

Antioxidant mechanisms of isoflavones in lipid systems: paradoxical effects of peroxy radical scavenging.

Patel RP, Boersma BJ, Crawford JH, et al. *Free Radic Biol Med* 2001;31:1570-1581.

Oxidation of lipids has been implicated in the pathophysiology of atherosclerosis. It has been suggested that scavenging of lipid peroxy radicals contribute to the antiatherosclerotic effects of naturally occurring compounds such as the isoflavones. This group of polyphenolics includes genistein and is present in relatively high concentrations in food products containing soy. Soy isoflavones are capable of inhibiting lipoprotein oxidation in vitro and suppressing formation of plasma lipid oxidation products in vivo. However, key aspects of the antioxidant mechanisms remain unknown. In this study the antioxidant effects of genistein and other soy isoflavones on lipid peroxidation initiated by mechanistically diverse oxidants was investigated. Although isoflavones inhibited lipid peroxidation stimulated by both metal-dependent and independent processes, the concentration required for these effects were relatively high compared to those found in vivo. Interestingly, however, isoflavones were not consumed and remained in the native state over the time during which inhibition of lipid peroxidation was observed. This was also the case under conditions where synergistic inhibition of LDL oxidation was observed with ascorbate. Furthermore, in an oxidation system driven solely by peroxy radicals, isoflavones were found to be relatively poor peroxy radical scavengers. Consistent with the apparent lack of reactivity with lipid-derived oxidants, isoflavones were also relatively resistant to oxidation mediated by the potent oxidant peroxynitrite. The potential antioxidant mechanisms of isoflavones are discussed in the context of possible reactivities of isoflavone-derived phenoxyl radicals.

Soy for breast cancer survivors: a critical review of the literature.

Messina MJ, Loprinzi CL. *J Nutr* 2001;131:3095S-3108S.

A variety of health benefits, including protection against breast cancer, have been attributed to soy food consumption, primarily because of the soybean isoflavones (genistein, daidzein, glycitein). Isoflavones are considered to be possible selective estrogen receptor modulators but possess nonhormonal properties that also may contribute to their effects. Concern has arisen over a possible detrimental effect of soy in breast cancer patients because of the estrogen-like effects of isoflavones. Genistein exhibits a biphasic effect on the growth of MCF-7 cells in vitro, stimulating proliferation at low concentrations but inhibiting it at high concentrations. In ovariectomized athymic mice implanted with MCF-7 cells, both genistein and soy protein stimulate tumor growth in a dose-dependent manner. In contrast, in intact mice fed estrogen, genistein inhibits tumor growth. Although two studies in premenopausal women suggested that soy exerts estrogenic-like effects on breast tissue, recently conducted year-long studies indicated that isoflavone supplements do not affect breast tissue density in premenopausal women and may decrease density in postmenopausal women. These latter effects are opposite to those of hormone replacement therapy (HRT). Importantly, substantial data suggest that the progestogen, not the estrogen, component of HRT increases risk of developing breast cancer. Furthermore, recently conducted studies have failed to find that even HRT reduces survival in breast cancer patients. Overall, the data are not impressive that the adult consumption of soy affects the risk of developing breast cancer or that soy consumption affects the survival of breast cancer patients. Consequently, if breast cancer patients enjoy soy products, it seems reasonable for them to continue to use them.

Ukrain(R), an alkaloid thiophosphoric acid derivative of *Chelidonium majus* L. protects human fibroblasts but not human tumour cells in vitro against ionizing radiation.

Cordes N, Plasswilm L, Bamberg M, Rodemann HP. *Int J Radiat Biol* 2002;78:17-27.

PURPOSE: Ukrain((R)), an alkaloid thiophosphoric acid derivative of *Chelidonium majus* L., has demonstrated a promising impact on chemotherapy in a variety of malignancies. The effects of the drug on cell survival, alteration of the cell cycle and induction of apoptosis were examined without and in combination with ionizing radiation (IR). The TP53 status of the cell lines used was also investigated. **MATERIALS AND METHODS:** Exponentially growing human tumour cell lines MDA-MB-231 (breast), PA-TU-8902 (pancreas), CCL-221 (colorectal), U-138MG (glioblastoma), and human skin and lung fibroblastic cells, HSF1, HSF2 and CCD32-LU were studied by colony assay, flow cytometry (cell-cycle, annexin-V staining for apoptosis) and Western blotting. Ukrain was used in concentrations from 0.1 to 50 mcg/ml(-1) for 1, 3 and 24 h and radiation as single doses of 1-10 Gy. Combined drug-radiation exposure employed 1 mcg/ml(-1) Ukrain for 24 h plus 2-8 Gy. **RESULTS:** Ukrain cytotoxicity was time- and dose-dependent. The combination of Ukrain plus IR gave enhanced toxicity in CCL-221 and U-138MG cells, but not in MDA-MB-231 and PA-TU-8902 cells. Most strikingly, a radioprotective effect was found in normal human skin and lung fibroblasts. Flow-cytometry analyses supported the differential and cell line-specific cytotoxicity of Ukrain. CCL-221 and U-138MG cells accumulated in G2 after 24-h Ukrain treatment, whereas no alterations were detected in the other tumour cells and normal fibroblasts tested. Western blotting of TP53 demonstrated non-functional overexpression in all tumour cell lines without affecting p21. HSF1 presented wild-type TP53 and a p21 response after IR. Flowcytometric analyses of annexin-V staining showed no induction of apoptosis after Ukrain treatment in comparison with untreated controls. **CONCLUSIONS:** Differential effects of

Ukrain in modulating radiation toxicity of human cancer cell lines and its protective effect in normal human fibroblasts suggest that this alkaloid may have potential properties for clinical radiochemotherapy.

Succimer treatment during ongoing lead exposure reduces tissue lead in suckling rats.

Varnai VM, Piasek M, Blanusa M, et al. *J Appl Toxicol* 2001;21:415-416.

There is a concern that oral treatment with succimer (meso-2, 3-dimercaptosuccinic acid, DMSA) can promote gastrointestinal lead absorption if not performed in a lead-safe environment. The scope of our investigation was to evaluate the efficacy of oral DMSA treatment during oral lead exposure on tissue lead in suckling rats. Six-day-old Wistar rats of both genders were divided into two groups-untreated (Pb) and treated (Pb + DMSA)-with 10 animals per group. Lead (as acetate) was given orally at a dose of 2 mg kg(-1) body weight day(-1) for eight consecutive days (total dose 16 mg kg(-1), i.e. 0.08 mmol kg(-1)). During this period the treated group received a daily dose of 0.5 mmol DMSA kg(-1) body weight p.o. six times on days 1-3 and 6-8 of the experiment (total dose 3 mmol kg(-1)). Tissue lead was determined by means of atomic absorption spectrometry. The DMSA efficiently reduced the lead concentration in the analysed tissues (carcass, liver, kidneys and brain) by approximately 50% compared with untreated controls. The pups' growth and organ weights were not affected. In conclusion, our results indicate that DMSA is an efficient oral lead chelator in sucklings even if challenged with ongoing lead exposure.

Prebiotic treatment of experimental colitis with germinated barley foodstuff: A comparison with probiotic or antibiotic treatment.

Fukuda M, Kanauchi O, Araki Y, et al. *Int J Mol Med* 2002;9:65-70.

There is increasing evidence that intestinal microflora play an important role in the pathogenesis of ulcerative colitis. Therefore, modification of the microflora by prebiotics, probiotics, and antibiotics may be a rational approach for controlling intestinal inflammation. Germinated barley food-stuff (GBF) is an insoluble mixture of glutamine-rich protein and hemicellulose-rich dietary fiber. GBF is utilized efficiently by *Bifidobacterium*, *Lactobacillus*, and *Eubacterium* and converted by them into lactate, acetate, and butyrate. These bacterial organic acids preserve a favorable intestinal condition. We have previously shown that GBF has attenuated intestinal inflammation in patients with ulcerative colitis and experimental colitis models through prebiotic actions. The aim of this study was to compare the effect of GBF with that of probiotics and antibiotics in an experimental colitis model. Colitis was induced by feeding male SD rats with a diet containing 3.0-3.5% dextran sodium sulfate (DSS). The therapeutic effect of oral administration of a prebiotic (GBF), probiotics (mixture of *Lactobacillus* and *Clostridium butyricum*), antibiotics (vancomycin, metronidazole), and the vehicle was determined by assessing clinical and pathological scores on day 6 after initiation of colitis. Butyrate concentrations in the cecal content were also determined. GBF treatment significantly reduced colonic inflammation as assessed by clinical scores with an increase in cecal butyrate levels. Probiotic treatment with a mixture of *Lactobacillus* and *Clostridium butyricum* did not show such an effect. Both antibiotic treatments significantly attenuated clinical and pathological scores. However, in contrast to GBF, this treatment led to a significant decrease in cecal butyrate levels. These data suggest that modification of the intestinal microflora by prebiotics, including GBF, may serve as a useful adjunct in the treatment of ulcerative colitis as well as antibiotic treatment.

Red wine decreases cyclosporine bioavailability.

Tsunoda SM, Harris RZ, Christians U, et al. *Clin Pharmacol Ther* 2001;70:462-467.

BACKGROUND: Many commonly ingested substances such as grapefruit juice and *Hypericum perforatum* (St John's wort) have been found to interact with important therapeutic agents such as cyclosporine (INN, ciclosporin). The mechanism for these interactions is thought to involve modulation of the activity of the drug-metabolizing enzyme cytochrome P4503A4 (CYP3A4) and/or the drug transport protein Pglycoprotein. In vitro data suggest that red wine may interact with CYP3A4 substrates such as cyclosporine. **METHODS:** We conducted a randomized, 2-way crossover study of 12 healthy individuals. Subjects received a single 8-mg/kg dose of oral cyclosporine with water (control) and with 12 oz of red wine (Blackstone Merlot, 1996; Blackstone Winery, Graton, Calif). Whole blood was analyzed for cyclosporine and 6 metabolites by specific fluorescence polarization immunoassay and tandem liquid chromatography-mass spectrometry. Blood levels of cyclosporine were compared between the 2 arms. **RESULTS:** Red wine caused a 50% increase in the oral clearance of cyclosporine. Systemic exposure as measured by the area under the concentration-versus-time curve (AUC) and peak concentration (C(max)) were significantly decreased by red wine. However, half-life was not affected, suggesting that red wine decreased cyclosporine absorption. In vitro, the solubility of cyclosporine in red wine appeared to be lower than in water. **CONCLUSIONS:** Administration of cyclosporine with red wine causes a significant decrease in cyclosporine exposure. Because cyclosporine is a narrow therapeutic range compound, caution may be warranted with concomitant intake of red wine and cyclosporine.

Docosahexaenoic acid regulated genes and transcription factors inducing apoptosis in human colon cancer cells.

Narayanan BA, Narayanan NK, Reddy BS. *Int J Oncol* 2001;19:1255-1262.

Epidemiological and preclinical studies demonstrate that consumption of diets high in omega-3 fatty acids (n-3 PUFAs) reduce the risk of colon cancer. Docosahexaenoic acid (DHA), a long chain polyunsaturated fatty acid (PUFAs) is a major constituent of nutrients rich in n-3 PUFAs. There are studies to indicate that colon tumor inhibition by n-3 PUFA-rich diets is, in part, mediated through modulation of signaling pathways that alter gene expression which are involved in colon tumor growth. In the present study using CaCo-2 colon cancer cell lines we examined the effects of DHA on the genetic precursors of human colon cancer at the transcription level using DNA oligonucleotide arrays. Our results indicated that DHA inhibits the growth of CaCo-2 cells and induces apoptosis. For gene expression analysis using DNA microarrays, total RNA extracted from DHA treated CaCo-2 cells was converted to cDNA, labeled with Cy5-dCTP (DHA-treated) and Cy3-dCTP (untreated cells) and used as probes for hybridization in human chip spotted with 3,800 oligonucleotides consisting of 156 functional categories. The expression profiles of genes indicated a reprogramming pattern of previously known and unknown genes and transcription factors that provided clues to the possible functional mechanism of DHA. An average of (ratios from triplicate experiments) 504 out of 3,800 genes expressed after 48 h of DHA treatment. Altered expression on the transcription factors includes down regulation of nine members of the RNA II polymerases, transcription co-repressor associated protein and enhancer binding proteins such as AP2, in addition to changes in the expression of zinc finger group of transcription factors. Activation of cytochrome c which triggers caspases was associated with the elevated expression of pro-apoptotic caspases 10, 13, 8, 5 and 9 in DHA treated cells. Activation of cyclin-dependent kinase inhibitors such as p21 (waf1/cip1), p27, p57, p19 and growth arrest specific proteins by more than 2-fold is consistent with the induction of apoptosis and

inactivation of antiapoptotic Bcl-2 family of genes. Inactivation of prostaglandin family of genes, lipoxygenases and altered expression of peroxisome proliferators (PPARalpha and gamma) by DHA seem to indicate a lipid peroxidation-induced apoptosis in addition to effect reflected on the modification of cell cycle regulatory genes. These findings support the conclusion that a genomewide expression profiling of human colon cancer precursor genes and transcription factors provides a set of novel regulatory mechanism(s) to determine the chemopreventive efficacy of DHA and thus to prevent the inflammation and neoplasia.

Cholesterol lowering benefits of soy and linseed enriched foods.

Ridges L, Sunderland R, Moerman K, et al. *Asia Pac J Clin Nutr* 2001;10:204-211.

Foods such as breads and breakfast cereals enriched with a combination of soy protein (soy grits and/or soy flour) and whole linseed are gaining popularity. Regular consumption of either whole grains or soy protein can lower risk factors for coronary heart disease. Furthermore, linseed is a rich source of the omega-3 fatty acid, alpha-linolenic acid (LNA), with purported cardiovascular benefits. The aim of this study was to determine the effect of daily consumption of soy and linseed containing foods and Canola (as an added source of LNA) on plasma lipid concentrations in 20 mildly hypercholesterolaemic postmenopausal women. Fasted blood samples were taken initially and after 3 and 8 weeks to assay plasma lipids and both plasma and erythrocyte membrane fatty acids. Urinary isoflavones were also measured. Data from 18 subjects were used for analysis. Plasma total, low-density lipoprotein (LDL) and non-high-density lipoprotein (HDL) cholesterol concentrations fell significantly (10, 12.5 and 12%, respectively) within 3 weeks. Although attenuated, there were still significant reductions in total and non-HDL cholesterol (5 and 6.5%, respectively) after 8 weeks of intervention. These reductions were associated with increases in urinary isoflavone excretion. This pilot study indicates that regular inclusion of foods containing soy and linseed in the diet may improve plasma lipids in subjects with hypercholesterolaemia.

Glyoxalase I deficiency is associated with an unusual level of advanced glycation end products in a hemodialysis patient.

Miyata T, Van Ypersele De Strihou C, Imasawa T, et al. *Kidney Int* 2001;60:2351-2359.

BACKGROUND: Advanced glycation of proteins and their attendant advanced glycation end products (AGEs) contribute to the complications associated with diabetes mellitus or uremia. Regulatory mechanisms of AGE formation in vivo remain an issue of particular interest. We investigated a role of the glyoxalase detoxification system of precursor reactive carbonyl compounds (RCOs) in the in vivo AGE formation. **METHODS:** Plasma levels of AGEs [pentosidine and Nepsilon-carboxymethyllysine (CML)], their RCO precursors, d-lactate (the final product resulting from the glyoxalase detoxification pathway), as well as of various compounds known to generate AGE precursors and surrogate markers for oxidative stress (antioxidant enzymes and glutathione), were measured in both hemodialysis (HD) patients and normal subjects. The activity and protein expression of glyoxalase I, an enzyme essential for the detoxification of alpha-oxoaldehydes, in red blood cells (RBC) were also examined. **RESULTS:** In one 69-year-old lady who had been on hemodialysis (HD) for three years and had suffered from recurrent cardiovascular complications despite the absence of significant risk factors, plasma levels of pentosidine (77.3 +/- 2.4 pmol/mg protein) and CML (330.8 +/- 8.2 pmol/mg protein) were markedly elevated as compared to other HD patients (N = 20: 26.6 +/- 11.8 pmol/mg protein for pentosidine and 224.4 +/- 51.7 pmol/mg protein for CML). The plasma level of RCO precursors for pentosidine and CML was also higher in this patient than in other HD patients. Further investigation disclosed a very low activity in RBC of glyoxalase I (1.5 +/- 0.4 mU/106 RBC), as compared to other HD patients (3.9 +/- 0.6 mU/106 RBC) or normal subjects (4.0 +/- 0.6 mU/106 RBC). The glyoxalase I protein level, assessed in RBC by immunoblot analysis with a specific antibody, was markedly lower than that observed in HD patients and normal subjects. The causes of this deficiency remain unknown. Nucleotide sequencing of the products of

reverse transcription-polymerase chain reaction from the patient's mononuclear cells revealed no genetic mutation within the coding region of the glyoxalase I gene. Plasma d-lactate level was also in the lower range (0.18 +/- 0.03 mg/dL) of the values measured in the other HD patients (0.27 +/- 0.09 mg/dL) and normal subjects (0.35 +/- 0.12 mg/dL). The plasma levels of various compounds known to generate AGE precursors (glucose, lipids and ascorbic acid) were either normal or low. The surrogate markers for oxidative stress such as antioxidant enzymes (glutathione peroxidases and superoxide dismutase) and glutathione were all within the range observed in the other HD patients. **CONCLUSION:** The unusually high levels of AGEs in this patient implicate a deficient glyoxalase detoxification of RCO precursors. The present clinical observation implicates, to our knowledge for the first time, the glyoxalase detoxification system and, in particular, glyoxalase in the actual level of AGEs in a uremic patient.

Effects of estrogen replacement therapy on human brain aging: An in vivo (1)H MRS study.

Robertson DM, van Amelsvoort T, Daly E, et al. *Neurology* 2001;57:2114-2117.

Estrogen replacement therapy (ERT) may preserve cognitive function in postmenopausal women, but the mechanism is unknown. Thus, the authors studied aging of parietal lobe and hippocampus using proton MR spectroscopy. ERT naive postmenopausal women had a significant increase in choline-containing compounds (Cho) compared to long-term ERT users and young women. Cho reflects increased neuronal/glial membrane turnover. Thus, ERT's "neuroprotective" effect may include modulating the effects of age on neural integrity in brain regions involved in cognitive function.

Alpha-lipoic acid inhibits TNF-alpha-induced NF-kappaB activation and adhesion molecule expression in human aortic endothelial cells.

Zhang WJ, Frei B. *FASEB J* 2001;15:2423-2432.

Endothelial activation and monocyte adhesion are initiating steps in atherogenesis thought to be caused in part by oxidative stress. The metabolic thiol antioxidant alpha-lipoic acid has been suggested to be of therapeutic value in pathologies associated with redox imbalances. We investigated the role of (R)-alpha-lipoic acid (LA) vs. glutathione and ascorbic acid in tumor necrosis factor alpha (TNF-alpha) -induced adhesion molecule expression and nuclear factor kappaB (NF-kappaB) signaling in human aortic endothelial cells (HAEC). Preincubation of HAEC for 48 h with LA (0.05-1 mmol/l) dose-dependently inhibited TNF-alpha (10 U/ml) -induced adhesion of human monocytic THP-1 cells, as well as mRNA and protein expression of E-selectin, vascular cell adhesion molecule 1 and intercellular adhesion molecule 1. LA also strongly inhibited TNF-alpha-induced mRNA expression of monocyte chemoattractant protein-1 but did not affect expression of TNF-alpha receptor 1. Furthermore, LA dose-dependently inhibited TNF-alpha-induced IkappaB kinase activation, subsequent degradation of IkappaB, the cytoplasmic NF-kappaB inhibitor, and nuclear translocation of NF-kappaB. In contrast, TNF-alpha-induced NF-kappaB activation and adhesion molecule expression were not affected by ascorbic acid or by manipulating cellular glutathione status with l-2-oxo-4-thiazolidinecarboxylic acid, N-acetyl-l-cysteine, or d,l-buthionine-S,R-sulfoximine. Our data show that clinically relevant concentrations of LA, but neither vitamin C nor glutathione, inhibit adhesion molecule expression in HAEC and monocyte adhesion by inhibiting the IkappaB/NF-kappaB signaling pathway at the level, or upstream, of IkappaB kinase.

Trigonellafoenum graecum (fenugreek) seed powder improves glucose homeostasis in alloxan diabetic rat tissues by reversing the altered glycolytic, gluconeogenic and lipogenic enzymes.

Raju J, Gupta D, Rao AR, et al. *Mol Cell Biochem* 2001;224:45-51.

Trigonella foenum graecum (fenugreek) seed powder has been suggested to have potential antidiabetic effects. The effect of oral administration of Trigonella whole seed powder (5% in the diet) for 21 days on glycolytic, gluconeogenic and NADP-linked lipogenic enzymes were studied in liver and kidney tissues of alloxan-induced diabetic Wistar rats. Diabetic rats were characterised by a 4-fold higher blood glucose level and a 0.7-fold lower body weight compared to normal controls. The activities of the glycolytic enzymes were significantly lower in the diabetic liver and higher in the diabetic kidney. The activities of gluconeogenic enzymes were higher in both liver and kidney during diabetes, however the activities of the lipogenic enzymes were decreased in both tissues during diabetes. Trigonella seed powder treatment to diabetic rats for 21 days brought down the elevated fasting blood glucose levels to control levels. The altered enzyme activities were significantly restored to control values in both the liver and kidney after Trigonella seed powder treatment. The therapeutic role of Trigonella seed powder in type-1 diabetes as exemplified in this study can be attributed to the change of glucose and lipid metabolising enzyme activities to normal values, thus stabilizing glucose homeostasis in the liver and kidney. These biochemical effects exerted by Trigonella seeds make it a possible new therapeutic in type-1 diabetes.

Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions.

Cheng AL, Hsu CH, Lin JK, et al.
Anticancer Res 2001;21:2895-2900.

Curcumin (diferuloylmethane), a yellow substance from the root of the plant *Curcuma longa* Linn., has been demonstrated to inhibit carcinogenesis of murine skin, stomach, intestine and liver. However, the toxicology, pharmacokinetics and biologically effective dose of curcumin in humans have not been reported. This prospective phase-I study evaluated these issues of curcumin in patients with one of the following five high-risk conditions: 1) recently resected urinary bladder cancer; 2) arsenic Bowen's disease of the skin; 3) uterine cervical intraepithelial neoplasm (CIN); 4) oral leucoplakia; and 5) intestinal metaplasia of the stomach. Curcumin was taken orally for 3 months. Biopsy of the lesion sites was done immediately before and 3 months after starting curcumin treatment. The starting dose was 500 mg/day. If no toxicity > or = grade II was noted in at least 3 successive patients, the dose was then escalated to another level in the order of 1,000, 2,000, 4,000, 8,000, and 12,000 mg/day. The concentration of curcumin in serum and urine was determined by high pressure liquid chromatography (HPLC). A total of 25 patients were enrolled in this study. There was no treatment-related toxicity up to 8,000 mg/day. Beyond 8,000 mg/day, the bulky volume of the drug was unacceptable to the patients. The serum concentration of curcumin usually peaked at 1 to 2 hours after oral intake of curcumin and gradually declined within 12 hours. The average peak serum concentrations after taking 4,000 mg, 6,000 mg and 8,000 mg of curcumin were 0.51 +/- 0.11 microM, 0.63 +/- 0.06 microM and 1.77 +/- 1.87 microM, respectively. Urinary excretion of curcumin was undetectable. One of 4 patients with CIN and 1 of 7 patients with oral leucoplakia proceeded to develop frank malignancies in spite of curcumin treatment. In contrast, histologic improvement of precancerous lesions was seen in 1 out of 2 patients with recently resected bladder cancer, 2 out of 7 patients of oral leucoplakia, 1 out of 6 patients of intestinal metaplasia of the stomach, 1 out of 4 patients with CIN and 2 out of 6 patients with

Bowen's disease. In conclusion, this study demonstrated that curcumin is not toxic to humans up to 8,000 mg/day when taken by mouth for 3 months. Our results also suggest a biologic effect of curcumin in the chemoprevention of cancer.

Does gastrointestinal *Candida albicans* prevent ubiquinone absorption?

Krone CA, Elmer GW, Ely JT, et al.
Med Hypotheses 2001;57:570-572.

Ubiquinones (coenzyme Qs (CoQ)) are essential for oxidative phosphorylation in yeasts and humans, although the isomers present in each are different. The human coenzyme Q, CoQ10, is administered orally for the treatment of heart disease and other disorders. Some patients, however, require much higher doses than others to attain a therapeutic CoQ10 blood level. We propose that one possible explanation for this variability is *Candida* colonization of the GI tract. Many common medical treatments including antibiotics and anti-hyperchlorhydric agents increase the risk of GI tract *Candida* colonization. Subsequent uptake and utilization of supplemental CoQ10 by the yeast could diminish availability for the human subject. Data from one patient and an in vitro pilot study using two pathogenic strains of *C. albicans* support this hypothesis. If *C. albicans* in the GI tract can hinder availability and interfere with therapeutic effects of CoQ10, it could be of clinical significance for large numbers of patients.

Low serum biotinidase activity in children with valproic acid monotherapy.

Schulpis KH, Karikas GA, Tjamouranis J, et al. *Epilepsia* 2001;42:1359-1362.

PURPOSE: Valproic acid (VPA) is an effective antiepileptic drug (AED), which is associated with dose-related adverse reactions such as skin rash, hair loss (alopecia), etc. Profound as well as partial biotinidase deficiency causes dermatologic manifestations similar to these. Therefore, it was of interest to evaluate serum biotinidase activity in patients receiving VPA monotherapy. **METHODS:** Seventy-five patients with seizures, mean age, 8.6 years (+/- 1.9 years) were divided into three groups. Group A (n = 25) was treated with VPA 28.7 +/- 8.5 mg/kg/24 h, group B (n = 25) with 41.6 +/- 4.9 mg/kg/24 h, and group C with 54.5 +/- 5.8 mg/kg/24 h. Their "trough" VPA serum levels were 40.9 +/- 13.2, 86.25 +/- 11.5, and 137 +/- 14.5 mcg/ml, respectively. Fifty healthy children were the controls. Patients and controls underwent clinical and laboratory evaluations including liver function data, complete blood counts, NH₃, and so on, after 45 days of VPA treatment. Biotinidase serum levels were evaluated fluorometrically. **RESULTS:** Liver function data were found elevated in the groups B and C. On the contrary, biotinidase activity was significantly lowered (p < 0.001) in groups B and C (1.22 +/- 1.11, 0.97 +/- 0.07 mmol/min/L respectively), as compared with controls (5.20 +/- 0.90 mmol/min/L). Strong inverse correlations were observed between liver enzymes and VPA blood levels with the activity of the enzyme. Additionally, no inhibitory effect on biotinidase activity was found, when the enzyme was incubated in vitro with high (1.2 mM) concentrations of the drug. Skin lesions (seborrheic rash, alopecia) were improved in our patients after biotin (10 mg/day) supplementation. **CONCLUSIONS:** It is suggested that VPA impairs the liver mitochondrial function, resulting in a low biotinidase activity and or biotin deficiency. Biotin supplementation could restore some of the side effects of the drug.

Reversibility of coronary endothelial vasomotor dysfunction in idiopathic dilated cardiomyopathy: acute effects of vitamin C.

Richartz BM, Werner GS, Ferrari M, Figulla HR. *Am J Cardiol* 2001;88:1001-1005.

In patients with idiopathic dilated cardiomyopathy, endothelium vasomotor function is disturbed. Increased oxidative stress and the consecutive formation of oxygen free radicals have been implicated as one possibility for this observation, suggesting that nitric oxide (NO) is inactivated by oxygen free radicals. We tested the hypothesis that the antioxidant, vitamin C, may improve endothelial function in idiopathic dilated cardiomyopathy. In 11 patients, the endothelium-dependent vasomotor response of the left anterior descending coronary artery to intracoronary acetylcholine (ACh) infusion (1/2 x 10⁻⁶ mol/L, 1/4 x 10⁻⁵ mol/L; respectively) was determined before and immediately after intravenous infusion of 3 g of vitamin C. Coronary cross-sectional diameter was obtained by quantitative coronary angiography, average peak velocity was measured by an intracoronary Doppler flow wire, and coronary blood flow (CBF) was calculated. Maximum cross-sectional diameter was determined after administration of nitroglycerin. Dose-dependent ACh showed a decrease in cross-sectional diameter (-5% to -7%, p < 0.05) and an increase in average peak velocity (+16% to +25%, p < 0.05); the CBF was unchanged (+1% to -2%, p = NS). After vitamin C infusion, the cross-sectional diameter increased in a dose-dependent manner from +11% to +15%, the average peak velocity increased from +20% to +41% (p < 0.05), and the CBF increased from +38% to +82% (p < 0.01, p < 0.001, respectively). Thus, patients with idiopathic dilated cardiomyopathy had endothelial dysfunction, and administration of vitamin C reversed endothelium-dependent dysfunction.

Human cobalamin deficiency: alterations in serum tumour necrosis factor-alpha and epidermal growth factor.

Peracchi M, Bamonti Catena F, Pomati M, et al. *Eur J Haematol* 2001;67:123-127.

Objectives: We have previously demonstrated that vitamin B12 (cobalamin)-deficient central neuropathy in the rat is associated with local overexpression of neurotoxic tumour necrosis factor (TNF)-alpha combined with locally decreased synthesis of neurotrophic epidermal growth factor (EGF). The aims of this study were to investigate whether a similar imbalance also occurs in the serum of adult patients with clinically confirmed cobalamin deficiency and whether it can be corrected by vitamin B12 replacement therapy. **Patients and methods:** We studied 34 adult patients with severe cobalamin deficiency, 12 patients with pure iron deficiency anaemia and 34 control subjects. Haematological markers of cobalamin deficiency and serum TNF-alpha and EGF levels were measured using commercial kits. Thirteen cobalamin-deficient patients were re-evaluated after 3 and 6 months of parenteral vitamin B12 treatment. **Results:** TNF-alpha was significantly higher ($p < 0.01$) and EGF significantly lower ($p < 0.01$) in the patients with cobalamin deficiency, but both were unchanged in patients with pure iron deficiency anaemia. In cobalamin-deficient patients the serum TNF-alpha levels correlated significantly with plasma total homocysteine levels ($r = 0.425$; $p < 0.02$). In the treated patients TNF-alpha and EGF levels normalised concomitantly with clinical and haematological disease remission. **Conclusions:** In humans, as in rats, cobalamin concentration appears to be correlated with the synthesis and release of TNF-alpha and EGF in a reciprocal manner, because cobalamin deficiency is accompanied by overproduction of TNF-alpha and underproduction of EGF.

Dietary boron supplementation enhanced the action of estrogen, but not that of parathyroid hormone, to improve trabecular bone quality in ovariectomized rats.

Sheng MH, Taper LJ, Veit H, et al. *Biol Trace Elem Res* 2001;82:109-123.

This study investigated whether boron would enhance the ability of 17beta-estradiol (E2) or parathyroid hormone (PTH) to improve bone quality in ovariectomized OVX rats. Adult OVX rats were treated for 5 wk with vehicle, boron (5 ppm as boric acid), E2 (30 microg/kg/d, sc), PTH (60 microg/kg/d, sc), or a combination of boron and E2 or PTH, respectively. The E2 treatment corrected many adverse effects of OVX on bone quality, increased bone Ca, P, and Mg contents, and decreased trabecular plate separation. Dietary boron supplementation had no effects on these bone parameters in OVX rats. When OVX rats were treated with boron and E2 together, trabecular bone volume (Tb.BS/TV) and plate density were increased significantly more than that caused by E2 alone. The boron and E2 combination also increased trabecular bone surface (Tb.BV/TV) and decreased trabecular plate separation in OVX rats. In contrast, whereas daily PTH injection also increased bone Ca, Mg, and P contents, Tb.BV/TV, Tb.BS/TV, trabecular plate density and thickness, and decreased trabecular plate separation in OVX rats, the combination of boron and PTH had no additional improvement in bone quality over that achieved by PTH alone. In summary, this study shows for the first time that boron enhanced the action of E2, but not that of PTH, to improve trabecular bone quality in OVX rats.

The protective effect of niacinamide on ischemia-reperfusion-induced liver injury.

Chen CF, Wang D, Hwang CP, et al. *J Biomed Sci* 2001;8:446-452.

Reperfusion of ischemic liver results in the generation of oxygen radicals, nitric oxide (NO) and their reaction product peroxynitrite, all of which may cause strand breaks in DNA, which activate the nuclear enzyme poly(ADP ribose)synthase (PARS). This results in rapid depletion of intracellular nicotinamide adenine dinucleotide and adenosine 5'-triphosphate (ATP) and eventually induces irreversible cytotoxicity. In this study, we demonstrated that niacinamide, a PARS inhibitor, attenuated ischemia/reperfusion (I/R)-induced liver injury. Ischemia was induced by clamping the common hepatic artery and portal vein of rats for 40 min. Thereafter, flow was restored and the liver was reperfused for 90 min. Blood samples collected prior to I and after R were analyzed for methyl guanidine (MG), NO, tumor necrosis factor (TNF-alpha) and ATP. Blood levels of aspartate transferase (AST), alanine transferase (ALT) and lactate dehydrogenase (LDH) which served as indexes of liver injury were measured. This protocol resulted in elevation of the blood NO level ($p < 0.01$). Inflammation was apparent, as TNF-alpha and MG levels were significantly increased ($p < 0.05$ and $p < 0.001$). AST, ALT and LDH were elevated 4- to 5-fold ($p < 0.001$), while ATP was significantly diminished ($p < 0.01$). After administration of niacinamide (10 mM), liver injury was significantly attenuated, while blood ATP content was reversed. In addition, MG, TNF-alpha and NO release was attenuated. These results indicate that niacinamide, presumably by acting with multiple functions, exerts potent anti-inflammatory effects in I/R-induced liver injury.

Effects of melatonin treatment in septic newborns.

Gitto E, Karbownik M, Reiter RJ, et al. *Pediatr Res* 2001;50:756-760.

Free radicals have been implicated in the pathogenesis of neonatal sepsis and its complications. This study was conducted to determine the changes in the clinical status and the serum levels of lipid peroxidation products [malondialdehyde (MDA) and 4-hydroxylalkenals (4-HDA)] in 10 septic newborns treated with the antioxidant melatonin given within the first 12 h after diagnosis. Ten other septic newborns in a comparable state were used as "septic" controls, while 10 healthy newborns served as normal controls. A total of 20 mg melatonin was administered orally in two doses of 10 mg each, with a 1-h interval. One blood sample was collected before melatonin administration and two additional blood samples (at 1 and 4 h) were collected after melatonin administration to assess serum levels of lipid peroxidation products. Serum MDA + 4-HDA concentrations in newborns with sepsis were significantly higher than those in healthy infants without sepsis; in contrast, in septic newborns treated with melatonin there was a significant reduction ($p < 0.05$) of MDA + 4-HDA to the levels in the normal controls at both 1 and 4 h ($p < 0.05$). Melatonin also improved the clinical outcome of the septic newborns as judged by measurement of sepsis-related serum parameters after 24 and 48 h. Three of 10 septic children who were not treated with melatonin died within 72 h after diagnosis of sepsis; none of the 10 septic newborns treated with melatonin died. To our knowledge, this is the first study where melatonin was given to human newborns.

Effects of oral glucosamine and chondroitin sulfate alone and in combination on the metabolism of SHR and SD rats.

Echard BW, Talpur NA, Funk KA, et al. *Mol Cell Biochem* 2001;225:85-91.

Glucosamine (G), often combined with chondroitin sulfate (CS), is a popular natural supplement used widely to treat osteoarthritis. However, use of glucosamine has been linked to development of insulin resistance. To assess the association between glucosamine and insulin resistance more closely, we challenged two rat strains highly sensitive to sugar-induced insulin resistance—Sprague-Dawley (SD) and Spontaneously Hypertensive (SHR) rats. Since elevations of systolic blood pressure (SBP) have been found to be an early and highly sensitive sign of insulin resistance in these two rat strains, we used this parameter as our primary endpoint. Four groups of both rat strains received either no agent (control), G, CS, or a combination of both for 9 weeks. The intake of each agent was calculated to be approximately 3-7 times comparable to human dose. Throughout the study, SBP of both strains consuming the two ingredients alone and in combination were not elevated. Rather, they were significantly lower than control, contrary to what is found in glucose-induced insulin resistance in rats. Over the study period, body weights of the four groups of SD and SHR did not vary significantly. Furthermore, no consistent trends in circulating glucose concentrations were found among the four different groups in the two strains after oral challenge with glucose. Finally, no significant histological differences were found in hearts, kidneys, and livers among the various groups of SHR and SD. From the above result, we conclude that glucosamine and chondroitin sulfate given alone or together do not produce insulin resistance or other related perturbations in two rat strains highly sensitive to sugar-induced insulin resistance.

Zinc supplementation in infants born small for gestational age reduces mortality: a prospective, randomized, controlled trial.

Sazawal S, Black RE, Menon VP, et al. *Pediatrics* 2001;108:1280-1286.

BACKGROUND: Low birth weight infants have been noted to have low zinc concentrations in cord blood, and zinc deficiency in childhood is associated with reduced immunocompetence and increased infectious disease morbidity. This study investigates whether zinc supplementation of infants born full term and small for gestational age affects mortality. **METHODS:** A randomized, double-blind, controlled trial with 2-by-2 factorial design enrolled 1154 full-term small for gestational age infants to receive in syrup 1 of the following: riboflavin; riboflavin and zinc (5 mg as sulfate); riboflavin, calcium, phosphorus, folate, and iron; or riboflavin, zinc, calcium, phosphorus, folate, and iron. A fixed dosage of 5 mL per child was given daily from 30 to 284 days of age. Household visits were made 6 days per week to provide the syrup and conduct surveillance for illness and death. When a child's death was reported, parental reports and medical records were used to ascertain the cause. The effects of zinc and of the combination of iron, folate, calcium, and phosphorus were analyzed by intent to treat. The mortality analysis was performed using a survival analytic approach that models time until death as the dependent variable; all models had 2 terms as independent variables: 1 for the zinc effect and 1 for the vitamin and mineral (calcium and phosphorus, folate and iron) effect. **RESULTS:** Zinc supplementation was associated with significantly lower mortality, with a rate ratio of 0.32 (95% confidence interval: 0.12-0.89). Calcium, phosphorus, folate, and iron supplementation was not associated with a mortality reduction, although a statistically nonsignificant trend toward reduction was observed with a rate ratio of 0.88 (95% confidence interval: 0.36-2.15). **CONCLUSIONS:** Zinc supplementation in small for gestational age infants can result in a substantial reduction in infectious disease mortality.

Vascular factors and metabolic interactions in the pathogenesis of diabetic neuropathy.

Cameron NE, Eaton SE, Cotter MA, Tesfaye S. *Diabetologia* 2001;44:1973-1988.

Diabetes mellitus is a major cause of peripheral neuropathy, commonly manifested as distal symmetrical polyneuropathy. This review examines evidence for the importance of vascular factors and their metabolic substrate from human and animal studies. Diabetic neuropathy is associated with risk factors for macrovascular disease and with other microvascular complications such as poor metabolic control, dyslipidaemia, body mass index, smoking, microalbuminuria and retinopathy. Studies in human and animal models have shown reduced nerve perfusion and endoneurial hypoxia. Investigations on biopsy material from patients with mild to severe neuropathy show graded structural changes in nerve microvasculature including basement membrane thickening, pericyte degeneration and endothelial cell hyperplasia. Arterio-venous shunting also contributes to reduced endoneurial perfusion. These vascular changes strongly correlate with clinical defects and nerve pathology. Vasodilator treatment in patients and animals improves nerve function. Early vasa nervorum functional changes are caused by the metabolic insults of diabetes, the balance between vasodilation and vasoconstriction is altered. Vascular endothelium is particularly vulnerable, with deficits in the major endothelial vasodilators, nitric oxide, endothelium-derived hyperpolarising factor and prostacyclin. Hyperglycaemia and dyslipidaemia driven oxidative stress is a major contributor, enhanced by advanced glycation end product formation and polyol pathway activation. These are coupled to protein kinase C activation and omega-6 essential fatty acid dysmetabolism. Together, this complex of interacting metabolic factors accounts for endothelial dysfunction, reduced nerve perfusion and function. Thus, the evidence emphasises the importance of vascular dysfunction, driven by metabolic change, as a cause of diabetic neuropathy, and highlights potential therapeutic approaches.

The effects of classic antipsychotic haloperidol plus the extract of ginkgo biloba on superoxide dismutase in patients with chronic refractory schizophrenia.

Zhou D, Zhang X, Su J, et al. *Chin Med J (Engl)* 1999;112:1093-1096.

OBJECTIVES: To explore the association between schizophrenic symptoms and superoxide dismutase (SOD), and to investigate the effect of classic antipsychotic haloperidol plus the extract of Ginkgo biloba (EGb) on SOD. **METHODS:** In 54 patients with chronic refractory schizophrenia, 27 were treated with haloperidol plus EGb (group 1), and the rest received haloperidol plus placebo (group 2). Superoxide dismutase (SOD) levels of these patients were measured before and after treatment and compared with the levels of 25 healthy volunteers. Therapeutic efficacy was equated with a change in clinical rating scores assessed by standardized measurement tools including the Scale for Assessment of Positive Symptoms (SAPS) and the Scale for Assessment of Negative Symptoms (SANS). **RESULTS:** Patients in group 1 improved significantly as demonstrated by scores from both SAPS and SANS, while those in group 2 only by scores from SANS. Assessed by SAPS, the response of patients receiving haloperidol plus EGb was more significant than those receiving haloperidol only. SOD levels before treatment in all patients were significantly higher than those in normal controls. After treatment, SOD levels decreased significantly in group 1 but not in group 2. In addition, before treatment, SOD levels in all patients correlated significantly with SAPS score. The levels of SOD measured before treatment were also correlated with the improvement of patients as measured by SAPS and SANS after 12 weeks. **CONCLUSIONS:** EGb may enhance the efficacy of classic antipsychotic haloperidol on schizophrenia, especially on positive symptoms. It may work through an antioxidant efficacy that is involved in the therapeutic mechanism.