Inhibition of Mammary Tumorigenesis by Conjugated Linoleic Acid: Characterization of PPAR-Related End Points in Various Human and Mouse Mammary Tumor Cell Lines. S. Shoaf, K. Specchio, M. Belury* and J. Vanden Heuvel. Department of Veterinary Science, Pathobiology Program, Pennsylvania State University, University Park, PA and *Department of Molecular Medicine, Northwest Hospital, Bothell, WA.

The dietary fatty acid conjugated linoleic acid (CLA) inhibits tumorigenesis in rats and decreases proliferation of tumor cell lines. We previously showed that CLA is a ligand and activator of peroxisome proliferator–activated receptors (PPAR), nuclear receptors that act as ligand-activated transcription factors for genes controlling cell growth and differentiation and lipid metabolism. In this study, we used several mammary cancer cell lines to characterize the effects of CLA on end points of cell proliferation, apoptosis and PPAR-responsive gene expression. The cell lines used were estrogen receptor (ER)-positive (MCF-7) and ER-negative (MDA-231) human mammary tumor cells and p53-null (TM2H) and p53-mutant (TM4) mouse mammary cells. All cell lines were treated with the 9Z11E- or 10E12Z-CLA isomers or a mixture of isomers (100 μmol/L), ciglitazone (5 μmol/L) or dimethylsulfoxide vehicle for 24 h. Cell cycle progression and apoptosis were examined as potential pathways of growth inhibition by CLA. Flow cytometry revealed that treatment with both isomers slowed down the cell cycle in serum-starved cells in an ER- and p53-dependent manner. CLA isomers increased annexin staining in all cell lines, suggesting the initiation of the apoptosis cascade. Quantitative reverse transcription-polymerase chain reaction was used to determine the mRNA levels of the cell cycle regulator c-myc, the differentiation marker MDGI, and the proliferation-related gene ODC, all of which are PPAR responsive. Both CLA isomers modulated expression of c-myc in all cell lines according to the ER and p53 status of the cell line. CLA also modulated expression of MDGI and ODC in the mouse cell lines in a p53-dependent manner. The ability of CLA to inhibit the cell cycle and induce apoptosis may be due to its effects as a PPAR ligand on cell cycle–related gene expression. The PPARγ subtype-specific ligand ciglitazone induced similar cell cycle–related gene expression and apoptosis, suggesting the role of the PPARγ subtype in mediating the effects of CLA. These in vitro results suggest possible mechanisms behind the antitumor effects of CLA and further characterize several cell lines that can be used to study these mechanisms.

Lack of Effect of a Long-Term, Low-Fat Dietary Intervention on Markers of Lipid Peroxidation in Women with Extensive Mammographic Densities. L. J. Martin, S. Agarwal,* V. A. Rao* and N. F. Boyd. Division of Epidemiology and Statistics, Ontario Cancer Institute, University Health Network, Toronto, ON, Canada and *Department of Nutritional Sciences, University of Toronto, Toronto, ON, Canada.

The wide variation in breast cancer incidence among countries and the increase in incidence observed in migrants moving from low to high risk countries suggest that environmental factors such as diet exert an important influence on breast cancer risk. Lipid peroxidation and oxidative damage may be involved in the development of breast cancer and influenced by diet, in particular dietary fat intake. Our aim was to study the effect of a long-term, low fat, high carbohydrate dietary intervention on markers of lipid peroxidation in women with extensive mammographic densities. Subjects were women in the low fat, high carbohydrate dietary intervention and control groups of a randomized controlled clinical trial who attended an annual clinic visit (from y 2 to 10 of follow-up) during the study period. Malondialdehyde (MDA) in serum and urine was measured by determination of thiobarbituric acid derivatives after separation by HPLC. Malondialdehyde-deoxyguanosine (dG-MDA) adduct will be determined by reverse-phase HPLC, C18 column and fluorescence detection and plasma antioxidants by reverse-phase HPLC. A total of 394 women participated in the study. Results for plasma MDA are available for 197 subjects (83 intervention and 113 controls) and urine results are available for 115 subjects (51 intervention and 64 controls). Plasma and total urinary MDA levels were positively correlated (r = 0.49). There was no difference in plasma or total urinary MDA levels or plasma antioxidant levels between subjects in the low fat intervention and control groups. Controlling for age, menopausal status, hormone use, length of time in trial and body weight did not affect these results. These preliminary results suggest that long-term intervention with a low fat, high carbohydrate diet does not affect lipid peroxidation in women at high risk for breast cancer because of extensive mammographic density. [Supported by American Institute for Cancer Research grant 98A040.]

Colon cancer

Risk Factors for Colorectal Cancer in a Portuguese Population: A Case-control Study. P. Ravasco,* I. M. Grillo*† and M. E. Camilo.* †Center of Nutrition and Metabolism of the Faculty of Medicine of the University of Lisbon, Lisbon, Portugal and *Center of Nutrition and Metabolism of the Faculty of Medicine of the University of Lisbon, Lisbon, Portugal.

The interaction between diet and colorectal cancer is still debated and the relevance of some nutrients is emerging. The aim of this prospective case-control study in a Portuguese population was to identify the prevalence of risk factors, i.e., diet, alcohol and smoking, for colorectal cancer. Over 30 mo,
Aging Alters the Inhibition of Colonic Aberrant Crypt Foci by Curcumin. Y. Kwon, J. Montgomery, M. Malik and B. Magnuson. Department of Nutrition and Food Science, University of Maryland, College Park, MD.

Curcumin, the yellow pigment of the rhizome of Curcuma longa Linn., has antioxidant, anti-inflammatory and chemopreventive activities. The effect of aging on the inhibition of early-stage colon cancer by curcumin was examined in young (6 wk), mature (12 mo) and old (22 mo) Sprague-Dawley male rats. Rats in each age group were fed either the AIN-93 containing 0.6% curcumin or AIN-93 control diet. Aberrant crypt foci (ACF) were induced by two weekly subcutaneous injections of azoxymethane (15 mg/kg body). After an additional 3 mo of consuming the diets, the number, multiplicity (number of crypts per focus) and distribution of ACF were evaluated. In rats fed the AIN-93 control diet, the number of ACF with a multiplicity ≥2 was 100.3 ± 28.1 in young rats, 76.2 ± 32.7 in mature rats and 76.7 ± 19.8 in old rats. Addition of curcumin to the diet reduced the number of ACF to 51.0 ± 14.5 (49% reduction) in young rats and 34.7 ± 15.0 (55% reduction) in old rats. However, surprisingly, no reduction of ACF (78.5 ± 28.0) was found in mature rats fed curcumin. Inhibition of large ACF also appeared to be affected by age, with the greatest reduction of large ACF occurring in old rats. Distribution of ACF was not altered by age. These results indicate that aging may play a significant role in the efficacy of chemoprevention of colon cancer by curcumin. Potential mechanisms responsible for these observations are currently under investigation in our laboratory. [Supported by the American Institute for Cancer Research.]

Dietary supplements are some of the most commonly used complementary and alternative therapies reported by cancer survivors and the general population. Baseline data (n = 730) from North Carolina Strategies for Improving Diet, Exercise and Screening (NC STRIDES), a health communications study, were used to document dietary supplement use by African-American and Caucasian colon cancer survivors and a comparison population in North Carolina. Of the study participants, 68% reported using some type of dietary supplement. Dietary supplement users were significantly (P < 0.05) more likely to be women, Caucasian, educated beyond high school, and be ≥65 y old; eat ≥5 daily servings of vegetables and fruits; follow a special diet; and have a body mass index (BMI) < 25 kg/m². Dietary supplement use did not differ significantly between colon cancer survivors and the comparison population. Respondents used different types of dietary supplements. The most frequently used dietary supplements were multivitamins (45%), single vitamins and minerals (44%), and herbs (10%). Multivitamin users and single vitamin and mineral users were significantly (P < 0.02) more likely to be women. Caucasian, educated beyond high school and to have a BMI < 25 kg/m². Herb users were more likely to be eating ≥5 daily servings of vegetables and fruits (P < 0.01) but associations were not found by gender, race or BMI. The findings from this study suggest that the use of different types of dietary supplements is associated with different sociodemographic and dietary profiles. Appropriate assessment of dietary supplement use by cancer survivors and the general population is critical for understanding how dietary supplement choices are associated with diet and dietary behaviors.

Reduction of Colon Tumorigenesis in Rats by Feeding Almonds. M. R. Bennink, J. M. Harkins and E. A. Rondini. Michigan State University, East Lansing, MI.

Previous research demonstrated that feeding almonds inhibited the very early stages of chemically induced colon carcinogenesis (1). The purpose of this research was to determine whether feeding almonds would also inhibit later stages of tumorigenesis. Colon tumorigenesis was initiated by two injections of azoxymethane (15 mg/kg) and dietary treatment commenced 1 wk after the second injection. Ground almonds constituted 41.2% of the almond diet, and the control diet was a modification of the AIN-93G diet. Both diets had comparable amounts of protein, mineral mixture, vitamin mixture, fiber and essential fatty acids (EFA) per 1000 kcal. After 32 wk of dietary treatment, tumor development was assessed. Colon tumor incidence was 73% for rats fed the control diet and 53% for rats fed the almond diet (a 27% reduction, P = 0.08). Tumor multiplicity (number of tumors/tumor-bearing rat) was reduced (P = 0.03, 1.22 for almond diet vs. 1.85 for control diet) and tumor burden (mg tumor/tumor-bearing rat) was also reduced (P = 0.04, 73 for almond diet vs. 224 for control diet) by feeding almonds. The three assessments of tumor development (tumor incidence, multiplicity and burden) and the previous research, taken together, provide strong evidence that feeding almonds results in an inhibition of colon tumorogenesis. Moreover, these data suggest that almonds can be part of a healthy diet aimed at reducing colon cancer. [Supported by the Almond Board of California and the Michigan Agricultural Experiment Station.]

Dietary Intake of Folate and Related Micronutrients, Genetic Polymorphisms in MTHFR and Colorectal Cancer: A Population-Based Case-Control Study in Scotland. L. Sharp,* J. Little,* N Brockton,* S. C. Cotton,* N. E. Haitem and J. Cassidy,** **Epidemiology Group, *Medical Genetics and **Oncology Group, Department of Medicine & Therapeutics, University of Aberdeen, Aberdeen, Scotland.

Colorectal cancer is the third most common cancer in males and the second most common in females in developed countries. High vegetable intake is associated with reduced colorectal cancer risk. Vegetables are a major source of folate. Intakes of folate and related vitamins are relatively low in Scotland. There are functional polymorphisms in the methyltetrahydrofolate reductase gene (MTHFR) that controls folate metabolism. These may be of public health relevance for conditions in which folate is etiologically important. We present results from a population-based case-control study of folate, related micronutrients, MTHFR and colorectal cancer. Eligible cases were northeast Scotland residents with histologically confirmed colorectal cancer diagnosed from September 1998 through February 2000. Control subjects were selected from National Health Service registers in the same area. Subjects completed a semiquantitative food-frequency questionnaire and provided a mouthwash DNA sample. C677T and A1298C polymorphisms were determined by polymerase chain reaction methods. Dietary data were converted into estimated nutrient intake and analyzed in quartiles, adjusted for total energy; 264 cases (62% of those eligible) and 408 controls (61%) participated. Overall, there was no association between dietary folate intake and colorectal cancer risk. For women, risk was reduced in the highest quartile [Q4 vs. Q1: odds ratio (OR) = 0.54, 95% confidence interval (CI) = 0.26–1.12]. The inverse was found in men. Patterns were inconsistent for other B vitamins. For C677T, compared with homozygous wild types, risk was modestly reduced for heterozygotes (OR = 0.93; 95% CI = 0.66–1.32) and TT homozygotes (OR = 0.72; CI = 0.41–1.29). For A1298C, compared with homozygous wild types, the OR for heterozygotes and CC homozygotes were 1.14 (0.80–1.64) and 0.67 (0.39–1.29), respectively. Genotype results were heterogeneous by cancer site and age. Interactions between dietary factors and genotype have been considered. For genotype, results had some consistency with other studies. For folate, the difference between the sexes, if confirmed, could have implications for the proposed fortification of food with folic acid in the United Kingdom.

Lung cancer

Low Fruit and Vegetable Intake Exacerbates the Risk of Lung Cancer Associated with Residential Radon Exposure. M. E. Wright, S. T. Mayne and M.C.R. Alavanja.* Yale University School of Medicine, Department of Epidemiology and Public Health, New Haven, CT and *Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD.

Effects of fruit and vegetable consumption on the association between residential radon exposure and lung cancer risk were examined using data from a population-based case-control study of lung cancer in Missouri women, most of whom were current or former smokers. Data were available for 356 newly diagnosed lung cancer cases and 470 controls frequency matched to cases on the basis of age. A modified food-frequency questionnaire was used to obtain information about usual diet 2–3 y before the interview. Twenty-year, time-weighted, average residential radon concentrations were assessed with CR-39 α-particle detectors (surface monitors), which appear to capture cumulative radon exposure more accurately than do standard indoor air radon detectors. Women exposed to high radon levels (greater than the median vs. less than or equal to the median value of the controls) had a significantly elevated risk of lung cancer in models adjusted for age, packyears of smoking, education level and previous lung disease [odds ratio (OR) 1.43, 95% confidence interval (CI), 1.07–1.92]. In stratified analyses, low consumption of several plant food groupings exacerbated the risk of lung cancer associated with high residential radon exposure. For example, the OR for high vs. low radon exposure was 1.66 (95% CI, 1.11–2.49) in women with low total vegetable, fruit and juice consumption and 1.15 (95% CI, 0.75–1.78) in women with high total vegetable, fruit and juice consumption. Joint effects of radon exposure with consumption of individual plant food groupings were not significant, however, in multiplicative interaction models. These results suggest that non-smoking and smoking women exposed to high levels of residential radon may have an even higher risk of lung cancer if they consume inadequate amounts of fruits and vegetables.

Prostate cancer

Feasibility and Efficacy of Low-Intensity Diet and Exercise Counseling for Overweight Men with Active Prostate Cancer. E. C. Miller, T.W.-M. Boileau,* T. Bray,* S. Schwartz† and S. K. Clinton. Department of Internal Medicine, Division of Hematology and Oncology, James Cancer Hospital and Solove Research Institute, Columbus, OH and *Department of Human Nutrition and †Department of Food Science and Technology, Ohio State University, Columbus, OH.

Men with asymptomatic, metastatic prostate cancer based on a rising prostate-specific antigen (PSA) level after local therapy are highly motivated to initiate low risk interventions to inhibit the progression of their disease. This population presents an opportunity for dietetics professionals to counsel patients on scientifically based dietary and lifestyle changes that may affect prostate cancer progression. We enrolled 11 overweight men [ages 58–79 y, body mass index (BMI) 25.0 kg/m², mean BMI = 31.5 ± 3.1 kg/m²] who had completed primary treatment for prostate cancer and had evidence of metastatic disease based on progressive rises in their PSA levels. The men were monitored for 9 wk, with dietary and exercise counseling occurring at enrollment and at wk 1 and 5. The goals of dietary counseling were based on American Institute for Cancer Research, National Cancer Institute and American Heart Association dietary guidelines for health promotion, including consuming at least 5 fruit and vegetable servings per day; increasing dietary fiber (>20 g/d); reducing total fat (<30% total energy), saturated fat (<10% total energy) and cholesterol (<300 mg/d); and energy restriction (to promote weight loss of 2.2 kg/wk). The goals for exercise counseling were based on the American College of Sports Medicine and the Centers for Disease Prevention and Control public health guideline encouraging 30 min of physical activity on most days of the week. Three-day diet records were completed at enrollment and midstudy and were analyzed with Diet Analysis Plus software (Wadsworth Publishing Group, Belmont, CA) and SPSS software (SPSS Chicago, IL). Physical activity was assessed by recall at each clinic visit. Weight loss was achieved in 8 of the 11 men. Average weight change
dietary fat, fruit and vegetable consumption or cholesterol intake was reduced from 308 to 149 mg/d to 219 ± 91 mg/d (P ≤ 0.041). Total minutes per week of programmed exercise increased from 11 ± 24 min/wk to 137 ± 77 min/wk (P ≤ 0.0001). Counseling did not significantly alter dietary fat, fruit and vegetable consumption or fiber intake. We conclude that overweight men with active prostate cancer can easily be induced to initiate tailored exercise programs and modest energy restriction to promote weight loss. Changes in dietary composition will require more intense and costly nutritional counseling and education.

In the prostate, zinc bound with high affinity to citrate inhibits the Krebs cycle enzyme aconitase, preventing citrate oxidation. However, in prostate cancer cells, zinc levels are dramatically lower, allowing citrate to be metabolized presumably to meet the higher energy demands of the tumor. We report here that treating prostate cancer cells with zinc produces a dose-dependent decrease in cell growth. LNCaP and PC-3 cells were treated with various doses of zinc chloride for 48 h. Cell viability was measured by trypan blue exclusion assay.

In contrast, treatment of LNCaP cells with 0.15 and 0.37 mM zinc/mL medium. Although the IC50 for [3H]-thymidine incorporation into DNA was ~8 µg zinc/mL in LNCaP cells, a 50% inhibitory concentration (IC50) for PC-3 cells was ~4 µg zinc/mL medium. The IC50 for [3H]-thymidine incorporation into DNA was ~8 µg zinc/mL in LNCaP cells, a 50% inhibitory concentration of zinc was not always achieved with doses in excess of 10 µg zinc/mL. Zinc increased the proportion of PC-3 cells in the S phase of the cell cycle (12–37%) over a range of 0.7–1 µg zinc/mL, with a concomitant decrease in G3/G4 (61–30%) and a small increase in G2/M (27–37%). In contrast, treatment of LNCaP cells with 0–22 µg zinc/mL produced only a slight increase in G2/M, a small decrease in G1 and no change in S. No satisfactory treatment exists for men with hormone-refractory prostate cancers. PC-3 cells are aggressive and androgen independent but were still inhibited by zinc treatment. Plasma concentrations of zinc are typically ~1 µg/mL (15.2 µmol/L). Therefore, obtaining a 50% reduction in cell growth with 4 µg zinc/mL suggests that this agent should be investigated further for cytostatic effects that may delay the progression of this disease. [Supported by American Institute for Cancer Research grant 08B022.]  

Boronic acid inhibits PSA and reduces the development and proliferation of prostate carcinomas. We tested this hypothesis using nude mice implanted subcutaneously with LNCaP cells in Matrigel. Male mice (n = 40) were divided into 4 groups: 3 groups were dosed with boracic acid solutions [1.7, 9.0 and 48.0 mg/(kg · d)] by gavage; the control group received only water. Tumor sizes were measured weekly. Serum PSA and IGF-1 levels were determined when the mice were killed. The size of tumors was decreased in mice exposed to the low and middle dose of boracic acid by 38 and 25%, respectively. Serum PSA levels decreased by 88.6 and 86.4%, respectively, compared with the control group. There were morphological differences between the tumors in control and boron-dosed mice, including a significantly lower incidence of mitotic figures in the boron-supplemented groups. Circulating IGF-1 levels were not different among groups, although immunohistochemical expression of IGF-1 in the tumors was markedly reduced by boron treatment. These data indicate that low level dietary variance in sensitivity to the antiproliferative effects of boronic acid in the human prostate cancer cell lines LNCaP and PC-3. M. T. Gallardo-Williams, M. W. Harris and R. R. Maronpot.* Laboratory of Molecular Toxicology and *Laboratory of Experimental Pathology, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Prostate-specific antigen (PSA) is a serine protease and one of the most abundant proteins secreted by the human prostate epithelium. PSA is used as a well-established marker of prostate cancer. The involvement of PSA in several early events leading to the development of malignant prostate tumors has been a major target for prevention and intervention. It is thought that PSA cleaves insulin-like growth factor (IGF) binding protein, providing increased local levels of IGF, leading to tumor growth. Separately, data suggest an enzymatic regulatory role for dietary boron. Boron reversibly inhibits several serine proteases. Our own in vitro results suggest that low concentrations (6 µg/mL) of boracic acid can partially inhibit the proteolytic activity of purified PSA toward synthetic substrates. We propose that dietary supplementation with boracic acid inhibits PSA and reduces the development and proliferation of prostate carcinomas.
boron supplementation reduced tumor size and content of a tumor trophic factor, IGF-1. This promising model is being evaluated in further studies.

**Using Target-Specific Arrays to Analyze Cell Cycle Control by PC-SPES in LNCaP Cells. X. Lu, J. Guo, T.-C. Hsieh and J. M. Wu. New York Medical College, Valhalla, NY.**

PC-SPES was a popular alternative herbal formulation for treating various forms of prostate cancer. Recent evidence regarding its contamination by prescribed medications has resulted not only in the discontinuation of PC-SPES but has also cast considerable doubt on the progress made in complementary and alternative medicine particularly on the effectiveness of herbal therapies. Because of the consistent and significant results of several clinical trials with PC-SPES, we consider it important to continue studies to elucidate the antiprostate cancer properties of this complex herbal mixture. Previous studies have reported the ability of ethanol extracts of PC-SPES to suppress prostate cancer cell growth by arresting cell cycling at specific checkpoints; however, the specific genes and gene products responsible for these effects of PC-SPES have not been well characterized. In this study, we used human cell cycle stage–specific cDNA array and Western blot analysis to assess PC-SPES–elicited changes in androgen-dependent LNCaP cells. Up-regulation of cyclin-dependent kinase inhibitor p21 was found after PC-SPES treatment, thereby confirming the cell cycle stage–specific effects of PC-SPES. In addition, PC-SPES attenuates the expression of the genes for proteins cyclin B, cyclin H, cdc2, CDC28 protein kinase 1 and 2, Cullin 4, E2F4, MPP2, mdm2, NEDD8, PCNA and p19, all of which are integrally involved in cell progression. Taken together, our results further support the effectiveness of PC-SPES as an alternative antiprostate herbal regimen. Our data also provide a framework that will allow identification and characterization of each bioactive ingredient in PC-SPES.

**Diets**

**Lack of Effect of a Low Fat High Carbohydrate Diet on Ovarian Hormones in Premenopausal Women: Results from a Randomized Trial. N. F. Boyd, C. Greenberg,* L. J. Martin, J. Stone, S. Minkin and G. Hammond. Canadian Diet and Breast Cancer Prevention Group, Ontario Cancer Institute, Toronto, London Regional Cancer Center, London, ON, Canada and *Princess Margaret Hospital, Toronto, ON, Canada.**

We are conducting a long-term, randomized controlled trial to find out whether intervention with a low fat, high carbohydrate diet reduces risk of breast cancer. This study examines the effects of 2 y of dietary intervention on blood levels of ovarian hormones in premenopausal women. Subjects with extensive mammographic densities were enrolled in a dietary intervention trial. The intervention involved intensive individual counseling aimed at reducing total fat intake to 15% of energy. Control subjects received general advice about diet but were not counseled to change their intake of fat. Serum levels of sex hormones were measured in 867 premenopausal subjects at entry and 2 y after randomization. Blood levels of estradiol were similar in intervention (n = 430) and control (n = 437) subjects at baseline and at 2 y. In the intervention group, the median change in estradiol levels was a 10% (24.5 pmol/L) reduction over 2 y compared with a reduction of 8% (20 pmol/L) in the control group. Blood levels of progesterone, follicle-stimulating hormone and sex hormone–binding globulin were also similar in intervention and control groups at baseline and 2 y. The changes in levels of these hormones in that time period were also similar in the intervention and control groups and none differed significantly. Partitioning hormone levels at 2 y according to the day of the menstrual cycle and progesterone level also were not significantly different between the groups. A low fat, high carbohydrate diet does not reduce blood levels of estradiol or progesterone in premenopausal women. If this dietary modification does reduce risk of breast cancer, it is unlikely to do so through an effect on blood levels of these hormones.

**Food Group Intake Assessed as Pyramid Servings in a Multi-ethnic Cohort. S. Sharma, S. Murphy, L. Wilkens, L. Shen and L. Kolonel. Cancer Research Center of Hawaii, University of Hawaii, Honolulu, HI.**

Awareness is growing that foods, in addition to their nutrient components, may have health benefits. Studies determining associations between chronic diseases such as cancer and food group intake are thus being encouraged. We have established a population-based multiethnic cohort of 215,000 in Hawaii and Los Angeles that comprises African Americans, Native Hawaiians, Japanese Americans, Mexican-born Hispanics, American-born Hispanics and Caucasians. Baseline dietary data included a quantitative food-frequency questionnaire consisting of >200 items. For each ethnic group, we evaluated mean food group intake using the USDA Food Guide Pyramid. Mean daily intake of the total meat group was highest for Mexican-born Hispanics (5.9 servings) and lowest for Caucasians (3.8 servings). Total mean daily intake of fruit and vegetables was highest for the Mexican-born Hispanics (10.1 servings) and lowest for African Americans (7.6 servings). Mean daily intake of discretionary fat varied greatly among the ethnic groups, i.e., Mexican-born Hispanics and Native Hawaiians had the highest intakes (70 g/d) whereas Japanese Americans had an intake 20 g lower (50 g/d). There are clearly ethnic differences in food consumption patterns, which could explain variations in cancer incidence among different ethnic groups.


Evidence exists that environmental factors are associated with the occurrence of prostate and breast cancer. Dietary factors have received special attention in this regard. The aim of this study was to identify the main dietary factors associated with the development of prostate and breast cancer. Articles in the MEDLINE database that were published from 1995 to 2000 showed results of case-control or cohort design studies, and the association among dietary factors and prostate and breast cancer were reviewed. Intakes of meat, butter, total fat, saturated fat and energy were associated with an increased risk of both tumors. The increase in prostate cancer risk was also seen with the consumption of meat and pork products, whereas alcohol intake was associated with breast cancer development. There was evidence that the risk of developing both tumors was
Behavioral Variables and Education Are Predictors of Dietary Change in the Women's Health Trial: Feasibility Study in Minority Populations. A. Bhargava, J. F. Guthrie* and J. Hays.† Department of Economics, University of Houston, Houston, TX; ‡Economic Research Service, U.S. Department of Agriculture, Washington, DC; and †Department of Medicine, Baylor School of Medicine, Houston, TX.

More widespread adoption of healthful diets can improve public health and reduce cancer risk. Nevertheless, the value of increased public investment in nutrition education has been questioned, because behavioral factors such as habit persistence, unhealthy eating patterns, perception of health risks, personal motivation and expectations are likely to influence dietary change. This paper analyzed data on dietary intakes, behavioral factors and anthropometric indicators at baseline and 6 and 12 mo for women in the control and intervention groups of the Women’s Health Trial: Feasibility Study in Minority Populations. The nutrition education program received by the intervention group led to greater changes in behavioral variables over the 1-y study. For the intervention group, education, concerns about health and participation motivation were significantly negatively associated with fat intakes and positively associated with fiber, β-carotene and ascorbic acid intakes. The results for the control group provide further insights into some of the behavioral factors affecting dietary change.

A Retrospective Case-Control Study of the Association among Antioxidant, Carcinogenic and Dietary Fat Intake and Cancer, A. Fatimah, S. Noorjah,* S. Suzana, T. Ruzita, M. Lingam† and F. N. Lau.** Department of Nutrition and Dietetics, Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia; *Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia; †National Cancer Council (MAKNA), Kuala Lumpur, Malaysia; and **General Hospital, Kuala Lumpur, Malaysia.

The objective of this study was to identify the dietary components in Malaysian diets that may contribute to the onset of cancer. A case-control retrospective study was conducted to evaluate the association between diet and cancer. The cases included in the study were 95 inpatients in the radiotherapy and oncology wards at Hospital Kuala Lumpur. These subjects were men and women of all races from Kuala Lumpur who had good cognitive status. The control subjects included individuals from the orthopedic, obstetric and gynecology wards, Bandar Kinrara residents and Klinik Kesihatan Petaling outpatients. Control subjects had never been diagnosed with cancer or other chronic diseases and were matched to cases by age, sex and ethnic origin. Dietary intake data were collected by recording a typical day’s intake and using a food-frequency questionnaire. A set of pretested questionnaires was used to obtain data on other risk factors including smoking and alcohol consumption. Anthropometric data such as weight, height, waist and hip circumference and the percentage of body fat were also obtained. Results show that body weight, body mass index, waist-hip ratio and the percentage of body fat were significantly lower among cancer patients. Family history of cancer was highly significant (P < 0.01). The mean intakes of total energy, total fat and different types of fatty acids were higher for breast cancer cases than controls (P < 0.01). Shrimp paste consumption was significantly higher for cases than controls. β-Carotene, selenium, anchovy and shrimp were found to have a positive association with cancer.

Novel Nutritional Strategies for Skewing the Immune System from Th2 to Th1: Implications for Cancer Prevention and Cancer Treatment. T. M. Fasy, L.-H. Wang and A. S. Sun.* Mount Sinai School of Medicine, New York, NY and *Connecticut Institute for Aging and Cancer, Milford, CT.

Even before treatment, cancer patients are invariably immunosuppressed; their immune systems are skewed toward Th2-type responses, which favor tumor tolerance. Achieving a strong shift toward a Th1 status is increasingly recognized as a highly desirable goal for cancer patients because Th1-type immunity favors effective antitumor responses. A strong shift of Th2 to Th1 can be induced by activating the innate immune system, the ancient surveillance network that is present in all eukaryotes and is designed to detect the presence of microbial pathogens. At least some of the conserved microbial molecules that can activate the innate immune system (e.g., modified muramyl dipeptides; modified peptidoglycans, β-glucans and oligodeoxynucleotides with unmethylated CpG motifs) are effective immunostimulants when administered orally. Moreover, some foods (e.g., certain edible mushrooms) are known to be rich sources of β-glucans, mannans and other fungal cell wall components that can activate the innate immune system. Other edible plants contain well-characterized immunostimulatory polysaccharides, which may act by mimicking microbial polysaccharides recognized by the innate immune system. Consequently, we propose that it is possible to design diets and dietary supplements for cancer patients that not only induce significant Th2-to-Th1 shifts but that are also nontoxic and free of adverse side effects. We recently described a dietary supplement that may promote a Th2-to-Th1 shift in the immune system (1,2). The daily ingestion of this supplement by nonsmall-cell lung cancer patients was associated with improved weight maintenance, improved performance status, reduced fatigue, prolonged survival and some partial and complete tumor responses.


Effects of Chitosan and Chitosan Oligomer on the Lipid Metabolic Disorders Induced by 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) in Rats. Y.-S. Lee, H.-S. Kang, Y.-J. Park and J.-H. Lee. Department of Food and Nutrition,
Various chitosan compounds are recognized as functional food components that improve lipid metabolism in hyperlipidemic animals. This study was designed to determine the effects of chitosan and chitosan oligomer on the lipid metabolic disorders induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Adult male Sprague-Dawley rats were divided into two groups and treated with either TCDD (40 μg/kg body) or vehicle solution by intraperitoneal injection. Rats in each group were divided into three subgroups and fed a basal, 5% chitosan or 5% chitosan oligomer diet for 4 wk. TCDD injection caused serum total cholesterol levels to increase, but a significant decrease was noted in the rats fed the 5% chitosan diet. In addition, the 5% chitosan diet apparently suppressed the liver lipid accumulation caused by TCDD. The effects of the 5% chitosan oligomer diet on serum and liver lipid levels were found to be weaker than those of 5% chitosan diet. Fecal lipids and cholesterol excretion increased more in the 5% chitosan-fed group than in the other groups. Serum phosphatidylcholine hydroperoxides and liver thiobarbituric acid reactive substances that were increased by TCDD decreased with the 5% chitosan and 5% chitosan oligomer diets. However, the hepatic cytosolic superoxide dismutase activities that decreased with TCDD did not recover with the chitosan and chitosan oligomer diets. The 5% chitosan and chitosan oligomer diets significantly decreased the hepatic microsomal cytochrome P450 (CYP), NADPH-CYP reductase, ethoxy-loumarin-o-deethylase and benzphetamine N-demethylase that were increased by TCDD. These results indicate that chitosan and chitosan oligomer have ameliorative effects on hypercholesterolemia, liver lipid accumulation, lipid peroxidation and oxidative stress induced by TCDD.

Obesity and physical exercise

Obesity and Colorectal Adenoma. Data from the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. C. P. Mayo, W. Y. Huang, R. B. Hayes and M. N. Fouad. University of Alabama at Birmingham, AL and National Cancer Institute, Bethesda, MD.

Obesity has been associated with increased risk for colorectal cancer. We investigated the association of body mass index with risk for colorectal adenoma, a neoplastic precursor. This cross-sectional cohort study analyzed data from 43,737 men and women who participated in the multicenter Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial from September 1993 to September 1999. All completed sigmoidoscopic screening (aged 55–74 y at screening) and a risk factor questionnaire and did not have a self-reported history of cancer (except basal cell skin carcinoma), colorectal polyps, ulcerative colitis, Crohn’s disease, familial polyposis or Gardner’s syndrome. There were 3467 cases of left-sided adenomas (descending or sigmoid colon or rectum) of whom 1368 were advanced and 34,132 participants who screened negative for abnormalities. Table 1 shows the prevalence odds ratio for adenomas according to current body mass index adjusted for age, gender, race, center, dietary fat intake, red meat consumption, exercise and smoking. Among participants, being overweight or obese was associated with increased risk for colon adenomas; risk was 20–40% greater than that for normal-weight individuals, depending on the site or aggressiveness of the adenoma. Obesity may increase risk for colorectal cancer.


Obesity and/or hyperinsulinemia (OB/HI) have been associated with augmented risk of developing colon cancer. Zucker obese/hyperinsulinemic rats were reported to be more sensitive to colon cancer than their lean counterparts. We and others have shown that energy restriction (ER) retards the development and growth of aberrant crypt foci (ACF) and colonic tumors. Whether ER could attenuate colon carcinogenesis associated with OB/HI is not known. We studied the effect of an 8-wk intervention with 20–25% ER or unlimited diets on the growth and development of ACF in Zucker obese (fa/fa) rats. A group of age-matched azoxymethane-injected lean (fa/−) rats consumed the unlimited diet for this period. In addition, the expression of cyclooxygenase (COX) isoforms at the protein and mRNA transcript levels was studied in the colonic mucosae of all experimental rats. At 8 wk, obese rats had significantly higher (P ≤ 0.001) average numbers of medium (crypt multiplicity between 4 and 6) and large (crypt multiplicity ≥ 7 or greater) ACF than did lean rats. A higher proportion of ACF of obese rats exhibited mild to moderate dysplasia than did the ACF of lean rats. Dietary ER in obese rats attenuated the appearance of large ACF without affecting the body weight or plasma insulin, glucose, lactate or cholesterol levels. The levels of COX-1 and -2 proteins were lower in colonic mucosae (P ≤ 0.05) in the ER than the non-ER obese rats. These observations suggest that augmented risk for colon cancer in Zucker obese rats may extend beyond the role of excessive body weight and plasma insulin level as pathological factors. Most importantly, our findings demonstrate that in Zucker obese rats, ER can modulate molecular events in the colonic mucosae such as the COX system, which is considered critical in colon carcinogenesis. [Supported by the Cancer Research Society Canada, and the Natural Sciences and Engineering Research Council of Canada.]

The Serum Metabolome of Dietary Restriction: Marker for Cancer Risk? B. S. Kristal,*† U. Paolucci1 and W. R. Mason.** *Departments of Biochemistry and Neuroscience, Cornell University Medical College, New York, NY; 1Dementia Research Service, Burke Medical Research Institute, White Plains, NY; and **ESA, Chelmsford, MA.

<table>
<thead>
<tr>
<th>Body mass index</th>
<th>Any left adenoma1</th>
<th>Left advanced2</th>
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<tbody>
<tr>
<td>18.5 (underweight)</td>
<td>0.9(0.5–1.7)</td>
<td>1.3(0.6–3.0)</td>
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<tr>
<td>&lt;18.5 to &lt;25 (normal weight)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>25 to &lt;30 (overweight)</td>
<td>1.2(1.1–1.3)</td>
<td>1.2(1.0–1.4)</td>
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<tr>
<td>≥30 (obese)</td>
<td>1.3(1.1–1.4)</td>
<td>1.4(1.1–1.8)</td>
</tr>
</tbody>
</table>

1Nonadvanced and advanced.
2Advanced: any 1 or more of size >1 cm, high-grade dysplasia, villous elements (including tubulovillous adenoma) or carcinoma in situ.
Dietary or energy restriction is the most potent and reproducible known means of reducing cancer risk in mammals. In the specific case of breast cancer, restriction is dominant with relation to genetic susceptibility, carcinogen exposure and specific components of the diet. Similarly, increased body mass index is associated with a twofold increase in postmenopausal breast cancer risk in humans. To further explore the relationship between energy intake and cancer risk, we have identified a biochemical profile that reflects the dietary restriction metabolome. Exploratory studies previously identified subsets of 20–63 redox-active small molecules from sera (measured by HPLC coupled with coulometric detector arrays) that distinguished dietary groups in both male and female rats. Expert system and pattern recognition–based approaches have 85–100% accuracy in distinguishing diet groups by using these profiles, indicating that the metabolites previously determined using principal component analysis and hierarchical cluster analysis contain sufficient information to construct classification models. We have now combined these disparate datasets into a single metabolic profile consisting of 104 chromatographically distinguishable peaks. These analyses bring to light fundamental differences between the metabolic response of females and males to energy restriction (e.g., more metabolites are significantly altered in females). Further analysis with projection methods shows that most samples fall within expected variation, but even most outliers are correctly classified. Significant components identified with projection methods (discriminant analysis) are sufficient to yield >95% accuracy in distinguishing metabolic type. These approaches have further highlighted a limited subset of metabolites that are important for this classification. [Supported by NIA AG15354.]

Effect of Psychiatric Diagnosis on Weight Loss in Obese Breast Cancer Survivors. Z. Djuric, I. Jenkins, N. DiLaura and W. Hryniuk. Karmanos Cancer Institute, Wayne State University, Detroit, MI.

Obesity is a major health problem in women who have had breast cancer, making development of effective methods for weight loss in this population a research priority. We started a pilot study to test an individualized approach for weight loss in obese breast cancer survivors (body mass index, 30–44 kg/m²). This approach was used either alone or combined with the Weight Watchers program in a study with a 2 x 2 design; 48 women were enrolled. A psychiatric evaluation was obtained at baseline. After 12 mo, 9.7 and 10.5% of initial body weight was lost using the individualized-approach (n = 9) and combined-approach groups (n = 9), respectively. Some weight regain occurred from 12 to 18 mo followed by stabilization of body weight from 18 to 24 mo in both groups. Mean weight loss at 24 mo was 5.1 and 6.8% of initial body weight using the individualized or combined approach, respectively. For women in either of these two study groups, weight loss at 24 mo was 1 and 1% of initial body weight for women with and without any psychiatric diagnosis, respectively. Weight loss achieved at 12 mo was much lower in the women who were diagnosed with either adjustment disorder or major depressive disorder than in the women with no diagnosis (5.2 vs. 12.3 kg, respectively, P = 0.025). This weight loss was achieved largely in the first 6 mo of intervention; from 6 to 12 mo, women with no diagnosis continued to lose weight (mean of 2.0 kg), whereas women with a psychiatric diagnosis had a mean weight gain of 1.6 kg (P = 0.009). The significant effect of psychiatric variables on weight loss in this study, despite its small sample size, indicates that some breast cancer survivors may benefit from psychiatric treatment as part of a weight loss program. [Supported by National Institutes of Health grant CA89761, the Ford Motor Company Fund and the Weight Watchers Group.]


Physical activity has been associated with a reduced risk of breast cancer, and one potential mechanism may be via a hormonal pathway. Two major metabolites of estradiol are 2-hydroxyestrone (2-OHE₁) and 16α-hydroxyestrone (16α-OHE₁); studies have shown that high urinary excretion of 2-OHE₁ relative to 16α-OHE₁ is associated with lower breast cancer risk. We conducted a randomized controlled clinical trial comparing the effect of a 1-y long moderate intensity aerobic exercise intervention vs. a stretching control on the sex hormone profile in postmenopausal women. A total of 173 women were randomly assigned to a study group and 170 women aged 50–75 y completed the study. Spot urine samples were collected at baseline and mo 3 and 12 and were analyzed for 2-OHE₁ and 16α-OHE₁ using the Estramat 2/16 enzyme immunoassay (Immunacare, Bethlehem, PA). Samples were also analyzed for creatinine (Cr), and excretion of the individual metabolites was expressed as ng/mg Cr. No significant differences were found between treatment groups at baseline for the excretion of either metabolite or their ratio (P > 0.05). From baseline to 12 mo, 2-OHE₁, 16α-OHE₁ and their ratio decreased for the aerobic exercisers. For the stretching control group, 2-OHE₁ and the ratio of 2-OHE₁ to 16α-OHE₁ increased and 16α-OHE₁ decreased. However, these changes were not significantly different between treatment groups (P > 0.05). Further analysis using a generalized estimating equation also suggested that there were no significant effects of treatment group over time on the excretion of either metabolite or their ratio (P > 0.05). We are currently investigating potential effects of study compliance and change in body adiposity. Our preliminary analyses suggest that, overall, a 12-mo moderate-intensity exercise intervention did not significantly alter the urinary excretion of 2OHE₁ or 16α-OHE₁; however, such changes may depend on study adherence and body fat loss.

Supplements

Nutritional Assessments for Cancer Patients Can Be Improved when Mineral Concentrations in Dietary Supplements Are Considered during Medical Nutrition Therapy Consultations. S. Meacham, J. Cizdziel, A. Sadik and N. Farrey. Department of Nutrition Sciences, *Harry Reid Center for Environmental Studies and †Clinical Laboratory Sciences Program, University of Nevada, Las Vegas, NV.

American consumers often purchase multiple dietary supplements, particularly consumers with diseases such as cancer. Nutrient assessments by health professionals do not consider the consumption of essential and nonessential elements from these products, often overlooking potential health hazards of mineral toxicities. Serious tissue damage such as atherosclerosis and carcinogenesis has been reported as a result of overconsumption of selected minerals. Also, the chemical interactions may have serious consequences; for example, a slight overload of manganese can cause or aggravate iron deficiency,
producing anemia. To identify potentially high mineral concentrations in dietary supplements, selected commercial products were analyzed using the Axiom, a high resolution inductively coupled plasma mass spectrometer, for concentrations of aluminum, arsenic, beryllium, boron, cadmium, chromium, cobalt, copper, lithium, magnesium, manganese, nickel, selenium, vanadium and zinc. Samples and certified reference materials [oyster tissue (NIST 1566b and tomato leaf) (NIST 1573)] were digested using nitric acid with a microwave digestion system. Preliminary data indicated that concentrations (μg/g) of magnesium, aluminum and manganese were 744, 11 and 21 in St. John’s wort; 723, 188 and 37 in Siberian ginseng; and 1000, 27 and 6 in dong quai, respectively. Patients prone to the overuse of nutritional supplements will consume vitamins and minerals in amounts that exceed the current safe recommended intakes. Health professionals require more information regarding the mineral content of commercially available dietary supplements in addition to food, beverage and drug use data to complete true daily nutrient analyses for cancer patients, thus preventing the potential health hazards of nutrient overconsumption and drug-nutrient interactions.

Phytochemicals


The primary responsibility of the USDA Nutrient Data Laboratory (NDL) is to provide authoritative nutrient composition data for foods eaten in the United States. Historically, NDL has made these data available in the form of handbooks. Today, the NDL Web site is our primary vehicle for dissemination of nutrient composition data (1). The site includes data sets, single-nutrient reports, previous USDA food composition releases, all of which can be downloaded, and an online search program to access the USDA Nutrient Database for Standard Reference (SR). In the near future, SR search applications for personal computers and personal digital assistants will also be included. Our aim is to make data retrieval easier while extending the usefulness of our Web site to specialized audiences, such as those involved in cancer research. We are able to aid researchers by delivering up-to-date nutrient data for use in their research or practice. Some nutrients of interest in current cancer research include fatty acids, antioxidants, phytosterogens and other phytochemicals. Data for these nutrients and others are available on the NDL Web site. To best serve our broad range of users and audiences, we evaluate use of our Web site to distinguish which types of data and what kinds of data access are most desired. We can then apply revisions to make the site and the data more accessible to these groups while addressing specific data needs.


Production of flavonoids in plants is enhanced in response to stresses such as fungal or bacterial infection or exposure to UV radiation. Recent interest of the scientific community in flavonoids in foods is attributed to the varied biological properties of flavonoids, which include antioxidative, antimicrobial and possibly anticarcinogenic and cardioprotective effects. Therefore a food composition database for flavonoids in foods is essential to evaluate associations between flavonoid intakes and risk factors for various diseases. We have conducted an exhaustive literature review that yielded ~475 articles on flavonoids published since 1970. Only 125 articles contained quantitative analytical data on flavonoids. After reviewing all of the data, we have analytical values for ~220 foods. Data will be presented for selected compounds in the 5 subclasses of flavonoids of interest (flavonol, flavones, flavanones, flavans and anthocyanidins). We observed that most of the available or existing data came from studies conducted in countries other than the United States. For many foods, there were only single values available and analysts frequently concentrated on 1 or 2 particular subclasses because a suitable analytical method was not available for separating and quantifying compounds from all 5 subclasses simultaneously. The full database will be released this fall on the Nutrient Data Laboratory’s Web site (1).


Perillic Acid, a Metabolite of Perillyl Alcohol, Inhibits Proliferation of Cancer Cells In Vitro. J. A. Elebede, M. Bakh- tary, D. Kinnear, G. Hilton and R. Wang,* University of Nevada, Las Vegas, NV and *University of Nevada School of Medicine, Las Vegas, NV.

Perillyl alcohol is a simple monoterpene found in a variety of plant oils. Earlier studies reported that perillyl alcohol exhibited anticarcinogenic activity in a variety of tumor models. Perillyl alcohol is currently being studied in human clinical trials. Although the mechanism of its antitumor activity is not clearly understood, it has been shown to exert a number of effects at the cellular level both in vivo and in vitro. Pharmacokinetic studies of perillyl alcohol administered to patients in human clinical trials showed that it is rapidly metabolized to perillic acid, its major circulating plasma metabolite. Based on the concentration of the circulating plasma metabolite, we hypothesized that the anticarcinogenic effects of perillyl alcohol may involve its primary metabolite. We studied the effects of perillic acid and perillyl alcohol on two head and neck squamous cell carcinoma (BroTo and HTB-43) and lung adenocarcinoma (A549) cell lines cultured in vitro. The cell lines were exposed to various concentrations of perillic acid or perillyl alcohol for up to 48 h and assayed for cell viability using the mitochondrial dehydrogenase activity assay and flow cytometry. Both perillic acid and perillyl alcohol inhibited proliferation of the cancer cells in a dose- and time-dependent manner although with different sensitivity. Flow cytometry analyses showed that the inhibition of proliferation correlated with arrest in the G2/M or G1 phase of the cell cycle and increased hypodiploidy, an indication that the cells might be dying by apoptosis. When perillic acid or perillyl alcohol were applied in combination treatment with cisplatin, preliminary data indicated that perillic acid elicited a synergistic effect. The mechanism of action of perillyl alcohol may involve its major metabolite, and perillic acid has potential for use in adjuvant therapy of human head and neck squamous carcinoma or lung...
Adenocarcinoma. [Supported by grants from 2001–02 UNLV Planning Initiative Award.]


Cigarette smoking predisposes individuals to lung and other cancers. The tar in smoke contains ~10^{17} electron spin resonance–detectable radicals per gram and many are stable for hours. This exposes smokers to increased oxidative stress and is reflected in patients having increased levels of 8-hydroxyguanosine present in the DNA of white blood cells and elevated levels of isoprostanes in their plasma and urine. Isoprostanes are biomarkers for increased lipoperoxidation. In an effort to reduce oxidative stress in smokers, we investigated the effect of dietary intervention with aged garlic extract on the plasma and urinary concentrations of 8-isoprostaglandin F_2alpha (8-iso-PGF_2alpha). A small trial was devised using age- and sex-matched individuals (n = 20). Both smokers and nonsmokers had plasma and urinary baseline 8-isoprostaglandin F_2alpha (PGF_2alpha) concentrations determined by immunoassay. The concentrations of isoprostanes in smokers were 58 and 85% greater in plasma and urine, respectively. The volunteers then received a 5-mL daily dose of aged garlic for 14 d. After this time, the isoprostane levels had decreased in both groups, with smokers showing a more marked decrease. The plasma and urinary concentrations in the smokers decreased by 35 and 48%, respectively. Fourteen days after cessation of dietary supplementation, the plasma and urinary concentrations of 8-iso-PGF_2alpha returned to levels that were not significantly different from baseline levels. This indicates that aged garlic may reduce oxidative stress in humans and particularly in smokers and may be beneficial in protecting against some of the detrimental effects of smoking.


Epidemiologic and experimental carcinogenesis studies provide evidence that components of garlic (Allium sativum) have anticancer activity. We recently reported that the water-soluble garlic derivative S-allylmercaptocteine (SAMC) inhibits growth, arrests cells in G_2/M and induces apoptosis in SW480 and HT29 human colon cancer cells.1. Because a fraction of the SAMC-treated cells are specifically arrested in mitosis, in the present study, we examined the mechanism of this effect. Indirect immunofluorescent staining for microtubules using an antibody to tyrosinated alpha-tubulin revealed that treatment of SW480 cells or NIH3T3 fibroblasts with SAMC at 150 micromol/L (the 50% inhibitory concentration) caused microtubule depolymerization and microtubule cytoskeleton disruption in interphase cells and interfered with the spindle assembly in mitotic cells. In vitro turbidity assays indicated that SAMC acted directly on tubulin to cause microtubule depolymerization when we used either pure tubulin or tubulin rich in microtubule-associated protein. These effects were similar to that caused by the tubulin-binding agent colchicine, but studies with fluorescent colchicine indicated that SAMC binds to a different site on tubulin than the colchicine-binding site. To investigate the signaling pathways involved in SAMC-induced apoptosis, we assayed Jun kinase (JNK) activity and found that treatment with SAMC caused a rapid and sustained induction of JNK1 activity from 15 min up to 12 h. The selective JNK inhibitor SP600125 blocked the apoptosis but not the G_2/M arrest induced by SAMC. SAMC also activated caspase-3, as evidenced by the cleavage of a fluorogenic tetrapeptide substrate and of poly(ADP-ribose) polymerase. We conclude that garlic-derived SAMC exerts antiproliferative effects, at least in part, by disrupting microtubule assembly, thus arresting cells in mitosis and triggering JNK1 and caspase-3 signaling pathways that lead to apoptosis.


We observed previously the induction of histone acetylation and inhibition of growth when DS19 mouse erythroleukemia cells were incubated with allicin and S-allylmercaptocteine (SAMC) in the low micromolar range. In this work, we sought to extend our understanding of the action of these compounds and garlic extracts on histone acetylation. The induction of histone acetylation by SAMC was observed in Caco-2 human colon cancer cells and T47D human breast cancer cells. In contrast to the effect on histone acetylation, the incorporation of [32P]-phosphate into histones decreased when DS19 cells were incubated with SAMC at 25 micromol/L. A similar degree of downregulation of both histone deacetylase (HDAC) and histone acetyltransferase (HAT) activities was observed when DS19 cells were incubated with either SAMC or allyl isothiocyanate. The induction of histone acetylation by SAMC was not blocked by a proteasome inhibitor, MG132. This observation suggests that the mechanism of induction of histone acetylation by SAMC differs from that reported for quinidine, which induces proteasomal degradation of HDAC. The proliferation of DS19 mouse erythroleukemia was inhibited by garlic extracts containing thiosulfinate concentration in the low micromolar range. Allyl sulfur compounds have been reported to react with cysteine and N-acetyl cysteine, but coinubcation of garlic extract with these compounds did not greatly affect the inhibitory action on cell growth. Induction of histone acetylation was demonstrated with garlic extracts at thiosulfinate concentrations that were similar to those associated with the inhibition of growth. There was a twofold increase in HAT activity in isolated nuclei by garlic extracts under conditions in which there was no change in HDAC activity by the garlic preparation. Although histone acetylation was induced by allicin, this compound did not increase HAT activity in isolated nuclei. We conclude, therefore, that histone acetylation and growth inhibition can be induced in cancer cells by allyl sulfur compounds that are derived from garlic and by crude garlic extracts, but different mechanisms may operate for different garlic constituents.
Anticancer Activity in Fractions of Cranberry Presscake Extract. P. J. Ferguson,* E. Kurowska,** N. Guthrie,** A. Chambers,† D. Freeman* and J. Koropatnick,*† *London Regional Cancer Center, London, ON, Canada; **University of Western Ontario, London, ON, Canada; and **Synergize, London, ON, Canada.

Our group previously showed that cranberry presscake (the material remaining after removing the juice), when fed to tumor-bearing mice as 5 g/100 g (wet weight) of their diet, delayed the growth of MDA-MB-435 breast tumors at primary injection sites and inhibited the establishment of distant metastatic tumors. We undertook to determine the active component of the presscake by fractionating a warm-water extract by C18 column chromatography based on hydrophobicity (Ocean Spray Cranberries, Lakeville-Middleboro, MA). The fractions obtained (with the percentage of the total presscake calculated as dry weight/weight) consisted of an initial water filtrate (5.5%), a water wash (0.50%), a 15% acidified methanol fraction (0.278%), a methanol eluant (0.521%) and several waxy components from various alcohol washes (total 0.1%). Significant in vitro antiproliferative activity was obtained from the acidified methanol fraction, which contains an assortment of organic acids, flavonoids and proanthocyanidins. Greater than 50% inhibition of proliferation was observed at 250 μg/mL of this fraction against human cell lines representing estrogen receptor–positive (MCF-7) and –negative (MDA-MB-435) breast carcinoma, androgen receptor–positive (LNCaP) and –negative (DU145) prostate carcinoma, colon carcinoma (HT-29), melanoma (SK-MEL-5), glioma (U87) and lung carcinoma (DMS114). This methanol fraction is currently being further fractionated and analyzed to identify the main contributors to the antiproliferative activity in vitro and in vivo. [Supported by the American Institute for Cancer Research.]

Estrogenic Activity in White and Red Wine Extracts. K. E. Risinger, V. Beck,* A. Jungbauer* and C. M. Klinge, Department of Biochemistry and Molecular Biology, University of Louisville School of Medicine, Louisville, KY and *Institute of Applied Microbiology, University of Agricultural Sciences, Vienna, Austria.

Epidemiologic evidence indicates that the consumption of diets rich in plant materials is associated with reduced cancer risk. Red-skinned grapes are enriched in resveratrol, trans-3,5,4-trihydroxystilbene, a compound that inhibits the development of preneoplastic lesions in mouse mammary tumor cells in culture and inhibits cancer cell proliferation in vitro. Grapes also contain other bioactive compounds including flavonoids, flavans and anthocyanins. We examined the estrogenic activity of extracts prepared from one white (Freie Weingärtnern Wachau, Grüner Veltliner, Austria) and two red (Woodbridge, Cabernet Sauvignon, California; and Lenz Moser Prestige, Blaunfränkisch Barrique, Austria) wines and compared these activities with that induced by estradiol and trans-resveratrol. First, the estrogenic activity of the concentrated solid-phase extraction (SPE)–bound and flow-through wine extracts were evaluated in the yeast estrogen screen assay in which yeast expresses copper-inducible estrogen receptor α (ERα) and a β-galactosidase reporter driven by estrogen response element. The white wine extract showed no estrogenic activity. In contrast, both red wine extracts showed estrogenic activity equivalent to that of estradiol at 0.2 nmol/L. Similarly, the white wine extract showed no transcriptional activity with either ERα or ERβ in transiently transfected CHO-K1 cells. Both red wine extracts stimulated activity of an estrogen response element reporter in a concentration-dependent manner that was inhibited by 4-hydroxytamoxifen, indicating that the observed transcriptional activity was ER mediated. The red wine extracts showed significantly higher ERβ than ERα agonist activity. Resveratrol showed no agonist activity in the yeast estrogen assay but activated ERα and ERβ in CHO-K1 cells in a concentration-dependent manner that was inhibited by 4-hydroxytamoxifen. This indicates that resveratrol requires mammalian cell components that are absent in yeast for estrogen agonist activity, whereas the estrogenic activity of wine extracts is mediated directly through ERα and does not require mammalian cell factors as activators. [Supported in part by NIH R01 DK53220 and AHA 0150818B to C.M.K.]

Grape Extracts Suppressed the Formation of Preneoplastic Foci and Activity of Fatty Acid Synthase in Rat Hepatocarcinogenesis. S. Kweon, Y. Kim, S. A. Kim and H. Choi, Department of Food and Nutrition, Seoul National University, Seoul, Republic of Korea.

Oxidative stress has been implicated in many chronic diseases including reperfusion injury, tumor promotion and carcinogenesis. Grapes contain many potent antioxidants such as resveratrol and quercetin. Several researchers have reported cancer preventive effects of red wine or grape polyphenols, but there have been few reports on liver cancer. This study was conducted to examine the effects of dietary grape extracts on preneoplastic foci formation in rat hepatocarcinogenesis and related hepatic enzymes. Male Sprague-Dawley rats were fed basal diet or grape diets containing 15% concentrated grape extracts (680 g/L). The grape diet groups were divided into GT (grape diet fed throughout experimental period) and GPT (grape diet fed from promotional stage) groups. Hepatocarcinogenesis was induced by diethylnitrosamine (200 mg/kg body) and two thirds partial hepatectomy in the basal diet group (CT) and the GT and GPT groups; the control (CC) group was treated with saline and a sham operation. The formation of placental glutathione S-transferase positive foci was significantly suppressed in the GT group but not in GPT group. The content of thiobarbituric acid reactive substances in the GT group was significantly lower than that of CT group. The activities of fatty acid synthase and glutathione (GSH) reductase were decreased by the grape diets. GSH and GSH peroxidase activities were increased by carcinogen treatment but not modulated by the grape diets. The activities of GSH S-transferase and catalase were not different among the groups. The inhibitory effect of the grape diet in preneoplastic foci formation may be affected by the different stages of carcinogenesis. A mechanism of hepatic cancer prevention by a grape diet might be via the inhibition of fatty acid synthase activity, which is highly expressed in tumor tissue.


Investigators have shown that green tea may decrease the risk of cancer. It is widely accepted that the main active component of green tea is epigallocatechin-3-gallate. In our previous study, we examined the effect of green tea on breast cancer growth and endothelial cells in both in vitro assays and animal models. Our
data show that mixed green tea extract as well as its individual catechin components is effective in inhibiting breast cancer and endothelial cell proliferation in vitro and that green tea extract suppresses breast cancer xenograft size and decreases the tumor blood vessel density in vivo. In the present study, we further demonstrate that green tea extract or epigallocatechin-3-gallate at 40 μg/mL can decrease the levels of the angiogenic basic fibroblast growth factor in the cells. This phenomenon is observed in both human umbilical vein endothelial cells (HUVEC) and human breast cancer cells MDA-MB231. This effect is dose dependent. Furthermore, green tea extract and epigallocatechin-3-gallate decrease the transcription levels of basic and acidic fibroblast growth factors in HUVEC and MDA-MB231 cells. Our findings suggest that the inhibition of the angiogenic fibroblast growth factors could account for one of the mechanisms of green tea’s actions. Because cancer is angiogenesis dependent, this may explain in part the antineoplastic effects associated with green tea consumption.

Changes in the Fecal Flora Composition of Human Volunteers in a Double-Blind Randomized Black Tea Feeding Study. V. Mai, H. Katki, B. Clevidence,* S. Hursting,† H. Harmsen** and A. Schatzkin. Division of Cancer Epidemiology and Genetics and †Division of Cancer Prevention, National Cancer Institute, Bethesda, MD; ‡Beltsville Human Nutrition Research Center, U.S. Department of Agriculture, Beltsville, MD; and **University of Groningen, Groningen, The Netherlands.

The importance of the microbial intestinal flora in human cancer and other disease has long been postulated. The effects of some dietary substances, such as prebiotics, on the composition of the flora have been established. The effects of diet on health may be mediated in part by changes in the composition of the intestinal flora. Black tea has been suggested to affect cancer risk, although evidence for this association is equivocal. Polyphenols in black tea could alter the bacterial flora, leading to an increased excretion of carcinogenic fecal bile acids. We analyzed changes in the fecal flora composition and in the fecal bile acid profile in 15 subjects in a black tea feeding study. Fecal samples were collected on d 1, 13 and 20 of the two periods (crossover design) for a total of six samples from each volunteer. We performed fluorescent in situ hybridization (FISH) and temporal gradient gel electrophoresis (TGGE) to analyze the bacterial flora and determine changes in the bile acid profile enzymatically and by HPLC. Large inter- and intrasubject variability prevented any small effects of diet or black tea on the flora composition. However, we did observe a decrease in the amounts of “other bacteria” detected only by the universal bacterial probe in the FISH analysis. TGGE analyses showed a distinct bacterial profile for each subject that was relatively stable over time. These results indicate that tea drinking affects some flora components, but more sensitive tools and larger studies are required to evaluate effects of diet on the intestinal flora.

Inhibition by Garlic Allyl Sulfides and Phenethylisothiocyanate of Methyl-n-Pentylnitrosamine Depentylation by P450 and Microsomes and of Liver DNA Alkylation by Dimethylnitrosamine in Rats. L. Zhou, C. R. Morris, S. C. Chen and S. S. Mirvish. Eppler Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, NE.

Garlic and cruciferae are associated with reduced risks of some human cancers, and several garlic allyl sulfides and phenethylisothiocyanate (PEITC) are anticarcinogenic in animal tests. Here we studied effects of garlic oil components allyl sulfide (DAS) and diallyl sulfide (DADS) and the crucifer constituent PEITC on the metabolism of the rat esophageal carcinogen methyl-n-pentylnitrosamine (MPN) and the rat liver carcinogen dimethylnitrosamine (DMN). We reported that MPN depentylation (its activation step) was inhibited by DAS (1). The method involved incubation of various [3H]MPN and DAS concentrations with cytochrome P450 (CYP) or microsomes and determination of resultant [3H]pentanaldehyde by HPLC of its 2,4-dinitrophenylhydrazone. Inhibitory rate constants (K) were 0.2–2.0 μmol/L for rat and human CYP2E1 and rat CYP2B1. Using similar techniques, we now report that DAS inhibition of MPN depentylation showed a K of 0.6 μmol/L for both rat CYP2A3 and rat esophageal microsomes; DADS showed a K of 7.60–80, 1.4–2.0 and 0.5–1.7 μmol/L for rat CYP2E1, human CYP2E1, rat CYP2A3 and rat esophageal microsomes, respectively; and PEITC showed a K of 4, 3–6 and 0.1–0.6 μmol/L for rat CYP2E1, CYP2A3 and esophageal microsomes, respectively. DAS and DADS (but not PEITC) were preincubated with P450s and microsomes for 15 min before the addition of MPN because this increased inhibition for rat CYP2E1 by 40–70%. We studied allyl sulfide inhibition of DNA guanine methylation by DMN, following similar studies with garlic powder by Milner et al. (2). We introduced allyl sulfides in 5 mL corn oil/kg or corn oil controls by gavage into male 9- to 12- wk-old Sprague-Dawley rats, wasted 2–18 h, injected DMN (20 mg in 5 mL saline/kg) and intraperitoneally, killed the rats 3 h later, homogenized and lysed ~350 mg liver, purified its DNA on Qiagen Genomic tips and hydrolyzed DNA by standard methods. O6-Methylguanine (O6-MG) and guanine were determined by HPLC on a SCX cation exchange column with fluorescence detection. Corn oil controls showed ratios of O6-MG-to guanine of 0.066 ± 0.005% (A ± B = mean ± SEM). Inhibition of O6-MG formation (expressed as ratios of O6-MG to guanine, mainly 6 rats/group) was 39 ± 5% for 200 mg DAS/kg given 2 h before DMN; 88 ± 2% down to 15 ± 7% for 200 down to 6 mg DAS/kg given 18 h before DMN; and 53 ± 15%, 41 ± 3% and 36 ± 4% for 125, 75 and 50 mg DAS/kg, respectively, given 18 h before DMN. The effective inhibition for DAS given 18 h before DMN and metabolic studies by Yang et al. (3) suggest that DAS is metabolized to diallyl sulfone, which persists and irreversibly destroys liver CYP2E1 that activates DMN. In support of this view, diallyl sulfoxide (90 mg/kg) and diallyl sulfone (100 mg/kg), given 18 h before DMN injection, inhibited O6-MG production in liver DNA from DMN by 72 ± 7% and 82 ± 10%, respectively, similar to the effect of DAS. Feeding 5% crushed garlic in a commercial diet for 3 d before DMN injection inhibited O6-MG production in liver DNA by 45 ± 5%. These results help explain the inhibition of nitrosamine carcinogenesis by vegetable constituents. [Supported by American Institute for Cancer Research grant 00A080 and National Institutes of Health core grant P30-CA-36727.]
**Fatty acids**

**Reduced Mammary Tumorigenesis in Rats Exposed Prepubertally to a Low-Fat (n-3) Polyunsaturated Fatty Acid Diet.**

S. Olivo, A. Cabanes, A. Foxworth, G. Khan, A. Zwart, R. Lee, R. Clarke and L. Hilakivi-Clarke. Department of Oncology, Lombardi Cancer Center, Georgetown University, Washington DC.

The timing of hormone exposure is essential in determining its effect on breast cancer risk. For example, estrogen is typically associated with increased breast cancer risk, but we have demonstrated in animals that an exposure to estrogens before puberty decreases breast cancer risk. Consequently, we attempted to alter prepubertal estrogen levels through dietary manipulation. Nursing Sprague-Dawley rats and their female offspring were fed low (16% energy) or high fat (39% energy) diets composed either of (n-3) or (n-6) polyunsaturated fatty acids (PUFA) in menhaden oil and corn oil, respectively, between postnatal d 5 and 25. Mammary glands and serum were collected from 6 rats per group at d 26 and 53 to evaluate mammary gland morphology, gene expression and circulating estradiol levels. At d 26, we observed that rats fed a low fat (n-3) PUFA diet had serum estradiol levels significantly higher than those of the rats fed the control low fat (n-6) PUFA diet (p < 0.01). In addition, the mammary glands of the former group contained a significantly higher number of differentiated structures called lobules (P < 0.0002). Mammary tumors were induced with dimethylbenz[a]anthracene at d 53. Final tumor incidence in rats fed a low fat (n-3) PUFA diet was 52%, whereas tumor incidence in the other 3 groups was < 80%.

Finally, gene microarray experiments demonstrated that the gene expression pattern in the mammary glands of rats fed a low fat (n-3) PUFA diet during prepuberty was clearly different from that seen in the other 3 groups. Currently, genes that are differentially expressed in the mammary glands are being determined. Taken together, the data suggest that prepubertal exposure to a low fat (n-3) PUFA diet alters the mammary gland morphology in a manner that reduces breast cancer risk. This lower risk is likely to be due to altered expression of genes involved in breast cancer susceptibility.

**Different Effects of Peroxidized (n-3) and (n-6) Fatty Acids on Cultured Colon Cells.**

N. Udilova, D. Jurek,* K. Stolze, R. Schulte-Hermann,* H. Nohl and B. Marian.* Institute of Pharmacology and Toxicology, Veterinary University of Vienna, Vienna, Austria and *Institute of Cancer Research, University of Vienna, Vienna, Austria.

A fat-rich diet is a major risk factor in colon carcinogenesis, although the molecular mechanisms are not fully understood. The composition of fat, especially the ratio of (n-3) to (n-6) fatty acids, seems to modulate the risk of colon cancer. Dietary polyunsaturated fatty acids (PUFA) may be readily oxidized to the respective hydroperoxides when heated or exposed to light and oxygen in air. In the presence of transition metals, lipid hydroperoxides yield lipid-derived radicals than can be detected by spin-trapping electron spin resonance. These radicals initiate lipid peroxidation in the cell membrane, resulting in toxic effects. We compared the effect of lipid hydroperoxides from (n-3) and (n-6) fatty acids (linoleic and linolenic acid, respectively) on proliferation of cultured colon carcinoma cells (SW480), colon adenoma cells (LT97) and intestinal epithelial cells (IEC18). Both peroxidized linoleic and linolenic acids were found to be toxic in all cultured cells under inves-

tigation. However, peroxidized linolenic acid was most toxic to SW480 cells followed by IEC18 and LT97, whereas peroxi-
dized linoleic acid was most toxic to IEC18 cells. Equimolar concentrations of nonoxidized fatty acids had no significant effects on cell proliferation. Cytotoxic effects of peroxidized fatty acids were accompanied by formation of reactive oxygen species as evidenced by the increased 2,7-dichlorofluorescin fluorescence and protection of cells by radical quenchers such as α-tocopherol. Thus, according to our data, peroxidized linoleic acid is most toxic to normal intestinal epithelial cells, whereas peroxidized linolenic acid is most toxic to carcinoma cells. These findings may partially explain protection by dietary (n-3) fatty acids against the development of sporadic colon cancer.

**Fatty acids**

**Fish Oil Suppressed the Preneoplastic Foci Formation and Modulated Antioxidative System in Rat Hepatocarcinogenesis.**


Although (n-3) fatty acids have been reported to be anticarci-

nogenic agents in many epidemiologic and animal studies, the reports on liver cancer have been few and controversial. This study was conducted to examine the effects of dietary fish oil, a rich source of (n-3) fatty acids, on preneoplastic foci formation in rat hepatocarcinogenesis. Male Sprague-Dawley rats were fed corn oil or fish oil diets at the level of 15%; in the fish oil diet, the ratio of (n-3) to (n-6) fatty acids was adjusted
to 1:1. Hepatocarcinogenesis was induced by diethylnitrosamine (DEN, 200 mg/kg body) and two thirds partial hepatectomy, whereas the control groups were treated with saline and a sham operation (2 treatment groups and 2 control groups). The formation of placental glutathione S-transferase-positive foci in the fish oil diet + DEN treatment group was significantly suppressed (by ~60%) compared with the corn oil diet + DEN-treatment group. The contents of thiobarbituric acid-reactive substances were decreased in the fish oil groups compared with the corn oil groups. Total glutathione content was increased by the fish oil diet. The glutathione reductase activities in the DEN-treatment groups were higher than those in the control groups. The activity of glutathione peroxidase was decreased in fish oil diet + DEN treatment group compared with fish oil diet + saline treatment group, but there was no difference between the corn oil groups. The activities of glutathione S-transferase, catalase and superoxide dismutase were not different among groups. The fish oil diet suppressed the formation of preneoplastic foci in rat liver, which could be related in part to the enhancement of host antioxidative defense system.

**Influence of Fatty Acids, Vitamin E and Selenium in the Diet on the Formation of 4-Hydroxy-2-Nonenal-DNA Adducts as Specific Markers for Carcinogenesis by Lipid Peroxidation.**

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Together with malondialdehyde, 4-hydroxy-2-nonenal (HNE) is the main product of lipid peroxidation. It forms promutagenic 1,2-propanoxygenoquinones adducts that are not repaired by base excision repair and only ineffectively by nucleotide excision repair. These adducts are highly specific for the genotoxic and carcinogenic effects of HNE because they still contain the entire chemical structural moiety of HNE. Oxidative stress and lipid peroxidation are considered to be important factors in carcinogenesis influenced by nutrition. In particular, (n-6) polyunsaturated fatty acids (PUFA) in the lipid membrane are susceptible to lipid peroxidation, whereas the antioxidative activities of vitamin E and selenium proteins (e.g., glutathione peroxidase) can suppress lipid peroxidation effectively. We developed a $^{32}$P-postlabeling method for sensitive measurement of these HNE-adducts in human and mammalian tissues and found that background adduct levels in nearly all human and rat tissues tested in the range of 5–600 adducts/10$^6$ nucleotides, depending on the type of tissue. Induction of lipid peroxidation by carbon tetrachloride clearly increased the adduct levels. Adduct levels were significantly increased in livers of F344 rats fed a selenium-deficient diet. Adduct levels were also higher in animals given different vegetable oils orally (i.e., 2 mL of coconut oil, olive oil, rapeseed oil or sunflower oil for 10 wk). The highest adduct levels were found after sunflower oil. In another study, we investigated the influence of a diet based on the different vegetable oils that was given for 4 wk. A clear dependence of the adduct levels on the content of (n-6) PUFA was found in the small intestine and liver but not in the colon mucosa, glandular stomach, kidney and lung. This means that the HNE-adduct levels in the organs in which the fatty acids are first absorbed (i.e., small intestine and liver) depend on the content of linoleic acid in the diet.

**Phytic acid**


Anticarcinogenic properties of phytic acid [inositol hexaphosphate (IP$_6$)], a natural dietary constituent present in grains, is gaining considerable importance. The objective of this study was to test the effect of IP$_6$ and inositol, both singly and in combination, at levels of 1 and 2% on azoxymethane-induced colonic aberrant crypt foci (ACF), which are preneoplastic lesions in Fisher 344 male rats. Six groups (n = 15/group) of Fisher 344 male weaning rats were assigned to AIN 93G (control; C), C + 1 or 2% IP$_6$, C + 1 or 2% inositol and C + 1% IP$_6$ + 1% inositol in water. All rats were fed 16 mg/kg body of azoxymethane dissolved in saline subcutaneously at age 7 wk followed by a second injection at age 8 wk. The animals were killed at age 17 wk. Inositol when fed in combination with IP$_6$ protected against the decrease in weight gain observed in the group fed 2% IP$_6$ alone. Results showed a significant (P < 0.05) reduction of colonic ACF. The group fed 1% IP$_6$ + 1% inositol showed a 42.7% reduction in total ACF compared with reductions of 32.5 and 42.1% for 1 and 2% IP$_6$ and 18.6 and 32.3% for 1 and 2% inositol, respectively. Although inositol acted by itself in reducing colonic ACF, it appeared to enhance the action of IP$_6$ and reduce the amount of IP$_6$ required by 50%. The results of this study indicate that inositol given in combination with IP$_6$ suppressed azoxymethane-induced ACF formation, an early preneoplastic marker in the process of colon carcinogenesis in Fisher 344 male rats.

**Inhibition of Growth and Induction of Differentiation by Inositol Hexaphosphate.**

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Inositol hexaphosphate (IP$_6$), also known as phytic acid, has shown effective anticancer activity in many different models. Although high concentrations of IP$_6$ induce death of malignant cells but not normal cells in a short time, lower doses of IP$_6$ over longer times are cytostatic and enhance differentiation and maturation of malignant cells, as evidenced by the appearance of differentiation markers, i.e., lactalbumin in breast, alkaline phosphatase in colon, acid phosphatase in prostate, actin in rhabdomyosarcoma and hemoglobin in erythroleukemia cell lines. However, the molecular mechanisms of the action of IP$_6$ are largely unknown. Flow cytometry measurements of DNA content and $[^3H]$thymidine incorporation assay showed G$_0$/G$_1$ arrest of breast and colon cancer cell lines and S phase inhibition by IP$_6$. Because IP$_6$ modulates inositol phosphates and phospholipids in cellular membranes and because cell signaling and regulators of cell cycle were recently recognized as molecular targets for cancer prevention and treatment, we investigated whether IP$_6$ can affect protein kinase C (PKC) and ras-Erk1/2 signaling, leading to alterations of the cell cycle. Using MCF-7 human breast cancer cells, we previously showed that IP$_6$ specifically increases the expression of PKCδ without affecting other major PKC isoforms and causes a transient decrease in Erk1/2 phosphorylation. We evaluated the regulators of G$_1$-S transition in a search for possible downstream targets of PKCδ and Erk1/2 potentially responsible for antiproliferative and differentiation
effects of IP₆. We found that IP₆ caused an increase in p27kip₁ protein levels, leading to a marked reduction of the retinoblastoma protein phosphorylation. We used the specific pharmacologic inhibitors of PKC (rollerin) and of the MEK/Erk pathway (U0126) to dissect the role of PKCδ and Erk1/2 in this IP₆-mediated upregulation of p27kip₁. The results show that PKCδ mediates IP₆-induced upregulation of p27kip₁. Our data suggest the potential usefulness of IP₆ as a novel therapeutic modulator of PKCδ and, consequently, p27kip₁, an important prognostic factor in human cancers.

Protein


This study describes the effect of a human milk protein on the activation of peripheral blood lymphocytes. A folding variant of α-lactalbumin from human milk has been isolated and characterized. The active complex precipitated with the casein fraction at pH 4.6 and was purified from casein by combination of anion exchange and gel chromatography. The fraction showed N-terminal and mass spectrometry identity with human milk α-lactalbumin, but monomeric, native α-lactalbumin had no effect on blood lymphocytes. The active fraction was previously shown to induce apoptotic cell death in immature undifferentiated cells but spared normal cells. This human milk fraction was identified as multimeric α-lactalbumin (MAL). Our continued studies with MAL have focused on the hypothesis that milk components may have a modulating effect on the development of the immune system in breast-fed infants very early in life. We compared the effect of short-term exposure to MAL, monomeric α-lactalbumin and human milk casein on mononuclear cell activation by flow cytometry using CD69 expression as the measure of early activation. In contrast to monomeric α-lactalbumin and casein, MAL proved to have a strong activating effect on lymphocytes leading to a fivefold increase in expression. Analysis of the lymphocyte activation response brings us one step closer to proving our hypothesis.

Reduced Hepatic MeCP2 Protein Level in Preneoplastic Methyl-Deficient Rats Is Associated with Reduced Sin3a and p53 Protein Levels. F. Esfandiari, R. F. Cotterman, R. Green and J. W. Miller. Department of Medical Pathology, University of California, Davis, School of Medicine, Davis, CA.

MeCP2 is a member of a family of proteins that bind specifically to methylated DNA (methyl-CpG-binding proteins) and induce chromatin remodeling and gene silencing. After binding to methylated DNA, MeCP2 recruits the corepressor protein, Sin3a, which in turn recruits histone deacetylase. Deacetylation of histones promotes chromatin compaction and transcriptional repression. Synthesis of S-adenosylmethionine, the methyl donor for DNA methylation, depends on adequate folate, choline and methionine. Dietary deficiency of these nutrients causes decreased tissue S-adenosylmethionine concentrations (methyl deficiency), global DNA hypomethylation and hepatic tumorogenesis in rodents. In this study we investigated the effect of methyl deficiency on preneoplastic hepatic expression of MeCP2 and Sin3a as well as the tumor suppressor gene, p53. Expression levels were determined by semiquantitative reverse transcription-polymerase chain reaction and Western blot analysis. After 9 wk of methyl deficiency, livers were enlarged, lipid-laden and fibrotic but showed no evidence of neoplastic foci. Hepatic mRNA levels for MeCP2, Sin3a and p53 (each normalized to GAPDH mRNA) were significantly higher in the deficient rats compared with replete controls (P ≤ 0.02). In contrast, protein levels for MeCP2, Sin3a and p53 (each normalized to β-actin protein) were significantly lower in the deficient rats compared with controls (P ≤ 0.03). Why these proteins are reduced in methyl deficiency is unclear but a possible explanation may be an increased rate of protein degradation. For the MeCP2-Sin3a repressor complex, methyl deficiency may reduce the number of methylated DNA binding sites, thus leaving unbound MeCP2 and Sin3a susceptible to proteolysis. Sin3a has been shown to bind and protect p53 from proteosome-mediated degradation. Therefore, the reduced p53 protein level in methyl deficiency may be the consequence of reduced Sin3a. The observed changes in MeCP2, Sin3a and p53 expression may influence the initiation and progression of cancer in methyl deficiency and may provide useful markers of preneoplastic change. [Supported by AICR grant 01A034-REV.]

Soy

Soy Isoflavones and Saponins Inhibit the Growth of Cultured Colon Adenocarcinoma Cells. R. S. Macdonald,† J. D. Browning, Jr.,* X. Li† and M. Berhow.** *Food Science and Human Nutrition, †Genetics Area Program University of Missouri, Columbia, MO and **National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture, Peoria, IL.

Populations that consume plant-based diets are generally found to have reduced colon cancer risk. We are investigating the components of soy that may influence colon cancer risk. Soy contains isoflavones, which have estrogenic activity, and saponins. Recent evidence suggests that estrogen plays a role in colon cancer risk; therefore, phytoestrogenic compounds may influence this relationship. The functional role of saponins has not been well described because of limited quantities of purified compounds. We obtained a purified fraction of soy group B saponins in both glycosylated and aglycone forms. Using the colon adenocarcinoma cell line CaCO-2 as a model, we examined the effects of the antiestrogen tamoxifen and various soy fractions on cell proliferation. Cells were cultured for 2–3 d in a complete medium containing the test compounds and proliferation was assessed by measurement of protein. We verified that cellular DNA and protein are highly correlated over the range of cell densities encountered. Tamoxifen at 1 mmol/L inhibited growth by 15%, suggesting that CaCO-2 cells are estrogen responsive. A commercial soy supplement (Novasoy, Archer Daniels Midland, Decatur, IL), which contains both isoflavones and saponins, inhibited growth by 20%. The isoflavones genistein and daidzein at 150 mmol/L inhibited growth by 40 and 20%, respectively. Glycosylated soy saponins had a minimal effect, whereas the aglycones at 100 g/L inhibited growth by 50%. These results suggest that CaCO-2 cells may be estrogen responsive and that soy isoflavones and saponins inhibit the growth of CaCO-2 cells. Hence, both soy isoflavones and saponins may be potential anticarcinogens. [Supported by the American Institute for Cancer Research and the MU Center for Phytonutrient and Phytochemical Studies.]
Baicalein and Genistein Act Differently on Estrogen Receptor Transactivation and Apoptosis in MCF-7 Cells. L. S. Po,* Z.-Y. Chen,† D.S.C. Tsang† and L. K. Leung.†*†Department of Biochemistry, Faculty of Medicine and †Food and Nutritional Sciences Program, Chinese University of Hong Kong, Hong Kong.

Genistein is a soy isoflavone that has attracted much attention for its chemopreventive properties. Baicalein is a flavone found in Scutellaria species, and its structure is analogous to that of genistein. We examined the antiestrogenicity of the two flavones using a transient transfection model. Our results indicated that baicalein could suppress 17β-estradiol–induced transactivation in cells expressing estrogen receptor-α, whereas no antagonistic activity was seen with genistein. Cell death assay and flow cytometry analysis demonstrated that baicalein was a stronger apoptosis-inducing agent than genistein. Considering all the criteria tested, baicalein could be a better chemopreventive agent than genistein.

Effect of Phytoestrogens on Normal Breast Tissue in Postmenopausal Breast Cancer Survivors. M. R. Palomares* and J. R. Gralow.*†Department of Medical Oncology, University of Washington and *Fred Hutchinson Cancer Research Center, Cancer Prevention Research Program, Seattle, WA.

Phytoestrogens have received media attention as a form of breast cancer prevention. Although epidemiologic studies support this claim, there are no prospective clinical trials demonstrating such a protective effect. This ongoing clinical trial, supported by an AICR Postdoctoral Award, aims to evaluate the effect of a phytoestrogen supplement on the breast tissue of postmenopausal breast cancer survivors. Disease-free posttherapy postmenopausal women (n = 60) with in situ or early invasive (Stage 0–II) breast cancer will be randomly assigned to receive either isoflavone tablets (100 mg/d) or placebo for 1 y. Biopsies of the uninvolved breast will be examined for proliferative changes in response to phytoestrogens as well as immunohistochemical breast cancer biomarkers. Mammographic density was assessed at the time of biopsy to correlate with histologic findings. As secondary end points, menopausal symptoms, vaginal epithelial changes, endometrial histology and serum steroid hormones are also being measured. We began to screen subjects in June 2001. Since then, 631 breast cancer patients have been screened through the Seattle Cancer Care Alliance, and we have received 53 additional self- or clinician referrals. A total of 467 were found to be ineligible, 52 refused participation and 15 have consented to participate so far. The primary reason for ineligibility at our institution is disease stage (76%). Of the women who stated their primary reason for refusal, the most common reasons have been invasiveness of the trial procedures (15%) and unwillingness to take phytoestrogen supplements (31%). Of the 10 women enrolled in the study, none have reported side effects attributable to the isoflavone tablets. One woman developed a small hematoma after one of her 3 biopsies and we received two complaints associated with the dressings used, but there have been no other biopsy-related complaints. To increase recruitment yield, a mechanism to see patients who receive their oncological care outside the sponsoring institution has been developed, and a community outreach and education campaign regarding phytoestrogens has begun. Mammographic density will be followed to see whether it can serve as a noninvasive end point for breast epithelial proliferation.

Effect of Dietary Intake of Estrogenic Isoflavones Genistein and Daidzein on a Carcinogen-Induced Mutation in Ovariectomized Transgenic Rats. A. Aidoo, M. E. Bishop, S. Shelton, L. Lyn-Cook, B. Delclos, T. Chen and M. G. Manjanatha. National Center for Toxicological Research, Food and Drug Administration, Jefferson AR.

Interest in the use of plant-derived estrogens such as soy isoflavones is increasing primarily for the reduction of menopausal symptoms. However, these compounds have not been evaluated extensively for their potential genotoxicity. In this study we examined the effects of soy isoflavones, genistein and daidzein, either singly or together on the mutagenicity induced by treating ovariectomized rats with 7,12-dimethylbenz[a]anthracene (DMBA), a potent mammary carcinogen. Groups of 50-d-old female Big Blue® (BB) rats, were treated by gavage with DMBA at 80 mg/kg, a carcinogenic dose suspended in sesame oil. The BB rats were ovariectomized 2 wk after DMBA treatment and were fed dietary genistein or daidzein at 0, 250 or 1000 μg/g and 17β-estradiol at 5 μg/g diet. Other animals were fed genistein, daidzein or 17β-estradiol alone. The experiments were terminated 16 or 20 wk post-DMBA treatment, and the rats were killed for tissue harvesting. Mutagenesis assays using the rat Hprt and lacI loci were conducted with lymphocytes isolated from the spleen. In the Hprt assay, there was no significant difference in the mutant frequency observed in the lymphocytes of rats fed either the isoflavones or estradiol alone compared with the vehicle. With the rats that were killed at 16 wk, the mutant frequency for DMBA alone was 108.45 ± 4.6 × 10⁻⁶. The addition of 250 μg/g genistein or daidzein did not appear to affect DMBA-mediated mutagenicity. However, 1000 μg/g of genistein and daidzein given either singly or together reduced DMBA response, with genistein displaying a significant reduction (P < 0.05). In contrast, in the rats killed at 20 wk, only the 1000 μg/g of daidzein showed a significant reduction in mutant frequency induced by DMBA exposure. Similar results in mutant frequency were observed in the lymphocyte lacI gene. These results indicate that the isoflavones used are antimutagenic at higher doses. Molecular analysis of the mutant clones from the high dose group now in progress should determine whether the agents alter the types of mutations induced by DMBA.


Insulin-like growth factors (IGF) are circulating hormones and paracrine signaling molecules. In epidemiologic studies, high IGF-I or low IGF binding protein (BP)-3 levels are associated with prostate, colon and other cancers. Isoflavone supplementation alters circulating IGF-I concentrations in animals. Our objective was to determine whether soy isoflavones modulate IGF in humans in a randomized, controlled, double-blind intervention. Polyp-positive participants (aged 50–80 y; hormone use excluded) were recruited from patients undergoing colonoscopy at two gastroenterology clinics and randomly assigned to consume 58 g soy beverage powder daily for 12 mo. Under the intervention condition, the powder contained 67 mg isoflavones, whereas the placebo contained negligible amounts. We hypothesized that isoflavone supplementation
would decrease serum IGF-I concentrations, increase IGFBP-3 concentrations and decrease the IGF-I/IGFBP-3 molar ratio. Blood samples were available from 149 of 150 fasting participants at baseline and 123 participants at 12 mo. Samples were assayed for IGF-I and IGFBP-3 using ELISA (Diagnostic Systems Laboratories, Webster, TX). Data were analyzed by linear regression. At baseline, the study population was 86% male (88% male at 12 mo), mean age was 64.8 y (sd 7.8), mean body mass index was 28.8 kg/m² (sd 4.6), mean serum IGF-I was 17.1 nmol/L (sd 4.9; 130.8 µg/L, sd 37.6) and mean IGFBP-3 was 80.6 nmol/L (sd 14.2; 2317 µg/L, sd 407). Differences in mean IGF at 12 mo were small: for the intervention group, the mean serum IGF-I concentration was 0.21 nmol/L (1.6 µg/L) higher [P = 0.74, 95% confidence interval (CI), −1.1, 1.5 nmol/L (−8.1, 11.3 µg/L)], mean difference in IGFBP-3 concentration −1.2 nmol/L (−35 µg/L) [P = 0.29, 95% CI, −3.5, +1.0 nmol/L (−101, 30 µg/L)] and mean IGF-I/IGFBP-3 molar ratio was 0.0051 higher (P = 0.46, 95% CI, −0.0085, 0.0188) than values in the placebo group. We found no evidence that a 12-mo, 67-mg/d isoflavone intervention modulates serum IGF-I, IGFBP-3 or the IGF-I/IGFBP-3 molar ratio in this population. [Supported by National Institute of Health grants U01 CA72035, R03 CA92772 and ES07262.]