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*Journal*  
**ABSTRACTS****Pregnenolone****A NEW MECHANISM OF SYNAPSE-SPECIFIC NEURONAL PLASTICITY.**

According to current concepts, long-term memory is based on structural-functional changes in particular synaptic connections between neurons in the brain (synapse-specific plasticity), which depend on the processes of translation and transcription. Studies on neurons in the mollusk *Aplysia* and the mammalian hippocampus have addressed a mechanism of synapse-specific plasticity which does not require synapse-specific molecular genetic processes. Stimulation of a synapse has been shown to lead to activation of intracellular second messengers in the synapse as well as "synaptic tagging"-the formation of mechanisms "recognizing" transcription products. In the neuron body, second messengers induce the synthesis of RNA and protein molecules which are widely distributed in neuron processes and which are inserted selectively only into stimulation-tagged synapses, evoking long-term changes in their functional and morphological characteristics. The results of our studies on common snail defensive behavior command neurons LPI1 and RPI1 suggest the existence of another mechanism controlling synapse-specific plasticity. On acquisition of sensitization, a number of second messengers and the genes controlled by them are involved in supporting the plasticity of defined synaptic inputs of these neurons in snails. The processes of induction of long-term facilitation in the sensory inputs of neurons from chemoreceptors on the head have been shown to involve cAMP and cAMP-dependent transcription factors of the immediate early gene C/EBP (CAAT/enhancer binding protein), while the mechanisms controlling the other sensory input of neurons LPI1 and RPI1-from mechanoreceptors on the head-involve protein kinase C and protein kinase C-dependent transcription factor SRF (serum response factor). The immediate early gene *zif268* is involved in controlling the inputs from both chemo-and mechanoreceptors on the head. These results are regarded as experimental support for the hypothesis that the molecular mechanisms of synapse-specific plasticity during learning may form on the basis of a selective neurochemical "projection" of the synaptic connections onto defined genes in the neuron.

Neurosci Behav Physiol. 2007 Jul;37(6):559-70

**PREGNENOLONE SULFATE ENHANCES POST-TRAINING MEMORY PROCESSES WHEN INJECTED IN VERY LOW DOSES INTO LIMBIC SYSTEM STRUCTURES: THE AMYGDALA IS BY FAR THE MOST SENSITIVE.**

Immediate post-training, stereotactically guided, intraparenchymal administration of pregnenolone sulfate (PS) into the amygdala, septum, mammillary bodies, or caudate nucleus and of PS, dehydroepiandrosterone sulfate, and corticosterone into the hippocampus was performed in mice that had been weakly trained in a foot-shock active avoidance paradigm. Intrahippocampal injection of PS resulted in memory enhancement (ME) at a lower dose than was found with dehydroepiandrosterone sulfate and corticosterone. Intraamygdally administered PS was approximately 10(4) times more potent on a molar basis in producing ME than when PS was injected into the hippocampus and approximately 10(5) times more potent than when injected into the septum or mammillary bodies. ME did not occur on injection of PS into the caudate nucleus over the range of doses tested in the other brain structures. The finding that fewer than 150 molecules of PS significantly enhanced post-training memory processes when injected into the amygdala establishes PS as the most potent memory enhancer yet reported and the amygdala as the most sensitive brain region for ME by any substance yet tested.

Proc Natl Acad Sci U S A. 1995 Nov 3;92(23):10806-10

**PREGNENOLONE SULFATE: A POSITIVE ALLOSTERIC MODULATOR AT THE N-METHYL-D-ASPARTATE RECEPTOR.**

The N-methyl-D-aspartate (NMDA) receptor is believed to play a major role in learning and in excitotoxic neuronal damage associated with stroke and epilepsy. Pregnenolone sulfate, a neurosteroid, specifically enhances NMDA-gated currents in spinal cord neurons, while inhibiting receptors for the inhibitory amino acids glycine and gamma-aminobutyric acid, as well as non-NMDA glutamate receptors. This observation is consistent with the hypothesis that neurosteroids such as pregnenolone sulfate are involved in regulating the balance between excitation and inhibition in the central nervous system.

### **NEUROSTEROID PREGNENOLONE SULFATE ENHANCES GLUTAMATERGIC SYNAPTIC TRANSMISSION BY FACILITATING PRESYNAPTIC CALCIUM CURRENTS AT THE CALYX OF HELD OF IMMATURE RATS.**

Pregnenolone sulfate (PREGS) is an endogenous neurosteroid widely released from neurons in the brain, and is thought to play a memory-enhancing role. At excitatory synapses PREGS facilitates transmitter release, but the underlying mechanism is not known. We addressed this issue at the calyx of Held in rat brainstem slices, where direct whole-cell recordings from giant nerve terminals are feasible. PREGS potentiated nerve-evoked excitatory postsynaptic currents (EPSCs) without affecting the amplitude of miniature EPSCs, suggesting that its site of action is presynaptic. In whole-cell recordings from calyceal nerve terminals, PREGS facilitated Ca<sup>2+</sup> currents, by accelerating their activation kinetics and shifting the half-activation voltage toward negative potentials. PREGS had no effect on presynaptic K<sup>+</sup> currents, resting conductance or action potential waveforms. In simultaneous pre- and postsynaptic recordings, PREGS did not change the relationship between presynaptic Ca<sup>2+</sup> influx and EPSCs, suggesting that exocytotic machinery downstream of Ca<sup>2+</sup> influx is not involved in its effect. PREGS facilitated Ba<sup>2+</sup> currents recorded from nerve terminals and also from HEK 293 cells expressed with recombinant N- or P/Q-type Ca<sup>2+</sup> channels, suggesting that PREGS-induced facilitation of voltage-gated Ca<sup>2+</sup> channels (VGCCs) is neither Ca<sup>2+</sup> dependent nor VGCC-type specific. The PREGS-induced VGCC facilitation was blocked by the PREGS scavenger (2-hydroxypropyl)-beta-cyclodextrin applied from outside, but not from inside, of nerve terminals. We conclude that PREGS facilitates VGCCs in presynaptic terminals by acting from outside, thereby enhancing transmitter release. We propose that PREGS may directly modulate VGCCs acting on their extracellular domain.

Eur J Neurosci. 2006 Oct;24(7):1955-66

### **PREGNENOLONE SULFATE ENHANCES LONG-TERM POTENTIATION IN CA1 IN RAT HIPPOCAMPUS SLICES THROUGH THE MODULATION OF N-METHYL-D-ASPARTATE RECEPTORS.**

Among the different steroids found in the brain, pregnenolone sulfate (3beta-hydroxy-5-pregnen-20-one-3-sulfate; PREGS) is known to enhance hippocampal-associated memory. The present study employs rat hippocampal slices to investigate the ability of PREGS to modulate long-term potentiation (LTP), a phenomenon considered as a model of synaptic plasticity related to memory processes. LTP (3 x 100 Hz/1 sec within 2 min), implicated essentially glutamatergic transmission, for which the different synaptic events could be pharmacologically dissociated. We show that PREGS enhances LTP in CA1 pyramidal neurons at nanomolar concentrations and exhibits a bell-shaped concentration-response curve. The maximal effect of PREGS on both induction and maintenance phases of LTP is observed at 300 nM and requires 10 min of superfusion. Although PREGS does not change the N-methyl-D-aspartate (NMDA) component of the field potentials (fEPSPs) isolated in the presence of 10 microM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) in Mg<sup>2+</sup>-free artificial cerebrospinal fluid, PREGS does enhance the response induced by NMDA application (50 microM, 20 sec). PREGS does not modify the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) component of the fEPSPs isolated in the presence of 100 microM DL-2-amino-7-phosphopentanoic acid (DL-AP5) or its potentiation induced by a single tetanic stimulation and the response induced by AMPA application (10 microM, 10 sec). Furthermore, PREGS does not affect the recurrent inhibition of the fEPSPs mediated by gamma-aminobutyric acid type A (GABA(A)) receptor. In conclusion, this study shows the ability of PREGS to enhance LTP in CA1 by accentuating the activity of NMDA receptors. This modulation of LTP might mediate the steroid-induced enhancement of memory.

J Neurosci Res. 2004 Dec 1;78(5):691-701

### **STEROID PREGNENOLONE SULFATE ENHANCES NMDA-RECEPTOR-INDEPENDENT LONG-TERM POTENTIATION AT HIPPOCAMPAL CA1 SYNAPSES: ROLE FOR L-TYPE CALCIUM CHANNELS AND SIGMA-RECEPTORS.**

Severe stress elevates plasma and CNS levels of endogenous neuroactive steroids that can contribute to the influence of stress on memory formation. Among the neuroactive steroids, pregnenolone sulfate (PREGS) reportedly strengthens memories and is readily available as a memory-enhancing supplement. PREGS actions on memory may reflect its ability to produce changes in memory-related neuronal circuits, such as long-term potentiation (LTP) of excitatory transmission in hippocampus. Here, we report a previously undiscovered pathway by which PREGS exposure promotes activity-dependent LTP of field excitatory postsynaptic potentials at CA1 synapses in hippocampal slices. Thus, application of PREGS, but not the phosphorylated conjugate of the steroid, selectively facilitates the induction of a slow-developing LTP in response to high-frequency (100 Hz) afferent stimulation, which is not induced in the absence of the steroid. The slow-developing LTP is independent of NMDA-receptor

function (i.e., dAP5 insensitive) but dependent on functional L-type voltage-gated calcium channels (VGCC) and sigma-receptors. By contrast, PREGS at the highest concentration tested produces a depression in NMDA-receptor-dependent LTP, which is evident when sigma-receptor function is compromised by the presence of a sigma-receptor antagonist. We found that at early times during the induction phase of L-type VGCC-dependent LTP, PREGS via sigma-receptors transiently enhances presynaptic function. As well, during the maintenance phase of L-type VGCC-dependent LTP, PREGS promotes a further increase in presynaptic function downstream of LTP induction, as evidenced by a decrease in paired-pulse facilitation. The identification of complex regulatory actions of PREGS on LTP, involving sigma-receptors, L-type VGCCs, NMDA-receptors, and inhibitory circuits will aid future research endeavors aimed at understanding the precise mechanisms by which this stress-associated steroid may engage multiple LTP-signaling pathways that alter synaptic transmission at memory-related synapses.

Hippocampus. 2007;17(5):349-69

### **ROLE OF PREGNENOLONE, DEHYDROEPIANDROSTERONE AND THEIR SULFATE ESTERS ON LEARNING AND MEMORY IN COGNITIVE AGING.**

Aging is a general process of functional decline which involves in particular a decline of cognitive abilities. However, the severity of this decline differs from one subject to another and inter-individual differences have been reported in humans and animals. These differences are of great interest especially as concerns investigation of the neurobiological factors involved in cognitive aging. Intensive pharmacological studies suggest that neurosteroids, which are steroids synthesized in the brain in an independent manner from peripheral steroid sources, could be involved in learning and memory processes. This review summarizes data in animals and humans in favor of a role of neurosteroids in cognitive aging. Studies in animals demonstrated that the neurosteroids pregnenolone (PREG) and dehydroepiandrosterone (DHEA), as sulfate derivatives (PREGS and DHEAS, respectively), display memory-enhancing properties in aged rodents. Moreover, it was recently shown that memory performance was correlated with PREGS levels in the hippocampus of 24-month-old rats. Human studies, however, have reported contradictory results. First, improvement of learning and memory dysfunction was found after DHEA administration to individuals with low DHEAS levels, but other studies failed to detect significant cognitive effects after DHEA administration. Second, cognitive dysfunctions have been associated with low DHEAS levels, high DHEAS levels, or high DHEA levels; while in other studies, no relationship was found. As future research perspectives, we propose the use of new methods of quantification of neurosteroids as a useful tool for understanding their respective role in improving learning and memory impairments associated with normal aging and/or with pathological aging, such as Alzheimer's disease.

Brain Res Brain Res Rev. 2001 Nov;37(1-3):301-12

**ENERGY BALANCE AND BREAST CANCER RISK: A PROSPECTIVE COHORT STUDY.**

While there is evidence that breast cancer risk is positively associated with body mass index (in postmenopausal women) and energy intake and inversely associated with physical activity, few studies have examined breast cancer risk in association with energy balance, the balance between energy intake and expenditure. Therefore, in the cohort study reported here, we studied the independent and combined associations of vigorous physical activity, energy consumption, and body mass index (BMI), with breast cancer risk. The investigation was conducted in 49,613 Canadian women who were participants in the National Breast Screening Study (NBSS) and who completed self-administered lifestyle and food frequency questionnaires between 1980 and 1985. Linkages to national mortality and cancer databases yielded data on deaths and cancer incidence, with follow-up ending between 1998 and 2000. During a mean 16.4 years of follow-up, we observed 2,545 incident breast cancer cases. Due to exclusions for various reasons, the analyses were based on 40,318 subjects amongst whom there were 1,673 incident cases of breast cancer. Participation in vigorous physical activity and body mass index were not independently associated with breast cancer risk in the total cohort. A statistically significant positive trend was observed, however, between energy intake and breast cancer risk ( $P$  (trend) = 0.01). Although there was some variation in risk associated with vigorous physical activity, and BMI when the analyses were stratified by menopausal status, these interactions were not statistically significant. The interaction between menopausal status and energy intake, however, was of borderline statistical significance ( $P$  (interaction) = 0.06), with a statistically significant increased risk of breast cancer associated with highest versus lowest quartile of energy intake among premenopausal women (Hazard Ratio [HR] = 1.45, 95% confidence interval [CI] = 1.13- 1.85,  $P$  (trend) = 0.001). There was evidence of an increased risk of breast cancer associated with a relatively high body mass index among postmenopausal women in the highest quartile level of energy intake (Hazard Ratio [HR] = 1.72, 95% confidence interval [CI] = 1.01- 2.93,  $P$  (trend) = 0.05). In addition, there was evidence of an increased risk of breast cancer among premenopausal, physically inactive, overweight/obese women who consumed  $\geq 1972$  kcal/day compared to physically active normal weight women who consumed  $< 1972$  kcal/day (HR = 1.60, 95% CI = 1.08-2.37). Our data suggest that obese premenopausal women with relatively high energy intake may be at increased risk of breast cancer. In addition, energy imbalance, represented by a relatively high energy intake, lack of participation in vigorous physical activity, and a relatively high body mass index, may be associated with increased breast cancer risk, particularly among premenopausal women.

Breast Cancer Res Treat. 2006 May;97(1):97-106

**URINARY ESTROGEN METABOLITES AND BREAST CANCER: A CASE-CONTROL STUDY.**

Preliminary studies suggest that the estrogen metabolite 16 alpha-hydroxyestrone is associated with breast cancer, whereas 2-hydroxyestrone is not. However, epidemiological studies evaluating this relationship and taking established risk factors for breast cancer into account are lacking. The purpose of this study was to examine the association of the ratio of the urinary estrogen metabolites (2-hydroxyestrone and 16 alpha-hydroxyestrone) and of the individual metabolites with breast cancer. A spot urine sample, a brief history, and clinical data were collected from breast cancer cases ( $n = 42$ ) and from women coming to the hospital for a routine mammogram or attending a free breast cancer screening ( $n = 64$ ). 2-Hydroxyestrone and 16 alpha-hydroxyestrone were measured by enzyme immunoassay, and the estrogen metabolite ratio (EMR; 2-hydroxyestrone:16 alpha-hydroxyestrone) was computed. Cases and controls were similar in terms of age (mean age of cases, 53.8  $\pm$  15.1 years, versus 54.2  $\pm$  10.4 years for controls;  $P = 0.9$ ) and demographics. Mean EMR was not associated with breast cancer overall (1.67  $\pm$  0.80 versus 1.72  $\pm$  0.66;  $P = 0.7$ ). However, in postmenopausal women, the mean EMR was significantly lower in cases compared to controls (1.41  $\pm$  0.73 versus 1.81  $\pm$  0.71;  $P = 0.05$ ). The multivariate adjusted odds ratios for the intermediate and lowest tertiles of the EMR relative to the highest among postmenopausal women were 9.73 (95% confidence interval, 1.27-74.84) and 32.74 (95% confidence interval, 3.36-319.09), respectively. The test for trend was highly significant ( $P = 0.003$ ). Analyses of the individual metabolites indicated that 16 alpha-hydroxyestrone was a strong risk factor. The EMR did not show any consistent associations with age, race/ethnicity, age at first birth, parity, body mass index, family history of breast cancer, smoking, or alcohol intake. These data suggest a strong, inverse association of the EMR and a strong positive association of 16 alpha-hydroxyestrone with breast cancer in postmenopausal women. Larger studies are needed to confirm these results and to assess the relationship of the EMR and of the individual metabolites with breast cancer, with attention to menopausal status and clinical factors and with adjustment for known breast cancer risk factors.

### **ESTROGEN METABOLISM AND BREAST CANCER.**

**BACKGROUND:** Specific pathways involved in estrogen metabolism may play a role in the etiology of breast cancer. We used data from a large population-based case-control study to assess the association of the urinary estrogen metabolites 2-hydroxyestrone (2-OHE1), 16 $\alpha$ -hydroxyestrone (16-OHE1), and their ratio (2/16) with both invasive and in situ breast cancer. **METHODS:** Study participants from the Long Island Breast Cancer Study Project provided a spot urine specimen and completed a comprehensive interviewer-administered questionnaire. Women who used exogenous hormones or who took tamoxifen in the 6 months before urine collection were excluded from the analysis, leaving 269 invasive cases, 158 in situ cases, and 326 controls. Unconditional logistic regression was used to obtain adjusted odds ratios (ORs) for invasive and in situ breast cancer, separately, in relation to tertiles of the individual metabolites (standardized for creatinine) and the 2/16 ratio, stratified by menopausal status. **RESULTS:** The OR for invasive breast cancer was inversely associated with the 2/16 ratio among premenopausal women (OR = 0.50 for extreme tertiles; 95% confidence interval = 0.25-1.01). ORs ranged from 0.32 to 0.60 when women were stratified by whether cases had received chemotherapy within 6 months before urine collection and by estrogen receptor status. In postmenopausal women, there was a slight reduction in the odds ratio for invasive cancer with high levels of the 2/16 ratio (OR = 0.78; 95% confidence interval = 0.46-1.33). Neither the individual metabolites nor the ratio were associated with in situ breast cancer. **CONCLUSION:** These data provide support for the hypothesis that the 2/16 ratio is associated with reduced breast cancer risk. The most consistent associations were observed with invasive cancer in premenopausal women.

Epidemiology. 2006 Jan;17(1):80-8

### **INDOLE-3-CARBINOL. A NOVEL APPROACH TO BREAST CANCER PREVENTION.**

The results show that all of the carcinogens, oncogenes, and tumor-associated viruses that we have studied profoundly affect the extent of 2- and 16  $\alpha$ -hydroxylation in a prorsik direction. All of the dietary and biological responses associated with increased cancer risk decrease 2-hydroxylation and increase 16  $\alpha$ -hydroxylation. Remarkably, although PAHs are reported to induce P450-1A1, we have found them to decrease 2-hydroxylation. Finally, using indole-3-carbinol to induce 2-hydroxylation results in the chemoprevention of mammary tumors in rodents and recurrences of laryngeal papillomas in humans. Also correlating with these studies in HPV is the decrease in the C-2/C-16  $\alpha$  metabolite ratio observed in women with CIN relative to control subjects. The greatest decrease was observed in women with the most severe form, CIN3. These findings are under further investigation.

Ann NY Acad Sci. 1995 Sep 30;768:180-200

### **HORMONAL PROFILES IN WOMEN WITH BREAST CANCER.**

The literature findings on endogenous hormonal profiles in women with breast cancer are reviewed in detail. It is concluded that four sets of findings are valid: (1) diminished adrenal androgen production, probably genetic, in women with premenopausal breast cancer; (2) ovarian dysfunction (luteal inadequacy plus increased testosterone production) in breast cancer at all ages; (3) increased 16  $\alpha$ -hydroxylation of estradiol in breast cancer at all ages; and (4) evidence that prolactin is a permissive risk factor for breast cancer, and that the pregnancy-induced decrease in prolactin levels may account for the protective effect of early pregnancy against breast cancer.

Obstet Gynecol Clin North Am. 1994 Dec;21(4):751-72

### **SOY ISOFLAVONES—BENEFITS AND RISKS FROM NATURE'S SELECTIVE ESTROGEN RECEPTOR MODULATORS (SERMS).**

Phytoestrogens have become one of the more topical areas of interest in clinical nutrition. These non-nutrient bioactive compounds are ubiquitous to the plant kingdom and possess a wide range of biological properties that contribute to the many different health-related benefits reported for soy foods and flaxseeds—two of the most abundant dietary sources of phytoestrogens. Reviewed is the recent knowledge related to their pharmacokinetics and clinical effects, focusing mainly on

isoflavones that are found in high concentrations in soy foods. Arguments are made for considering soy isoflavones as natural selective estrogen receptor modulators (SERMs) based upon recent data of their conformational binding to estrogen receptors. Rebuttal is made to several key and important issues related to the recent concerns about the safety of soy and its constituent isoflavones. This article is not intended to be a comprehensive review of the literature but merely highlight recent research with key historical perspectives.

J Am Coll Nutr. 2001 Oct;20(5 Suppl):354S-362S

**WATERCRESS SUPPLEMENTATION IN DIET REDUCES LYMPHOCYTE DNA DAMAGE AND ALTERS BLOOD ANTIOXIDANT STATUS IN HEALTHY ADULTS.**

**BACKGROUND:** Cruciferous vegetable (CV) consumption is associated with a reduced risk of several cancers in epidemiologic studies. **OBJECTIVE:** The aim of this study was to determine the effects of watercress (a CV) supplementation on biomarkers related to cancer risk in healthy adults. **DESIGN:** A single-blind, randomized, crossover study was conducted in 30 men and 30 women (30 smokers and 30 nonsmokers) with a mean age of 33 y (range: 19-55 y). The subjects were fed 85 g raw watercress daily for 8 wk in addition to their habitual diet. The effect of supplementation was measured on a range of endpoints, including DNA damage in lymphocytes (with the comet assay), activity of detoxifying enzymes (glutathione peroxidase and superoxide dismutase) in erythrocytes, plasma antioxidants (retinol, ascorbic acid, alpha-tocopherol, lutein, and beta-carotene), plasma total antioxidant status with the use of the ferric reducing ability of plasma assay, and plasma lipid profile. **RESULTS:** Watercress supplementation (active compared with control phase) was associated with reductions in basal DNA damage (by 17%;  $P = 0.03$ ), in basal plus oxidative purine DNA damage (by 23.9%;  $P = 0.002$ ), and in basal DNA damage in response to ex vivo hydrogen peroxide challenge (by 9.4%;  $P = 0.07$ ). Beneficial changes seen after watercress intervention were greater and more significant in smokers than in nonsmokers. Plasma lutein and beta-carotene increased significantly by 100% and 33% ( $P < 0.001$ ), respectively, after watercress supplementation. **CONCLUSION:** The results support the theory that consumption of watercress can be linked to a reduced risk of cancer via decreased damage to DNA and possible modulation of antioxidant status by increasing carotenoid concentrations.

Am J Clin Nutr. 2007 Feb;85(2):504-10

**SULFORAPHANE INDUCES CELL TYPE-SPECIFIC APOPTOSIS IN HUMAN BREAST CANCER CELL LINES.**

Sulforaphane, an isothiocyanate found in cruciferous vegetables, has been shown to induce phase 2 detoxication enzymes and inhibit the growth of chemically induced mammary tumors in rats, although the exact mechanisms of action of sulforaphane are not understood. In this study, we evaluated the effects of sulforaphane on cell growth and death in several human breast cancer cell lines and examined the hypothesis that sulforaphane acts as a histone deacetylase (HDAC) inhibitor in these cell lines. Sulforaphane treatment inhibited cell growth, induced a G(2)-M cell cycle block, increased expression of cyclin B1, and induced oligonucleosomal DNA fragmentation in the four human breast cancer cell lines examined, MDA-MB-231, MDA-MB-468, MCF-7, and T47D cells. Activation of apoptosis by sulforaphane in MDA-MB-231 cells seemed to be initiated through induction of Fas ligand, which resulted in activation of caspase-8, caspase-3, and poly(ADP-ribose) polymerase, whereas apoptosis in the other breast cancer cell lines was initiated by decreased Bcl-2 expression, release of cytochrome c into the cytosol, activation of caspase-9 and caspase-3, but not caspase-8, and poly(ADP-ribose) polymerase cleavage. Sulforaphane inhibited HDAC activity and decreased the expression of estrogen receptor-alpha, epidermal growth factor receptor, and human epidermal growth factor receptor-2 in each cell line, although no change in the acetylation of H3 or H4 was seen. These data suggest that sulforaphane inhibits cell growth, activates apoptosis, inhibits HDAC activity, and decreases the expression of key proteins involved in breast cancer proliferation in human breast cancer cells. These results support testing sulforaphane in vivo and warrant future studies examining the clinical potential of sulforaphane in human breast cancer.

Mol Cancer Ther. 2007 Mar;6(3):1013-21

**CRUCIFEROUS VEGETABLES AND HUMAN CANCER RISK: EPIDEMIOLOGIC EVIDENCE AND MECHANISTIC BASIS.**

Cruciferous vegetables are a rich source of glucosinolates and their hydrolysis products, including indoles and isothiocyanates, and high intake of cruciferous vegetables has been associated with lower risk of lung and colorectal cancer in some epidemiological studies. Glucosinolate hydrolysis products alter the metabolism or activity of sex hormones in ways that could inhibit the development of hormone-sensitive cancers, but evidence of an inverse association between cruciferous vegetable intake and breast or prostate cancer in humans is limited and inconsistent. Organizations such as the National Cancer Institute

recommend the consumption of five to nine servings of fruits and vegetables daily, but separate recommendations for cruciferous vegetables have not been established. Isothiocyanates and indoles derived from the hydrolysis of glucosinolates, such as sulforaphane and indole-3-carbinol (I3C), have been implicated in a variety of anticarcinogenic mechanisms, but deleterious effects also have been reported in some experimental protocols, including tumor promotion over prolonged periods of exposure. Epidemiological studies indicate that human exposure to isothiocyanates and indoles through cruciferous vegetable consumption may decrease cancer risk, but the protective effects may be influenced by individual genetic variation (polymorphisms) in the metabolism and elimination of isothiocyanates from the body. Cooking procedures also affect the bioavailability and intake of glucosinolates and their derivatives. Supplementation with I3C or the related dimer 3,3'-diindolylmethane (DIM) alters urinary estrogen metabolite profiles in women, but the effects of I3C and DIM on breast cancer risk are not known. Small preliminary trials in humans suggest that I3C supplementation may be beneficial in treating conditions related to human papilloma virus infection, such as cervical intraepithelial neoplasia and recurrent respiratory papillomatosis, but larger randomized controlled trials are needed.

Pharmacol Res. 2007 Mar;55(3):224-36

### **HEAD AND NECK CANCER: A CASE FOR INHIBITION BY ISOTHIOCYANATES AND INDOLES FROM CRUCIFEROUS VEGETABLES.**

Chemical carcinogens derived from cigarettes and other tobacco products, as well as betel quid, paan, and alcohol consumption, are commonly associated with head and neck cancer risk. This is a particularly debilitating cancer, with a high recurrence rate and long-term treatment comorbidities affecting health and lifestyle. Controlling tobacco access or use may be an ideal prevention strategy but may also be challenging or undesired. Individuals, however, may be able to reduce their risk through simple and focused dietary change. Results from epidemiologic studies, basic research, and clinical investigations suggest that a diet rich in cruciferous vegetables may increase carcinogen metabolism, induce apoptosis, and reduce the risk of developing a primary head and neck tumor. This review briefly summarizes head and neck cancer nutritional epidemiology, and then describes the biochemical and epidemiologic literature describing the effects of crucifer consumption on head and neck carcinogenesis. To translate these findings, the strengths and limitations of specific intervention models are discussed, including differences in target populations and the choice of a food-based or pill-based approach for intervention. Addressing these factors in a future intervention may define a low-cost and non-toxic approach to reduce the burden of head and neck cancer.

Eur J Cancer Prev. 2007 Aug;16(4):348-56

### **PHENETHYL ISOTHIOCYANATE, A CANCER CHEMOPREVENTIVE CONSTITUENT OF CRUCIFEROUS VEGETABLES, INHIBITS CAP-DEPENDENT TRANSLATION BY REGULATING THE LEVEL AND PHOSPHORYLATION OF 4E-BP1.**

Phenethyl isothiocyanate (PEITC), a constituent of many edible cruciferous vegetables, exerts significant protection against chemically induced cancer in animal models and inhibits growth of cancer cells in culture and in vivo by causing cell cycle arrest and apoptosis induction. In this study, we report a novel response to PEITC involving the regulation of translation initiation at pharmacologically achievable concentrations. Treatment of human colorectal cancer HCT-116 cells and human prostate cancer PC-3 cells, but not a normal prostate epithelial cell line (PrEC), with PEITC caused an increase in expression of the eukaryotic translation initiation factor 4E (eIF4E) binding protein (4E-BP1) and inhibition of 4E-BP1 phosphorylation. Results from pull-down assay using 7-methyl-GTP Sepharose 4B beads indicated that PEITC treatment reduced cap-bound eIF4E, confirming that increased 4E-BP1 expression and inhibition of 4E-BP1 phosphorylation indeed reduced the availability of eIF4E for translation initiation. Accordingly, results from in vivo translation using luciferase reporter assay indicated that PEITC treatment inhibited cap-dependent translation, in particular the translation of mRNA with secondary structure (stem-loop structure). Ectopic expression of eIF4E prevented PEITC-induced translation inhibition and conferred significant protection against PEITC-induced apoptosis. These results indicate that PEITC modulates availability of eIF4E for translation initiation leading to inhibition of cap-dependent translation. The present study also suggests that inhibition of cap-dependent translation may be an important mechanism in PEITC-induced apoptosis.

Cancer Res. 2007 Apr 15;67(8):3569-73

### **MOLECULAR BASIS FOR CHEMOPREVENTION BY SULFORAPHANE: A COMPREHENSIVE REVIEW.**

The consumption of cruciferous vegetables has long been associated with a reduced risk in the occurrence of cancer at various sites, including the prostate, lung, breast and colon. This protective effect is attributed to isothiocyanates present in these

vegetables, and sulforaphane (SF), present in broccoli, is by far the most extensively studied to uncover the mechanisms behind this chemoprotection. The major mechanism by which SF protects cells was traditionally thought to be through Nrf2-mediated induction of phase 2 detoxification enzymes that elevate cell defense against oxidative damage and promote the removal of carcinogens. However, it is becoming clear that there are multiple mechanisms activated in response to SF, including suppression of cytochrome P450 enzymes, induction of apoptotic pathways, suppression of cell cycle progression, inhibition of angiogenesis and anti-inflammatory activity. Moreover, these mechanisms seem to have some degree of interaction to synergistically afford chemoprevention.

Cell Mol Life Sci. 2007 May;64(9):1105-27

### **ASSESSMENT OF THE ANTI-GENOTOXIC, ANTI-PROLIFERATIVE, AND ANTI-METASTATIC POTENTIAL OF CRUDE WATERCRESS EXTRACT IN HUMAN COLON CANCER CELLS.**

Although it is known to be a rich source of the putative anti-cancer chemicals isothiocyanates, watercress has not been extensively studied for its cancer preventing properties. The aim of this study was to investigate the potential chemoprotective effects of crude watercress extract toward three important stages in the carcinogenic process, namely initiation, proliferation, and metastasis (invasion) using established in vitro models. HT29 cells were used to investigate the protective effects of the extract on DNA damage and the cell cycle. The extract was not genotoxic but inhibited DNA damage induced by two of the three genotoxins used, namely hydrogen peroxide and fecal water, indicating the potential to inhibit initiation. It also caused an accumulation of cells in the S phase of the cell cycle indicating (possible) cell cycle delay at this stage. The extract was shown to significantly inhibit invasion of HT115 cells through matrigel. Component analysis was also carried out in an attempt to determine the major phytochemicals present in both watercress leaves and the crude extract. In conclusion, the watercress extract proved to be significantly protective against the three stages of the carcinogenesis process investigated.

Nutr Cancer. 2006;55(2):232-41

### **BROCCOLI AND WATERCRESS SUPPRESS MATRIX METALLOPROTEINASE-9 ACTIVITY AND INVASIVENESS OF HUMAN MDA-MB-231 BREAST CANCER CELLS.**

A high dietary intake of cruciferous vegetables has been associated with a reduction in numerous human pathologies particularly cancer. In the current study, we examined the inhibitory effects of broccoli (*Brassica oleracea* var. *italica*) and watercress (*Rorripa nasturtium aquaticum*) extracts on 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced cancer cell invasion and matrix metalloproteinase-9 activity using human MDA-MB-231 breast cancer cells. Aberrant overexpression of matrix metalloproteinases, including metalloproteinase-9, is associated with increased invasive potential in cancer cell lines. Our results demonstrate that extracts of broccoli and *Rorripa* suppressed TPA-induced MMP-9 activity and invasiveness in a concentration dependent manner as determined by zymographic analysis. Furthermore, fractionation of individual extracts followed by liquid chromatography mass spectroscopy analysis (LC-MS) revealed that the inhibitory effects of each vegetable were associated with the presence of 4-methylsulfinylbutyl (sulforaphane) and 7-methylsulphinylheptyl isothiocyanates. Taken together, our data indicate that isothiocyanates derived from broccoli and *Rorripa* inhibit metalloproteinase 9 activities and also suppress the invasive potential of human MDA-MB-231 breast cancer cells in vitro. The inhibitory effects observed in the current study may contribute to the suppression of carcinogenesis by diets high in cruciferous vegetables.

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### **BETA-PHENYLETHYL AND 8-METHYLSULPHINYLOCTYL ISOTHIOCYANATES, CONSTITUENTS OF WATERCRESS, SUPPRESS LPS INDUCED PRODUCTION OF NITRIC OXIDE AND PROSTAGLANDIN E2 IN RAW 264.7 MACROPHAGES.**

Beta-phenylethyl (PEITC) and 8-methylsulphinyl octyl isothiocyanates (MSO) represent two phytochemical constituents present in watercress *Rorripa nasturtium aquaticum*, with known chemopreventative properties. In the present investigation, we examined whether PEITC and MSO could modulate the inflammatory response of Raw 264.7 macrophages to bacterial lipopolysaccharide (LPS) by assessment of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expression. Overproduction of both nitric oxide (NO) and prostaglandins (PGE) has been associated with numerous pathological conditions including chronic inflammation and cancer. Our results demonstrate that LPS (1 microg/ml approximately 24 h) induced nitrite and prostaglandin E2 (PGE-2) synthesis in Raw 264.7 cells was attenuated by both isothiocyanates (ITCs) in a concentration-dependent manner. Both PEITC and MSO decreased (iNOS) and (COX-2) protein expression levels leading to reduced secretion of both pro-

inflammatory mediators. Interestingly, the reduction in both iNOS and COX-2 expression were associated with the inactivation of nuclear factor-kappaB and stabilization of IkappaBalph. Taken together our data gives further insight into the possible chemopreventative properties of two dietary derived isothiocyanates from watercress.

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