime. [Supported by the World Cancer Research Fund (2001/38)].

The Effects of Weight Loss on Biomarkers of Breast Cancer Risk. Michelle Harvie,* Tom Mercer,† Riz Malik,** Judith Adams,** Alan Flyvbjerg,* Robert Chatterton,†† Sue M. Astley,*** Alan P. Hufton,* Caroline R. Boggs,* Ruth Warren,‡ and Anthony Howell.* South Manchester University Hospitals Trust, Manchester, UK; † Departments of Exercise and Sports Science, University of Wales, Bangor, UK; ‡ Imaging Science and Biomedical Engineering, University of Manchester, Manchester, UK; †† Aarhus University Hospital, Aarhus, Denmark; and † Department of Obstetrics and Gynaecology, Northwestern University Chicago, Chicago, IL; and ‡ Department of Radiology, Addenbrooke’s Hospital, Cambridge, UK.

Weight gain over the pre- and menopausal years increases risk of breast cancer, whereas weight loss (≥5%) reduces risk. We determined the effect of weight loss over 12 mo through an energy-restricted and exercise weight-loss program on biomarkers of breast cancer risk in 79 premenopausal women (aged 35–45) with relatively large adult weight gain (>7 kg since age 20) and a family history of breast cancer (≥1 in 6 lifetime risk). Body weight; percent body fat (dual-energy X-ray absorptiometry); intraabdominal fat (magnetic resonance imaging); sex hormone binding globulin (SHBG); testosterone; prolactin; insulin sensitivity (homeostasis model assessment); and total and free insulin-like growth factor-1; IGF binding protein (IGFBP)-1,2,3; salivary estradiol and progesterone over 1 mo; and percentage mammographic density:volume (step wedge) and area (visual readings) were determined. We report changes from baseline between subjects with significant weight loss (≥5%, n = 26) and <5% weight loss (n = 48) at 6 and 12 mo. At 6 mo, the >5% weight loss group experienced significant declines in testosterone and prolactin; improved insulin sensitivity; and increased SHBG, IGFBP-1, and IGFBP-2 (P < 0.05), but no change in salivary estradiol or progesterone or free IGF-1. At 12 mo, subjects with >5% weight loss maintained lower levels of testosterone (P < 0.001), but the beneficial effects on insulin sensitivity, SHBG, IGFBP-1, and prolactin were not maintained. Mammographic data at 12 mo linked weight loss >5% to loss of breast fat, no change in breast gland volume, and increased percentage gland volume in the breast (P < 0.05). Weight loss of >5% is associated with significant reduction in some biomarkers at 6 mo. The beneficial effect of >5% weight loss on testosterone is maintained at 12 mo, but the effects on SHBG and insulin are not maintained beyond 6 mo, despite weight stabilization. These data may suggest the role of energy restriction rather than energy balance for cancer prevention.