

### **Distal procto-colitis and n-3 polyunsaturated fatty acids: the mechanism(s) of natural cytotoxicity inhibition.**

Almallah YZ, El-Tahir A, Heys SD, et al. *Eur J Clin Invest* 2000;30:58-65.

**BACKGROUND:** Altered natural killer (NK) and lymphokine-activated killer (LAK) cell activities have been reported with ulcerative colitis (UC). Previously, we have shown that in patients with UC, the n-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), specifically inhibit natural cytotoxicity with clinical improvement in disease activity. The aim of this study therefore was to evaluate the possible mechanism(s) involved in this inhibition, and in particular the alteration of production of interleukin 2 (IL2) and the arachidonic acid metabolite leukotriene B4 (LTB4), both known to modulate NK cell activity. **MATERIALS AND METHODS:** Each patient with procto-colitis received either fish oil extract (EPA 3.2 g, DHA 2.4 g; n = 9) or placebo (n = 9) daily for 6 months. Monthly assessment included disease activity using clinical and sigmoidoscopic scores. Peripheral blood mononuclear (PBMN) cells were isolated and NK cell cytotoxic activity in vitro was measured. Monthly serum samples were analysed for LTB4, IL2 and soluble IL2 receptors (sIL2R). **RESULTS:** The n-3 PUFAs group had significantly reduced NK cell activity, compared with the placebo group ( $P < 0.05$ , Mann-Whitney U-test). In the n-3 PUFA group, incubation of PBMN cells for 72 h with recombinant interleukin 2 (rIL2) reversed the NK inhibition. In patients with active proctocolitis, serum levels of LTB4 correlated positively with NK cell cytotoxicity ( $r = 0.873$ ,  $P < 0.05$ , Kendall's correlation coefficient). After six months of n-3 PUFAs supplementation, serum levels of LTB4 were undetectable with concurrent significant reduction in NK cell cytotoxic activity. The latter was associated with significant reduction of serum IL2 and sIL2R levels ( $P < 0.05$ ). **CONCLUSION:** This study has demonstrated both evidence of suppression of immune reactivity and concurrent reduction in disease activity in patients with procto-colitis receiving n-3 PUFAs supplementation. This may have important implications for therapy in patients with UC.

### **Genistein induced molecular changes in a squamous cell carcinoma of the head and neck cell line.**

Alhasan SA, Ensley JF, Sarkar FH. *Int J Oncol* 2000;16:333-338.

Epidemiological studies have shown lower incidence of breast and prostate cancers in Asian populations consuming a traditional diet rich in soy. Protection from these cancers was attributed to the isoflavones, particularly genistein and daidzein found in vivo as the major metabolites of soy isoflavones. However, the role of isoflavones in head and neck cancer is less clear. In our previous studies we reported that genistein can induce cell growth inhibition by arresting the cells at S/G2-M phases, and also induces apoptosis in HN4 squamous cell carcinoma of the head and neck cell line (HNSCC). In this report we show that these changes are accompanied by the down-regulation of Cdk1, and CyclinB1, and up-regulation of the cyclin dependent kinase (Cdk) inhibitor p21WAF1, which may be responsible for the induction of cell cycle arrest and apoptosis. The evidence for the induction of apoptosis was supported by the appearance of DNA ladder as reported previously, and further supported by our current results on the cleavage of poly-ADP-ribose polymerase (PARP), hallmark of apoptosis. This was also accompanied by the up-regulation of Bax, with modest down-regulation of Bcl-2 protein expression, which changes the balance between pro- and anti-apoptosis molecules in favor of pro-apoptosis. Furthermore, we also observed down-regulation and degradation of Cdc25C, which is a marker of cell proliferation, and plays important role in CyclinB-Cdk1 complex activation. The down-regulation followed by the degradation of Cdc25C is an indicator of G2/M arrest and anti-proliferation effects of genistein. Collectively, these data provide strong molecular evidence for the anti-tumor activity of genistein in HNSCC cells.

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### **The effect of estrogen replacement therapy on total plasma homocysteine in healthy postmenopausal women.**

Berger PB, Herrmann RR, Dumesic DA. *Mayo Clin Proc* 2000;75:18-23.

**OBJECTIVE:** To clarify the effect of estrogen on total plasma homocysteine concentration and on the concentration of vitamins required for homocysteine metabolism (folate, vitamin B12, and vitamin B6). **METHODS AND RESULTS:** We measured total fasting plasma homocysteine in 16 healthy postmenopausal women before and 6 hours after a methionine load (100 mg/kg); fasting concentrations of folate, vitamin B12, vitamin B6, total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol were also determined. After 6 months of estrogen replacement therapy with estradiol, 2 mg daily, and 1 cycle of quarterly methoxyprogesterone acetate, 5 mg daily administered on the 91st through 100th days, measurements were repeated. There was no significant change in mean  $\pm$  SD fasting homocysteine concentration (8.8 $\pm$ 2.5 vs 8.5 $\pm$ 2.0 micromol/L;  $P=.30$ ); homocysteine concentrations after methionine load increased from 38.8 $\pm$ 12.3 to 51.1 $\pm$ 12.5 micromol/L ( $P=.01$ ). During this time period, no significant changes occurred in the concentrations of folate (11.7 $\pm$ 4.4 vs 9.8 $\pm$ 4.1 nmol/L;  $P=.06$ ), vitamin B12 (394 $\pm$ 182 vs 411 $\pm$ 155 pmol/L;  $P=.40$ ), or vitamin B6 (pyridoxal phosphate) (26 $\pm$ 21 vs 36 $\pm$ 25 nmol/L;  $P=.15$ ). The mean  $\pm$  SD concentration of low-density cholesterol declined 20% (from 147 $\pm$ 32 to 118 $\pm$ 37 mg/dL) and high-density lipoprotein increased 16% (from 40 $\pm$ 13 to 46 $\pm$ 19 mg/dL) during the study period. **CONCLUSIONS:** Six months of estrogen replacement therapy did not lower fasting plasma total homocysteine concentrations and raised homocysteine concentrations following a methionine load. Lipid profiles improved significantly during the study period. A reduction in homocysteine concentrations is not likely to contribute to the reduction in cardiovascular events seen with estrogen replacement therapy.

**Protection against cis-diamminedichloroplatinum-induced nephrotoxicity by 2,3-dimercaptosuccinic acid in rats.**

Mishima K, Hidaka S,  
Takamura N, Shinozawa S.  
*Ren Fail* 1999;21:593-602.

The present study was designed to examine the usefulness of 2,3-dimercaptosuccinic acid (DMSA) for the purpose of reducing cis-diamminedichloroplatinum (DDP)-induced nephrotoxicity and effective clinical use of DDP and safe. The effectiveness of DMSA on the DDP-excretion in rat kidney was observed by measuring the platinum concentration using Atomic Absorption Instrument. Co-administration of DMSA (1.0 or 2.0 mmol/kg) 1 hour after DDP injection (20  $\mu$ mol/kg) showed more decrease in the platinum concentration than that immediately after DDP injection. The alleviating effect of DMSA on DDP toxicity was evaluated by lipid peroxidation, enzymatic antioxidants, and glutathione levels. The administration of DDP alone caused a significant increase in lipid peroxidation and significant decreases in enzymatic antioxidants and glutathione levels in the kidney. Co-administration of DMSA (2.0 mmol/kg) 1 hour after DDP injection showed the most effective reduction of these enzymatic damages caused by DDP. These findings suggested that the co-administration of DMSA (2.0 mmol/kg) 1 hour after DDP injection leads DDP to effectively excrete from renal tissue and suppresses the lipid peroxide reaction and results in reduction of nephrotoxicity.

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### **A conservative triple antioxidant approach to the treatment of hepatitis C. Combination of alpha lipoic acid (thioctic acid), silymarin, and selenium: three case histories.**

Berkson BM. *Med Klin* 1999;94:S84-S89.

**BACKGROUND:** There has been an increase in the number of adults seeking liver transplantation for hepatitis C in the last few years and the count is going up rapidly. There is no reliable and effective therapy for chronic hepatitis C since interferon and antivirals work no more than 30% of the time, and liver transplant surgery is uncertain and tentative over the long run. This is because, ultimately, residual hepatitis C viremia infects the new liver. Furthermore, liver transplantation can be painful, disabling and extremely costly. **TREATMENT PROGRAM:** The author describes a low cost and efficacious treatment program in 3 patients with cirrhosis, portal hypertension and esophageal varices secondary to chronic hepatitis C infection. This effective and conservative regimen combines 3 potent antioxidants (alpha-lipoic acid [thioctic acid], silymarin, and selenium) that possess antiviral, free radical quenching and immune boosting qualities. **CONCLUSION:** There are no remarkably effective treatments for chronic hepatitis C in general use. Interferon and antivirals have less than a 30% response rate and because of the residual viremia, a newly transplanted liver usually becomes infected again. The triple antioxidant combination of alpha-lipoic acid, silymarin and selenium was chosen for a conservative treatment of hepatitis C because these substances protect the liver from free radical damage, increase the levels of other fundamental antioxidants, and interfere with viral proliferation. The 3 patients presented in this paper followed the triple antioxidant program and recovered quickly and their laboratory values remarkably improved. Furthermore, liver transplantation was avoided and the patients are back at work, carrying out their normal activities, and feeling healthy. The author offers a more conservative approach to the treatment of hepatitis C, that is exceedingly less expensive. One year of the triple antioxidant therapy described in this paper costs less than \$2,000, as compared to more than \$300,000 a year for liver transplant surgery. It appears reasonable, that prior to liver transplant surgery evaluation, or during the transplant evaluation process, the conservative triple antioxidant treatment approach should be considered. If there is a significant betterment in the patient's condition, liver transplant surgery may be avoided.

### **Alpha-lipoic acid in the treatment of diabetic polyneuropathy in Germany: current evidence from clinical trials.**

Ziegler D, Reljanovic M, Mehnert H, Gries FA. *Exp Clin Endocrinol Diabetes* 1999;107:421-430.

Diabetic neuropathy represents a major health problem, as it is responsible for substantial morbidity, increased mortality, and impaired quality of life. Near-normoglycaemia is now generally accepted as the primary approach to prevention of diabetic neuropathy, but is not achievable in a considerable number of patients. In the past two decades several medical treatments that exert their effects despite hyperglycaemia have been derived from the experimental pathogenetic concepts of diabetic neuropathy. Such compounds have been designed to improve or slow the progression of the neuropathic process and are being evaluated in clinical trials, but with the exception of alpha-lipoic acid (thioctic acid) which is available in Germany, none of these drugs is currently available in clinical practice. Here we review the current evidence from the clinical trials that assessed the therapeutic efficacy and safety of thioctic acid in diabetic polyneuropathy. Thus far, 15 clinical trials have been completed using different study designs, durations of treatment, doses, sample sizes, and patient populations. Within this variety of clinical trials, those with beneficial effects of thioctic acid on either neuropathic symptoms and deficits due to polyneuropathy or reduced heart rate variability resulting from cardiac autonomic neuropathy used doses of at least 600 mg per day. The following conclusions can be drawn from the recent controlled clinical trials. 1.) Short-term treatment for 3 weeks using 600 mg of thioctic acid i.v. per day appears to reduce the chief symptoms of diabetic polyneuropathy. A 3-week pilot study of 1800 mg per day given orally indicates that the therapeutic effect may be independent of the route of administration, but this needs to be confirmed in a larger sample size. 2.) The effect on symptoms is accompanied by an improvement of neuropathic deficits. 3.) Oral treatment for 4-7 months tends to reduce neuropathic deficits and improves cardiac autonomic neuropathy. 4.) Preliminary data over 2 years indicate possible long-term improvement in motor and sensory nerve conduction in the lower limbs. 5.) Clinical and postmarketing surveillance studies have revealed a highly favourable

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### **Short-term vitamin E supplementation before marathon running: a placebo-controlled trial.**

Buchman AL, Killip D, Ou CN, et al. *Nutrition* 1999;15:278-283

Gastrointestinal complaints and occult bleeding have been commonly described in marathon runners. We hypothesized that these complaints may arise from intestinal ischemia caused by the shunting of blood away from the splanchnic circulation during endurance racing followed by reperfusion injury. Studies in animal models have suggested prophylactic vitamin E supplementation may prevent this type of injury. We sought to determine if prerace vitamin E supplementation would prevent intestinal ischemia/reperfusion injury in humans. Forty subjects who planned to complete the 1996 Houston-Tennaco Marathon were randomized to receive vitamin E (1000 IU daily) or placebo

(soya lecithin) for 2 wk before the race in a double-blinded trial. Inclusion criteria included no use of non-steroidal anti-inflammatory drugs (NSAIDs) within 24 d of the race or vitamin or mineral supplements containing vitamins C or E or selenium within 30 d of the race. Subjects were studied 2 wk before the race and immediately following the race. Blood was obtained for serum vitamin E and total lipid and salicylate concentrations. A solution of lactulose (5 g) and mannitol (2 g) was consumed and urine was collected for 6 h. Aliquots were assayed for lactulose and mannitol concentration. Stool samples were tested for occult blood and following the race subjects rated their nausea, abdominal pain, and cramping on a 1-5 scale. Twenty-six subjects (24 male, 2 female) completed the marathon. Finish times ranged between 2 h 43 min and 5 h 28 min. All subjects had heme-negative stool prerace and four developed heme-positive stool postrace, with no difference between vitamin E and placebo groups (Fisher's exact = 0.63). All had non-detectable salicylate concentrations pre- and postrace. Serum vitamin E concentration increased in both  $P = 0.02$  in the vitamin E group and  $1.45 \pm 0.40$  to  $1.66 \pm 0.48$  mg/dL in the placebo group,  $P = 0.02$ ). However, the serum vitamin E: total lipid ratio increased significantly in the vitamin E-supplemented group ( $0.0022 \pm 0.0002$  to  $0.0051 \pm 0.0015$ ,  $P = 0.02$ ), but not in the placebo group ( $P = 0.25$ ). Overall, the urinary lactulose:mannitol ratio increased from  $0.03 \pm 0.02$  to  $0.06 \pm 0.08$  postrace ( $P = 0.06$ ) without difference between vitamin E or placebo groups. Intestinal permeability increased significantly more in those who developed occult bleeding. More subjects in the placebo group developed abdominal cramping (Fisher's exact = 0.04) and abdominal pain (Fisher's exact = 0.06), although there was no difference in severity between groups. There was no difference in the incidence of nausea and no diarrhea was reported by any subject. Intestinal permeability tends to increase and occult gastrointestinal bleeding occurs during endurance running, suggesting the occurrence of intestinal ischemia/reperfusion injury. Prerace supplementation with the antioxidant vitamin E had no effect on performance, intestinal injury, occult bleeding, or the severity of postrace gastrointestinal complaints. Vitamin E supplementation was associated with a decreased incidence of these complaints but had no effect on their severity.

### **Efficacy of vitamin B-6 in the treatment of premenstrual syndrome: systematic review.**

Wyatt KM, Dimmock PW, Jones PW, Shaughn O'Brien PM. *BMJ* 1999;318:1375-1381.

**OBJECTIVE:** To evaluate the efficacy of vitamin B-6 in the treatment of premenstrual syndrome. **DESIGN:** Systematic review of published and unpublished randomised placebo controlled trials of the effectiveness of vitamin B-6 in the management of premenstrual syndrome. **SUBJECTS:** Nine published trials representing 940 patients with premenstrual syndrome. **MAIN OUTCOME MEASURES:** Proportion of women whose overall premenstrual symptoms showed an improvement over placebo. A secondary analysis was performed on the proportion of women whose premenstrual depressive symptoms showed an improvement over placebo. **RESULTS:** Odds ratio relative to placebo for an improvement in overall premenstrual symptoms was 2.32 (95% confidence interval 1.95 to 2.54). Odds ratio relative to placebo for an improvement in depressive symptoms was 1.69 (1.39 to 2.06) from four trials representing 541 patients. **CONCLUSION:** Conclusions are limited by the low quality of most of the trials included. Results suggest that doses of vitamin B-6 up to 100 mg/day are likely to be of benefit in treating premenstrual symptoms and premenstrual depression.

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### **Essential fatty acid metabolism and its modification in atopic eczema.**

Horrobin DF. *Am J Clin Nutr* 2000;71:367S-372S.

Research from the 1930s to the 1950s established that a deficit of n-6 essential fatty acids (EFAs) leads to an inflammatory skin condition in both animals and humans. In a common inherited skin condition, atopic dermatitis (eczema), there was evidence of low blood EFA concentrations and of a therapeutic response to exceptionally high doses of linoleic acid. More recently, it has been established that there is no deficit of linoleic acid in atopic eczema. Concentrations of linoleic acid instead tend to be elevated in blood, milk, and adipose tissue of patients with atopic eczema, whereas concentrations of linoleic acid metabolites are substantially reduced. This suggests reduced conversion of linoleic acid to gamma-linolenic acid (GLA). In most but not all studies, administration of GLA has been found to improve the clinically assessed skin condition, the objectively assessed skin roughness, and the elevated blood catecholamine concentrations of patients with atopic eczema. Atopic eczema may be a minor inherited abnormality of EFA metabolism.

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### **Vascular nitric oxide, sex hormone replacement, and fish oil may help to prevent Alzheimer's disease by suppressing synthesis of acute-phase cytokines.**

McCarty MF. *Med*

*Hypotheses* 1999;53:369-374.

The neurodegenerative plaques of Alzheimer's disease (AD) are characterized by a self-sustaining acute-phase reaction in which both interleukin-1 (IL-1) and interleukin-6 (IL-6) are up-regulated. The fact that IL-6 is detectable in early stage diffuse plaques encourages the speculation that the acute-phase process is crucial to the pathogenesis of AD. The epidemiological association of AD with estrogen deficiency, as well as with various disorders characterized by vascular endotheliopathy, suggest a protective role for vascular nitric oxide (NO). NO has an autocrine anti-inflammatory impact on endothelium, owing in part to antagonism of NF-kappaB activity; since induction of IL-6 is dependent on NF-kappaB, this may explain recent evidence that NO inhibits macrophage IL-6 production. It is reasonable to postulate that, analogously, cerebrovascular NO decreases IL-6 production in the brain. Vascular NO may also have direct neuroprotective activity. Estrogen, in addition to promoting vascular NO synthesis, can block IL-6 production by a more direct mechanism in cells expressing estrogen receptors; since such receptors have been reported in brain glia and astrocytes, estrogen has the potential to limit brain IL-1 activity. Testosterone likewise can inhibit IL-6 induction in androgen-responsive cells, which may include brain glia and astrocytes. Since fish oil and gamma linolenic acid (GLA) suppress IL-1 production by stimulated monocytes, they conceivably could exert this effect in the brain as well; the comparatively low prevalence of AD in elderly Japanese is intriguing in this regard. These considerations suggest that a healthy cerebrovascular endothelium, sex hormone activity, and dietary fish oil/GLA may slow or prevent AD onset by dampening acute-phase mechanisms in the brain.

**Polyunsaturated fatty acids and inflammatory bowel disease.**

Belluzzi A, Boschi S, Brignola C, et al. *Am J Clin Nutr* 2000;71:339S-342S.

The rationale for supplementation with n-3 fatty acids to promote the health of the gastrointestinal tract lies in the antiinflammatory effects of these lipid compounds. The first evidence of the importance of dietary intake of n-3 polyunsaturated fatty acids was derived from epidemiologic observations of the low incidence of inflammatory bowel disease in Eskimos. The aim of this paper was to briefly review the literature on the use of n-3 fatty acids in inflammatory bowel disease (ulcerative colitis and Crohn disease), the results of which are controversial. The discrepancies between studies may reside in the different study designs used as well as in the various formulations and dosages used, some of which may lead to a high incidence of side effects. Choosing a formulation that lowers the incidence of side effects, selecting patients carefully, and paying strict attention to experimental design are critical when investigating further the therapeutic potential of these lipids in inflammatory bowel disease.

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### **Efficacy of succimer chelation for reducing brain lead in a primate model of human lead exposure.**

Cremin JD Jr, Luck ML, Laughlin NK, Smith DR.  
*Toxicol Appl Pharmacol*  
1999;161:283-293.

The extent to which succimer (meso-2,3-dimercaptosuccinic acid [DMSA], Chemet) reduces brain lead (Pb) levels may be a primary consideration in evaluating its efficacy for reducing neurotoxicity. Clinical research in this area has been hampered by the need to use blood Pb levels as the index of treatment efficacy, despite the fact that brain Pb level is the exposure parameter of greater relevance to cognitive outcomes. Here, a nonhuman primate model of human Pb exposure was used to determine: (1) The efficacy of oral succimer for reducing brain Pb derived from chronic or recent exposures, and (2) The extent to which blood Pb levels reflect brain Pb prior to and following chelation. Adult rhesus monkeys were chronically exposed to Pb orally for 5 weeks to reach and maintain a target blood Pb level of 35-40 microg/dL. Chelation of Pb from recent exposures was assessed using a stable (204)Pb isotope tracer administered over 4 days prior to treatment. Immediately prior to chelation, a prefrontal cortex (PFC) biopsy was collected to determine pretreatment brain Pb levels. Subsequently, monkeys were assigned to vehicle (n = 5) or succimer (n = 6, 30 mg/kg/day x 5 days followed by 20 mg/kg/day x 14 days) groups. Blood and brain PFC, frontal lobe (FL), hippocampus (H), and striatum (S) were analyzed for total Pb and (204)Pb tracer concentrations by magnetic sector inductively coupled plasma-mass spectrometry. There were no measurable differences in brain Pb concentrations between the succimer and vehicle groups, indicating that succimer treatment was not efficacious in reducing brain Pb levels. In contrast, the cessation of Pb exposure significantly reduced brain (PFC) Pb (approximately 34%) when compared to pretreatment levels (succimer and vehicle groups). Pb concentrations also varied among brain regions (PFC > FL approximately H > S). Finally, pretreatment PFC Pb concentrations were significantly correlated with the integrated blood Pb level (AUC) over the Pb exposure period, but not with the single pretreatment blood Pb collected concurrently with the PFC biopsy. Following treatment, blood Pb levels correlated only with Pb in the PFC, and not the other brain regions measured (FL, H, S). These data indicate that, under the conditions of this study, succimer treatment did not reduce brain Pb levels beyond the cessation of Pb exposure alone. Moreover, a single blood Pb measurement may be a poor predictor of brain Pb levels, reflecting limitations in the use of blood Pb level as an indicator of treatment efficacy.

### **Niacinamide therapy for osteoarthritis - does it inhibit nitric oxide synthase induction by interleukin 1 in chondrocytes?**

McCarty MF, Russell AL.  
*Med Hypotheses*  
1999;53:350-360.

Fifty years ago, Kaufman reported that high-dose niacinamide was beneficial in osteoarthritis (OA) and rheumatoid arthritis. A recent double-blind study confirms the efficacy of niacinamide in OA. It may be feasible to interpret this finding in the context of evidence that synovium-generated interleukin-1 (IL-1), by inducing nitric oxide (NO) synthase and thereby inhibiting chondrocyte synthesis of aggrecan and type II collagen, is crucial to the pathogenesis of OA. Niacinamide and other inhibitors of ADP-ribosylation have been shown to suppress cytokine-mediated induction of NO synthase in a number of types of cells; it is therefore reasonable to speculate that niacinamide will have a comparable effect in IL-1-exposed chondrocytes, blunting the anti-anabolic impact of IL-1. The chondroprotective antibiotic doxycycline may have a similar mechanism of action. Other nutrients reported to be useful in OA may likewise intervene in the activity or synthesis of IL-1. Supplemental glucosamine can be expected to stimulate synovial synthesis of hyaluronic acid; hyaluronic acid suppresses the anti-catabolic effect of IL-1 in chondrocyte cell cultures, and has documented therapeutic efficacy when injected intra-articularly. S-adenosylmethionine (SAM), another proven therapy for OA, upregulates the proteoglycan synthesis of chondrocytes, perhaps because it functions physiologically as a signal of sulfur availability. IL-1 is likely to decrease SAM levels in chondrocytes; supplemental SAM may compensate for this deficit. Adequate selenium nutrition may down-regulate cytokine signaling, and ample intakes of fish oil can be expected to decrease synovial IL-1 production; these nutrients should receive further evaluation in OA. These considerations suggest that non-toxic nutritional regimens, by intervening at multiple points in the signal transduction pathways that promote the synthesis and mediate the activity of IL-1, may provide a substantially superior alternative to NSAIDs (merely palliative and often dangerously toxic) in the treatment and perhaps prevention of OA.

### **Estrogen synthesis in human colon cancer epithelial cells.**

Fiorelli G, Picariello L, Martineti V, et al. *J Steroid Biochem Mol Biol* 1999;71:223-230.

Epidemiological and experimental data suggest an involvement of estrogen in the development and progression of colorectal cancer. In order to determine whether local synthesis of estrogen occurred in human colonic cancer cells, two colorectal cancer cell lines, HCT8 and HCT116, were evaluated for gene expression and enzyme activity of cytochrome P450 aromatase. In addition, the effect on aromatase expression of charcoal-stripped fetal calf serum, of quercetin and genistein and of tamoxifen and raloxifene was investigated in both cell lines. RT-PCR analysis revealed that colorectal adenocarcinoma cell lines contain aromatase as a major component. The conversion of [(3)H]-androstenedione to estrone and labeled water was dose-dependently inhibited by 4-hydroxyandrostenedione and obeyed Michaelis-Menten kinetic with apparent Km values of approximately 20 nM and V(max) values of approx. 200 and 500 fmol/mg protein/h for HCT8 and HCT116 cells, respectively. After 24 h incubation, genistein (1 microM) significantly increased aromatase activity in HCT8 cells, with no effect on HCT116 cells. In accord with previous observation in reproductive tissues, quercetin (1 microM) significantly inhibited the enzyme activity in both cell lines. Also tamoxifen (100 nM) acted as inhibitor, while raloxifene (10 nM) decreased the enzyme activity only in HCT116 cells. The aromatase gene expression modulation by these effective agents was consistent with their effects on enzyme activity. These findings demonstrate for the first time that colorectal adenocarcinoma cell lines express aromatase. Interestingly, the enzyme activity was inhibited by quercetin, one major dietary flavonoid, by tamoxifen, a hormonal therapeutic agent for breast cancer, and by raloxifene, used in the prevention of postmenopausal osteoporosis.

**Plant phenolics decrease intestinal tumors in an animal model of familial adenomatous polyposis.**

Mahmoud NN, Carothers AM, Grunberger D, et al. *Carcinogenesis* 2000;21:921-927.

Epidemiological studies consistently indicate that consumption of fruits and vegetables lowers cancer risk in humans and suggest that certain dietary constituents may be effective in preventing colon cancer. Plant-derived phenolic compounds manifest many beneficial effects and can potentially inhibit several stages of carcinogenesis in vivo. In this study, we investigated the efficacy of several plant-derived phenolics, including caffeic acid phenethyl ester (CAPE), curcumin, quercetin and rutin, for the prevention of tumors in C57BL/6J-Min/+ (Min/+) mice. These animals bear a germline mutation in the Apc gene and spontaneously develop numerous intestinal adenomas by 15 weeks of age. At a dietary level of 0.15%, CAPE decreased tumor formation in Min/+ mice by 63%. Curcumin induced a similar tumor inhibition. Quercetin and rutin, however, both failed to alter tumor formation at dietary levels of 2%. Examination of intestinal tissue from the treated animals showed that tumor prevention by CAPE and curcumin was associated with increased enterocyte apoptosis and proliferation. CAPE and curcumin also decreased expression of the oncoprotein beta-catenin in the enterocytes of the Min/+ mouse, an observation previously associated with an antitumor effect. These data place the plant phenolics CAPE and curcumin among a growing list of anti-inflammatory agents that suppress Apc-associated intestinal carcinogenesis.

### **Critical evaluation of the effect of valerian extract on sleep structure and sleep quality.**

Donath F, Quispe S, Diefenbach K, et al. *Pharmacopsychiatry* 2000;33:47-53.

A carefully designed study assessed the short-term (single dose) and long-term (14 days with multiple dosage) effects of a valerian extract on both objective and subjective sleep parameters. The investigation was performed as a randomised, double-blind, placebo-controlled, cross-over study. Sixteen patients (4 male, 12 female) with previously established psychophysiological insomnia (ICSD-code 1.A.1.), and with a median age of 49 (range: 22 to 55), were included in the study. The main inclusion criteria were reported primary insomnia according to ICSD criteria, which was confirmed by polysomnographic recording, and the absence of acute diseases. During the study, the patients under-

went 8 polysomnographic recordings: i.e., 2 recordings (baseline and study night) at each time point at which the short and long-term effects of placebo and valerian were tested. The target variable of the study was sleep efficiency. Other parameters describing objective sleep structure were the usual features of sleep-stage analysis, based on the rules of Rechtschaffen and Kales (1968), and the arousal index (scored according to ASDA criteria, 1992) as a sleep microstructure parameter. Subjective parameters such as sleep quality, morning feeling, daytime performance, subjectively perceived duration of sleep latency, and sleep period time were assessed by means of questionnaires. After a single dose of valerian, no effects on sleep structure and subjective sleep assessment were observed. After multiple-dose treatment, sleep efficiency showed a significant increase for both the placebo and the valerian condition in comparison with baseline polysomnography. We confirmed significant differences between valerian and placebo for parameters describing slow-wave sleep. In comparison with the placebo, slow-wave sleep latency was reduced after administration of valerian (21.3 vs. 13.5 min respectively,  $p < 0.05$ ). The SWS percentage of time in bed (TIB) was increased after long-term valerian treatment, in comparison to baseline (9.8 vs. 8.1% respectively,  $p < 0.05$ ). At the same time point, a tendency for shorter subjective sleep latency, as well as a higher correlation coefficient between subjective and objective sleep latencies, were observed under valerian treatment. Other improvements in sleep structure - such as an increase in REM percentage and a decrease in NREM1 percentage - took place simultaneously under placebo and valerian treatment. A remarkable finding of the study was the extremely low number of adverse events during the valerian treatment periods (3 vs. 18 in the placebo period). In conclusion, treatment with a herbal extract of radix valerianae demonstrated positive effects on sleep structure and sleep perception of insomnia patients, and can therefore be recommended for the treatment of patients with mild psychophysiological insomnia.

**Quercetin inhibits benzo[a]pyrene-induced DNA adducts in human Hep G2 cells by altering cytochrome P-450 1A1 gene expression.**

Kang ZC, Tsai SJ, Lee H.  
*Nutr Cancer* 1999;35:175-179.

Quercetin is one of the most abundant of the naturally occurring flavonoids. It has been estimated that about 25-50 mg of quercetin are consumed from the daily diet. The chemopreventive effect of quercetin on dietary carcinogen has been intensely studied in animal models; however, knowledge regarding the molecular mechanism is still limited. In this study, the human hepatoma Hep G2 cell line was used to investigate how quercetin prevents benzo[a]pyrene (B[a]P)-induced DNA adducts. The Hep G2 cells were treated with 10 microM B[a]P for 18 hours in the presence or absence of quercetin. The DNA adduct levels, evaluated by 32P postlabeling, decreased in a dose-dependent manner after treatment with quercetin. Cytochrome P-450 1A1 (CYP1A1) and glutathione S-transferase involvement have been well demonstrated in the modulation of B[a]P-induced DNA damage. From the assays of both enzyme activities, quercetin inhibits CYP1A1-linked ethoxyresorufin O-dealkylase activity more effectively than glutathione S-transferase activity. To elucidate the molecular mechanisms, reverse transcriptase-polymerase chain reaction and Western blot were used to evaluate whether the decrease in CYP1A1 enzyme activity by quercetin is mediated because of alterations of CYP1A1 transcription or mRNA stability. The results indicated that quercetin significantly inhibits B[a]P-induced CYP1A1 mRNA and protein expression. From these findings, we conclude that quercetin suppresses B[a]P-induced DNA damage in human Hep G2 cells by altering CYP1A1 gene expression. Thus we suggest that dietary quercetin may have a long-term preventive effect on chemical carcinogenesis, especially in people who eat a diet rich in fruits and vegetables.

### **Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial.**

Heymsfield SB, Greenberg AS, Fujioka K, et al. *JAMA* 1999;282:1568-1575.

**CONTEXT:** The protein hormone leptin is important to the homeostatic regulation of body weight. Treatment with exogenous leptin may affect weight loss. **OBJECTIVE:** To determine the relationship between increasing doses of exogenous leptin administration and weight loss in both lean and obese adults. **DESIGN:** A randomized, double-blind, placebo-controlled, multicenter, escalating dose cohort trial conducted from April 1997 to October 1998. **SETTING:** Four university nutrition and obesity clinics and 2 contract clinical research clinics. **PARTICIPANTS:** Fifty-four lean (body mass index, 20.0-27.5 kg/m<sup>2</sup>; mean [SD] body weight, 72.0 [9.7] kg) and 73 obese (body mass

index, 27.6-36.0 kg/m<sup>2</sup>; mean [SD] body weight, 89.8 [11.4] kg) predominantly white (80%) men (n = 67) and women (n = 60) with mean (SD) age of 39 (10.3) years. **INTERVENTIONS:** Recombinant methionyl human leptin self-administered by daily morning subcutaneous injection (0 [placebo], 0.01, 0.03, 0.10, or 0.30 mg/kg). In part A, lean and obese subjects were treated for 4 weeks; in part B, obese subjects were treated for an additional 20 weeks. Lean subjects consumed a eucaloric diet to maintain body weight at the current value, and obese subjects were prescribed a diet that reduced their daily energy intake by 2100 kJ/d (500-kcal/d) from the amount needed to maintain a stable weight. **MAIN OUTCOME MEASURES:** Body weight, body fat, and incidence of adverse events. **RESULTS:** Weight loss from baseline increased with increasing dose of leptin among all subjects at 4 weeks (P = .02) and among obese subjects at 24 weeks (P = .01) of treatment. Mean (SD) weight changes at 4 weeks ranged from -0.4 (2.0) kg for placebo (n = 36) to -1.9 kg (1.6) kg for the 0.1 mg/kg dose (n = 29). Mean (SD) weight changes at 24 weeks ranged from -0.7 (5.4) kg for the 0.01 mg/kg dose (n = 6) to -7.1 (8.5) kg for the 0.30 mg/kg dose (n = 8). Fat mass declined from baseline as dose increased among all subjects at 4 weeks (P = .002) and among obese subjects at 24 weeks of treatment (P = .004); more than 95% of weight loss was fat loss in the 2 highest dose cohorts at 24 weeks. Baseline serum leptin concentrations were not related to weight loss at week 4 (P = .88) or at week 24 (P = .76). No clinically significant adverse effects were observed on major organ systems. Mild-to-moderate reactions at the injection site were the most commonly reported adverse effects. **CONCLUSIONS:** A dose-response relationship with weight and fat loss was observed with subcutaneous recombinant leptin injections in both lean and obese subjects. Based on this study, administration of exogenous leptin appears to induce weight loss in some obese subjects with elevated endogenous serum leptin concentrations. Additional research into the potential role for leptin and related hormones in the treatment of human obesity is warranted.