

ABSTRACTS

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I3C/Cancer

Inhibition of cigarette smoke-related DNA adducts in rat tissues by indole-3-carbinol.

Indole-3-carbinol (I3C) found in various cruciferous vegetables has been shown to exert anti-carcinogenic activity in several target organs. In this study, we have investigated the effects of I3C on cigarette smoke-related lipophilic DNA adduct formation, potentially a key step in chemical carcinogenesis. Female Sprague-Dawley rats were exposed to sidestream cigarette smoke in a whole-body exposure chamber for 6 h per day, 7 days a week for 4 weeks. Control animals received only vehicle while the intervention groups received I3C (1.36 or 3.40 mmol/kg, b.wt.) daily by gavage starting from 1 week prior to smoke initiation until the end of the experiment. Analysis of tissue DNA by nuclease P1-mediated ³²P-postlabeling showed one major and several minor smoke-related adducts in lung, trachea, heart and bladder. The high dose of I3C significantly inhibited the major adducts in lung (#5) and trachea (#3) by 55% each; minor adducts were slightly inhibited (20-40%). The low dose of I3C showed lesser degree of inhibition (30-40%) in both lung and trachea; however, it was found statistically significant in lung only. The major smoke-related adduct in bladder (#2) was strongly inhibited (>65%) by high dose of I3C approaching adduct levels achieved in sham-exposed rats. A small but statistically significant decrease in the smoke-related DNA adduct (#5) in heart tissue was also observed by intervention with high dose I3C. Low levels (30-50 adducts/10(10) nucleotides) of I3C-derived DNA adducts were also found in all the tissues examined although their significance remains unknown. These data show significant inhibition of cigarette smoke-related DNA adducts by I3C, particularly in the lung, trachea and bladder.

Mutat Res 2000 Jul 20;452(1):11-18

Placebo-controlled trial of indole-3-carbinol in the treatment of CIN.

OBJECTIVE: Most precancerous lesions of the cervix are treated with surgery or ablative therapy. Chemoprevention, using natural and synthetic compounds, may intervene in the early precancerous stages of carcinogenesis and prevent the development of invasive disease. Our trial used indole-3-carbinol (I-3-C) administered orally to treat women with CIN as a therapeutic for cervical CIN. **METHODS:** Thirty patients with biopsy proven CIN II-III were randomized to receive placebo or 200, or 400 mg/day I-3-C administered orally for 12 weeks. If persistent CIN was diagnosed by cervical biopsy at the end of the trial, loop electrocautery excision procedure of the transformation zone was performed. HPV status was assessed in all patients. **RESULTS:** None (0 of 10) of the patients in the placebo group had complete regression of CIN. In contrast 4 of 8 patients in the 200 mg/day arm and 4 of 9 patients in the 400 mg/day arm had complete regression based on their 12-week biopsy. This protective effect of I-3-C is shown by a relative risk (RR) of 0.50 ((95% CI, 0.25 to 0.99) P = 0.023) for the 200 mg/day group and a RR of 0.55 ((95% CI, 0.31 to 0.99) P = 0.032) for the 400 mg/day group. HPV was detected in 7 of 10 placebo patients, in 7 of 8 in the 200 mg/day group, and in 8 of 9 in the 400 mg/day group. **CONCLUSIONS:** There was a statistically significant regression of CIN in patients treated with I-3-C orally compared with placebo. The 2/16 alpha-hydroxyestrone ratio changed in a dose-dependent fashion.

Gynecol Oncol 2000 Aug;78(2):123-129

Effects of dietary indole-3-carbinol on estradiol metabolism and spontaneous mammary tumors in mice.

Indole-3-carbinol (I3C) is a potent inducer of cytochrome P450 enzymes in many species, including humans. We therefore studied alterations in the cytochrome P450-dependent metabolism of estradiol in different strains of mice consuming I3C in semisynthetic powdered diets at doses ranging from 250 to 5000 p.p.m. (34-700 mg/kg/day) for different periods of time. In short-term metabolic studies (3 weeks), wet liver weight increased in SW and C3H/OuJ mice in a dose-responsive manner. Dietary I3C increased the cytochrome P450 content measured in hepatic microsomes, as well as the extent of estradiol 2-hydroxylation, up to 5-fold. In a long-term feeding experiment (8 months), female C3H/OuJ mice consumed synthetic diets containing I3C at 0, 500 or 2000 p.p.m. Mammary tumor incidence and multiplicity were significantly lower at both doses of I3C, and tumor latency was prolonged in the high-dose group. We conclude that I3C is an inducer of hepatic P450-dependent estrogen metabolism in mice, and that it is chemopreventive in the C3H/OuJ mouse mammary tumor model. This protective effect may be mediated in part by the increased 2-hydroxylation and consequent inactivation of endogenous estrogens.

Cigarette smoking and the risk of endometrial cancer.

Because of evidence of reduced estrogen excretion in the urine of women who smoke cigarettes and evidence linking estrogen levels to the risk of cancer of the female reproductive system, we evaluated the risk of endometrial cancer in relation to cigarette use in a hospital-based case-control study of 510 women with endometrial cancer (cases) and 727 women with other cancers (controls). The rate-ratio estimate (relative risk) for current smokers as compared with women who had never smoked was 0.7 (95 per cent confidence interval, 0.5 to 1.0), and for former smokers the estimate was 0.9 (0.6 to 1.2). For women currently smoking 25 or more cigarettes per day, the rate-ratio estimate was 0.5 (0.3 to 0.8). The effect of current smoking of at least 25 cigarettes per day appeared to be confined to postmenopausal women, among whom the estimate was 0.5 (0.2 to 0.9). Among premenopausal women the estimate was 0.9 (0.4 to 2.2), but the difference between these two estimates could have been due to chance. The data suggest that women who smoke heavily may have a lower risk of endometrial cancer than nonsmokers. The present findings do not have direct public health importance since cigarettes, overall, have serious deleterious effects. However, if these results are confirmed, elucidation of the underlying mechanisms whereby smoking reduces the risk would be of interest and might be useful in the development of strategies for preventing endometrial cancer.

N Engl J Med 1985 Sep 5;313(10):593-596

Changes in levels of urinary estrogen metabolites after oral indole-3-carbinol treatment in humans.

BACKGROUND: The oxidative metabolism of estrogens in humans is mediated primarily by cytochrome P450, many isoenzymes of which are inducible by dietary and pharmacologic agents. One major pathway, 2-hydroxylation, is induced by dietary indole-3-carbinol (I3C), which is present in cruciferous vegetables (e.g., cabbage and broccoli). **PURPOSE:** Because the pool of available estrogen substrates for all pathways is limited, we hypothesized that increased 2-hydroxylation of estrogens would lead to decreased activity in competing metabolic pathways. **METHODS:** Urine samples were collected from subjects before and after oral ingestion of I3C (6-7 mg/kg per day). In the first study, seven men received I3C for 1 week; in the second study, 10 women received I3C for 2 months. A profile of 13 estrogens was measured in each sample by gas chromatography-mass spectrometry. **RESULTS:** In both men and women, I3C significantly increased the urinary excretion of C-2 estrogens. The urinary concentrations of nearly all other estrogen metabolites, including levels of estradiol, estrone, estriol, and 16 α -hydroxyestrone, were lower after I3C treatment. **CONCLUSIONS:** These findings support the hypothesis that I3C-induced estrogen 2-hydroxylation results in decreased concentrations of several metabolites known to activate the estrogen receptor. This effect may lower estrogenic stimulation in women. **IMPLICATIONS:** I3C may have chemopreventive activity against breast cancer in humans, although the long-term effects of higher catechol estrogen levels in women require further investigation.

J Natl Cancer Inst 1997 May 21;89(10):718-723

Screening of potential chemopreventive agents using biochemical markers of carcinogenesis.

Ninety potential chemopreventive agents were screened using 6 chemoprevention-associated biochemical end points. These compounds were tested using rodent (tracheal epithelial or liver) cells and human cells [neonatal foreskin fibroblasts, bronchial epithelial cells, or human leukemic cells (HL-60)]. The effects measured were: (a) inhibition of 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced tyrosine kinase activity in HL-60 cells; (b) inhibition of TPA-induced ornithine decarboxylase (ODC) activity in rat tracheal epithelial cells; (c) inhibition of poly(ADP-ribose)polymerase in propane sultone-treated primary human fibroblasts; (d) inhibition of benzo[a]pyrene(B[a]P)-DNA binding in human bronchial epithelial cells; (e) induction of reduced glutathione in Buffalo rat liver cells; and (f) inhibition of TPA-induced free radical formation in primary human fibroblasts or HL-60 cells. Fifty compounds were highly effective in inhibiting TPA-induced tyrosine kinase activity. This assay identified compounds from a wide variety of chemical classes as effective inhibitors, including all the vitamins, retinoic acid analogues, protein kinase C inhibitors, and chemicals belonging to the amino acid category. Fifty-two chemicals were classified as highly positive compounds when examined for their ability to inhibit TPA-induced ODC activity. These agents showed a dose-dependent inhibition or inhibition at all doses. Retinoids, in general, exhibited strong inhibition of ODC activity. A category of compounds showing dose-dependent inhibition were the sulfur compounds, especially the thiols and thiones. Among the natural products, terpenes were strong inhibitors of ODC. Forty-seven compounds were classified as strong inhibitors of poly(ADP-ribose)polymerase. In the carcinogen-DNA binding inhibition assay, 21 compounds were identified as strong inhibitors, which include phenolic compounds as well as sulfur compounds. Vitamins and their analogues were also good inhibitors. Testing for induced glutathione yielded 19 compounds that were good inducers. Sulfur-containing compounds and most of the phenolic compounds were also inducers of glutathione. Twenty compounds were highly positive for inhibition of TPA-induced free radical formation. A significant number of phenolic and sulfur compounds were again strong oxygen radical scavengers. Some antiinflammatory agents were also identified as free radical inhibitors. In general, retinoids were quite active in all the assays. Eight compounds were positive in all of the six assays; these were vitamin C (ascorbic acid), bismuththiol, esculetin, etoperidone, folic acid, hydrocortisone, indole-3-carbinol, and tocopherol succinate. Agents that were positive in these assays may inhibit the carcinogenesis process by similar mechanisms in humans

and are identified as candidates for development as chemopreventive agents.

Cancer Res 1994 Nov 15;54(22):5848-5855

Influence of smoking on the development of lung metastases from breast cancer.

BACKGROUND. This study examined the association between cigarette smoking status and the development of lung metastases in a group of 835 women diagnosed with primary malignant unilateral breast cancer. **METHOD.** Female patients with breast cancer diagnosed between 1982 and 1991 at Roswell Park Cancer Institute (RPCI) in Buffalo, New York, who provided information on their cigarette smoking history at the time of their diagnosis were included. The subsequent disease status of patients was monitored by the RPCI Tumor Registry. The Cox regression model was used to estimate the relationship between smoking status and the development of lung metastases, adjusting for the patient's age, stage of disease at diagnosis, and body weight. **RESULTS.** Of those patients who developed lung metastases, 8.7% were nonsmokers, 14.1% were former smokers and 14.3% were current smokers. Tests showed that nonsmokers had significantly fewer lung metastases than either of the two smoking groups ($P < 0.01$). The estimated relative rates of lung metastases developing adjusting for age, stage, and body weight in women who smoked less than 10,000, between 10,001 and 20,000, and more than 20,000 packs over their lifetimes compared with nonsmokers were 1.06 (95% CI, 0.51-2.20), 3.10 (95% CI, 1.5-6.3), and 3.73 (95% CI, 1.6-8.9) respectively. The Cox regression model showed that every 1000 packs of cigarettes consumed over a lifetime increased a woman's risk of developing lung metastases by about 3% to 7% ($P < 0.001$). **CONCLUSION.** This study found a significant association between cigarette smoking history and risk of lung metastases developing in women diagnosed with primary invasive unilateral breast cancer. The risk of lung metastases developing increased as the number of cigarettes smoked in a lifetime increased.

Cancer 1995 Jun 1;75(11):2693-2699

Induction by estrogen metabolite 16 alpha-hydroxyestrone of genotoxic damage and aberrant proliferation in mouse mammary epithelial cells.

BACKGROUND: Estrogens are potent mammary tumor promoters influencing post-initiation events via epigenetic mechanisms. The upregulation (i.e., induction) of the C16 alpha-hydroxylation pathway during 17 beta-estradiol (E2) biotransformation has been associated with mammary cell transformation. The action of E2 metabolites on tumorigenic transformation, however, is poorly understood. **PURPOSE:** The newly established mammary epithelial cell line C57/MG, derived from the C57BL mouse strain, was used to examine whether E2 or its metabolites, 16-hydroxyestrone (16 alpha-OHE1) and estriol (E3), function as initiators of mammary cell transformation. **METHODS:** DNA repair (hydroxyurea-insensitive thymidine uptake), estrogen metabolism (3H exchange to form 3H₂O), hyperproliferation (increased cell number), and acquisition of anchorage-independent growth (soft-agar colonies) were used as quantitative end points to measure the relative extent of transformation. **RESULTS:** Treatment of cells with 200 ng/mL 16 alpha-OHE1 resulted in a 55.2% increase in DNA repair synthesis, a 23.09% increase in proliferative activity, and a 18-fold increase in the number of soft-agar colonies, relative to the solvent controls (P less than .0001). The extent of upregulation of the three end points was similar to that induced by the genotoxic mammary carcinogen 7, 12-dimethylbenz[a]anthracene (DMBA, positive control). DMBA treatment also upregulated the ratio of 16 alpha/C2 hydroxylation of E2 leading to increased formation of 16 alpha-OHE1. E2 and E3 were not effective in upregulating these markers for transformation. **CONCLUSION:** These results demonstrate that in nontransformed C57/MG cells, 16 alpha-OHE1 may function as an initiator, perturbing the intermediate biomarkers for preneoplastic transformation.

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Increased urinary catechol estrogen excretion in female smokers.

Premenopausal female smokers show significantly increased estrogen 2-hydroxylation, which may account in part for the anti-estrogenic effects of cigarette smoking. We have measured five major urinary estrogens, including estradiol (E2), estrone (E1), 16 alpha-hydroxyestrone (16 alpha OHE1), estriol (E3), and 2-hydroxyestrone (2OHE1), in premenopausal female smokers and non-smokers, to determine whether increased C-2 hydroxylation affected the urinary excretory patterns in these subjects. While total measured estrogen excretion in the follicular phase did not differ significantly between the two groups, urinary 2OHE1 among the smokers constituted a significantly greater proportion of the total (31.1 vs 18.2%, P less than 0.02). This difference was largely caused by significantly increased urinary 2OHE1 and decreased E3 observed in smokers. A urinary catechol estrogen index, defined by $[2OHE1]/[E3]$, was significantly elevated in smokers compared with non-smokers (1.67 ± 0.21 vs 0.56 ± 0.08 , P less than 0.001), and this urinary index correlated strongly with radiometrically determined estrogen 2-hydroxylation ($r = 0.84$, P less than 0.01). Ratios of the various estrogen metabolites did not vary substantially throughout the menstrual cycle. Urinary estrogen indices as described here may therefore be useful in demonstrating differences in estrogen metabolism, specifically 2-hydroxylation vs 16 alpha-hydroxylation, in selected populations.

Steroids 1988 Jul;52(1-2):69-83

Translocation of Bax to mitochondria induces apoptotic cell death in indole-3-carbinol (I3C) treated breast cancer cells.

Epidemiological studies have suggested that the consumption of fruits and vegetables that provide several classes of compounds, including Indole-3-carbinol (I3C), may have chemopreventive activity against breast cancer. Several in vitro and in vivo animal studies also provide convincing evidence for the anti-tumor activity of I3C, however, the molecular mechanism(s) by which I3C exerts its biological effects on breast cancer cells has not been fully elucidated. In this study, we investigated the effects of I3C in Her-2/neu over-expressing MDA-MB-435 breast cancer cells and compared these results with parental cells transfected with control vector. We focused our investigation in elucidating the molecular mechanism(s) by which I3C induces apoptosis in breast cancer cells. Our data show that I3C inhibits breast cancer cell growth in a dose dependent manner in Her-2/neu over-expressing and in normal Her-2/neu expressing cells. Induction of apoptosis was also observed in these cell lines when treated with I3C, as measured by poly (ADPribose) polymerase (PARP) and caspase-3 activation. In addition, we found that I3C up-regulates Bax, down-regulates Bcl-2 and, thereby, increased the ratio of Bax to Bcl-2 favoring apoptosis. These results suggest that the alteration in the expression of these genes may play an important role in mediating the biological effects of I3C. Moreover, we also show the cellular localization of Bax by confocal microscopy, which showed diffuse distribution of Bax throughout the cytoplasmic compartment in breast cancer cells in control culture. However, in I3C treated cells, Bax showed a punctate pattern of distribution that was localized in the mitochondria. From these results, we conclude that the over-expression and translocation of Bax to mitochondria causes mitochondrial depolarization and activation of caspases, which may be one of the mechanism(s) by which I3C induces apoptotic processes in I3C treated breast cancer cells. Overall, our present data provide a novel molecular mechanism (s) by which I3C elicits its biological effects on both Her-2/neu over-expressing and with normal Her-2/neu expressing breast cancer cells, suggesting that I3C could be an effective agent in inducing apoptosis in breast cancer cells.

Oncogene 2000 Nov 23;19(50):5764-5771

Suppression of breast cancer invasion and migration by indole-3-carbinol: associated with up-regulation of BRCA1 and E-cadherin/catenin complexes.

Indole-3-carbinol (I3C) is a compound occurring naturally in cruciferous vegetables and has been indicated as a promising agent in preventing breast cancer development and progression. In the present study we have investigated the effect of I3C on the cell migration and invasion behavior in estrogen receptor positive MCF-7 and estrogen receptor negative MDA-MB-468 human breast cancer cell lines. Both MCF-7 and MDA-MB-468 were poorly invasive cell lines and exhibited modest invasion and migration capacity in the presence of fibronectin as the chemoattractant. I3C (50 or 100 microM) elicited a significant inhibition of in vitro cell adhesion, migration, and invasion as well as in vivo lung metastasis formation in both cell lines. I3C also suppressed the 17beta-estradiol stimulated migration and invasion in estrogen-responsive MCF-7 cells. These results indicate that anti-invasion and antimigration activities of I3C occur via estrogen-independent and estrogen-dependent pathways. Moreover, I3C significantly caused a dose-dependent increase in E-cadherin, three major catenins (alpha, beta and gamma-catenin) and BRCA1 expression. Our current finding is the first demonstration that I3C can activate the function of invasion suppressor molecules associated with the suppression of invasion and migration in breast cancer cells. Thus, clinical application of I3C may contribute to the potential

benefit for suppression of breast cancer invasion and metastasis.

J Mol Med 2000;78(3):155-165

Indole-3-carbinol and tamoxifen cooperate to arrest the cell cycle of MCF-7 human breast cancer cells.

The current options for treating breast cancer are limited to excision surgery, general chemotherapy, radiation therapy, and, in a minority of breast cancers that rely on estrogen for their growth, antiestrogen therapy. The naturally occurring chemical indole-3-carbinol (I3C), found in vegetables of the Brassica genus, is a promising anticancer agent that we have shown previously to induce a G1 cell cycle arrest of human breast cancer cell lines, independent of estrogen receptor signaling. Combinations of I3C and the antiestrogen tamoxifen cooperate to inhibit the growth of the estrogen-dependent human MCF-7 breast cancer cell line more effectively than either agent alone. This more stringent growth arrest was demonstrated by a decrease in adherent and anchorage-independent growth, reduced DNA synthesis, and a shift into the G1 phase of the cell cycle. A combination of I3C and tamoxifen also caused a more pronounced decrease in cyclin-dependent kinase (CDK) 2-specific enzymatic activity than either compound alone but had no effect on CDK2 protein expression. Importantly, treatment with I3C and tamoxifen ablated expression of the phosphorylated retinoblastoma protein (Rb), an endogenous substrate for the G1 CDKs, whereas either agent alone only partially inhibited endogenous Rb phosphorylation. Several lines of evidence suggest that I3C works through a mechanism distinct from tamoxifen. I3C failed to compete with estrogen for estrogen receptor binding, and it specifically down-regulated the expression of CDK6. These results demonstrate that I3C and tamoxifen work through different signal transduction pathways to suppress the growth of human breast cancer cells and may, therefore, represent a potential combinatorial therapy for estrogen-responsive breast cancer.

Cancer Res 1999 Mar 15;59(6):1244-1251

Indole-3-carbinol inhibits the expression of cyclin-dependent kinase-6 and induces a G1 cell cycle arrest of human breast cancer cells independent of estrogen receptor signaling.

Indole-3-carbinol (I3C), a naturally occurring component of Brassica vegetables such as cabbage, broccoli, and Brussels sprouts, has been shown to reduce the incidence of spontaneous and carcinogen-induced mammary tumors. Treatment of cultured human MCF7 breast cancer cells with I3C reversibly suppresses the incorporation of [3H]thymidine without affecting cell viability or estrogen receptor (ER) responsiveness. Flow cytometry of propidium iodide-stained cells revealed that I3C induces a G1 cell cycle arrest. Concurrent with the I3C-induced growth inhibition, Northern blot and Western blot analyses demonstrated that I3C selectively abolished the expression of cyclin-dependent kinase 6 (CDK6) in a dose- and time-dependent manner. Furthermore, I3C inhibited the endogenous retinoblastoma protein phosphorylation and CDK6 phosphorylation of retinoblastoma in vitro to the same extent. After the MCF7 cells reached their maximal growth arrest, the levels of the p21 and p27 CDK inhibitors increased by 50%. The antiestrogen tamoxifen also suppressed MCF7 cell DNA synthesis but had no effect on CDK6 expression, while a combination of I3C and tamoxifen inhibited MCF7 cell growth more stringently than either agent alone. The I3C-mediated cell cycle arrest and repression of CDK6 production were also observed in estrogen receptor-deficient MDA-MB-231 human breast cancer cells, which demonstrates that this indole can suppress the growth of mammary tumor cells independent of estrogen receptor signaling. Thus, our observations have uncovered a previously undefined antiproliferative pathway for I3C that implicates CDK6 as a target for cell cycle control in human breast cancer cells. Moreover, our results establish for the first time that CDK6 gene expression can be inhibited in response to an extracellular antiproliferative signal.

J Biol Chem 1998 Feb 13;273(7):3838-3847

Effects of pesticides on the ratio of 16 alpha/2-hydroxyestrone: a biologic marker of breast cancer risk.

Xenobiotic estrogens are external compounds with estrogenic activity that may thereby affect the risk of breast cancer. This paper describes a mechanism by which xeno-estrogens may affect the development of breast cancer. Estradiol metabolism proceeds by hydroxylation at one of two mutually exclusive sites at C-2 and C-16 alpha. The catechol pathway yields the weakly estrogenic 2-hydroxyestrone (2-OHE1), which inhibits breast cell proliferation. In contrast, the alternative pathway yields the genotoxic 16 alpha-hydroxyestrone (16 alpha-OHE1), which enhances breast cell growth, increases unscheduled DNA synthesis, and oncogene and virus expression, and increases anchorage-independent growth. Using a radiometric assay that measures the relative formation of 16 alpha-OHE1 versus 2-OHE1 from specifically tritiated estradiol in (ER+) MCF-7 cells, we compared the ratio of 16 alpha-OHE1/2-OHE1 observed after treatment with the known rodent carcinogen 7,12-dimethylbenz[a]anthracene (DMBA) with the ratios after treatment with DDT, atrazine, gamma-benzene hexachloride, kepone, coplanar PCBs, endosulfans I and II, linoleic and eicosapentenoic acids, and indole-3-carbinol (I3C). These pesticides significantly increase the ratio of 16 alpha-OHE1/2-OHE1 metabolites to values comparable to or greater than those observed after DMBA. In contrast, the antitumor agent I3C increased 2-OHE1 formation and yielded ratios that are 1/3 of those found in unexposed control cells and 1/10th of those found in DMBA-treated cells. Thus the ratio of 16 alpha-OHE1/2-OHE1 may provide a marker for the risk of breast cancer. Assays of this ratio, which can be measured in spot urines, may prove useful for a variety of in vitro and in vivo studies bearing on breast cancer risk.

Interactions of indoles with specific binding sites for 2,3,7,8-tetrachlorodibenzo-p-dioxin in rat liver.

In order to identify some of the structural requirements for binding of indoles to the receptor for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), we have investigated the capacity of various indoles to inhibit specific [¹²⁵I]-TCDD binding in rat liver cytosol, as analyzed by electrofocusing in polyacrylamide gel. Of these indoles, indolo[3,2-b]carbazole was the most active. The IC₅₀ value for receptor binding of indolo[3,2-b]carbazole as well as for 2,3,7,8-tetrachlorodibenzofuran was 3.6 nM, whereas that of 5,6-benzoflavone was 26 nM. Both indolo[3,2-b]carbazole and 2,3,7,8-tetrachlorodibenzofuran competitively inhibited the binding of [³H]TCDD to the receptor. The well-known microsomal enzyme inducer 3,3'-diindolymethane did not interact significantly with the TCDD receptor. Previous concepts of structure-activity relationships for binding of chlorinated dioxins to the TCDD receptor fail to account for the receptor binding of unhalogenated aryl hydrocarbon hydroxylase inducers such as 5,6-benzoflavone. We have instead considered the true three-dimensional space occupied by some receptor ligands by means of a computer using crystallographic data as inputs. When the atomic van der Waals radii were included, all potent receptor ligands studied could be fitted into a rectangle of 6.8 X 13.7 Å.

Mol Pharmacol 1985 Oct;28(4):357-363

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Caloric Restriction

Effects of calorie restriction on polymicrobial peritonitis induced by cecum ligation and puncture in young c57bl/6 mice.

Calorie restriction (CR) is known to prolong the life span and maintain an active immune function in aged mice, but it is still not known if rodents under CR can respond optimally to bacterial infection. We report here on the influence of CR on the response of peritoneal macrophages to lipopolysaccharide, splenic NF-kappaB and NF-interleukin-6 (IL-6) activities, and mortality in polymicrobial sepsis induced by cecal ligation and puncture (CLP). Macrophages from 6-month-old C57BL/6 mice on a calorie-restricted diet were less responsive to lipopolysaccharide, as evidenced by lower levels of IL-12 and IL-6 protein and mRNA expression. Furthermore, *in vitro* lipopolysaccharide-stimulated macrophages from mice under CR also expressed decreased lipopolysaccharide receptor CD14 levels as well as Toll-like receptor 2 (TLR2) and TLR4 mRNA levels. In addition, the phagocytic capacity and class II (I-A(b)) expression of macrophages were also found to be significantly lower in mice under CR. Mice under CR died earlier ($P < 0.005$) after sepsis induced by CLP, which appeared to be a result of increased levels in serum of the proinflammatory cytokines tumor necrosis factor alpha and IL-6 and splenic NF-kappaB and NF-IL-6 activation 4 h after CLP. However, mice under CR survived significantly ($P < 0.005$) longer than mice fed *ad libitum* when injected with paraquat, a free radical-inducing agent. These data suggest that young mice under CR may be protected against oxidative stress but may have delayed maturation of macrophage function and increased susceptibility to bacterial infection.

Clin Diagn Lab Immunol 2001 Sep;8(5):1003-11

Caloric restriction and cancer.

In 1909 Moreschi observed that tumors transplanted into underfed mice did not grow as well as those transplanted into mice fed *ad libitum*. His finding stimulated a decade of research which showed that caloric restriction also affected negatively the growth of spontaneous tumors. Between 1920 and 1940 little work was done in this area, possibly because of limiting methodology. In the 1940s the laboratories of Tannenbaum (Chicago) and Baumann (Wisconsin) were able to design studies using defined diets and showed that the observed effect was due to caloric content of the diet independently of the source of calories. After another active decade research activity in the calorie-cancer area declined until it was reborn in the 1980s. By the 1980s knowledge of physiology and molecular biology had advanced enough to allow investigators to probe mechanisms underlying the calorie-cancer phenomenon. We now know that caloric expenditure (as work or exercise) will lead to reduced risk. Energy restriction enhances DNA repair and moderates oxidative damage to DNA. Energy restriction reduces oncogene expression as well. Over a half century ago, Boutwell noted that energy restriction in female rats resulted in adrenal hypertrophy and reduced weight of ovaries and uterus. He suggested that energy restriction resulted in "pseudohypophysectomy." We now know that adrenalectomy can negate the effects of caloric restriction. Caloric restriction also affects insulin metabolism and may influence gene expression. These recent observations should help us understand some of the basic mechanisms involved in establishment and proliferation of tumors.

J Nutr Sci Vitaminol (Tokyo) 2001 Feb;47(1):13-9

Brain-derived neurotrophic factor mediates an excitoprotective effect of dietary restriction in mice.

Dietary restriction (DR; reduced calorie intake) increases the life span of rodents and increases their resistance to cancer, diabetes and other age-related diseases. DR also exerts beneficial effects on the brain including enhanced learning and memory and increased resistance of neurons to excitotoxic, oxidative and metabolic insults. The mechanisms underlying the effects of DR on neuronal plasticity and survival are unknown. In the present study we show that levels of brain-derived neurotrophic factor (BDNF) are significantly increased in the hippocampus, cerebral cortex and striatum of mice maintained on an alternate day feeding DR regimen compared to animals fed *ad libitum*. Damage to hippocampal neurons induced by the excitotoxin kainic acid was significantly reduced in mice maintained on DR, and this neuroprotective effect was attenuated by intraventricular administration of a BDNF-blocking antibody. Our findings show that simply reducing food intake results in increased levels of BDNF in brain cells, and suggest that the resulting activation of BDNF signaling pathways plays a key role in the neuroprotective effect of DR. These results bolster accumulating evidence that DR may be an effective approach for increasing the resistance of the brain to damage and enhancing brain neuronal plasticity.

J Neurochem 2001 Jan;76(2):619-626

Effects of calorie restriction on thymocyte growth, death and maturation.

We previously reported that calorie restriction (CR) significantly delays the spontaneous development of thymic lymphomas and other neoplasms in p53-deficient mice and their wild-type littermates. The purpose of the present study was to further characterize the anti-lymphoma effects of CR by assessing thymocyte growth, death and maturation in response to acute (6 day) and chronic (28 day) CR regimens. Male C57BL/6J mice fed a CR diet (restricted to 60% of control ad libitum intake) for 6 days displayed a severe reduction in thymic size and cellularity, as well as a decrease in splenic size and cellularity; these declines were sustained through 28 days of CR. Mice maintained on a CR diet for 28 days also displayed a significant depletion in the cell numbers of all four major thymocyte subsets defined by CD4 and CD8 expression. Analysis within the immature CD4(-)8(-) thymocyte subset further revealed an alteration in normal CD44 and CD25 subset distribution. In particular, CR for 28 days resulted in a significant decrease in the percentage of the proliferative CD44(-)25(-) subset. In addition, a significant increase in the percentage of the early, pro-T cell CD44(+)25(-) population was detected, indicative of a CR-induced delay in thymocyte maturation. Taken together, these findings suggest that CR suppresses (through several putative mechanisms) lymphomagenesis by reducing the pool of immature thymocytes that constitute the lymphoma-susceptible subpopulation.

Carcinogenesis 2000 Nov;21(11):1959-64

Calorie restriction and aging: a life-history analysis.

The disposable soma theory suggests that aging occurs because natural selection favors a strategy in which fewer resources are invested in somatic maintenance than are necessary for indefinite survival. However, laboratory rodents on calorie-restricted diets have extended life spans and retarded aging. One hypothesis is that this is an adaptive response involving a shift of resources during short periods of famine away from reproduction and toward increased somatic maintenance. The potential benefit is that the animal gains an increased chance of survival with a reduced intrinsic rate of senescence, thereby permitting reproductive value to be preserved for when the famine is over. We describe a mathematical life-history model of dynamic resource allocation that tests this idea. Senescence is modeled as a change in state that depends on the resources allocated to maintenance. Individuals are assumed to allocate the available resources to maximize the total number of descendants. The model shows that the evolutionary hypothesis is plausible and identifies two factors, both likely to exist, that favor this conclusion. These factors are that survival of juveniles is reduced during periods of famine and that the organism needs to pay an energetic "overhead" before any litter of offspring can be produced. If neither of these conditions holds, there is no evolutionary advantage to be gained from switching extra resources to maintenance. The model provides a basis to evaluate whether the life-extending effects of calorie-restriction might apply in other species, including humans.

Evolution Int J Org Evolution 2000 Jun;54(3):740-50

Effects of age and dietary restriction on life span and oxidative stress of SAMP8 mice with learning and memory impairments.

This study was to evaluate the effect of dietary restriction (DR) on life span and oxidative stress of dementia mouse model SAMP8 with impaired learning and memory. SAMP8 female mice were fed either ad libitum (AL) or fed 60% of food intake of AL. Results showed that basal metabolic rates (BMR) were significantly lower (15 to 22%) in DR with increased median and maximum life spans, suggesting feed and gross efficiencies were significantly lower in DR than in AL. Grading score of senescence resulted in a marked improvement about 2-fold by DR compared with AL. The amounts of lipofuscin at 12 months were significantly lowered 16% in DR than that of AL. Median and maximal life spans significantly increased (28.5% and 16.4%, respectively) by DR, and also lowered superoxide radical about 15 approximately 45% in DR compared with AL at 4, 8 and 12 months of age. On the other hand, superoxide dismutase (SOD) activities were higher (about 15 approximately 30%) in DR than those in AL group of SAMP8 except for 4 months of age. Our results suggest that 40% calorie restricted SAMP8 can effectively suppress dementia-related abnormalities during aging.

J Nutr Health Aging 2000;4(3):182-6

Calorie restriction and spontaneous hepatic tumors in C3H/He mice.

Caloric restriction started at the young adult (YA) stage and the full adult (FA) stage in mice was compared, specifically focussing on whether there would be a delay in the onset time of spontaneous hepatoma or a reduction in its frequency. Caloric restriction lengthened the life spans of both groups, the YA and FA. Both groups showed striking reductions of spontaneous hepatomas, from 70.9 +/- 3.5% for non-restricted controls down to 35.7 +/- 5.7 and 30.4 +/- 4.0%, for mice restricted from young adult, and from full adult stages, respectively; further, the numbers of tumor-free mice in the restricted groups increased by 45.7% and 38.5%, respectively, from 11.5%, in the non-restricted control. The cumulative incidences of hepatoma in the caloric restricted groups showed a delayed and lower incidence compared with those of the non-restricted group; a parallel delay might result from a weakened activity in tumor-promotion, whereas a lower frequency, might reflect a possible reduction of target cells for hepatomata development. Both effects can be assumed to have resulted from caloric restriction. When cumulative incidences of small hepatomas were compared between the two restricted groups, restriction started at the young adult stage is assumed to have

caused fewer initiation stresses, as well as to have delayed promotion, as clearly evidenced by a flatter curve of incidence with a lower total incidence. Thus, the time at which caloric restriction is started plays a critical role in its subsequent effects.

J Nutr Health Aging 1999;3(2):121-6

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ABSTRACTS

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Short-term calorie restriction improves disease-related markers in older male rhesus monkeys (*Macaca mulatta*).

Calorie restriction (CR) is widely known for its effects on life span, physiological aging and age-related disease in laboratory rats and mice. Emerging data from CR studies in rhesus monkeys suggest that this nutritional intervention paradigm may also have beneficial effects in long-lived mammals. Studies from our laboratory and others have suggested that young- or adult-onset CR might have beneficial effects on cardiovascular disease and diabetes. For example, long-term CR reduced body fat and serum triglycerides, and increased a subfraction of HDL cholesterol associated with decreased cardiovascular disease risk. These studies suggested that long-term CR begun in young or adult animals might have important effects on markers relevant to age-related disease. Few studies have examined the effects of CR initiated in older animals (rodents or monkeys), and the temporal nature of some potentially beneficial effects of CR is unknown. The present study examined several markers related to diabetes and cardiovascular disease in thirteen older adult (> 18 year) non-obese (body fat < 22%), male rhesus monkeys during a short-term CR paradigm. Specifically, we collected these data at baseline (ad libitum feeding), 10, 20, and 30% CR, and at 6 and 12 months on 30% CR. Fasting and peak insulin were significantly reduced as were the acute and second-phase insulin responses. CR also marginally reduced triglycerides (50% reduction), but had no effect on total serum cholesterol or blood pressure. Interestingly, the observed glucoregulatory changes emerged prior to any evidence of a change in body composition suggesting that certain effects of CR may not be wholly dependent on changes in body composition in older monkeys.

Mech Ageing Dev 2000 Jan 10;112(3):185-96

Controlling caloric consumption: protocols for rodents and rhesus monkeys.

One approach for investigating biological aging is to compare control-fed animals with others restricted in calorie intake by 20% or more. Caloric restriction (CR) is the only intervention shown to extend the maximum life span of several invertebrates and vertebrates including spiders, fish, rats and mice. The capacity of CR to retard aging in nonhuman primates is now being explored. The rodent studies show that CR opposes the development of many age-associated pathophysiological changes, including changes to the brain and changes in learning and behavior. One goal of studying CR in rodent is to determine the mechanisms by which it retards aging to design interventions that duplicate those effects. The methods that we use for conducting CR studies on mice and rhesus monkeys are described. We employ procedures designed to achieve a high degree of caloric control for all animals in the study. As used in our studies, this control includes the following features: 1) animals are individually housed, and 2) all individuals in the control group eat the same number of calories (i.e., they are not fed ad lib). Although this method results in strict caloric control for all animals, there seems to be considerable procedural flexibility for the successful conduct of CR studies.

Neurobiol Aging 1999 Mar-Apr;20(2):157-65

The effects of aging and calorie restriction on plasma nutrient levels in male and female Emory mice.

We examined the effect of diet, age (4.5, 13 and 23 months), and sex on plasma levels of retinol, tocopherol, ascorbate, cholesterol, glucose and glycohemoglobin in male and female Emory mice which were fed control (C) and 50% calorie restricted (R) diets. Results showed that C fed animals tended to have higher levels of plasma ascorbate (50-71%), cholesterol (23-71%), glucose (38-81%) and glycohemoglobin (50%). However, these diet differences varied with the age and sex of the animals. Plasma retinol levels were lower only in R males vs. C males (50%). Novel sex-related differences in levels of plasma retinol (2-fold higher in C male mice than in C or R female mice) are described. Aging was associated with trends towards lower levels of plasma ascorbate (14-25%), glucose (34-36%) and glycohemoglobin (47-57%) from 4.5 to 23 months of age. However, these age differences depended upon the diet and sex of the animals. These data suggest that lower plasma levels of glucose, glycosylated hemoglobin and cholesterol may be causally related to the life extension noted in R animals since elevated levels of these moieties have been related to aging. Since oxidative stress is thought to be causally related to aging it appears unlikely that retinol, tocopherol and ascorbate are causally related to R-induced life extension.

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Antioxidant enzyme activities in lens, liver and kidney of calorie restricted Emory mice.

Dietary calorie restriction extends both mean and maximum life span and retards age-related diseases, including eye lens cataract in Emory mice. The beneficial effects of calorie restriction have been hypothesized to reflect enhanced tissue antioxidant capacity.

As a test of this hypothesis, we reared male and female Emory mice on control (C) or 40% calorie-restricted (R) diets. We then determined activities of total superoxide dismutase (T-SOD), Cu/Zn-SOD, Mn-SOD, glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT) in eye lens, liver and kidney of young (4.5 or 6 months), mature (11 or 12 months) and old (22 months) animals. Effects of diet, age and sex were evaluated by multi-factor ANOVA. Only kidney GR activities (mean \pm S.E.M.) were significantly enhanced with the R diet (R, 61 \pm 2 vs. C, 54 \pm 3 U/mg protein; $P = 0.03$). More frequently, we noted reduced antioxidant enzyme activity in R as compared with C animals, including reduced activities of T-SOD in lens, liver and kidney, Cu/Zn-SOD in liver and kidney, liver Mn-SOD and liver CAT ($P < 0.05$). Effects of age on antioxidant enzyme activity in C mice included age-dependent decreases in lens and kidney CAT and in liver Mn-SOD. There was also an age-dependent increases in liver and kidney Cu/Zn-SOD and liver GR. None of these age-dependent alterations in antioxidant enzyme function were attenuated in tissues of mice fed the R diet. Values for liver CAT were significantly lower in females than in males ($P = 0.05$). These results indicate that antioxidant enzyme activities in Emory mouse tissues are influenced by diet, age and sex. However, it is unlikely that increased lifespan and attenuation of cataract (and perhaps other age-dependent debilities), which are associated with the R diet in the Emory mouse, are due to enhanced antioxidant enzyme capabilities.

Mech Ageing Dev 1997 Dec 30;99(3):181-92

Effects of changes in calorie intake on intestinal nutrient uptake and transporter mRNA levels in aged mice.

In aged, chronically calorie-restricted (CR) mice, intestinal nutrient uptake is significantly higher than in same-age ad libitum controls. Can this chronic restriction-induced enhancement of uptake be reversed by ad libitum feeding? We addressed this question by switching 32-mo-old chronically CR mice to ad libitum feeding for 4 wk (CRAL). Intestinal transport rate and total intestinal absorptive capacity for D-sugars and several nonessential L-amino acids decreased significantly in CRAL mice. In contrast, switching CR mice to an ad libitum regimen for only 3 d had no effect on intestinal nutrient transport, indicating that the negative effects of ad libitum feeding require a duration longer than the 3-d lifetime of most enterocytes. Permeability of the intestinal mucosa to L-glucose was independent of the switches in diet. Levels of the brushborder glucose transporter SGLT1, brushborder fructose transporter GLUT5, and basolateral sugar transporter GLUT2 mRNA as determined by reverse transcriptase-polymerase chain reaction in 6-, 24- and 32-mo-old mice were each apparently independent of caloric restriction and age. We conclude that the high rates of intestinal nutrient uptake exhibited by chronically CR mice can be reversed by ad libitum feeding of only 1 month duration. These decreases in uptake were due mainly to specific decreases in transport per unit weight of intestine and not to nonspecific decreases in intestinal mass. Changes in rates of sugar uptake induced by chronic CR and age are apparently not accompanied by changes in steady-state levels of mRNA coding for those transporters.

J Gerontol A Biol Sci Med Sci 1997 Nov;52(6):B300-10

Effects of dehydroepiandrosterone and calorie restriction on the Bcl-2/Bax-mediated apoptotic pathway in p53-deficient mice.

Modulation of apoptosis through altered expression of Bcl-2 and/or Bax may be a mechanism by which dehydroepiandrosterone (DHEA) administration and calorie restriction (CR) exert their chemopreventive effects in p53-deficient (p53^{-/-}) mice. Using immunohistochemical detection we found that treatment with both DHEA and CR resulted in decreased expression of the PCNA proliferation marker in the thymus. In addition, treatment with DHEA also increased the rate of apoptosis in the thymus, resulting in marked thymic atrophy. Thus, both DHEA and CR appear to shift cell number homeostasis by favoring apoptosis. To further understand the molecular mechanisms by which DHEA and CR exert their effects, we examined two components of the apoptotic pathway, Bcl-2 and Bax. We found that p53^{-/-} mice have much higher levels of Bcl-2 mRNA in the thymus than wild-type (p53^{+/+}) mice. Treatment of p53^{-/-} animals with DHEA resulted in decreased Bcl-2 but not Bax mRNA levels in the thymus. In contrast, CR did not change either Bcl-2 or Bax mRNA expression. The present study provides molecular evidence that DHEA and CR may modulate tumorigenesis through alterations in the apoptotic and/or proliferative pathways.

Cancer Lett 1997 Jun 3;116(1):61-9

Calorie restriction inhibits the age-related dysregulation of the cytokines TNF-alpha and IL-6 in C3B10RF1 mice.

TNF-alpha and IL-6 are generally increased in the sera of aged humans and mice. The dysregulation of these cytokines may be critical in autoreactivity and immune dysfunction. In earlier studies we demonstrated that production of TNF-alpha and IL-6 following in vitro stimulation of peritoneal macrophages by LPS was reduced in old compared to young mice, and that dietary caloric restriction (CR) had no effect on the induction of TNF-alpha in this system. In the present study we examined the effects of age and calorie restriction on the constitutive production of both TNF-alpha and IL-6. Serum levels of both cytokines were significantly higher in old versus young mice. However, in old mice subjected to long term CR the serum levels were comparable to those of young mice. The potential involvement of normalization of TNF-alpha and IL-6 levels in the life extension effect of CR are discussed.

Mech Ageing Dev 1997 Feb;93(1-3):87-94

Effects of calorie restriction and aging on the expression of antioxidant enzymes and ubiquitin in the liver of Emory mice.

We studied the effect of age and calorie restriction on the expression of genes involved in antioxidant defenses in livers of young (4.5 to 6 months) and old (22 months) Emory mice fed a control (C) or restricted (R) diet. Specifically examined were catalase (CAT), glutathione peroxidase (Gpx), Cu/Zn and Mn superoxide dismutase (Cu/ZnSOD and MnSOD). As an indicator of oxidative damage to the tissues we measured lipid peroxidation. As indicators of oxidative stress we determined ubiquitin mRNA levels and endogenous high molecular weight (HMW) ubiquitin conjugates. Lower mRNA levels of ubiquitin ($P < 0.05$), CAT ($P < 0.01$) and Gpx ($P < 0.01$) were observed in tissues from young R versus C animals. The old C group had a lower CAT mRNA level ($P < 0.0001$) compared with young C. In the R group, age did not affect the CAT mRNA levels or Gpx mRNA levels; however, ubiquitin mRNA levels were higher ($P < 0.05$). No significant changes in Cu/Zn or MnSOD mRNA were observed with age or diet. Cu/ZnSOD protein levels were lower in the young R at 4.5 months ($P < 0.05$) than young C, and higher in the old R group versus old C ($P < 0.05$). CAT protein levels were higher in the old C versus old R ($P < 0.05$). Changes of HMW ubiquitin conjugates with age or diet were not significant. Of the four groups, the old R group showed the highest levels of lipid peroxidation.

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