

## Poster Abstracts

**The Second WCRF International Expert Report: Food, Nutrition, Physical Activity and the Prevention of Cancer: A Global Perspective. To be published in 2006. Steven Heggie,\* Elaine Stone,\* Lisa Miles,\* Ritva Butrum,† Rachel Thompson,\*\* Geoffrey Cannon,\* and Martin Wiseman.\*\*\***  
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In 1997, the World Cancer Research Fund (WCRF) and its affiliate in the United States, the American Institute for Cancer Research (AICR) jointly published the ground breaking report *Food, Nutrition and the Prevention of Cancer: a Global Perspective*. It remains the most authoritative report in the field of nutrition and cancer. Over 30,000 copies each of the report and of its summary have been produced, distributed, and sold worldwide. Since the publication of the 1997 report, further evidence has been published and there have been developments in techniques of synthesizing research evidence. The WCRF global network has decided to publish a new report and will be using a formal, standardized, and transparent process based on systematic reviews of the relevant literature. A crucial part of the process is the separation of the process of review of the literature from judgments based on the evidence. The process for producing the new report takes place in three overlapping stages. The first stage involves the development—guided by a panel of independent experts—of an appropriate method for systematic reviewing of the voluminous scientific literature in the light of increasing standards expected from such reports. The second stage is to outsource the literature reviews, based on the methodology developed in stage one, to scientific institutes in various countries. In the third stage a separate panel of experts will consider the evidence, formulate judgments, draw conclusions, and make recommendations. The project is expected to take 5 years, starting with stage 1 in 2001 and concluding with the launch and distribution of the report in 2006.

### Breast cancer

**Controlling for Intervention-Associated Response Set Bias in Dietary Change in Postmenopausal British Women with Previously Diagnosed Breast Cancer. J. M. Lawrence,\* B. Parry,†**

**R. M. Rainsbury,† and B. M. Margetts.\*\*** \*EIHMS, University of Surrey, †Women's Intervention Nutrition Study (UK), Royal Hampshire County Hospital, Winchester, and \*\*Institute of Human Nutrition, University of Southampton, UK.

The rationale for supporting an evaluation of dietary fat reduction in the secondary prevention of breast cancer has been reviewed (1) but concerns regarding measurement error have made interpretation of epidemiologic studies difficult (2). In an attempt to control for intervention-associated response set bias, we investigated the ability of postmenopausal women with breast cancer to follow either healthy eating or low-fat dietary advice for 2 y. Women completed 4-d food diaries at baseline, 1 y, and 2 y; these were analyzed using Dietplan 5 (Forestfield Software, UK). Data are presented with 95% confidence intervals and *F* values from a 2-way ANOVA with repeated measures factor (Table 1). Women from both groups significantly reduced their fat intake. The difference in reduction in fat intake between the two groups suggests that it is possible to control for the effects of the intervention and still show reduction in fat intake. [Supported by the World Cancer Research Fund.]

1. Chlebowski, R. T., Rose, D., Buzzard, I. M., Blackburn, G. L., Insull, W., Jr., Grosvenor, M., Elashoff, R. & Wynder, E. L. (1992) Adjuvant dietary fat intake reduction program in postmenopausal breast cancer patient management. The Women's Intervention Nutrition Study (WINS). *Breast Cancer Res. Treat.* 20: 73–84.

2. Prentice, R. L. (1996) Measurement error and results from analytic epidemiology: dietary fat and cancer. *J. Natl. Cancer Inst.* 88: 1738–1747.

**Nutritional Pattern of Women with Respect to the Estrogen and Progesterone Receptor Status of Their Breast Cancer. M. B. Krawinkel,\* U. Ringsdorf,\* A. Hebestreit,\* M. Mann,† and R. H. Boedecker.†** \*Institute of Nutritional Science and †Institute of Medical Documentation and Information, Justus-Liebig-University Giessen, Germany.

Breast cancer is the leading cause of death of women in industrialized countries. Studies indicate some evidence for an etiologic or pathogenetic role of a number of nutrients and food

TABLE 1

	Energy (kcal/d)		Energy from fat (%)		Fat (g/d)	
	Low fat	Healthy eating	Low fat	Healthy eating	Low fat	Healthy eating
Baseline, mean (95% CI)	2091 (1893–1290)	1884 (1691–2076)	39 (37–42)	34 (32–37)	92 (82–103)	72 (61–82)
1 y, mean (95% CI)	1476 (1306–1646)	1560 (1396–1725)	26 (23–30)	30 (26–33)	43 (35–51)	50 (42–58)
2 y, mean (95% CI)	1382 (1247–1517)	1562 (1431–1693)	26 (23–29)	29 (26–32)	40 (33–47)	51 (44–57)
<i>F</i> within subjects	(2,58) = 40.4, <i>p</i> < 0.001		(2,58) = 55.7, <i>p</i> < 0.001		(2,58) = 78.2, <i>p</i> < 0.001	
<i>F</i> between subjects	(1,29) = 1.97, <i>p</i> = 0.171		(1,29) = 2.3, <i>p</i> = 0.139		(1,29) = 4.0, <i>p</i> = 0.056	

energy. The highest significance has been found for an association between alcohol intake and the development of cancer of the mammary gland. Because estrogen receptor (ER) status has a far-reaching prognostic effect on the course of the disease, the study of the association between dietary aspects and hormone receptor presentation of the tumor deserved further investigation. We investigated nutrition before disease outbreak in 100 women with breast cancer, of whom 50 were ER positive and 50 were ER negative. Age at menarche, number of children, and cumulative duration of breast-feeding episodes were not significantly different between groups. Food intake data were processed in a data bank facilitating the analysis of the nutrient content (Nutrisurvey ©), which is based on the German Federal Food Database (Bundeslebensmittelschlüssel). Descriptive and analytical statistics were performed using SPSS ©. Results revealed an association between an eating pattern with intakes of carotenoid, fiber, and polyunsaturated fatty acids above the median and a higher chance of an ER-positive tumor (OR 4.0, 95% CI 1.736–10.27). If these results can be confirmed either in a study with a greater number of patients or in the prospective design of one of the large epidemiologic cohort studies (e.g., EPIC), they will further underscore the importance of a diet rich in fruits and vegetables for the prevention of cancer and for the prevention of the worse type of ER-negative breast cancer.

**Breast Cancer and Diet in the Kilimanjaro Region of Tanzania.** A. Hebestreit,\* B. Swai,<sup>†</sup> and M. Krawinkel.\* \*Institute of Nutritional Science, University of Giessen, Germany, and <sup>†</sup>Pathology Department, KCMC, Moshi, Tanzania.

Because breast cancer is one of the most prevalent malignant diseases in Tanzania, patients with this tumor were enrolled for a case-control study. The investigation of the association between the incidence of breast cancer and nutrition is facilitated by the monotonous diet of people in East Africa. Patients with primary mammary carcinoma who were not HIV infected were included. For each case ( $n = 40$ ), 2 individually matched controls were studied ( $n = 80$ ). Structured interviews were conducted about nutritional habits and lifestyle, such as use of contraceptives and reproductive habits. Nutrition was recorded by the food-frequency method and evaluated by using Nutrisurvey ©. Multivariate analyses of nutrients reveals a correlation between alcohol intake and breast cancer (odds ratio [OR] = 2.997;  $p = 0.0205$ ). A total fat intake of  $\geq 30\%$  of the daily energy intake had a weak inverse effect (adjusted OR = 0.618; n.s.), saturated fatty acids showed no effect (adjusted OR = 0.913; n.s.). A high intake of soluble dietary fiber showed a weak protective effect (adjusted OR = 0.553; n.s.). Carotene intake showed no effect on the cancer risk (OR = 1.101; n.s.). There was a higher risk associated with an early menarche (OR = 1.512) and a lower risk associated with a first pregnancy at younger age (OR = 0.460). The results of this case-control study in Tanzania further indicate alcohol consumption as a risk factor for breast cancer. The higher fat intake of the controls may be less detrimental because of the type of fat (sunflower oil, maize germ oil). The higher fruit, vegetable, and legume intake of the control group led to a higher intake of fiber, vitamins, and minerals. Further research is needed to clearly identify associations between breast cancer incidence and nutrition. An analysis of the fat quality of the food should be included.

**Epigenetic Basis of Vitamin A Resistance in Breast Cancer. Implications for Prevention and Treatment.** Silvia Pozzi,\*

Mingqiang Ren,\* Giulia Somenzi,\* Gaia Bistulfi,\* Riccardo Ghidoni,<sup>†</sup> Silvia M. Sirchia,<sup>†</sup> and Nicoletta Sacchi.\* \*Roswell Park Cancer Institute, Buffalo, NY, USA, and <sup>†</sup>San Paolo University Hospital, School of Medicine, University of Milan, Italy.

Understanding the relationship between genes and diet may improve cancer prevention and treatment. Genes and genomes seem able to adapt to dietary cues by epigenetic changes via postsynthetic modifications of either the DNA itself or the histone proteins that are intimately associated with DNA. We are beginning to understand the relationship among diet, individual gene differences (polymorphisms), and epigenetic modulation of genes and genomes and the direct influence of diet on the epigenetic status of genes and genomes. Vitamin A and its natural bioactive derivative retinoic acid (RA) have received a lot of attention for their potential anticancer effects on epithelial cells. However, *in vitro* studies and clinical trials using RA as a differentiation agent have shown that cancer cells are refractory to RA. RA action is normally mediated by retinoid receptors (RARs and RXR). RARs are transcriptionally active in the presence of both RA and activator-coactivator complexes with histone acetyltransferase activity. In contrast, they are repressed in the absence of RA by corepressor complexes with histone deacetylase activity. We found that RAR-beta is methylated in breast cancer cell lines and tumors (1) and that breast cancer patients with RAR-beta-methylated tumors do not respond to RA differentiation therapy (2). Recently, we found that RAR-beta methylation is present in normal epithelial cells adjacent to tumor cells in breast tissue, suggesting that epigenetic modifications leading to epigenetic RA resistance are likely an early event in the process of breast tumorigenesis. We hypothesized that factors leading to RAR-beta inactivity in human breast epithelial cells may predispose them to development of epigenetic RA resistance. When we forced RAR-beta into transcriptional inactivity, we observed the appearance of epigenetic modifications at RAR-beta (DNA methylation, histone modifications) leading to RA resistance in breast epithelial cells. Our studies suggest that we may prevent the development of RA resistance in breast epithelial cells by preventing transcriptional inactivation of vitamin A signaling. Moreover, our studies show that we can reverse epigenetic changes leading to RA resistance and restore RA sensitivity in breast cancer cells. These studies may have a general validity for prevention and treatment of breast cancer as well as other epithelial cancers where we also observed the molecular signatures of epigenetic RA resistance. [Supported by U.S. Army IDEA Awards DAMD17-99-1-9241, DAMD17-02-1-0432, and AIRC Award 2001 to NS.]

1. Sirchia, S. M., Ferguson, A. T., Sironi, E., Subramanyan, S., Orlandi, R., Sukumar, S. & Sacchi, N. (2000) Evidence of epigenetic changes affecting the chromatin state of the retinoic acid receptor beta 2 promoter in breast cancer cells. *Oncogene* 19: 1556–1563.

2. Sirchia, S. M., Ren, M., Pili, R., Sironi, E., Somenzi, G., Ghidoni, R., Roma, S., Nicolo, G. & Sacchi, N. (2002) Endogenous reactivation of the RARbeta2 tumor suppressor gene epigenetically silenced in breast cancer. *Cancer Res.* 62: 2455–2461.

**Impairment of Retinoic Acid Receptor—Ceramide Signaling in Retinoic Acid-Resistant Breast Cancer Cells. Implications for Retinoid Differentiation Therapy of Breast Cancer.** G. Somenzi,\* M. Ren,\* G. Sala,<sup>†</sup> R. Ghidoni,<sup>†</sup> and N. Sacchi.\* \*Roswell Park Cancer Institute, Buffalo, NY, USA, and <sup>†</sup>San Paolo University Hospital, School of Medicine, University of Milan, Italy.

Despite promising preclinical animal studies, retinoic acid (RA), the bioactive derivative of vitamin A, has given disappointing results in clinical trials of human breast cancer because of the hurdle of RA resistance. RA resistance seems to have an heterogeneous molecular basis. One form of RA resistance in breast cancer has been traced to epigenetic silencing of RA receptors (RARs). In the breast cancer cell line T47D, RA treatment (1  $\mu\text{mol/L}$ , 72 h) induced impaired cell growth and apoptosis concomitant with a 2.3-fold increase of endogenous ceramide, a sphingolipid with a recognized role as proapoptotic second messenger. In contrast, in the breast cancer cell line MDA-MB-231 the same treatment failed to induce both growth arrest and accumulation of proapoptotic ceramide. The two cell lines differ in their RAR profiles in that T47D cells are RAR- $\alpha$  positive and RAR- $\beta$  inducible whereas MDA-MB-231 cells are RAR- $\alpha$  and RAR- $\beta$  negative. Thus we hypothesized that ceramide accumulation may be mediated by RARs. To test this hypothesis we engineered T47D cells to stably express an RAR- $\alpha$  dominant-negative (DN) construct. DN T47D cells present an RA-resistant phenotype and fail to accumulate ceramide in response to RA treatment. In addition, by inhibiting RAR- $\alpha$  in T47D cells with RAR- $\alpha$  antagonists, we observed a failure to accumulate endogenous ceramide. Altogether these data indicate that RA-induced ceramide in T47D cells is mediated by RAR- $\alpha$  signaling. The synthetic retinoid fenretinide, 4-HPR, whose action is largely independent of RARs, was able to induce cell death and a concomitant ceramide increase in the MDA-MB-231 cell line, thus indicating that the biochemical machinery required for endogenous ceramide production is intact. In conclusion, we show that RA resistance associated with a lack of functional RARs in breast cancer cells can be overcome by using synthetic retinoids such as 4-HPR. [Supported by U.S. Army IDEA Awards DAMD17-99-1-9241 and DAMD17-02-1-0432, AIRC Award 2001, and RPCI Alliance to NS.]

### Colorectal cancer

**Risk of Colorectal Cancer Associated with Dietary Pattern in a Portuguese Population.** P. Ravasco,\* I. Monteiro Grillo,\*<sup>†</sup> and M. E. Camilo.\* \*Center of Nutrition and Metabolism, Faculty of Medicine, University of Lisbon, and <sup>†</sup>Radiotherapy Department, Santa Maria Hospital, Lisbon, Portugal.

**INTRODUCTION:** Food and nutrition as major causes of colorectal cancer (CRC) are still debatable. **AIM:** This prospective study in a Portuguese population investigated the association between dietary patterns and CRC. **METHODS:** We evaluated 70 patients with CRC, 34 men and 36 women, aged  $66 \pm 11$  y (33–94 y) and 70 age- and sex-matched controls. Usual diet (diet history), alcohol consumption (amount, type, years), and smoking habits (number packages/year, years) were evaluated. Before estimating the relative risk (RR) and 95% confidence intervals, we performed logistic regression to adjust for family history of CRC and smoking habits. **RESULTS:** The intake of the following foods was inversely associated with the risk of CRC for the highest versus the lowest quartile of intake: green leafy vegetables, RR 0.25 (0.19–0.32); fruits, RR 0.31 (0.21–0.41); fish, RR 0.21 (0.15–0.23); and whole-grain cereals, RR 0.33 (0.25–0.40); values for legumes were not significant: RR 0.41 (0.27–0.50). Positive associations with CRC risk regarding the lowest versus the highest quartiles of intake were found for meat, RR 2.80 (2.75–2.92); smoked salted pork meat products, RR 2.10 (1.98–2.22); refined cereal products, RR 1.70

(1.55–1.79); and alcohol, RR 1.60 (1.58–1.69). **CONCLUSIONS:** Our results support the protective effect of a balanced diet conveying adequate amounts of macronutrients, dietary fiber, micronutrients, and other bioactive components. A high and frequent intake of meat and processed meat products, available sugars, and alcohol, prevalent among CRC patients, appears to entail a significant risk whereas a preferential intake of fish and vegetables was associated with a decreased risk and was prevalent in individuals without CRC.

**Antimetastatic Activity of Epican Forte™ in Human Colon Cell Line HCT 116.** M. W. Roomi, V. Ivanov, S. P. Netke, M. Rath, and A. Niedzwiecki. Matthias Rath, Inc., R&D, Cancer Division, Santa Clara, CA.

**OBJECTIVE:** Colon cancer, the second leading cause of cancer death in United States, causes 55,000 deaths per year, most commonly in men and women at and over the age of 50 y. Over 90% of all cancer fatalities result from metastasis to other parts of the body. Matrix metalloproteinases (MMPs, -2 and -9) have been identified as bulldozers that disintegrate the extracellular matrix and assist cancer cell invasion and spread to distal organs. We have developed strategies to inhibit cancer development and spread by using naturally occurring nutrients, such as lysine, proline, arginine, ascorbic acid, and green tea extract. Such a specific formulation, Epican Forte™ (EF), was shown to exert potential synergistic anticancer activity by inhibiting MMPs and invasion. **METHODS:** We tested the effect of EF on colon cancer cell line HCT 116 by expression of MMPs (by zymography), invasion (through Matrigel), cell proliferation and cytotoxicity (MTT assay), and morphology (hematoxylin and eosin stains). **RESULTS:** EF inhibited the expression of MMP-9 in a dose-dependent fashion, with significant inhibition with EF at 100  $\mu\text{g/mL}$ . The invasion of colon cancer cells (HCT 116) through Matrigel was reduced by 54%, 66%, 76%, and 100% with 10, 50, 100, and 500  $\mu\text{g/mL}$  concentrations of EF, respectively. Interestingly, EF was not toxic to colon cancer cells even at 1000  $\mu\text{g/mL}$ . Hematoxylin and eosin stains did not show any alterations in morphology with different doses of EF. **CONCLUSIONS:** These observations suggest that EF is a valuable and promising agent with potential antimetastatic activity by inhibiting both invasion and MMP-9 activity.

**The Association between Calcium and Apoptosis in Human Rectal Epithelium.** Eric A. Miller, Temitope O. Keku, Christopher F. Martin, Pauline K. Lund, John T. Woosley, and Robert S. Sandler. University of North Carolina at Chapel Hill, Chapel Hill, NC.

**PURPOSE:** Evidence exists that calcium intake is associated with a decreased risk of colorectal adenomas. We investigated whether increased calcium intake is associated with increased apoptosis in the colonic epithelium as a possible mechanism. **METHODS:** In a cross-sectional study designed to examine risk and etiologic factors for colorectal adenomas, we recruited consecutive patients undergoing colonoscopies at a large referral hospital. Eligible patients underwent rectal mucosal pinch biopsies during their procedure. Apoptosis rates were scored as the number of apoptotic cells per crypt by using standard morphologic criteria from biopsy sections stained with hematoxylin and eosin. Information on diet and lifestyle factors was collected by phone interview. Diet was assessed using a food frequency questionnaire assessing the patients' usual diet over

the past year. Bivariate analysis, multiple regression, stratified analysis, and multivariate logistic regression were used to examine the association between dietary calcium and apoptosis scores. RESULTS: Data were available for 498 patients (174 with adenomas and 324 without). We dichotomized apoptosis scores above and below the median to calculate odds ratios for elevated apoptosis in relation to calcium intake. The association between calcium intake and apoptosis was modified by adenoma case status. In a logistic regression model adjusted for race, sex, total caloric intake, vitamin C, and total fiber intake, adenoma patients in the highest versus lowest tertile of dietary calcium intake were 3.6 times (95% confidence interval 1.0–12.6) more likely to have elevated apoptosis scores. In adenoma-free patients, high calcium intake was not related to apoptosis (odds ratio = 1.3 [0.6–2.8]). CONCLUSIONS: This analysis provides evidence that increased calcium intake is associated with increased apoptosis in patients with adenomas who have lower levels of apoptosis. The protective effect of calcium on adenoma development may be mediated through apoptosis.

**Prebiotic Synergy2 and Sulindac, a Nonsteroidal Anti-Inflammatory Drug: Effect on Azoxymethane-Induced Aberrant Crypt Foci in Fisher Rats.** M. Verghese,\* I. A. Bonsi,\* L. T. Walker,\* L. Shackelford,\* C. B. Chawan,\* and Jan Van Loo.† \*Nutrition and Carcinogenesis Laboratory, Department of Food and Animal Sciences, Alabama A & M University, Normal, AL, and †ORAFIT, Tienen, Belgium.

Cancer of the colon is one of the leading causes of cancer morbidity and mortality among men and women in the Western countries, including the United States. The objective of this study was to test the possible combinational effects of different fractions of chicory inulin–Raftilose@Synergy1 (HP-Inulin, Oligofructose: 1/1); Raftilose@Synergy2 (HP-Inulin, Oligofructose: 2/1); and Sulindac, a nonsteroidal anti-inflammatory drug, on azoxymethane (AOM)-induced aberrant crypt foci (ACF), an early preneoplastic marker in the process of colon carcinogenesis in Fisher 344 male rats. After an acclimatization period of 1 wk, 65 male weanling rats were divided into 6 groups and fed AIN93G (Control-C) and 5 experimental diets that contained C + 10% Synergy1, C + 10% Synergy2, C + 200 ppm Sulindac, C + 10% Synergy1 + 200 ppm Sulindac, and C + 10% Synergy2 + 200 ppm Sulindac. All the rats received 16 mg/kg body weight of AOM dissolved in saline subcutaneously at age 7 wk followed by a second injection at age 8 wk. The rats continued to receive the assigned diets until being killed by CO<sub>2</sub> asphyxiation at age 17 wk. Dietary administration of Synergy1, Synergy2, and Sulindac significantly ( $p < 0.05$ ) suppressed induction of colonic ACF, both in total number and crypt multiplicity. The reductions in ACF in the experimental groups compared with the control were 52.8% for C + 10% Synergy1, 64.3% for C + 10% Synergy2, 53.1% for C + 200 ppm Sulindac, 67.1% for C + 10% Synergy1 + Sulindac, and 78.05% for C + 10% Synergy2 + Sulindac. The results of this study indicate that the prebiotic Synergy1 and Synergy2, when given in combination with sulindac, had a significantly ( $p < 0.05$ ) higher effect in reducing AOM-induced ACF formation than did either one given alone.

#### Head and neck cancer

**Risk Factors for Head and Neck Cancer in a Portuguese Population: A Case-Control Study.** P. Ravasco,\* I Monteiro

Grillo,\*† and M. E. Camilo.\* \*Center of Nutrition and Metabolism, Faculty of Medicine University of Lisbon, and †Radiotherapy Department Santa Maria Hospital, Lisbon, Portugal.

**INTRODUCTION:** The role of nutrition on the incidence of head and neck cancer (HNC) is still under debate whereas the relevance of some nutrients is emerging. **AIM:** The aim of this prospective case-control study in a Portuguese population was to identify the prevalence of dietary constituents, alcohol, and smoking as risk factors for HNC. **METHODS:** We evaluated 65 cases of HNC, 52 men and 13 women, aged  $60 \pm 11$  y (36–84 y) and 60 age- and sex-matched controls. Data were collected on alcohol consumption (amount, type, years), smoking habits (number packages/year, years), and diet history further analyzed to detail nutrient intake (Dietplan5, Forestfield Software Ltd., UK). Relative risk (RR) and 95% confidence intervals were estimated. **RESULTS:** Significant risk was associated with smoking, RR 4.20 (4.16–4.26); among nutrients, positive associations regarding higher-than-recommended versus adequate intakes emerged for alcohol, RR 3.30 (3.25–3.36); sodium, RR 2.01 (1.96–2.05); chloride, RR 1.98 (1.93–2.03); and available starch, RR 1.25 (1.20–1.29). Risk was inversely associated with lower-than-recommended versus adequate intakes for flavonoids, RR 0.15 (0.11–0.19); selenium, RR 0.20 (0.16–0.24); n-3 fatty acids, RR 0.23 (0.18–0.27);  $\beta$ -carotene, RR 1.74 (1.69–1.77); vitamin C, RR 1.65 (1.61–1.69); and isoflavones, RR 1.63 (1.58–1.68); values for calcium were not significant, RR 0.90 (0.87–0.93). **CONCLUSIONS:** HNC patients reported having a diet deficient in protective nutrients concurrent with smoking and marked excesses of alcohol, sodium, chloride, and starch for a prolonged period. Our results are consistent with well-established worldwide nutritional risk factors—alcohol and smoking—and advance the relevance of several emerging nutrients, for example, flavonoids, n-3 fatty acids, and isoflavones. The expected Mediterranean diet was seldom reported, although controls were closer to that pattern than were cases.

**Does Dietary Pattern Influence the Risk of Head and Neck Cancer?** P. Ravasco,\* I Monteiro Grillo,\*† and M. E. Camilo.\* \*Center of Nutrition and Metabolism, Faculty of Medicine University of Lisbon, and †Radiotherapy Department Santa Maria Hospital, Lisbon, Portugal.

**INTRODUCTION:** Despite the established association between nutrients and the incidence of head and neck cancer (HNC), the relevance of dietary components remains controversial. **AIM:** This prospective study in a Portuguese population investigated the association between dietary patterns and HNC. **METHODS:** We evaluated 65 cases of HNC, 52 men and 13 women, aged  $60 \pm 11$  y (36–84 y) and 65 age- and sex-matched controls. Usual diet (diet history), alcohol consumption (amount, type, years), and smoking habits (number packages/year, years) were evaluated. Before estimating the relative risk (RR) and 95% confidence intervals, we performed logistic regression to adjust for family history of HNC and smoking habits. **RESULTS:** The intake of the following foods was inversely associated with the risk of HNC for the highest versus the lowest quartile of intake: vegetables, RR 0.31 (0.26–0.36); fruits, RR 0.33 (0.29–0.37); and fish, RR 0.35 (0.30–0.39); there was a trend for dairy products: RR 0.51 (0.57–0.55). Positive associations with HNC risk for the lowest versus the highest quartile of intake were found for alcoholic beverages, RR 3.30

(3.25–3.36); smoked salted meat products, RR 2.20 (2.17–2.25); salt, RR 2.01 (1.96–2.05); and refined cereal products, RR 1.10 (1.03–1.16). Smoking was one of the most significant risk factors: OR 4.20 (4.16–4.26); the effect of smoking increased when combined with excessive alcohol intake: OR 4.93 (4.87–4.97). **CONCLUSIONS:** Our results agree with established lifestyle risk factors—alcohol and smoking—and further denote the negative influence of smoked products, salt, and refined cereals. Conversely, our results show that a diet rich in fish, vegetables, and fruits may prove protective.

### Brain cancer

**A Rare Brain Tumor in Childhood: Germinoma of Pituitary Stalk with Arachnoid Cyst: Nutritional Aspects, Diagnostic Aspects, and Therapy.** Mustafa Metin Donma,\* Işın Çelik,\* and Cihat Kaynar.† \*Republic of Turkey, Ministry of Health, Suleymaniye Education and Research Hospital, Istanbul, and †Ministry of Social Security, Ankara Education and Research Hospital, Ankara, Republic of Turkey.

Germ-cell tumors including germinomas of the central nervous system constitute a small group of all primary brain neoplasms. Germinoma of pituitary stalk with arachnoid cyst is infrequent in children with diabetes insipidus (DI). We report the clinical course of a prepubertal 8-y-old girl with DI and subsequent germinoma. The diagnosis of DI was established and magnetic resonance imaging (MRI) revealed a thickened pituitary stalk. Repeated brain MRI scans were performed. Thickness of pituitary stalk, which was 3 mm during the first and second hypophyseal MRI, increased to 7 and 8 mm at the fourth and fifth hypophyseal MRIs, respectively. At cranial MRI evaluation, an arachnoid cyst was detected. One year after the beginning of complaints, the patient underwent surgery (pterional craniotomy plus biopsy) for diagnostic purposes. The pathology report was grade III germinoma. Standard chemotherapy was performed 3 wk after surgery and cranial irradiation was performed 2 wk later. Desmopressin acetate was also administered. At the end of the therapy protocol, the seventh MRI revealed total remission. The patient had 40 d of breast-feeding with concomitant formula feeding starting at the first day of life. Additional foods and cow milk were administered at the third and sixth months of life, respectively. She had fast food twice per month from age 2 y. The relationship between childhood cancers and the type of feeding has been widely investigated. Comprehensive studies may elucidate dietary factors that affect the risk of brain tumors in children. Not only the infant's and child's diet but also the maternal diet during gestation may be associated with tumor occurrence. Reports on nutritional features of the individual should accompany such rare cases of brain tumors so that the possible relationship among food, nutrition, and childhood brain tumors may be presented clearly in the future.

### Antimutagenicity and Antioxidative Activities

**Antimutagenicity and Antioxidative Activity of Traditional Japanese Herbs.** Naoko Hiramatsu,\* Wang Xiufen,† and Ryusuke Takechi.\* \*Laboratory of Nutritional Science, School of Humanities for Environmental Policy and Technology, Himeji Institute of Technology, Himeji, Japan, and †Department of Food Science, Jinan University, Guangzhou, People's Republic of China.

The multistage induction theory is generally regarded as the mechanism of carcinogenesis. The initiation stage is caused either by carcinogens themselves or by the attack of active oxygens on genes in a cell. Another possibility for initiation is the action of the ultimate metabolically activated cytochrome P450s. In order to prevent the initiation stage of carcinogenesis, it is meaningful to find out the functional components in edible plants. The objective of this research was to test the antimutagenicity and antioxidative activity of the functional components from several traditional herbs used in Japan. The traditional herbs gennoshoko (*Geranium nepalense* var. thunbergii), yomogi (*Artemisia vulgaris* var. indica), senburi (*Swertia japonica*), iwa-tobacco (*Conandron ramondioides*), sarunokoshikake (*Elfvigia applanata*), kanzo (*Glycyehiza uralensis* Fisch), and matatabi (*Actinidia polygama*) were examined by the Ames mutagenesis assay test with *Salmonella typhimurium* TA98 and TA100 against mutagens Trp-P-1, Trp-P-2, and B(a)P. We used both HPLC and a colorimetric method for evaluating the free radical-scavenging activity of the herbs by using 1,1-diphenyl-2-picrylhydrazyl. The water-soluble components and volatile oil of the herbs were extracted in boiling water. The extracts of gennoshoko showed strong antimutagenicity against B(a)P with *S. typhimurium* TA98 and TA100 as well as Trp-P-1 and Trp-P-2 with *S. typhimurium* TA98. Yomogi, senburi, and iwa-tobacco were also proved to have good antimutagenicity against Trp-P-1 and Trp-P-2 with *S. typhimurium* TA98 but weaker antimutagenicity against B(a)P. Other herbs did not show any obvious antimutagenicity against these mutagens. The extracts of gennoshoko, yomogi, and iwa-tobacco also showed significant radical-scavenging activity whereas the sarunokoshikake and other herbs showed weak radical-scavenging activity. In addition, the volatile oil of yomogi also had a remarkable antimutagenic effect against the mutagens used with *S. typhimurium* TA98 and the strong antioxidative effect as well.

**Evaluation of Potential Mutagenic and Antimutagenic Properties of Foods in Dynamic In Vitro Models of the Gastrointestinal Tract.** Robert Havenaar,\* Cyrille Krul,\* Ronald Schothorst,† Koen Venema,\* and Christele Humblot.\*\* \*TNO Nutrition and Food Research, Zeist, Netherlands, †National Institute of Public Health and the Environment (RIVM), Bilthoven, Netherlands, and \*\*National Institute for Agricultural Research (INRA), Jouy-en-Josas, France.

TNO gastrointestinal models (TIM) are dynamic systems that simulate human physiological conditions in the stomach and small intestine (TIM-1) and large intestine (TIM-2). They regulate body temperature, pH, peristaltic movements, secretion of enzymes and bile, absorption of digestion products and water, and the large intestine microflora. We studied the activity of food mutagens and antimutagens by using these models in combination with in vitro assays (Ames, Comet, TEAC) and chemical and microbial analyses. In the gastric compartment, significant levels of nitrosamine (NDMA) were formed after the intake of nitrite and dimethylamine via various species of fish. Levels of NDMA were related to the level of nitrite and to the species of fish (codfish>herring>pollack, plaice>mackerel>salmon). NDMA formation was inhibited by the intake of spinach, tea, and orange juice. The antimutagenicity of tea was studied in TIM-1. Simultaneous addition of green or black tea and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) resulted in efficient inhibition of mutagenic activity of MeIQx in the Ames test. The addition of different

types of milk to the tea extracts reduced antimutagenicity. In TIM-2 we investigated the metabolism of glucosinolates by an active microflora of human origin with physiological density and composition. TIM was "fed" with sinigrin, a glucosinolate occurring in *Brassica*. The breakdown of sinigrin (HPLC analysis) to allyl isothiocyanate (solid-phase microextraction and gas chromatography analysis) was measured. Sinigrin was totally degraded between 6 and 12 h after feeding. Allyl isothiocyanate, the putative antimutagenic metabolite, was detected from 3 to 36 h, reaching its highest concentrations between 6 and 12 h. Thus, the TIM systems in combination with other in vitro (genotoxicity) assays and analyses can be applied in mechanistic studies of the mutagenic and antimutagenic properties of food.

**Involvement of Oxidative Stress in the Etiology of Esophageal Cancer in a South African Black Population.** K. S. A. Mossanda,\* C. F. Van der Merve,<sup>†</sup> and H. F. Joubert.\* Chemical Pathology Department, Medical University of Southern Africa, and <sup>†</sup>Gastro-enterology Department, Medical University of Southern Africa, South Africa.

**INTRODUCTION:** Squamous cell carcinoma of the esophagus has become the most common cancer in black South African men (incidence 14.5%) and is second only to carcinoma of the cervix incidence in black women whereas for whites this cancer is ranked ninth. Most patients attend the oncology clinic at Garankuwa Hospital when it is too late for chemotherapy and surgery treatment. The best form of cancer prevention is early detection, which would be based on heartburn, difficulty in swallowing, and severe long-standing reflux symptoms justifying an endoscopy (gastroscope) for confirmation, at least, of a premalignant lesion of the esophagus (Barrett's esophagus). The risk of esophageal adenocarcinoma with Barrett's esophagus is reported to be approximately 30–125-fold greater than that of the general population in Western countries; in black South African patients, the risk of squamous cell carcinoma is neither established nor associated with any premalignant lesion. Our preliminary results demonstrated that the dietary intake of antioxidants by patients with proven esophageal carcinoma was low. These data correlated with high level of thiobarbituric acid substances, a decrease of superoxide dismutase (SOD) activity, and a slight modification of glutathione peroxidase (GPx) activity. To confirm these results, we investigated the implication of oxidative stress in the etiology of this squamous cell esophageal carcinoma in a black South African population. **METHODS AND SUBJECTS:** Thirty-five patients were recruited from the Ga-Rankuwa Hospital, South Africa, where they were seen for diagnostic endoscopy; 15 control patients were recruited from those undergoing endoscopy for other gastroenterologic conditions. Blood and urine were collected for biochemical determinations, especially those related to the evaluation of antioxidant status and oxidative stress: lipid peroxides, 8-isoprostane, SOD, GPx, 8-hydroxy-deoxyguanosine (8-OHdG), and 8-isoprostane. Biopsies were taken from cancer patients for histological analysis and immunohistochemistry examination of 8-OHdG, a biomarker of DNA damage. **RESULTS:** A significant difference between patients with proven squamous cell carcinoma and control subjects was observed only for lipoperoxides, 8-isoprostane level, and GPx activity, whereas an increase was noted as a result of oxidative stress and a decreased level of antioxidants. In contrast, SOD activity was low in the patients. The histological findings and the immunohistochemical evidence of DNA damage by the

presence in situ (biopsies) of 8-OHdG provided a consistent correlation between low intake of dietary antioxidant and the occurrence of carcinoma. Our data on antioxidant status in esophageal cancer patients confirmed our previous results and established a correlation between the imbalance of this antioxidant status and the high level of lipid peroxides, the decrease of SOD as a result of an increase of oxidative stress. **CONCLUSION:** Although we did not find the accurate predictive factors that distinguish patients who are likely to progress to squamous cell carcinoma from patients who will follow a benign course, this case-control study demonstrates the involvement of oxidative stress in the occurrence of esophageal cancer. High levels of reactive oxygen and nitrogen species inducing a depletion of the antioxidant defense system combined with other unknown factors are probable etiological factors of the observed high incidence of esophageal cancer in black South African populations. The limited area of occurrence of this type of cancer and the sudden rise in incidence over the past few decade imply exposure to certain specific carcinogens: polycyclic aromatic hydrocarbons and mycotoxin-contaminated food alone or in conjunction with alcohol intake, tobacco smoking, or both. The interaction of this complex mixture of components may activate some nuclear transcription factors that induce an immune response leading to cancer. A multidisciplinary project including larger populations of patients and controls is indicated for adequate statistical analysis. Our understanding of the intrinsic mechanism underlying the full expression of the antioxidant defense system may explain the lack of a total reduction in the mortality rates of cancer, especially those related to gastrointestinal cancers, despite adequate supplementation in micronutrients and antioxidants.

**Antioxidant, Anti-Inflammatory/Immunomodulatory, and Chemopreventive Activities of Some African Foodstuffs and Medicinal Plants.** K. S. A. Mossanda,\* Hye-kyung Na,<sup>†</sup> Chun Kyung-Soo,<sup>†</sup> H. L. Ko,\*\* J. Beuth,\*\* H. F. Joubert,\* W. J. Du Plooy,<sup>‡</sup> and Young-Joon Surh.<sup>†</sup> \*Chemical Pathology Department, Medical University of Southern Africa, South Africa, <sup>†</sup>College of Pharmacy, Seoul National University, Shimlin-dong, Seoul, South Korea, \*\*Institute for Scientific Evaluation of Naturopathy, University of Cologne, Germany, and <sup>‡</sup>Pharmacology & Therapeutics Department, Medical University of Southern Africa, South Africa.

**INTRODUCTION:** A variety of natural compounds present in our traditional medicinal plants and diet have been reported to bear antimutagenic, antioxidant, or antimutagenic activities. The World Health Organization's 1993 guidelines on the evaluation of herbal medicines consider that clinical evaluation is ethical where drugs have long been in traditional use. However, our understanding of their molecular mechanism will help to predict adverse effects not detected before their approval and to modify our classical strategies for treatment of various diseases. Epidemiologic investigations as well as animal model cell culture experiments show that those foodstuffs and medicinal plants may play a role in the prevention of cancer and inflammatory diseases through their antimutagenic, antioxidant, and anti-inflammatory/immunomodulatory properties. **MATERIAL AND METHODS:** Antimutagenic activity against the mutagen daunomycin was evaluated by a *Salmonella typhimurium* strain TA 102 reversion test. Antioxidant activity was demonstrated in plant and vegetable extracts by their ability to scavenge superoxide anion, bleach the stable 1,1-diphenyl-2-picrylhydrazyl radical, and protect DNA supercoiled after strand

scission induced by UV-photolysis of H<sub>2</sub>O<sub>2</sub>. Immunomodulatory effect was tested using inhibition of zymosan-induced chemiluminescence generation in macrophages RAW 264.7 cells and in human granulocytes. To estimate the anti-inflammatory and anticarcinogenic activity of those foodstuffs and medicinal plants and to evaluate to what extent they contain factors that can down-regulate cyclooxygenase (COX)-2 expression through suppression of nuclear factor- $\kappa$ B activation, Western and Northern blotting analyses were performed for measurement of COX-2 mRNA expression after incubation of ethanolic plant extracts with the immortalized human breast epithelial (MCF-10A) cells in vitro and with mouse skin in vivo. An electrophoretic mobility shift assay of the nuclear fraction evaluated nuclear factor- $\kappa$ B DNA binding activity. RESULTS: Our data show that cancer bush (*Sutherlandia frutescens*), used as a medicinal plant in South Africa; devil's claw (*Harpagophytum procubens*), used as anti-inflammatory and antiarthritis drugs in Botswana; bambara groundnut (*Vigna subterranea*); and *Warburgia salutaris* possess antimutagenic, antioxidant, immunomodulatory, and anticarcinogenic activities at some stages. *W. salutaris* and *H. procubens* showed the greatest antioxidant and immunomodulatory activities of the tested plants and vegetables. However, significant chemopreventive and anti-inflammatory activities detected by the inhibition of tetradecanoyl phorbol-3 acetate-stimulated cyclooxygenase-2 expression in MCF-10A cells in vitro and in mouse in vivo was observed with cancer bush. CONCLUSION: Additional in vitro and in vivo experiments are needed to 1) enable us to fully understand the mechanism involved in the modulation of the antimutagenic/anticarcinogenic and antioxidant response by African foodstuffs and medicinal plants against specific carcinogens (e.g., benzopyrene, aflatoxin, fumonisin) present in most African diets because of storage conditions and 2) explain the immunomodulatory and antiproliferative activities. It is only in this way that we will be able to recommend the extensive use of those African materials in the diet to reduce the incidence of particular types of cancer. Such experiments will allow a comparison of traditional medicine used as complementary medicine and conventional methods for treating the same pathological conditions with optimal safety and efficacy and a minimum of adverse effects.

### Phytochemicals

**Brassica Vegetables and Prevention of Colorectal Cancer: The Role of Isothiocyanates as Cell Cycle Inhibitors.** Elizabeth K. Lund, Tracy K. Smith, and Ian T. Johnson. Institute of Food Research, Norwich, UK.

Epidemiologic studies suggest a particularly strong link between consumption of brassica vegetables and reduced risk of colorectal cancer (1). These effects have predominantly been linked to induction of phase 2 enzymes by glucosinolates, a group of chemicals specific to brassica vegetables. However, we previously showed, using the rat 1,2-dimethylhydrazine (DMH) model, that Brussels sprouts or their major glucosinolate, sinigrin, act postinitiation to decrease mitosis and increase apoptosis (2,3), changes associated with a decrease in the formation of aberrant crypt foci 10 wk postdosing. The active metabolites of glucosinolates are believed to be the isothiocyanates (ITCs). We have therefore chosen to use two colorectal cancer cell lines—HT-29 and LoVo cells—to investigate both cell cycle and induction of apoptosis in response to a number of ITCs. Cells in logarithmic growth were exposed to equimolar

concentrations of allyl-ITC (AITC), benzyl-ITC (BITC), phenethyl-ITC (PEITC), and methylsulfinylbutyl-ITC (sulforaphane). All treatments except sulforaphane led to cell detachment within 24 h, with AITC and BITC being more effective than PEITC. Flow cytometry analysis indicated that AITC caused a block at G2/M that occurred before cell detachment and was more apparent in LoVo cells. BITC and PEITC also induced a block in G2/M but to a lesser extent. Annexin V staining of the detached cells after exposure was not detectable above control levels except in LoVo cells exposed to PEITC. Different brassicas may have complementary effects. Those, such as Brussels sprouts, most able to cause deletion of precancerous cells may suppress the accumulation of such cells with age whereas vegetables such as broccoli may be of most benefit in blocking the effect of any carcinogen before it causes any genetic damage. PEITC-containing plants, such as watercress, may be beneficial in both respects.

1. Van Poppel, G., Verhoeven, D. T., Verhagen, H. & Goldbohm, R. A. (1999) Brassica vegetables and cancer prevention. *Epidemiology and mechanisms. Adv. Exp. Med. Biol.* 472: 159–168.

2. Smith, T. K., Lund, E. K. & Johnson, I. T. (1998) Inhibition of dimethylhydrazine-induced aberrant crypt foci and induction of apoptosis in rat colon following oral administration of the glucosinolate sinigrin. *Carcinogenesis* 19: 267–273.

3. Smith, T. K., Mithen, R. & Johnson, I. T. (2003) Effects of *Brassica* vegetable juice on the induction of apoptosis and aberrant crypt foci in rat colonic mucosal crypts in vivo. *Carcinogenesis* 24: 491–495.

**The Role of the Nrf2 Transcription Factor in the Prevention of Colon Cancer by Cruciferous Vegetables.** Gail K. McWalter, J. Hayes, Lesley McLellan, and Cliff Elcombe. Biomedical Research Centre, Ninewells Hospital and Medical School, University of Dundee, Scotland.

Consumption of vegetables, especially cruciferous vegetables such as broccoli, Brussels sprouts, cauliflower, and cabbage, has been shown to confer some protection against colon cancer, although the mechanisms by which this occurs are not known with certainty. Evidence suggests that glucosinolates within cruciferous vegetables are hydrolyzed by the enzyme myrosinase into isothiocyanates, indoles, and other products, which act as either bifunctional (phase 1 and 2 enzymes) or monofunctional (phase 2 enzyme) inducers and lead to protection against toxicity and carcinogenesis. It has been proposed that transcriptional activation of detoxification and antioxidant genes such as glutathione S-transferases and NAD(P)H:quinone oxidoreductase by phytochemicals is in part mediated by transcription factor Nrf2, a cap'n'collar bZIP transcription factor, being recruited to the antioxidant response element (ARE) within promoter regions of inducible genes. Our hypothesis is that cruciferous vegetables and their phytochemicals confer protection against colon cancer through activation of Nrf2 and subsequent induction of protective antioxidant and detoxification genes via binding of Nrf2 to the ARE enhancer within their promoters. We have shown induction of ARE-regulated genes in wild type female mice exposed to allyl isothiocyanate (the main isothiocyanate in Brussels sprouts) and reduced constitutive and inducible expression in knockout mice. A cell culture system has been set up to assess the effect of cruciferous vegetables and their phytochemicals on cells in vitro. The ability of cruciferous vegetables and their phytochemicals to inhibit chemically initiated colon cancer via induction of antioxidant and detoxification enzymes in wild type and knockout mice will be assessed.

**Molecular Targets of Sulforaphane Using Oligonucleotide Microarray: Chemoprevention and Anti-Inflammatory Role.** Rajesh K. Thimmulappa, Kim H. Mai, Sorachai Srisuma, and Shyam Biswal. Department of Environmental Health Sciences, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD.

Electrophiles formed during metabolic activation of chemical carcinogens and reactive oxygen species play a significant role in carcinogenesis. Cancer chemoprevention by induction of phase 2 proteins to counteract the insults of these reactive intermediates has gained considerable attention. Sulforaphane, an isothiocyanate derived from broccoli, has been widely studied because of its strong chemopreventive efficacy against various cancers. This study shows that sulforaphane through activation of transcriptional factor Nrf2 leads to transcriptional induction of genes that can counteract oxidative stress and a broad spectrum of carcinogens. Nrf2-wild type and Nrf2-deficient mice were fed with sulforaphane (9  $\mu$ mol/d per mouse) for 7 d and gene expression changes were evaluated in small intestine using the murine genome U74Av2 oligonucleotide array (representing ~6000 well-characterized genes and nearly 6000 expressed sequence tags). Sulforaphane induced numerous genes vital for cellular protection against carcinogens via Nrf2 activation. The list of sulforaphane-induced Nrf2-dependent genes includes NAD(P)H:quinone reductase, glutathione S-transferase,  $\gamma$ -glutamylcysteine synthetase, UDP-glucuronosyltransferases, epoxide hydrolase, cellular NADPH-regenerating enzymes (glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, malic enzyme), xenobiotic metabolizing enzymes, antioxidants (glutathione peroxidase, glutathione reductase, ferritin, haptoglobin), and biosynthetic enzymes of the glutathione and glucuronidation conjugation pathways. By an Nrf2-independent mechanism, sulforaphane repressed a subset of genes encoding proteins involved in inflammation and progression of neoplastic lesions such as tumor necrosis factor super family 3-like, interferon-induced protein, NOD/Lt] small inducible cytokine A5, spasmolytic polypeptide gene, nuclear protein 1, and thioredoxin interacting protein-1. The molecular targets of sulforaphane suggest its cancer chemoprevention and anti-inflammatory role and provide the rationale for dietary intervention to counteract carcinogens. [Supported by the NIEHS center grant # P30 ES 03819 and FAMRI award (SB).]

**Flavonoids—Keep it Simple: Mechanistic Studies of Antioxidant Activity.** Jennifer Dean and Phillip Musich. Department of Biochemistry and Molecular Biology, James H. Quillen College of Medicine, East Tennessee State University, Johnson City, TN.

Flavonoids exist ubiquitously in all fruits and vegetables. The average consumption of flavonoid-rich foods (2–3 g/d) correlates with overall health and disease prevention. However, the molecular basis for any biological benefit to flavonoid consumption remains obscure. Deemed antioxidants, flavonoids reportedly scavenge free radicals, bind DNA, and chelate metals to prevent oxidative damage. All flavonoids are based on a 15-carbon skeleton (chromane ring bearing a phenyl substituent) and are characterized by modifications to this rudimentary structure to confer antioxidant functions. Hydroxyl and sugar attachments found in quercetin and its glycosidic analog rutin are considered essential to antioxidant activity. To further evaluate the molecular mechanisms of flavonoid antioxidant activity, we examined the simplest flavonoid. Flavone is an

electron-rich planar compound modified from the base structure by a 2,3 conjugated double bond and an oxo group attachment. Select flavonoids were evaluated by the supercoiled-to-nicked circular conversion (SNCC) assay in which DNA was exposed to Fenton-generated hydroxy radicals. Although void of any hydroxyl or sugar groups, flavone exhibited significant antioxidant activity against DNA damage induced by hydroxy radical. In contrast, quercetin increased oxidative DNA damage in the SNCC assay, and rutin afforded neither anti- nor prooxidant effects. Flavone's molecular interactions were monitored by spectroscopy. Fluorescence and absorption studies indicate a direct flavone-DNA interaction. Ethidium's intercalation was decreased by flavone, suggesting that, like quercetin, flavone intercalates. Flavone also interacts with iron and alters DNA-iron binding, indicating an antioxidant mechanism via disruption of hydroxy radical generation in the Fenton reaction. These and other studies suggest that the antioxidant mechanism of flavone includes DNA binding and iron association and warrants future studies on the mechanistic features of simple flavonoids.

**Flavonoid Content of Vegetables.** D. B. Haytowitz, A. L. Eldridge, S. Bhagwat, S. E. Gebhardt, J. M. Holden, G. R. Beecher, J. Peterson, and J. Dwyer. Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD.

Flavonoids are biologically active polyphenolic compounds widely distributed in plants. Intake of flavonoids may be associated with a decreased risk of cancer, cardiovascular disease, and inflammatory disease in humans. Vegetables are one source for flavonoid compounds in the diet. The U.S. Department of Agriculture's Nutrient Data Laboratory evaluated the quality of existing literature from sources around the world and compiled a database containing five subclasses (flavonols, flavones, flavanones, flavan-3-ols, and anthocyanidins) of flavonoids. Fifty-eight different vegetables, 28 herbs and edible leaves, and 4 vegetable recipes were included as part of the development of the USDA flavonoid database for foods (1). Many vegetables, including onions, hot peppers, broccoli, snap beans, kale, and lettuce, contain the flavonol compounds quercetin and kaempferol. Onions, followed by lettuce, are the major vegetable contributors of quercetin to the diet whereas broccoli is the major contributor of kaempferol. Broadbeans and marrowfat peas provide catechins, the flavan-3-ols. Parsley, rutabagas, and celery provide high levels of apigenin, a flavone. Parsley, followed by celery, is the major vegetable contributor of apigenin to the diet. In compiling the database, analytically valid data were assigned confidence codes (A = most confidence, D = least confidence) based on the quality of the sampling procedures, sample handling, analytical methods, and analytical quality control. Although there were no A-quality data for any flavonoid values for vegetables in the database, most vegetables had B- or C-quality data. This database is the first step in evaluating the need and directing research for obtaining new analytical data on the flavonoid content of vegetables.

1. Nutrient Data Laboratory. Agricultural Research Service, U.S. Department of Agriculture. USDA Database for the Flavonoid Content of Selected Foods, 2003. Available at <http://www.nal.usda.gov/fnic/foodcomp/Data/Flav/flav.html> (accessed July 7, 2003).

**Antiproliferation Effects of Tomato Polyphenols in Hep-1c1c7 and LNCaP Cell Lines.** Jessica K. Campbell,\*



**Jennifer L. King,\* Mary Ann Lila,<sup>†</sup> and John W. Erdman, JR.\*** \*Department of Food Science and Human Nutrition and <sup>†</sup>Department of Natural Resources and Environmental Sciences, University of Illinois, Urbana, IL, USA.

Epidemiologic evidence suggests a decreased risk of prostate cancer with increased tomato consumption. Tomatoes contain a variety of phytochemicals including carotenoids and polyphenols, which have been shown to have antioxidant and perhaps anticancer effects. To evaluate the anticancer properties, a variety of polyphenols found in tomatoes were screened using a mouse carcinoma hepatic cell line and a human prostate cancer cell line. Hepa1c1c7 cells and LNCaP cells were maintained in  $\alpha$ -MEM or RPMI 1640 growth media, respectively, at 37°C in 5% CO<sub>2</sub>. Cells were plated with growth medium in 96-well plates and treated 24 h later with a known micromolar concentration of tomato polyphenolic compounds. The cell viabilities of Hepa1c1c7 and LNCaP were determined after a 48- or 72-h treatment, respectively, using a colorimetric MTS cell proliferation assay. Results show that tomato aglycone polyphenols such as quercetin, kaempferol, and naringenin inhibited cancer cell proliferation in both cell lines in a dose-dependent manner (10–50  $\mu$ mol/L). In contrast, glycone polyphenols such as rutin, quercetrin, and naringin did not decrease cell growth, suggesting that the glycones are not readily taken up by either Hepa1c1c7 or LNCaP cells. In addition, combination treatments (25–50  $\mu$ mol/L) of quercetin, kaempferol, and naringenin produce additive and perhaps synergistic inhibition of growth in both cell lines. [Supported by U.S. Department of Agriculture Initiative for Future Agriculture and Food Systems Program Project grant #00-52101-9695 and AICR grant #01B061.]

**Chemopreventive Effect of Canola Phenolic Acid-Enriched Extract on 7,12-Dimethylbenz[a]anthracene/12-O-Tetradecanoylphorbol 13-Acetate (DMBA/TPA)-Induced Mouse Skin Carcinogenesis.** E. M. Kurowska,\* N. Guthrie,\* A. Muir,<sup>†</sup> N. Westcott,<sup>†</sup> P. J. Ferguson,\*\* and K. Morley.\*\* \*KGG Synergize Inc., London, Ontario, <sup>†</sup>Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, Saskatchewan, and \*\*London Regional Cancer Centre and University of Western Ontario, London, Ontario, Canada.

Canola meal, a residual fraction obtained from the processing of canola oil, is a rich source of phytochemicals that may possess anticancer properties. Recently, we showed that hydrolyzed extract from canola meal enriched in free phenolic acids, largely sinapic acid (CPA), can inhibit the proliferation of human cancer cell lines, especially skin cancer cells SK-MEL5. To verify this observation, a large-scale CPA extraction method was developed and the anticancer potential of 4 extracts was assessed in the SK-MEL5 cell line by an alamarBlue® viability assay. The most active formulation was tested in the mouse model of 7,12-dimethylbenz[a]anthracene (DMBA)-initiated and 12-O-tetradecanoylphorbol 13-acetate (TPA)-promoted skin cancer. For the animal study, female ICR mice (20 animals/group) were given a semipurified diet with CPA at 0, 0.06, or 0.2 g/100 g for 24 wk starting 1 wk before DMBA initiation. Additional groups (10 animals each) served as DMBA- and TPA-vehicle controls and as a CPA initiator-promoter control. In SK-MEL5 cells, the most active CPA extract, containing 52.9%, had an IC<sub>50</sub> value of 140  $\mu$ g/mL. Consistently, in mice, dietary supplementation with either 0.2 or 0.06 g/100g of the

most active CPA moderately delayed the onset of skin tumors (median 3 d) and significantly reduced tumor multiplicity (by 34%). This was associated with reduced tumor incidence (by 17%) during early stages of treatment (weeks 6–9). Supplementation with the 0.2 g/100 g dose but not with the 0.06 g/100g dose of CPA also significantly reduced total tumor volume during the last 7 wk of treatment (by 26%) and significantly reduced the final weight of dissected tumors by 36%. These beneficial effects of 0.2% CPA were not associated with toxicity, as confirmed by the normal growth and food consumption. In conclusion, a novel dietary supplement, CPA, and its active constituents have potential benefit as cancer chemopreventive and anticarcinogenic agents.

**Curcumin Down-Regulates Cyclooxygenase-2 by Inhibiting Activation of Extracellular Signal-Regulated Kinase 1/2 and Nuclear Factor- $\kappa$ B in Phorbol Ester Stimulated in Mouse Skin.** Kyung-Soo Chun, and Young-Joon Surh. College of Pharmacy, Seoul National University, Seoul, Korea.

Recently, considerable efforts have been made to search for naturally occurring substances that will intervene in carcinogenesis. Curcumin, a yellow coloring ingredient of turmeric (*Curcuma longa* L., Zingiberaceae), has been shown to inhibit experimental carcinogenesis and mutagenesis, but the molecular mechanisms underlying its chemopreventive activities remain unclear. In the present work, we assess the effects of curcumin on 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced expression of cyclooxygenase-2 (COX-2) in mouse skin. When topically applied onto shaved backs of female ICR mice 30 min before TPA was applied, curcumin significantly inhibited the expression of COX-2 and its mRNA in a dose-related manner. Immunohistochemical analysis of TPA-treated mouse skin revealed enhanced expression of COX-2 localized primarily in the epidermal layer, which was markedly suppressed by curcumin pretreatment. Multiple lines of evidence support the role of the eukaryotic transcription factor nuclear factor- $\kappa$ B (NF- $\kappa$ B) in regulation of COX-2 expression. In agreement with this notion, the NF- $\kappa$ B inhibitor pyrrolidine dithiocarbamate suppressed not only NF- $\kappa$ B activation but also the induction of COX-2 in mouse skin. Curcumin treatment attenuated TPA-stimulated epidermal NF- $\kappa$ B activation, which was associated with its blocking of degradation of the inhibitory protein I $\kappa$ B $\alpha$  and subsequent translocation of the p65 subunit to the nucleus. TPA treatment resulted in rapid activation via phosphorylation of extracellular signal-regulated kinase (ERK)1/2 and p38 mitogen-activated protein kinases, which are upstream of NF- $\kappa$ B. The mitogen-activated protein kinase kinase/ERK kinase (MEK1/2) inhibitor U0126 strongly inhibited NF- $\kappa$ B activation whereas p38 inhibitor SB203580 failed to block TPA-induced NF- $\kappa$ B activation in mouse skin. Furthermore, U0126 blocked the I $\kappa$ B $\alpha$  phosphorylation by TPA, thereby blocking the nuclear translocation of NF- $\kappa$ B. Curcumin inhibited the catalytic activity of ERK1/2 in mouse skin. Taken together, suppression of COX-2 expression by inhibiting ERK activity and NF- $\kappa$ B activation may represent molecular mechanisms underlying previously reported anti-tumor-promoting effects of this phytochemical in mouse skin tumorigenesis.

**Ceramide Mediates Apoptosis Induced by Resveratrol in Metastatic Breast Cancer Cells.** Francesca Scarlatti,\* Giusy Sala,\* Giulia Somenzi,\*<sup>†</sup> Paola Signorelli,\* Nicoletta

**Sacchi,<sup>†</sup> and Riccardo Ghidoni.\*** \*Laboratory of Biochemistry and Molecular Biology San Paolo University Hospital, School of Medicine, University of Milan, Italy, and <sup>†</sup>Roswell Park Cancer Institute, Buffalo, NY.

Resveratrol (3,4',5-*trans*-trihydroxystilbene), a phytoalexin present in grapes and red wine, is emerging as a natural compound with potential anticancer properties although its mode of action is still under investigation. Here we show that resveratrol can induce growth inhibition and apoptosis in MDA-MB-231, a highly invasive and metastatic breast cancer cell line, in concomitance with a dramatic increase of endogenous ceramide. To understand the resveratrol-induced pathway, we investigated the mechanism leading to ceramide formation and proved that ceramide is responsible for the apoptotic effect of resveratrol. By metabolic labeling of cellular sphingolipids with either palmitic acid or dihydrosphingosine, we found that accumulation of ceramide derives from both de novo ceramide synthesis and sphingomyelin hydrolysis. More specifically we demonstrated, by in vitro evaluation of enzymatic activities of microsomal fractions obtained from treated cells, that resveratrol activates serine-palmitoyl-transferase (SPT), the key enzyme of de novo ceramide biosynthetic pathway, and neutral sphingomyelinase (nSMase), a key enzyme of the sphingomyelin-ceramide cycle. However, the use of specific inhibitors of SPT, myriocin, and L-cycloserine counteracted the apoptotic effects induced by resveratrol whereas nSMase inhibitors, glutathione and manumycin, did not. From these data we conclude that resveratrol-induced apoptosis of metastatic breast cancer MDA-MB-231 cells is mediated by ceramide endogenously induced by the activation of the de novo synthesis pathway.

**Edible *Vernonia amygdalina* Leaf Extract Inhibits Extracellular Signal-Regulated Kinases and Human Breast Cancer Cell Growth. Ernest B. Izevbigie,\* Joseph L. Bryant,<sup>†</sup> and Alice Walker.\*** \*Molecular Genetics and Molecular and Cellular Signaling Laboratory, Department of Biology, and National Institutes of Health Center for Environmental Health, Jackson State University, Jackson, MS, and <sup>†</sup>Institute of Human Virology, Animal Model Division, University of Maryland Biotechnology Institute, Baltimore, MD.

Breast cancer is the most commonly diagnosed cancer in women, representing ~30% of all types of cancer in women. One of every 8 women will be diagnosed with breast cancer in her lifetime. It is estimated that in the United States, breast cancer accounted for >15% of all new cancer cases in 2002 and 7% of cancer-related deaths in the United States in 2002. A water-soluble extract of edible *Vernonia amygdalina* leaves was recently reported to be a potent inhibitor of cultured human breast cancer cells (MCF-7 cells). The mechanisms by which *V. amygdalina* extract inhibits MCF-7 cell growth have not been studied. The objective of this study was to evaluate the effects of *V. amygdalina* extract on extracellular signal-regulated protein kinase 1/2 (ERK 1/2) activities, DNA synthesis, and subsequent cell growth. Cell growth was determined by standard methods of [<sup>3</sup>H]thymidine incorporation and confirmed by cell counts using a hemocytometer. ERK activities were determined by immunoprecipitation with immobilized phospho-p44/42 kinase (Thr202/Tyr204) monoclonal antibody and using ELK fusion protein as a substrate. Treatment of cells with *V. amygdalina* extract (3–100 µg/mL) potently inhibited ERK activity, DNA synthesis ( $P < 0.005$ ), and cell growth ( $P < 0.01$ ) in

a concentration-dependent fashion both in the absence and presence of serum. Pretreatment of cells with *V. amygdalina* extract at 10 µg/mL for 48 h before transfer to extract-free medium did not affect subsequent growth rate compared with untreated cells ( $P > 0.05$ ). These results suggest that *V. amygdalina* extract (10 µg/mL) exhibits cytostatic actions to retard the growth MCF-7 cells. In addition, the ERK signaling pathways may be one of the intracellular targets for *V. amygdalina* antineoplastic actions. [Supported by Research Centers in Minority Institutions/National Institutes of Health grant #G122RR13459 and Center for University Scholars of Jackson State University.]

**Eupatilin Inhibits Growth of H-ras-Transformed Human Breast Epithelial Cells through Cell Cycle Arrest. Dohee Kim, Hye-Kyung Na, and Young-Joon Surh.** College of Pharmacy, Seoul National University, South Korea.

Extracts of *Artemisia asiatica* Nakai (Asteraceae) possess anti-inflammatory and antioxidative activities. Eupatilin (5,7-dihydroxy-3,4,6-tri-methoxy-flavone), one of the pharmacologically active ingredients derived from *A. asiatica*, was shown to induce apoptosis in human promyelocytic leukemia (HL-60) cells (1). In this study we examined the cytostatic effects of eupatilin in H-ras-transformed human breast epithelial (MCF10A-ras) cells. Eupatilin inhibited the growth of MCF10A-ras cells in a concentration-dependent and time-related manner as assessed by MTT reduction and [<sup>3</sup>H]thymidine incorporation assays. To determine whether the inhibitory effects of eupatilin on the growth of MCF10A-ras are mediated through cell cycle blockade, DNA content was analyzed by flow cytometry. Eupatilin (100 µmol/L) blocked the cell cycle progression in the G2/M phase. Moreover, eupatilin inhibited the expression of Cdk2, Cdc2, cyclin B1, and cyclin D1, which are responsible for mediating cell cycle progression, while it increased the expression level of cyclin-dependent kinase inhibitors such as p27<sup>kip1</sup> and p53. However, expression of p21<sup>waf/Cip1</sup> was decreased at both the protein and mRNA levels. It has been known that p21<sup>waf/Cip1</sup> expression is regulated by extracellular signal-regulated kinase (ERK)1/2. Eupatilin also inhibited the activation of ERK1/2 as well as the expression of Raf-1 and Ras in MCF10A-ras cells. Therefore, the inhibitory effect of eupatilin on p21<sup>waf/Cip1</sup> expression is likely to be mediated by targeting the Raf/MEK/ERK signaling pathway. Eupatilin failed to inhibit activation of Akt, an important component of prosurvival signaling pathways, which is constitutively activated in tumors harboring mutant ras. In conclusion, the antiproliferative effect of eupatilin is likely to be associated with its inhibition of ERK1/2 activation and subsequent blocking of the G2/M phase of cell cycle progression in MCF10A-ras cells.

1. Seo, H. J. & Surh, Y. J. (2001) Eupatilin, a pharmacologically active flavone derived from *Artemisia* plants, induces apoptosis in human promyelocytic leukemia cells. *Mutat. Res.* 496: 191–198.

**Early Exposure to Flaxseed and Its Lignan Reduced Mammary Cancer Risk at Adulthood. Lilian U. Thompson, Jianmin Chen, Kah Poh Tan, and Wendy E. Ward.** Department of Nutritional Sciences, University of Toronto, Toronto, Ontario, Canada.

Our previous studies showed that feeding flaxseed or its lignan, secoisolariciresinol diglucoside (SDG), to rat dams during

lactation enhances the differentiation of mammary glands in their female offspring. This study elucidated the mechanisms by which the mammary gland differentiation was enhanced and determined whether exposure to flaxseed or SDG during suckling could protect against dimethylbenzanthracene (DMBA)-induced mammary tumorigenesis at adulthood without adverse effects on selected reproductive indexes. Dams were fed the basal diet (BD) throughout pregnancy. After delivery, dams were fed the BD or BD supplemented with flaxseed at 10 g/100 g or its equivalent amount of SDG during lactation. After weaning, all offspring were fed BD. At postnatal day (PND) 21, mammary glands in the offspring from the flaxseed and SDG groups had more terminal end buds and terminal ducts, higher epithelial cell proliferation, and higher expression of epithelial epidermal growth factor receptor and stromal fibroblast epidermal growth factor but lower epithelial estrogen receptor- $\alpha$  and - $\beta$  than did the control. Conversely, at PND 49–51, fewer terminal end buds but a higher ratio of lobules to terminal end buds with decreased expression of epidermal growth factor receptor and epidermal growth factor were observed in the treatment groups. Compared with the BD group, offspring in the flaxseed and SDG groups given DMBA by gavage at PND 49–51 had a significantly lower tumor incidence, total tumor load, mean tumor size, and tumor number per rat at week 21 post-DMBA. There were no significant differences in selected reproductive indexes among dams or offspring. In conclusion, exposure to flaxseed or SDG during suckling enhanced mammary gland morphogenesis through modulation of epidermal growth factor receptor, epidermal growth factor, and estrogen receptor- $\alpha$  and - $\beta$ , which resulted in more differentiated mammary glands at PND 49–51. This suppressed DMBA-induced rat mammary tumorigenesis without causing adverse reproductive effects in dams or their offspring. The effect of flaxseed is primarily due to its SDG. [Supported by AICR.]

**The Plant Lignan 3-O-Methyl-Nordihydroguaiaretic Acid Induces Apoptosis in Human Papilloma Virus-Positive Cervical Cancer Cells by Stabilizing p53 Tumor Suppressor Protein.** Kristi L. Allen and Angelo L. DeLucia. Department of Microbiology/Immunology, Northeastern Ohio Universities College of Medicine, Rootstown, OH.

Persistent human papilloma virus (HPV) infection may lead to cancer of the uterine cervix, a leading cause of cancer mortality in women worldwide. High-risk oncogenic HPVs partly exert their carcinogenic effect on the infected cell after viral DNA integration by uncontrolled expression of the viral gene products E6 and E7. These proteins reactivate the cellular machinery for DNA replication and interfere with the cellular tumor suppressor proteins, p53 and retinoblastoma protein (Rb). The E6 oncoprotein accelerates the degradation of p53 protein, reducing its levels and hindering its protective response to cellular stress. Studies in our laboratory show that a plant lignan from the creosote bush (*Larrea tridentata*), 3-O-methyl-nordihydroguaiaretic acid, inhibits gene expression from the early promoter of HPV-16 and endogenous HPV E6/E7 gene expression within cervical cancer cells. Western blot analysis has shown that p53 is stabilized within lignan-treated HPV-positive cervical tumor cells. The induction of the bax gene and appearance of the proapoptotic bax protein within lignan-treated cells indicated that the stabilized p53 is transcriptionally active. Apoptosis, as measured by caspase-3 activation, was induced in HPV-positive cervical cancer cells. HPV-negative cervical tumor cells did not show caspase-3 activation and did

not undergo apoptosis when treated with lignan. The lack of p21 induction as shown by Western blot analysis in the presence of stabilized p53 protein indicates that apoptosis is favored over cellular growth arrest within treated tumor cells. The combination of the lignan with a conventional chemotherapeutic agent, actinomycin D, showed p53 protein to be considerably more stabilized than after treatment with each drug alone. Thus, the ability of the lignan alone or in combination with known chemotherapy to induce p53-dependent apoptosis within HPV-positive tumor cells makes it a potential small-molecule therapy against HPV-induced cancers.

**Chemopreventive Effects of Herbal Supplements in Rat Colon Aberrant Crypt Foci Assay.** S. R. Volate, D. M. Davenport, M. E. S. Zander, R. S. Hudson, C. J. Woods, S. J. Muga, and M. J. Wargovich. University of South Carolina, School of Medicine; South Carolina Cancer Center, Columbia, SC.

Colorectal cancer is the second most frequent cause of cancer deaths in the United States. Numerous studies suggest that increased consumption of polyphenolic flavonoids in the diet reduces the incidence of colorectal cancer. This study attempts to determine the ability of some of the most common dietary herbal supplements (silymarin, quercetin, curcumin, rutin, and ginseng), which contain polyphenolic flavonoids, to suppress aberrant crypt foci, a precursor for colorectal cancer, and induce apoptosis in an azoxymethane-induced rat colon cancer model. Results of this study show that none of the herbal supplements induced aberrant crypt foci formation without azoxymethane. Of the 5 agents tested, 4 agents (silymarin, quercetin, curcumin, and ginseng) significantly reduced the incidence of aberrant crypt foci in at least 1 of 2 doses tested ( $p < 0.01$ ) and 3 agents (silymarin, quercetin, and ginseng) were effective at both doses tested ( $p < 0.05$ ). Immunohistochemical analysis of colon mucosa indicates that all of the herbal supplements tested were effective in inducing apoptosis, with quercetin and curcumin being the most potent. The ability of ginseng and silymarin to induce apoptosis increased with time, whereas the ability of quercetin and curcumin diminished. Western blot analysis of bax (proapoptotic) and bcl-2 (antiapoptotic) proteins from the colon scrapings reveal that these herbal supplements induce apoptosis, with ginseng being the most potent and curcumin being the least potent. These results indicate that dietary herbal supplements containing silymarin, quercetin, curcumin, and ginseng are effective in preventing aberrant crypt foci and modulating apoptosis. The effects of these compounds on cyclooxygenase are being investigated. [Supported by AICR grant 00B041-REV.]

**Chemopreventive and Chemotherapeutic Effects of Ginseng and Ginkgo biloba.** M. E. S. Zander and M. J. Wargovich. University of South Carolina, Columbia, SC.

Habitual use of nonsteroidal anti-inflammatory drugs (NSAIDs) can reduce the risk of colon cancer by 50%, but this use is associated with many serious side effects. This study seeks to evaluate commonly used herbal supplements that may have the benefits of NSAIDs without the negative side effects. Because NSAIDs are proposed to work by inhibiting the cyclooxygenase (COX) enzymes, herbals with these same potential mechanisms were studied. The objectives of this study were to determine the effects of ginseng (*Panax quinquefolius*) and its constituents and a standardized *Ginkgo biloba* extract on COX-1 and COX-2

expression, rates of proliferation, and apoptosis in the human colon cancer cell lines HT-29 and HCT-15. These compounds were compared with the effects of curcumin, a botanical that has proven chemopreventive effects; ibuprofen, a nonselective COX inhibitor; and nimesulide, a selective COX-2 inhibitor. The concentrations of supplements required to inhibit colon cancer cell growth by 50% (IC<sub>50</sub>s) were determined using the MTS assay, and these levels of supplements were used for the remaining assays. Initial COX analysis has been performed, confirming that HT-29 cells express both COX-1 and COX-2 whereas HCT-15 cells do not. Furthermore, results from Northern blot analysis indicate that ginseng treatment selectively decreases levels of COX-2 mRNA expression in HT-29 cells. Additionally, studies indicate that ginseng treatment increases levels of apoptosis as seen with the TUNEL assay, and Western blot analysis indicates that caspase-3 and bax may be involved in this increase. Future studies will examine the effects of ginsenosides on COX expression and apoptosis, and the results from the in vitro experiments will be verified in a clinically relevant in vivo model.

### Phytoestrogens

**Derivation and Validation of a Biological Marker for Phytoestrogen Intake.** M. R. Ritchie,\* M. S. Morton,<sup>†</sup> N. Deighton,\*\* A. Blake,\*\* A. M. Thompson,<sup>‡</sup> J. H. Cummings,\* and C. M. Steel.<sup>††</sup> \*Department of Molecular and Cellular Pathology University of Dundee, Ninewells Hospital and Medical School, Dundee, Scotland, <sup>†</sup>Department of Medical Biochemistry, University of Wales College of Medicine, Heath Park, Cardiff, Wales, \*\*Scottish Crop Research Institute, Invergowrie, Dundee, <sup>‡</sup>Department of Surgery and Molecular Oncology, University of Dundee, Ninewells Hospital and Medical School, Dundee, and <sup>††</sup>School of Biology, Bute Medical School, University of St. Andrews, Fife, Scotland.

Phytoestrogens (PE) are naturally occurring bioactive compounds capable of provoking an estrogenic response in humans. In addition, their potential antioxidant and anticancer properties have been the basis for extensive research in this area of phytochemistry and nutrition. Two of the principal PE in food are genistein and daidzein. Previous studies have assessed dietary PE exposure by using food diaries and food frequency questionnaires, where intake values were estimated from measured genistein and daidzein concentrations in certain foods and entered onto a database. The databases in such studies do not appear to have been validated. Furthermore, although previous studies indicated a relationship between PE intake and urinary excretion of PE or concentrations of PE in plasma, there appears to have been no investigation of this relationship over a range of dietary PE intakes that would allow a biomarker of intake to be established. We constructed a comprehensive PE database that was subsequently validated by duplicate diet analysis. A biomarker of PE intake was then developed by investigating the relationship between dietary PE intake and 24-h urinary excretion of PE, concentrations of PE in a timed plasma sample, and PE in a timed spot urine sample. Twenty-four-hour urinary excretion of PE, timed plasma concentrations of PE, and spot urine PE were identified as suitable biomarkers of PE intake, with 24-h urinary PE > timed plasma PE > spot urine PE. Variation in PE intake over time was also monitored using a biomarker. The results validated the use of a biomarker of PE intake. The use of such a biomarker will assist future epi-

demologic studies by more accurately assessing dietary PE exposure. This may be of benefit in further disease-related research where the relationship between dietary PE exposure and relative risk of disease can be investigated. In addition, subject compliance can be measured during long-term PE intervention studies by the use of a biomarker.

**Soy Isoflavones Inhibit Cell Adhesion and Motility of Highly Invasive Breast Cancer Cells by a Urokinase Plasminogen Activator-Dependent and -Independent Mechanism.** Daniel Sliva,\*<sup>†</sup> Tatiana Valachovicova,\* Veronika Slivova,\* Heidi Bergman,\* Jennifer Shuherk,\* and F. P. Lloyd, Jr.\*<sup>†</sup> \*Methodist Research Institute Clarian Health Partners Inc., Indianapolis, IN, and <sup>†</sup>School of Medicine, Indiana University, Indianapolis, IN.

High consumption of soy products in some Asian countries has been linked to the low incidence of breast cancer in women. The chemopreventive effect of the soy isoflavone genistein has been observed through the suppression of cell proliferation, inhibition of angiogenesis, and stimulation of apoptosis in breast carcinoma cells. Cancer metastasis consists of interdependent processes, including cell adhesion, migration, and invasion. In this study we compare the effect of soy isoflavones in the form of aglycones (genistein, daidzein, and glycitein) and glucosides (genistin, daidzin, and glycitin) on the behavior of highly invasive breast cancer cells. We demonstrate that genistein suppresses cell adhesion and migration by inhibiting the constitutively active transcription factors nuclear factor (NF)- $\kappa$ B and activator protein (AP)-1, resulting in the suppression of secretion of urokinase plasminogen activator (uPA) in breast cancer cells MDA-MB-231. In addition, we show that all tested soy isoflavone aglycones and glucosides markedly reduced motility of MDA-MB-231 cells. However, only genistein and daidzein inhibited constitutively active NF- $\kappa$ B and AP-1 and suppressed secretion of uPA from breast cancer cells. Furthermore, genistein and daidzein inhibited adhesion of MDA-MB-231 cells to vitronectin (VN), which binds to  $\alpha$ <sub>v</sub> $\beta$ <sub>3</sub> integrin receptor, suggesting that the suppression of uPA secretion resulted also in the inhibition of the formation of uPA-uPAR-VN- $\alpha$ <sub>v</sub> $\beta$ <sub>3</sub> complex. Taken together, our results demonstrate that dietary soy isoflavones inhibit adhesion and motility of highly invasive breast cancer cells by a uPA-dependent and -independent mechanism.

### Folate

**Folate Bioaccessibility from Various Food Products Studied in a Dynamic In Vitro Gastrointestinal Model.** Robert Havenaar,\* Miriam Verwei,<sup>†</sup> Ana Belén Olivares,\*\* Karin Arkbåge,<sup>‡</sup> Gaspar Ros,\*\* Cornelia Witthöft,<sup>‡</sup> Caroline Walker,<sup>‡‡</sup> Emilia Carnovale,<sup>‡‡</sup> Susanna Kariluoto,<sup>#</sup> and Paul Finglas.<sup>§</sup> \*TNO Nutrition and Food Research, Zeist, The Netherlands, <sup>†</sup>Wageningen University and Research, The Netherlands, \*\*University of Murcia, Spain, <sup>‡</sup>Swedish University of Agricultural Sciences, Uppsala, <sup>††</sup>Brewing Research International, Surrey, UK, <sup>‡‡</sup>Istituto Nazionale della Nutrizione, Rome, <sup>#</sup>University of Helsinki, and <sup>§</sup>Institute for Food Research, Norwich, UK.

Folate may be associated with reduced risks of vascular disease and cancer. A limiting factor can be the bioavailability from

various food sources. We studied the bioaccessibility of folate (5MTHF) and folic acid (PGA) from dairy products, drinks, rye bread, and vegetables fortified or processed under various conditions. The experiments were performed in a validated, computer-controlled gastrointestinal model (TIM), simulating physiological conditions in stomach and small intestine of humans. From nonfortified pasteurized milk with 7  $\mu\text{g}$  folate/100 g, 5MTHF was 86%  $\pm$  2% bioaccessible. From pasteurized and ultra-high-temperature milk fortified with 40  $\mu\text{g}$ /100 g, PGA was 74–77%  $\pm$  2% bioaccessible and 5MTHF was 90%  $\pm$  2% bioaccessible. From fortified fermented milk, PGA and 5MTHF were 82–90% bioaccessible. However, folate bioaccessibility was significantly lowered by the addition of folate binding protein (1:1 to folate) in PGA-enriched milk and the addition of 5MTHF and PGA in enriched fermented milk. The bioaccessibility of folates from orange juice (40  $\mu\text{g}$ /100 g, heat or high-pressure pasteurized) and beer (11  $\mu\text{g}$ /100 g, with or without alcohol) was 87–89%  $\pm$  3% and 83–87%  $\pm$  1%, respectively. From peas (canned, 38  $\mu\text{g}$ /100 g; frozen, 65  $\mu\text{g}$ /100 g), spinach (soup, 53  $\mu\text{g}$ /100 g; leaves, 110  $\mu\text{g}$ /100 g), rye bread (78  $\mu\text{g}$ /100 g), and gazpacho (49  $\mu\text{g}$ /100 mL), the availability for absorption was 78%  $\pm$  3%, 82%  $\pm$  4%, 88%  $\pm$  2%, and 94%  $\pm$  5%, respectively. Because of high folate bioaccessibility, dairy products are good sources for folate fortification if folate binding protein is not added. Because of the high content and bioaccessibility of folates, processed food products such as spinach, peas, gazpacho, rye bread, and orange juice seem good sources of folates. However, complex matrices and mucosal folate transport could lower bioavailability. Further research should focus on combined food products and intestinal transport. [Supported by EU project "Folate: from food to functionality and optimal health" QLK1-1999-00576.]

**Bioavailability: Why Studies of the Association between Low-Folate Dietary Intake and Risk of Cervical Cancer Are Inconsistent.** Capri-Mara Fillmore Medical College of Wisconsin, City of Milwaukee Department of Health, Milwaukee, WI.

Cervical cancer (and dysplasia) studies of the association of low folate as measured by dietary intake generally concluded that no association exists. However, biomarkers of folate status often suggest an association. Folate bioavailability varies greatly between foods as well as between food and fortification/supplements. The dietary folate equivalent (DFE) was designed to take this into account. The DFE, however does not take into account differences in percentage of absorption between foods (e.g., the absorption of lima beans is 4.5% whereas that of beef liver is 72%) but assumes that absorption of all foods is 50%. The Block98.2 Questionnaire was completed by 67 women of reproductive age; 9 questionnaires were discarded because of abnormally high or low intake. Information on folate food bioavailability is unknown for many foods, so estimates of availability by food group were made (e.g., fortified folate availability is 62.5%). Depending on which measurement used, mean folate values varied greatly: 305  $\mu\text{g}/\text{d}$  ( $\pm$ 161; range 86–670) if one uses the folate measurement methodology found in most studies; 637  $\mu\text{g}$  ( $\pm$ 404; range 86–1248) and 786  $\mu\text{g}$  ( $\pm$ 387; range 127–1475), respectively, for DFEs before and after fortification; and 466  $\mu\text{g}/\text{g}$  ( $\pm$ 197; range 143–938) for mean bioavailable folate after fortification. Four women, one from each quartile of prefortification DFEs, varied greatly in assigned quartile depending on the measure used. This misclassification may account for inconsistencies between folate intake and

cervical cancer as well as other diseases, suggesting that bioavailability may be an important confounder. Additionally, caution may also be needed for other nutrients, such as iron and calcium (well known for variability in bioavailability by type of food), when dietary intake analysis is used to determine disease associations.

**The Relationship between Dietary Intake and Genetic Stability, as Measured by the Amount of Oxidative DNA Damage and Uracil Misincorporation in the DNA of Single Lymphocytes.** M. Kapiszewska,\* M. Kalemba,\* U. Wojciech,\* A. Cierniak,\* and T. Milewicz.<sup>†</sup> \*Faculty of Biotechnology Jagiellonian University, Krakow, and <sup>†</sup>Collium Medicum, Jagiellonian University, Krakow, Poland.

We investigated the associations of base DNA damage level with nutritional status in female students before and after 4 wk of folic acid supplementation (400  $\mu\text{g}/\text{d}$ ). Nineteen subjects recorded their food, beverage, herb, and spice intake for 2 weekdays and 1 weekend day for 3 wk in a winter month (9 records). All individual questionnaires were computerized, and the amount of dietary compounds were estimated with a specifically developed program linked to recently updated Polish food tables. The alkaline single-cell gel electrophoresis assay (comet assay) with enzyme modification was used for the detection of endogenous DNA damage in individual cells. Blood samples were taken during the study for the evaluation of DNA oxidative damage and uracil misincorporation into DNA. After 4 wk of folate supplementation, blood samples were collected and the comet assay analysis was performed. In addition to estimating the folate intake from daily food intake, we measured plasma concentrations of folate and vitamin B-12. The statistical analysis showed that the measured DNA damage was not associated with energy, complex carbohydrate, or saturated fatty acid intake. However, a positive correlation ( $P < 0.05$ ) between DNA oxidative damage and flavonoid intake, particularly flavons and flavonols, was observed only after folate supplementation; a negative correlation ( $P < 0.05$ ) between flavonoid intake and uracil misincorporation was detected before folate supplementation. Moreover, the concentration of folate in serum was negatively associated with uracil misincorporation into DNA. DNA damage was also studied in lymphocytes in vitro after incubation with quercetin.

#### *Polyunsaturated fatty acids*

**The Mechanism of Skin Cancer Induced by n-6 Polyunsaturated Fatty Acids and HPV16E6/E7.** Dazhi Chen, Mei Qi, Walter Ralph, JR., and Karen Auburn. North Shore-Long Island Jewish Research Institute, Manhasset, NY.

**BACKGROUND:** Skin cancer is the most common cancer among Caucasians in the United States, with an estimated one-half million new cases per year. Many sets of data indicate that infection with certain types of human papillomaviruses (HPVs) occur early and become latent and that prolonged UV irradiation is needed to activate viral gene functions. Recent studies indicate that fat is also a factor in the development of nonmelanoma skin cancer (NMSC) and that recurrences can be prevented by a low-fat diet. In our early studies, we demonstrated that both HPV type 16 and a high-fat diet (n-6 polyunsaturated fatty acids [PUFAs]) are cofactors during skin cancer induction in a mouse model that constitutively expresses

HPV type 16 transgenes for E6/E7 driven by the human keratin 14 promoter. **PURPOSE:** Our purpose was to investigate the mechanism for skin cancer induced by linoleic acid (an n-6 PUFA) and HPV16E6/E7. **METHODS:** Immunohistochemistry was done on tissues from benign and malignant skin lesions from our mouse study using antibody against cyclooxygenase-2 (COX-2), an enzyme that increases proliferation and inflammation. Other studies used HPV16E6/E7 immortalized human skin cells (HEK-001). A Western blot was used for detecting COX-2 and GADD153, a growth-arrest protein produced during differentiation of mouse skin. Real-time reverse transcription-polymerase chain reaction was used to evaluate COX-2 expression at the RNA level. A luciferase assay was used to determine the expression driven by the GADD153 promoter. Immunostaining was used to detect active MAP kinase (antibody against active MAP kinase) and proliferating cell nuclear antigen (for cell proliferation). **RESULTS:** The mouse lesions, both benign and malignant, expressed COX-2. n-6 PUFA increased proliferation of HEK-001 cells. Similarly, n-6 PUFA increased COX-2 at both the RNA and protein levels and decreased expression driven by the GADD153 promoter and GADD153. Active MAP kinase was increased. **CONCLUSION:** Our results indicated that n-6 PUFA up-regulated COX-2 and down-regulated GADD153. Furthermore, our results suggest that the MAP kinase pathway may be involved in skin cancer induced by n-6 PUFA and HPV16E6/E7.

#### Vitamin D

**Nutritional Soy Rescues the Mouse Colon from Hyperproliferation and Supports Colonic Vitamin D Synthesis.** H. S. Cross, E. Kallay, E. Bajna, and D. Lechner. Department of Pathophysiology, University of Vienna Medical School, Austria.

Epidemiologic studies have suggested a beneficial effect of sunshine against colorectal cancer incidence. Cholecalciferol (vitamin D<sub>3</sub>) is produced in the skin by UV energy. The active hormonal metabolite 1,25-dihydroxyvitamin D<sub>3</sub> (1,25-D) is known to be antimetabolic and prodifferentiating in cancer cells. We were the first to demonstrate presence of 1,25-D synthesizing (CYP27B1) and degrading (CYP24) enzymes as well as of the vitamin D receptor (VDR) in human colon cancer cells and tissues. Interestingly, expression of enzymes and of the steroid hormone receptor at the mRNA and protein level is up-regulated early during tumor progression whereas expression in late-stage high-grade tumors is diminished. Our studies in VDR knockout mice have demonstrated that the lack of genomic action of 1,25-D via VDR would result in enhanced proliferation and in DNA lesions. Such data suggest that the vitamin D system may be a physiological defense against tumor progression, losing its efficacy during late-stage malignancy. Proper functioning of this defense would necessitate high levels of 1,25-D synthesizing enzymes and low levels of catabolic enzymes in the colon. We were able to demonstrate in a mouse model that soy feeding or genistein gavage results in enhanced expression of CYP27B1 and reduced expression of CYP24. When mice were fed a low-calcium diet (0.04% vs. 0.9% of normal calcium levels), expression of CYP24 mRNA was increased at least 14-fold in parallel to enhanced proliferation of the colon mucosa, whereas mice on the low-calcium diet fed with 10% soy showed a reduction of CYP24 mRNA expression to normal low levels. Our data demonstrate that the raised catabolism of 1,25-D occurring during increased proliferation of the colon mucosa can

be counteracted by soy nutrition. Decreased catabolism would result in the enhanced presence of the steroid hormone in tissues. This in turn could support normal colonic growth and could inhibit tumor progression. [Supported by the World Cancer Research Fund, London, UK.]

**Enhanced Nutritional Calcium Up-Regulates Extrarenal Synthesis of the Tumor-Preventing Steroid Hormone 1,25-Dihydroxycholecalciferol in the Mouse Colon.** Enikő Kállay,\* Maya Khorchide,\* Manuel Zeitelhofer,\* Ilse Steffan,<sup>†</sup> and Heide S. Cross.\* \*Department of Pathophysiology, University of Vienna Medical School, and the <sup>†</sup>Institute of Analytical Chemistry, University of Vienna, Austria.

Colonic epithelial cell hyperproliferation and hyperplasia induced in mice fed a Western-style diet can be reversed by the addition of calcium to the diet. We propose that calcium in the intestinal lumen can mediate its growth-regulatory and cancer-preventing properties not only by binding and eliminating harmful bile acids but also by a direct effect on colonocytes. Therefore we investigated its concentration-dependent action on proliferating cell nuclear antigen (PCNA) expression by immunohistochemistry and on expression of CYP27B1 (25-cholecalciferol-1 $\alpha$ -hydroxylase) and of estrogen receptors alpha and beta (ER- $\alpha$ , ER- $\beta$ ) by real-time reverse-transcription polymerase chain reaction. Mice were fed a modified AIN-76 diet containing 20% lactose and 0.9%, 0.1%, or 0.04% calcium. We measured the calcium content of the feces, which decreased depending on the calcium concentration of the diet (53.4, 9.7, and 2.4 mg/g feces, respectively). When we evaluated PCNA expression, we found that only 0.1% calcium (compared with 0.9% calcium) had a consistently promitotic effect on crypts. Crypts of mice fed the diet containing 0.04% calcium displayed almost no positive reaction for PCNA, both in proximal and distal colon. CYP27B1 expression in ascending colon was higher than in descending colon. Low-calcium diets significantly reduced CYP27B1 mRNA expression in both segments of the colon. ER- $\alpha$  gene expression was elevated both in colon ascendens and descendens in mice fed the 0.1% calcium-containing diet but not in mice fed 0.04% calcium. There are much higher levels of ER- $\beta$  in colon descendens than in colon ascendens regardless of the calcium concentration. In the distal colon, feeding 0.04% calcium also results in considerable induction of ER- $\beta$ . We conclude that colonic synthesis of 1,25-dihydroxycholecalciferol is, in contrast to 1 $\alpha$ -hydroxylation in the kidney, down-regulated by low calcium. Low dietary calcium levels increased proliferation of colonocytes. This high proliferation rate correlated with up-regulated ER- $\alpha$  and, at least in the distal colon, also with upregulation of ER- $\beta$ . [Supported by the Austrian National Bank.]

**Hormone Regulation of Vitamin D Receptor Promoter Activity in Breast Cancer Cells.** J. A. Wietzke, E. C. Ward, and J. E. Welsh. Department of Biological Sciences, University of Notre Dame, Notre Dame, IN.

1,25-Dihydroxycholecalciferol (1,25-D), the biologically active form of vitamin D, binds to the nuclear vitamin D receptor (VDR) to transactivate target genes involved in cell cycle arrest, differentiation, and apoptosis. We previously identified a novel promoter region upstream of exon 1c in the human VDR gene and demonstrated that hormones and growth factors up-regulated activity of this VDR promoter. We hypothesize that

upregulation of VDR will increase cell sensitivity to the effects of 1,25-D and vitamin D analogs used in the treatment of breast cancer. In these studies we used T47D, an estrogen receptor-positive human breast cancer cell line. Estrogen and phytoestrogens significantly up-regulated VDR promoter activity in these cells and Western blot analysis confirmed that VDR protein expression was consistently increased in response to doses that up-regulated the promoter. We used site-directed mutagenesis and gel shift analysis to determine that the mechanism by which hormones up-regulate VDR promoter activity involve several Sp-1 sites within the promoter region. We show that upregulation of the VDR by phytoestrogens increased transactivation of target genes and increased cellular sensitivity to vitamin D analogs. We also determined that estrogens had no effect on the activity of the VDR promoter in HKC-8 kidney cells. This suggests that sensitization would not also lead to increased calcemic side effects even though the VDR promoter is regulated by other hormones and treatments in HKC-8 cells. Cell-type-specific regulation of VDR may enable optimization of tumor response to vitamin D analogs while minimizing responses to calcium mediated through VDR in the kidney. Our studies offer proof that dietary components can sensitize cells to the effects of vitamin D analogs and may lead to better recommendations for women on vitamin D analog therapy for breast cancer. [Supported by the Department of Defense and the National Institutes of Health, Department of Health and Human Services].

### Tocopherols

**$\gamma$ -Tocopherol Up-Regulates Peroxisome Proliferator Activated Receptor  $\gamma$  Expression and Achieves a Higher Intracellular Concentration in SW480 Colon Cancer Cell Lines.** Sharon Campbell, Sarah Whaley, William Stone, and Koyamangalath Krishnan. Department of Internal Medicine and Pediatrics, East TN State University, and James H. Quillen VA Medical Center, Johnson City, TN.

**BACKGROUND:** Tocopherols are lipid-soluble antioxidants. Eight structurally different isoforms of vitamin E are recognized. The dietary intake of  $\gamma$ -tocopherol is higher than  $\alpha$ -tocopherol in the U.S. diet. The results of the effects of vitamin E as a potential cancer preventive agent have been mixed. All published clinical trials with vitamin E have used the  $\alpha$ -tocopherol form. Recent epidemiologic, experimental, and molecular studies suggest that  $\gamma$ -tocopherol may be the more potent form of vitamin E. In this investigation we tested the effects of both  $\alpha$ - and  $\gamma$ -tocopherol on the expression of peroxisome proliferator activated receptor (PPAR) mRNA and protein in SW480 colon cancer cell lines. We also measured the intracellular concentrations of vitamin E in SW 480 colon cancer cell lines by HPLC. **RESULTS:** We have discovered that the alpha and gamma isoforms of vitamin E up-regulate PPAR  $\gamma$  RNA and protein expression in the SW480 colon cancer cell lines.  $\gamma$ -Tocopherol is a better modulator of PPAR expression than  $\alpha$ -tocopherol at the concentrations tested. Intracellular concentrations increased as the vitamin E concentration added to the media was increased. Furthermore, cells treated with  $\gamma$ -tocopherol have higher intracellular tocopherol concentrations than do those treated with the same concentrations of  $\alpha$ -tocopherol. **CONCLUSION:** Our data suggest that both  $\alpha$ - and  $\gamma$ -tocopherol can up-regulate the expression of PPAR  $\gamma$ , considered an important target for colon cancer chemoprevention. We show that both the expression of the mRNA

and protein are increased and the effects are more pronounced with the  $\gamma$ -tocopherol. We also show that the intracellular concentrations of  $\gamma$ -tocopherol are severalfold higher than  $\alpha$ -tocopherol. Further work on other colon cancer cell lines is required to quantitate differences in ability of these forms of vitamin E to induce apoptosis and suppress cell proliferation and its effects in conjunction with other chemopreventive agents. Ligand binding assays are being pursued to determine whether vitamin E is a synthetic ligand for PPAR activity.

**$\gamma$ -Tocopherol Is Superior to  $\alpha$ -Tocopherol in the Induction of Growth Arrest of Human Colon Cancer Cell Lines HCT 116 and SW 480.** Devin Sherman, Sharon Campbell, Sarah Whaley, William Stone, and Koyamangalath Krishnan. Department of Internal Medicine and Pediatrics, East TN State University, and James H. Quillen VA Medical Center, Johnson City, TN.

**BACKGROUND:**  $\gamma$ -Tocopherol is a major component of tocopherols in the U.S. diet and the second most common tocopherol in human serum. Until recently, most interest in the antioxidant and protective effects of tocopherols was focused only on  $\alpha$ -tocopherol.  $\alpha$ -Tocopherol is the main form of vitamin E in supplements.  $\gamma$ -Tocopherol is present in rich amounts in plant seeds (corn, soybean, sesame), vegetable oils, and nuts (walnuts, pecans, peanuts).  $\gamma$ -Tocopherol (vitamin E) may be more efficient than  $\alpha$ -tocopherol in arresting cancer progression. Epidemiologic and experimental data show that  $\gamma$ -tocopherol is more efficient at prostate cancer prevention than is  $\alpha$ -tocopherol. **METHODOLOGY:** We looked at the effects of the vitamin E isoforms at different concentrations (5, 10, 25, and 100  $\mu$ mol/L) on cell viability in SW 480 (adenomatous polyposis coli [APC] truncated, cyclooxygenase [COX]-2 negative) and HCT 116 (APC wild type, COX-2 inducible) colon cancer cell lines. We used the Live/Dead Viability/Cytotoxicity Assay Kit (Molecular Probes, Eugene, OR), which provides a 2-color fluorescence based on the simultaneous determination of live and dead cells with 2 probes that measure 2 recognized parameters of cell viability (intercellular esterase activity and plasma membrane integrity). In addition, standard cell counts and the trypan blue exclusion assay were used to determine the percentage of cell death over hours. **RESULTS:** In both HCT116 and SW480 cell lines, maximal cell death occurred at a concentration of 100  $\mu$ mol/L for both  $\alpha$ -tocopherol and  $\gamma$ -tocopherol. In HCT 116 cell lines,  $\gamma$ -tocopherol was able to kill 80% and 90% of the cells at days 2 and 5, respectively;  $\alpha$ -tocopherol was able to kill only 5% and 20%. In SW480 cell lines,  $\gamma$ -tocopherol was able to kill 60% of the cells at day 2 compared with 21% by  $\alpha$ -tocopherol. **CONCLUSIONS:** Our work adds to the growing body of evidence that supports the idea that  $\gamma$ -tocopherol may be the more relevant form of vitamin E in cancer prevention. We show a differential effect of  $\gamma$ -tocopherol and  $\alpha$ -tocopherol in their ability to induce cell death in 2 human colon cancer cell lines with different molecular characteristics. In both cell lines at the time and concentration tested,  $\gamma$ -tocopherol is more efficient in inducing cell death. The molecular basis of these differences is being explored.  $\gamma$ -Tocopherol needs to be given more attention in colon cancer prevention strategies and in human nutrition models.

### Dietary metals

**Methylselenol, the Active Metabolite of Selenium, Selectively Inhibits Protein Kinase C in Precancer Cells at the Pro-**

**motional Stage of Carcinogenesis. Rayudu Gopalakrishna, and Usha Gundimeda.** Department of Cell and Neurobiology, Keck School of Medicine, University of Southern California, Los Angeles, CA.

Besides scavenging free radicals, antioxidants such as selenium inhibit cell signaling and inhibit tumor promotion. However, it is not clear how selenium interferes with cell signaling selectively in precancer cells but not in normal cells. Methylselenol was previously postulated to be the active metabolite that mediates cancer-preventive actions of selenium. Nevertheless, it is a volatile compound and its mechanism of action, especially that occurring at bioavailable low concentrations, is not known. We previously showed that redox-active selenocompounds directly inactivate protein kinase C (PKC) by inducing sulfhydryl oxidation. Because selenium is required at low concentrations to inhibit tumor promotion, the oxidants present in promoting cells may enhance cellular retention and the preventive actions of volatile methylselenol. Methylselenol, benzeneselenol, and selenol metabolites of ebselen at catalytically low concentrations induced PKC inactivation only in the presence of peroxides such as arachidonic acid hydroperoxide and *t*-butyl hydroperoxide. By reducing peroxides, selenol was oxidized to seleninic acid, which in turn oxidized protein sulfhydryls and thus was reduced back to selenol. Although this peroxidatic redox-cycling process nonspecifically oxidizes many thiols including glutathione, the lipophilic selenols and organic peroxides present in the membrane can selectively oxidize sulfhydryls of key membrane-bound enzymes such as PKC. Application of sublethal doses of these peroxides and organic selenols to JB6 epidermal cells resulted in inactivation of PKC  $\alpha$  isoenzyme, induction of apoptosis, and inhibition of cell transformation. Conceivably, the reaction of selenols with tumor-promoting peroxides provides one of the specific mechanisms by which cancer-preventive selenocompounds selectively interfere with signaling in the promoting cells but not in the normal cells.

**The Relationship among Selenium in Nutrition, Selenoproteins, Oxidative Stress, Lipid Peroxidation, and Initiation of Cancer. E. Eder,\* D. Kratzin,\* P. Wanek,\* Z. Zhou,\* J. Köhrle,<sup>†</sup> and R. Brigelius-Flohe.\*\*** \*University of Würzburg Germany, <sup>†</sup>Charite, Humboldt University of Berlin, Germany, and \*\*German Institute of Human Nutrition, Bergholz-Rehbrücke, Germany.

Selenium plays a prominent role in glutathione peroxidase (GPx) selenoproteins for the cellular antioxidant defense system and thus in the prevention of DNA damage, cancer initiation, and cancer progression by reactive oxygen species and lipid peroxidation. The promutagenic 1,*N*<sup>2</sup>-propanodeoxyguanosine adducts of the lipid peroxidation product 4-hydroxy-2-nonenal are highly specific markers for initiation of cancer cells and of cancer progression induced by oxidative stress and lipid peroxidation. The role of selenium and selenoproteins in the reduction of oxidative DNA damage is clearly reflected by the *in vivo* levels of these hydroxynonenal DNA adducts. There is a clear relationship between a decrease in the selenium and tocopherol content of the animal diet and an increase of these DNA adduct levels induced by carbon tetrachloride-initiated lipid peroxidation in the small intestine and in the liver of F 433 rats. In addition, a clear positive relationship was found between the selenium content in the livers of rats and enzyme activities of the GPx and the thioredoxin reductase and an inverse

correlation was found between these parameters (selenium content and GPx activities) and the DNA adduct levels in the rat livers. These results are confirmed by studies in HepG2 cells stably transfected with the phospholipid hydroperoxide GPx selenoprotein. In nontransfected cells we found a background of  $196 \pm 36$  hydroxynonenal adducts/ $10^9$  nucleotides whereas in transfected cells the adduct levels were in the range of the detection limit of 2 adducts/ $10^9$  nucleotides. This is a dramatic decrease in adduct levels in transfected cells as compared with normal HepG2 cells. Adduct levels in HepG2 cells transfected with gastrointestinal GPx (GI-GPx) and cytosolic GPx (cGPx) are being measured to compare the potency of different GPx in suppression of lipid peroxidation-induced DNA adducts.

**Dietary Metals, Phytochemical, and Cancer. Orkide Donma\* and M. Metin Donma.<sup>†</sup>** \*Istanbul University, Cerrahpasa Medical Faculty, Istanbul, and <sup>†</sup>Ministry of Health, Suleymaniye Education and Research Hospital, Istanbul, Turkey.

Normal human diet contains both essential and toxic metals. Some metals are essential for human nutrition, others are found as contaminants in foodstuffs. The deficiencies and overload of essential elements result in adverse effects, which is also the case for some phytochemicals recently introduced. Among physiologically important metals exhibiting double action, iron and copper are involved in DNA damage and mutagenesis. Free iron favors reactions that form free radicals. Both DNA and telomeric sequence are susceptible to damage from copper-mediated reactive oxygen species. Selenium is another essential metal whose antioxidant function is well known. The protective role of selenium with respect to genetic damage and cancer is important. However, intake of excessive doses of selenium may cause oxidative damage leading to genomic instability. There is some doubt on the antioxidant nature of foods or beverages containing phytochemicals because of the other constituents in the foods. Red wine and purple grape juice are recommended because of their high polyphenol content whereas wine appears to contain also appreciable amounts of arsenic, which originates from arsenic-containing insecticides used on the grapes. Arsenic is a well-documented carcinogen and also appears to be a valuable therapeutic tool in cancer treatment. This is the paradox of arsenic. Evidence from epidemiologic and experimental data has linked high intake of fruits and vegetables to lower cancer risk. Controversial data, however, show that substances with presumed anticarcinogenic properties may not only exert protective effects. These substances, as in Cruciferae family, can also be involved in the induction of multistep carcinogenesis by acting as genotoxic agents; cocarcinogens; or promoters, stimulating procarcinogen activating enzymes. In this context, healthy and harmful effects of some nutrients are debated. In the meantime, as the benefits of a diet rich in fruits and vegetables are emphasized, attempts for regular mass administration of single phytochemical or metal should be done prudently.

#### *Insulin-like growth factor 1*

**The Relation between Nutritional Factors and Insulin-Like Growth Factor I in Premenopausal Women of Different Ethnicity. Gertraud Maskarinec,\* Yumie Takata,\* and Rudolph Kaaks.<sup>†</sup>** \*Cancer Research Center of Hawaii, Honolulu, HI, and <sup>†</sup>International Agency for Research on Cancer, Lyon, France.



Insulin-like growth factor-I (IGF-I) and one of its binding proteins (IGFBP-3) have been proposed as a link between nutritional factors and cancer risk. This study explored possible dietary determinants of IGF-I levels in healthy premenopausal women who participated in a nutritional intervention and did not take hormones during the previous year. At baseline, all subjects donated blood early in the morning ~5 d after ovulation and completed a validated food frequency questionnaire. Serum concentrations of IGF-I and IGFBP-3 were measured by double-antibody enzyme-linked immunosorbent assay. C-peptide concentration was assessed in a subgroup of 115 women. After creating quartiles of 16 nutrients and 12 foods, least-square means of IGF-I, IGFBP-3, and the IGF-I/IGFBP-3 molar ratio were calculated by quartile while adjustments were made for age, body mass index, year of analysis, and energy intake. The majority of the 97 Caucasian, 61 Japanese, 21 Chinese, 14 Filipino, 31 Hawaiian, and 34 other/mixed subjects were born in the United States. The mean age of the 258 subjects was 43 years. Whereas body mass index and dietary intakes varied significantly by ethnicity, IGF-I, IGFBP-3, and their ratio did not differ among groups. We observed weak positive associations of dietary fiber and fat and oil intake with IGFBP-3 as well as a negative association between fish consumption and the IGF-I/IGFBP-3 ratio. Adjustment for ethnicity did not change these findings. Among Caucasians, legume consumption was positively associated with IGF-I and IGFBP-3 whereas vitamin B-12 intake was weakly associated with IGF-I in Asian women. After adjustments were made for C-peptide levels in the subgroup, vitamin A and iron were positively associated with IGF-I and vitamin C was positively associated with the ratio whereas protein, saturated fat, cholesterol, zinc, grains, and meat correlated directly with IGFBP-3. In conclusion, this study detected no ethnic differences in IGF levels and observed a small number of weak correlations with dietary variables that require further investigation.

#### Weight control

**ACTIVATE: A Childhood Overweight Prevention Initiative.** Susan T. Borra, Elizabeth P. Davenport, Richard B. Elder, and Allison J. Esser. International Food Information Council Foundation, Washington, D.C.

**PURPOSE:** Overweight/obesity is a major public health problem, particularly among children. Effective partnerships and communication programs are needed to develop childhood overweight prevention strategies that incorporate nutrition and physical activity and affect children at home, in school, and in the community. **SIGNIFICANCE:** ACTIVATE, a unique partnership of the American Academy of Family Physicians, American College of Sports Medicine, American Dietetic Association, International Food Information Council Foundation, International Life Sciences Institute Center for Health Promotion, and National Recreation and Park Association developed an overweight prevention communications program (Kidnetic.com) targeted to children ages 9–12 and their parents. **METHODS:** Two and a half years of in-depth qualitative consumer research—focus group, ethnographic, in-home interviews and quantitative—was used to track consumer knowledge and perceptions of the overweight problem, define appropriate audiences for messages, and develop customized program elements. The research included 16 focus group interviews among children (grades 3–7), parents, and teachers and miniethnographic studies that included in-home interviews,

observation, and diaries of six families. Reactions to communication concepts were obtained during 25 one-on-one interviews conducted with 17 sixth and seventh graders and 8 parent pairs. **RESULTS:** Based on the results of this research, the ACTIVATE partnership developed an innovative, interactive, and educational Web site that delivers healthy eating and physical activity messages to children and their families in ways that are engaging, relevant, and meaningful to help prevent childhood overweight. Kidnetic.com was launched in June 2002. With over 975,000 visits to the site since June 2002, Kidnetic.com is effectively reaching kids and parents. **CONCLUSION:** Healthy lifestyle information can be successfully delivered to children and families through a Web site designed around their needs and interests. Kidnetic.com is a useful childhood overweight prevention tool that can be useful in cancer prevention education.

**Diet-Dependent Metabolic Serotypes: Markers for Cancer Risk?** Bruce S. Kristal,\*† Ugo Paolucci,† Yevgeniya Shurubor,† and Wayne R. Matson.\*\* \*Departments of Biochemistry and Neuroscience, Cornell University Medical College, NY, †Dementia Research Service, Burke Medical Research Institute, White Plains, NY, and \*\*ESA, Inc., Chelmsford, MA.

Dietary or caloric restriction (DR) 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. Likewise, increased body mass index is associated with a 2-fold increase in postmenopausal breast cancer risk in humans. To further explore the relationship between caloric intake and cancer risk, we have identified a biochemical profile that reflects the DR metabolome. Exploratory studies previously identified 93 redox-active small molecules from sera (measured by HPLC coupled with coulometric detector arrays) with potential to distinguish dietary groups in both male and female rats. Classification power was addressed using megavariate data analysis approaches. Discrimination of samples from freely fed and DR rats by principal components analysis (PCA) was weak because of noise resulting from intercohort sampling. Soft independent modeling of class analogy, which builds independent PCA models of each class of interest, distinguished groups with 95% accuracy but overfit models built on single cohorts. In contrast, partial least squares projection to latent structures discriminant analysis (PLS-DA), a projection method optimized for class separation, built models with >95% accuracy in distinguishing groups without obvious cohort interference. Data processing choices of transformation, scaling, and outlier removal (winsorizing) each affected the strength of the models and, in some cases, revealed distinct metabolites of importance in building these models, often in gender-specific ways. Approaches based on computational biology suggest that the models have potential for >99.5% accuracy in larger cohorts. Diets varying in extent and duration of DR were used to develop models for intermediate caloric intakes, which are more relevant for human studies. We will present the models, their ability to distinguish sera based on caloric intake, and the potential for moving these markers to human sera.

**Limited Diet and Cancer Frequency in Poor Rural Indigenous Communities.** Miriam Muñoz,\* Adolfo Chávez,† Roxana

Rosales,<sup>†</sup> and Virginia Melo.\* \*Universidad Autónoma del Estado de Morelos, México, and <sup>†</sup>Instituto Nacional de Ciencias Médicas y Nutrición, Mexico City, México.

An epidemiologic study was carried out for 4.5 y in 6 indigenous communities. A sample of 1165 families with 2635 adults were surveilled. Information on cancer cases throughout the region for the past 10 y was collected during interviews with the families and from health personnel and statistics. In the region and in most indigenous communities, the diet has started to change because of the consumption of more fats, mainly from lard, some pork products, and salty and sugary packed products. This change has begun an epidemiologic transition, because obesity and diabetes are increasing. The objective of the study was to evaluate whether the transition is already promoting the incidence of cancer. Among the 2635 adults only 5 cases of cancer were found: 3 of the cervix and 2 (in men) of the stomach. Among the 2800 families of the region, only 2 more cases were detected: 1 of the cervix and 1 of the stomach. The incidence of cancer found was 0.42 per 1000 adults per year in the sample surveilled and 0.14 per 1000 adults per year in the region. Although the cancer incidence rates were low, the population is young, the facilities in the area for diagnosing cancer are not good, and the types of cancer found are more linked to infection than dietary factors. We conclude that the epidemiologic transition regarding cancer has not started. The difference in other chronic diseases such as obesity and diabetes must be due to the limited dietary changes and possibly to genetic characteristics. The communities should be revisited periodically to survey the changes in lifestyle and cancer incidence.

#### Functional foods

**Functional Food Labels: Experimental Evidence of the Role of Claims and Endorsements.** Ratapol P. Teratanavat,\* Derek D. Rucker,<sup>†</sup> Curtis P. Haugtvedt,\*\* and Neal H. Hooker.\* \*Department of Agricultural, Environmental, Development Economics, <sup>†</sup>Department of Psychology, and \*\*Department of Marketing and Logistics, Ohio State University, Columbus, OH.

Experimental data evaluating the role of various aspects of diet-health related information provided on functional food labels are presented. Subjects (285 students) were randomly assigned to receive different label information from the front of a product and the nutrition facts for a hypothetical food (tomato juice with added soy protein) with potential cardiovascular and anticancer benefits. The packaging of the product contained health claims, phytochemical content claims, or simple product information. In addition, an argument quality manipulation (an adjustment for the Nutrition Fact Panel)—a healthy and an unhealthy nutritional profile—was included. The role of product endorsement and the presence of medical association names and logos

(e.g., American Heart Association, American Institute for Cancer Research) validating the diet to the health link with phytochemicals was assessed. After exposure to the particular stimulus material, attitudes toward the product, purchase intentions, participant personality characteristics, demographics, and health-related knowledge were measured. This poster reports measures of overall attitudes toward the product and intent to purchase the product and discusses moderation of these measures by the manipulated variables and personal characteristics. The effect of real and hypothetical claims and endorsements is discussed. The argument quality manipulation is used to examine the possibility that health claims can mask an otherwise bad nutritional profile and is therefore related to the recent delisting of American Heart Association–endorsed beef products because of excessive fat content. The results suggest that the level of health information on a product label affects consumer attitude and product label evaluation.

**Beneficial Effects of Chitosan on the Nutritional Disorders in Rats Exposed to Various Levels of Lead.** Yeon-Sook Lee,\* Joo-Ran Park,\* and Meehye Kim.<sup>†</sup> \*Department of Food and Nutrition, Seoul National University, Seoul, Korea, and <sup>†</sup>Department of Food Evaluation, Korea Food & Drug Administration, Seoul, Korea.

With increasing industrialization, concerns have arisen about nutritional disorders induced by environmental contaminants such as lead. The aim of this study was to determine the preventive effects of *chitosan*, which is recognized as a functional food compound, on the nutritional damage in rats exposed to various levels of lead. Male Sprague-Dawley rats were divided into 8 groups ( $n = 64$ ) and then fed diets containing 3% cellulose (control) or 3% *chitosan*, each with 4 different lead doses (0, 20, 50, or 100 mg/d) for 4 wk. Lead doses were given 3 times per week by oral administration. There was no significant difference in weight gain and food intake among groups of rats. Serum lead levels in rats increased depending on the administered doses of lead. Rats fed *chitosan* diets showed lower serum lead concentration than did their respective controls. The effect of *chitosan* on the serum lead was more beneficial in rats exposed to lower lead (20 mg/d) than in rats exposed to higher lead (50 and 100 mg/d). Histological changes in erythrocytes and liver were also examined. *Chitosan* tended to reduce numbers of basophilic stippling erythrocytes and improve the histological liver changes in rats given various lead doses. The preventive effects of *chitosan* on liver damage were stronger in rats with higher lead than those with lower lead. These results indicate that *chitosan* has beneficial effects on both serum toxicological response and histological damage of erythrocytes and liver induced by the administration of various lead doses. [Supported by grant R01-2002-000-00321-0 from the Basic Research Program of the Korea Science & Engineering Foundation.]