

**1,500 Scientific Abstracts**

**Evidence-Base to Support  
Food Supplement Health Claims**

**Maintenance of Wellness  
Restoration of Wellness**

**Prevention of Disease  
Disease Risk Reduction  
Treatment of Disease**

**Presented to  
Codex Committee on Food Labelling**

**By South African Government**

**May 2004**

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# 1. Acne

Preventative and curative options include:

Chromium, zinc, vitamin B6, vitamin E, vitamin A

## **Acne vulgaris: therapy directed at pathophysiologic defects.**

Ayres S Jr, Mihan R

Cutis 1981 Jul;28(1):41-2

An effective therapeutic regimen for the treatment of acne vulgaris is presented. The emphasis is based upon correcting a defect in keratinization of the sebaceous follicles with a combination of vitamins A and E. This prevents the formation of milia and comedones, thus depriving the *Propionibacterium acnes* of a culture medium. Vitamin E also prevents irritating lipid peroxidation of sebum, damaged by bacterial growth, which may be responsible for the inflammatory aspects of acne. No antibiotics were employed in the series of 98 consecutive cases examined herein.

## **Endotoxin-induced changes in copper and zinc metabolism in the Syrian hamster.**

Etzel KR, Swerdel MR, Swerdel JN, Cousins RJ

J Nutr 1982 Dec;112(12):2363-73

The temporal response of zinc and copper metabolism to endotoxin administration was examined in Syrian hamsters over a 144-hour period. Serum copper was significantly elevated at 12, 24 and 72 hours after endotoxin, whereas serum zinc was reduced 4-48 hours after treatment. A brief elevation (8 hours) in liver copper concentration and a sustained (72 hours) increase in liver zinc concentration were also observed. The amount of zinc associated with liver metallothionein (MT) progressively increased with time, to a plateau by 24 hours and persisted at the elevated level until 72 hours after endotoxin treatment. In vitro translation of poly (A)<sup>+</sup> RNA from liver polyribosomes showed that following endotoxin treatment MTmRNA activity was maximally elevated 6 hours after endotoxin administration and remained elevated 24 and 48 hours thereafter. Slab gel electrophoresis of serum proteins indicated changes in a stainable protein comigrating with purified ceruloplasmin after endotoxin administration. Pooled gingival tissue from endotoxin-treated hamsters demonstrated a consistently elevated copper content 12-144 hours after treatment. Endotoxin isolated from *Bacteroides melaninogenicus* was more effective in elevating gingival and serum copper and gingival zinc than *Escherichia coli* endotoxin. It was concluded that endotoxin administration elicits responses that result in enhanced metallothionein

mRNA activity. In addition, Cu and Zn concentrations in serum, liver and gingival tissue are influenced by different endotoxins to different degrees.

**[Current aspects about the role of zinc in nutrition].** [Article in French]

Favier A Groupe de recherche sur les pathologies oxydatives, Universite de Grenoble, La Tronche.

Rev Prat 1993 Jan 15;43(2):146-51

The role played by zinc in biology is now better known, and numerous biochemical mechanisms, such as immunity or actions on several hormones and more than 200 enzymes, have proved to be zinc-dependent. Thus, many functions are disturbed when this trace metal is deficient, including, for example, taste and appetite, cell multiplication, growth, pregnancy, fertility, defence against bacteria and brain functions. Zinc intake has been found to be unexcessive and indeed, at the limit of sufficiency in the French population. Groups at risk, such as neonates, growing children, pregnant women and elderly people, should have a higher zinc intake provided by dietary measures or supplementation. Zinc supplementation has been shown to exert a beneficial effect in randomized studies concerning children's growth, acne, old people's immunity or low female fertility. Such supplementation must be balanced and given in moderate doses since zinc interacts with other foodstuffs, and an excess of zinc can be as bad as its deficiency in our nutrition.

**Nutrition-endocrine interactions: induction of reciprocal changes in the delta 4-5 alpha-reduction of testosterone and the cytochrome P-450-dependent oxidation of estradiol by dietary macronutrients in man.**

Kappas A, Anderson KE, Conney AH, Pantuck EJ, Fishman J, Bradlow HL

Proc Natl Acad Sci U S A 1983 Dec;80(24):7646-9

The in vivo biotransformations of drugs known to be metabolized by enzymes localized in the endoplasmic reticulum of liver can be greatly altered by diet in humans, as we have shown previously. Steroid hormones also are metabolized extensively by hepatic microsomal enzymes; therefore, we examined the possibility that testosterone and estradiol biotransformations, as assessed with radiolabeled tracer methods, could be influenced by dietary macronutrients. Normal males were fed a high-protein diet for 2 weeks, followed by a high-carbohydrate diet for an additional 2 weeks. The delta 4-5 alpha-reduction of testosterone was considerably diminished, while the cytochrome P-450-dependent hydroxylation of estradiol at the C2 position was substantially enhanced during ingestion of the high-protein diet as compared with the high-carbohydrate diet. These results indicate that dietary macronutrients can significantly alter major metabolic pathways for testosterone and estradiol in man. The mechanism by which reciprocal changes in the delta 4-5 alpha-reduction of testosterone and the cytochrome P-450-mediated oxidation of estradiol are produced by diets is not known. Similar changes in steroid delta 4-5 alpha-reduction and cytochrome P-

450-dependent chemical oxidations have been observed in circumstances in which the mixed-function oxidase system in liver is induced by agents such as phenobarbital, hexachlorobenzene, dioxin, and polyhalogenated biphenyls. Thus, the alterations in steroid hormone metabolism produced by dietary macronutrients in man mimic those that can be produced by drugs and environmental chemicals.

### **Oral vitamin A in acne vulgaris. Preliminary report.**

Kligman AM, Mills OH Jr, Leyden JJ, Gross PR, Allen HB, Rudolph RI

Int J Dermatol 1981 May;20(4):278-85

Oral vitamin A (retinol) is generally not considered useful in the treatment of acne vulgaris. We conducted a study which showed that retinol was indeed ineffective at the usual doses of 50,000 to 100,000 IU daily. Retinol was highly efficacious in doses of 300,000 units for women and 400,000 to 500,000 units for men, toxicity was slight and limited mainly to skin (xerosis) and mucous membranes (cheilitis). The danger of hypervitaminosis A in this dosage range has been exaggerated. Retinol is a valuable drug for treating stubborn, severely inflammatory acne vulgaris. It is administered until the disease is brought under control, usually within three to four months. Then the dosage is progressively reduced relying on conventional drugs to keep the disease in abeyance.

### **Pantothenic acid deficiency as the pathogenesis of acne vulgaris.**

Leung LH. Department of General Surgery, Hong Kong Central Hospital, Hong Kong.

Med Hypotheses 1995 Jun;44(6):490-2

For years, the pathogenesis of acne vulgaris has been known to be strongly influenced by hormonal factors. However, the exact role of and the interrelationship among the various hormones in question have not been well elucidated. Here, I wish to suggest a radically different theory for its pathogenesis and relate its basic pathology to a deficiency in pantothenic acid, a vitamin hitherto not known to cause any deficiency syndrome in humans. Hence, the effect of hormonal factors in this disease entity becomes secondary to that of the availability of pantothenic acid. A complete cure of this condition is effected by a very liberal replacement therapy with the vitamin.

### **High-chromium yeast for acne?**

McCarty M

Med Hypotheses 1984 Jul;14(3):307-10

Many dermatologists have reported that insulin and tolbutamide are therapeutically effective in acne. This rationalizes a recent observation that high-chromium yeast appears to have value as an acne treatment.

### **Effects of oral zinc and vitamin A in acne.**

Michaelsson G, Juhlin L, Vahlquist A

Arch Dermatol 1977 Jan;113(1):31-6

The effects of oral zinc sulfate (corresponding to 135 mg of zinc daily) alone and in combination with vitamin A (300,000 international units) daily on acne lesions have been compared with those of vitamin A alone and of a placebo. The number of comedones, papules, pustules, and infiltrates were counted at each visit. After four weeks, there was a significant decrease in the number of papules, pustules, and infiltrates in the zinc-treated groups. The effect of zinc plus vitamin A was not better than zinc alone. After 12 weeks of treatment, the mean acne score had decreased from 100% to 15%. The mechanism for the effect of zinc therapy in acne, to our knowledge, is not presently known.

### **Serum zinc and retinol-binding protein in acne.**

Michaelsson G, Vahlquist A, Juhlin L

Br J Dermatol 1977 Mar;96(3):283-6

The serum levels of zinc and retinol-binding protein (RBP) have been determined in 173 patients with acne and compared with those of a control group. The RBP is a specific transport protein and its level in plasma reflects the amount of vitamin A available to the tissues. Patients with severe acne were found to have lower levels of RBP than either patients with mild acne or healthy subjects of the same age. In the case of males with severe acne, the mean serum zinc level was significantly lower than that of the control group. No such difference was observed for girls. The observed condition of low levels of zinc and vitamin A in the serum of patients with severe acne may provide a rationale for the clinically good effect of oral zinc treatment.

### **A double-blind study of the effect of zinc and oxytetracycline in acne vulgaris.**

Michaelsson G, Juhlin L, Ljunghall K

Br J Dermatol 1977 Nov;97(5):561-6

With a double-blind technique, the effects of oral zinc and tetracyclines were compared in 37 patients with moderate and severe acne. No difference in effect between the treatments was seen and no side-effects were noted in any group. After 12 weeks of treatment, the average decrease in the acne score was about 70% in both groups.

### **Erythrocyte glutathione peroxidase activity in acne vulgaris and the effect of selenium and vitamin E treatment.**

Michaelsson G, Edqvist LE

Acta Derm Venereol 1984;64(1):9-14

The glutathione-peroxidase (GSH-Px) activity in erythrocytes was determined in 42 men with severe acne and 47 women with acne--26 of a moderate degree and 21 severe. The male acne patients had significantly lower GSH-Px levels than the controls. The women with acne did not differ significantly from the controls in this respect when patients and controls using oral contraceptives were excluded. Both the female controls and the women with acne using oral contraceptives had significantly higher GSH-Px values than the corresponding groups not using the pill. The pubertal acne girls had the same high GSH-Px activity as women on oral contraceptives. In an open trial 29 patients were given 0.2 mg of selenium (as Na<sub>2</sub>Se O<sub>3</sub>) + 10 mg of tocopheryl succinate for their acne twice daily for 6-12 weeks. A good result was obtained, especially in patients with pustular acne and low GSH-Px activity, and the beneficial effect was usually paralleled by a slow rise of the GSH-Px activity. Some 6-8 weeks after withdrawal of the treatment the GSH-Px values had returned to the pretreatment levels.

**A double-blind controlled evaluation of the sebosuppressive activity of topical erythromycin-zinc complex.**

Pierard-Franchimont C, Goffin V, Visser JN, Jacoby H, Pierard GE Department of Dermatopathology, University of Liege, Belgium.

Eur J Clin Pharmacol 1995;49(1-2):57-60

In a double-blind randomised study, 14 volunteers applied 4% erythromycin plus 1.2% zinc (Zineryt lotion) and 4% erythromycin lotions, each on half of the forehead twice daily for 3 months. The sebum output was evaluated at 3-week intervals using the photometric and the lipid-sensitive film methods. Evaluations of casual level (CL) and sebum excretion rate (SER) were made with a Sebumeter, and total area of lipid spots (TAS) was measured on Sebutapes. Compared to baseline values, the formulation of the erythromycin-zinc complex induced significant reductions in SER after 6 and 9 weeks, and in CL and TAS at 3, 6, 9 and 12 weeks. The mean reduction in TAS was over 20% for four successive 1-h samplings on completion of the study. Significant reductions in CL, SER and TAS were observed for the erythromycin-zinc formulation compared to the control lotion at 6 and 9 weeks, and also at 3 weeks for SER and TAS, and at 12 weeks for CL and TAS. This study indicates that sebum output is significantly reduced by the erythromycin-zinc complex. This reduction is theoretically beneficial for the acneic patient.

**Increased target tissue uptake of, and sensitivity to, testosterone in the vitamin B6 deficient rat.**

Symes EK, Bender DA, Bowden JF, Coulson WF

J Steroid Biochem 1984 May;20(5):1089-93

Six-week old male rats were maintained for 4 weeks on a vitamin B6-free diet to cause a moderately severe degree of vitamin B6 depletion. This led to a significant reduction in the circulating concentration of testosterone in plasma (control = 8.36 +/- 1.68, deficient = 2.13 +/- 0.54 nmol/l), but had no effect on circulating concentrations of luteinizing hormone, or, in intact males, on the weight of the prostate relative to body weight. In both intact and 24-h castrated animals vitamin B6 deficiency resulted in a significant increase in the uptake of [3H]testosterone into the prostate, and both increased and prolonged the specific nuclear retention of the steroid, as assessed by the ratio of radioactivity in the nuclear pellet: the high speed supernatant fraction. The results suggest that vitamin B6 has a function in the action of testosterone (and other steroid hormones), possibly in the recycling of receptors from the nucleus back into the cytosol after initial translocation. Vitamin B6 deficient animals have either a reduced rate of synthesis of testosterone or an increased rate of metabolic clearance compared with vitamin B6 supplemented controls, and this appears to be associated with enhanced target organ response to the hormone.

### **High-dose vitamin A therapy for Darier's disease.**

Thomas JR 3d, Cooke JP, Winkelmann RK

Arch Dermatol 1982 Nov;118(11):891-4

Three patients with Darier's disease were treated with 1 X 10<sup>6</sup> IU of orally administered vitamin A daily for 14 days. In all patients, 50% to 80% improvement in the skin lesions was noted. Desquamation was minimal, and side effects consisted of drowsiness, mild frontal headache, dry lips and dry nose. During therapy, all patients had a transient, mild increase in the serum triglyceride level, and two patients had a minimal increase in the serum cholesterol concentration.

### **Zinc sulfate in acne vulgaris.**

Weimar VM, Puhl SC, Smith WH, tenBroeke JE

Arch Dermatol 1978 Dec;114(12):1776-8

The effects of orally administered zinc sulfate in 52 patients with mild to moderate acne vulgaris were compared to those of a placebo capsule. The numbers of comedones, papules, pustules, infiltrates, and cysts were counted at each visit over a 12-week period. Forty patients completed the study. Zinc appeared to have a somewhat beneficial effect on pustules but not on comedones, papules, infiltrates, or cysts. Fourteen patients (50%) in the zinc group had side effects of nausea, vomiting, or diarrhea. Six patients (21%) in the zinc group could not tolerate the nausea and withdrew from the study.

### **Inhibition of erythromycin-resistant propionibacteria on the skin of acne patients by topical erythromycin with and without zinc.**

Bojar RA, Eady EA, Jones CE, Cunliffe WJ, Holland KT Department of Microbiology, University of Leeds, U.K.

Br J Dermatol 1994 Mar;130(3):329-36

Propionibacteria resistant to high concentrations of erythromycin [minimal inhibitory concentration (MIC)  $\leq$  0.5 mg/ml] are now commonly isolated from the skin of antibiotic-treated acne patients. This double-blind study was carried out to assess the ability of 4% w/v erythromycin with and without 1.2% w/v zinc acetate to reduce the numbers of erythromycin-resistant propionibacteria *in vivo*, and also to monitor the acquisition of resistant strains *de novo* during therapy. Under laboratory conditions, erythromycin-resistant propionibacteria were shown to be as sensitive to zinc acetate as fully sensitive strains. *In vivo*, the erythromycin/zinc complex and erythromycin alone produced highly significant reductions in total propionibacteria ( $P < 0.001$ ) and in the number of erythromycin-resistant strains ( $P < 0.001$  at 8 weeks). After 12 weeks, resistant propionibacteria were reacquired, or acquired *de novo*, by three patients treated with erythromycin alone and four patients treated with the erythromycin/zinc complex. In contrast, changes in numbers of Micrococcaceae were slight and, after 12 weeks, erythromycin-resistant strains were predominant in both treatment groups. *In vitro* MIC determinations suggested that this finding might be explained by the exceptionally high degree of erythromycin resistance displayed by some staphylococcal strains (MIC  $< 4$  mg/ml) and by the relative insensitivity of all staphylococcal strains to zinc acetate. Erythromycin with and without zinc was clinically effective, and both preparations produced significant reductions in acne grade, and inflamed and non-inflamed lesion counts ( $P < 0.001$ ).

### **Endotoxin-induced changes in copper and zinc metabolism in the Syrian hamster.**

Etzel KR, Swerdel MR, Swerdel JN, Cousins RJ

J Nutr 1982 Dec;112(12):2363-73

The temporal response of zinc and copper metabolism to endotoxin administration was examined in Syrian hamsters over a 144-hour period. Serum copper was significantly elevated at 12, 24 and 72 hours after endotoxin, whereas serum zinc was reduced 4-48 hours after treatment. A brief elevation (8 hours) in liver copper concentration and a sustained (72 hours) increase in liver zinc concentration were also observed. The amount of zinc associated with liver metallothionein (MT) progressively increased with time, to a plateau by 24 hours and persisted at the elevated level until 72 hours after endotoxin treatment. *In vitro* translation of poly (A)<sup>+</sup> RNA from liver polyribosomes showed that following endotoxin treatment MTmRNA activity was maximally elevated 6 hours after endotoxin administration and remained elevated 24 and 48 hours thereafter. Slab gel electrophoresis of serum proteins indicated changes in a stainable protein comigrating with purified ceruloplasmin after endotoxin administration. Pooled gingival tissue from endotoxin-treated hamsters demonstrated a consistently elevated copper content 12-144 hours after treatment. Endotoxin isolated from

*Bacteroides melaninogenicus* was more effective in elevating gingival and serum copper and gingival zinc than *Escherichia coli* endotoxin. It was concluded that endotoxin administration elicits responses that result in enhanced metallothionein mRNA activity. In addition, Cu and Zn concentrations in serum, liver and gingival tissue are influenced by different endotoxins to different degrees.

**The effect of zinc on the 5 alpha-reduction of testosterone by the hyperplastic human prostate gland.**

Leake A, Chisholm GD, Habib FK

J Steroid Biochem 1984 Feb;20(2):651-5

The present studies were performed to evaluate the role of zinc in the regulation of testosterone 5 alpha-reduction by the 800 g supernatants prepared from human benign prostate hyperplasia specimens. The results show that when zinc is added at low concentrations the 5 alpha-reduction of testosterone is increased but at higher cation concentrations the metabolism is significantly inhibited. This decrease was mediated by both a non-competitive inhibition of the binding of testosterone to the 5 alpha-reductase enzyme and by a reduction in the formation of the NADPH cofactor. We have also demonstrated that the decreased synthesis of NADPH was produced by a competitive inhibition of both G6P and NADP binding to the G6PD enzyme. The data also suggests that the increase in testosterone metabolism observed at low zinc concentrations does not produce any changes in the binding of testosterone to the 5 alpha-reductase enzyme. In spite of the above observations we were unable to establish any correlation between the endogenous zinc content of the tissue and the in vitro capacity of the BPH samples to 5 alpha-reduce testosterone. The present study suggests a possible physiological role for the regulation of testosterone metabolism by zinc in the human prostate gland.

**Toxic doses of vitamin A for pityriasis rubra pilaris.**

Randle HW, Diaz-Perez JL, Winkelmann RK

Arch Dermatol 1980 Aug;116(8):888-92

Seven patients who were disabled by pityriasis rubra pilaris were given toxic doses of oral vitamin A (1 million IU/day in six of the seven patients) for five to 14 days. Within 72 hours, the patients began to exfoliate the hyperkeratotic and keratodermatous lesions. The desquamative process was completed between ten and 14 days. The skin remained erythematous for several months before assuming a normal color. The skin of six of the seven patients was virtually cleared by the treatment, and none suffered a relapse of the pityriasis rubra pilaris. Serial skin biopsy specimens showed evidence suggestive of an accelerated turnover rate of epidermal cells during treatment. Transient abnormalities of liver function test results were noted in two patients.

**[Retinotherapy of skin diseases].** [Article in French]

Saurat JH

Presse Med 1994 Nov 5;23(34):1551-3

The discovery of retinoid receptors has contributed greatly to our understanding of the mechanism of action of vitamin A. The organism produces at least two ligands from ingested vitamin A which act as hormones modulating the activity of numerous genes via their nuclear receptor. These ligands are produced locally by target cells from retinol and retinaldehyde. These advances do not respond to the clinicians' interrogation as to why 13cis retinoic acid blocks sebaceous secretion and cures severe acne while other known retinoids are ineffective. Current research would suggest that the expression of nuclear receptors is not altered in skin diseases but that upstream anomalies in the intracrine system (enzymes and binding proteins) could be involved. Clinically, teratogenic risks are a major obstacle to the oral administration of retinoids and the future in skin diseases lies most likely in topical applications.

## 2. Allergies

Preventative and curative options include:

Omega 3 and 6 fatty acids, co enzyme Q10, vitamin C, vitamin E, magnesium, DHEA, n-acetyl cysteine, bifido bacteria, grape seed extract, ginkgo biloba, glutamine, nettle leaf, aloe vera, pantothenic acid, quercitin,

### **Thiols decrease cytokine levels and down-regulate the expression of CD30 on human allergen-specific T helper (Th) 0 and Th2 cells.**

Bengtsson A, Lundberg M, Avila-Carino J, Jacobsson G, Holmgren A, Scheynius A. Department of Medicine, Unit of Clinical Allergy Research, Karolinska Institutet, Stockholm, Sweden. asa.bengtsson@mb.ki.se

Clin Exp Immunol 2001 Mar;123(3):350-360

The thiol antioxidant N-acetyl- L-cysteine (NAC), known as a precursor of glutathione (GSH), is used in AIDS treatment trials, as a chemoprotectant in cancer chemotherapy and in treatment of chronic bronchitis. In vitro, GSH and NAC are known to enhance T cell proliferation, production of IL-2 and up-regulation of the IL-2 receptor. The 120-kD CD30 surface antigen belongs to the tumour necrosis factor (TNF) receptor superfamily. It is expressed by activated T helper (Th) cells and its expression is sustained in Th2 cells. We have analysed the effect of GSH and NAC on the cytokine profile and CD30 expression on human allergen-specific T cell clones (TCC). TCC were stimulated with anti-CD3 antibodies in the presence of different concentrations of GSH and NAC. Both thiols caused a dose dependent down-regulation of IL-4, IL-5 and IFN-gamma levels in Th0 and Th2 clones, with the most pronounced decrease of IL-4. Furthermore, they down-regulated the surface expression of CD30, and the levels of soluble CD30 (sCD30) in the culture supernatants were decreased. In contrast, the surface expression of CD28 or CD40 ligand (CD40L) was not significantly changed after treatment with 20 m M NAC. These results indicate that GSH and NAC favour a Th1 response by a preferential down-regulation of IL-4. In addition, the expression of CD30 was down regulated by GSH and NAC, suggesting that CD30 expression is dependent on IL-4, or modified by NAC. In the likely event that CD30 and its soluble counterpart prove to contribute to the pathogenesis in Th2 related diseases such as allergy, NAC may be considered as a future therapeutic agent in the treatment of these diseases.

### **Effects of Orally consumed aloe vera juice on gastrointestinal function in normal humans**

Jeffrey Bland Linus Pauling Institute of Science & Medicine

Preventive Medicine March/April 1985

This study evaluated the effect of oral Aloe Vera juice supplemented on gastric pH, stool specific gravity, protein digestion/absorption, and stool microbiology. Results indicate that supplemental oral Aloe Vera juice is well tolerated by most individuals and has a favorable effects upon a number of gastrointestinal parameters. A discussion of the potential role of Aloe Vera juice on inflammatory bowel disorders based upon this work presented.

**Effect of vitamin C on histamine bronchial responsiveness of patients with allergic rhinitis**

Bucca C.; Rolla G.; Oliva A.; Farina J.-C. Clinica Medica I, Dpt. Scienz Biomediche e Oncologia Umana, Via Genova3, 10126 Torino Italy

Ann. Allergy (USA), 1990, 65/4 (311-314)

The effect of acute oral administration of 2 g vitamin C on bronchial responsiveness to inhaled histamine in 16 patients with allergic rhinitis was compared with placebo on two consecutive days in double-blind, crossover design. The PC15FEV1 was significantly increased one hour after treatment with vitamin C but not after placebo.

**Pretreatment of skin with a Ginkgo biloba extract/sodium carboxymethyl-beta-1,3-glucan formulation appears to inhibit the elicitation of allergic contact dermatitis in man**

Castelli D.; Colin L.; Camel E.; Ries G. D. Castelli, RoC Laboratoires de Dermo-esthetique, 50 Rue de Seine, 92704 Colombes France

Contact Dermatitis (Denmark), 1998, 38/3 (123-126)

The clinical efficiency of mitigating contact dermatitis with a Ginkgo biloba extract and carboxymethyl-beta-1,3-glucan formulation was investigated in a double-blind versus placebo study using 22 subjects (Caucasian women aged 22-55 years) with allergic contact dermatitis from various substances in the European standard series. The formulation was applied to intact skin 2 x a day for 2 weeks ('in use' application) prior to a single application of a selected contact allergen under a Finn Chamber for 24 h. Readings were carried out in a blind study by a dermatologist 2 and 3 days after patch removal. Representative photographs were taken of treated, placebo and untreated test areas. 68.2% of the panelists showed significantly reduced skin reactivity ( $p = 0.037^*$ ) on the treated site 2 days after patch removal, versus untreated and/or placebo sites. This finding indicates that the Ginkgo biloba/carboxymethyl-beta-1,3-glucan formulation can mitigate against allergic contact dermatitis.

### **The potential role of tocopherol in asthma and allergies: modification of the leukotriene pathway.**

Centanni S, Santus P, Di Marco F, Fumagalli F, Zarini S, Sala A. Respiratory Unit, San Paolo Hospital, University of Milan, Milan, Italy.  
stefano.centanni@unimi.it

BioDrugs 2001;15(2):81-86

Metabolism of arachidonic acid via the 5-lipoxygenase (5-LO) pathway leads to the formation of hydroperoxyeicosatetraenoic acids (HPETEs) and leukotriene (LT) A<sub>4</sub>. This unstable allylic epoxide can be further converted by secondary enzymes into LTB<sub>4</sub> and cysteinyl LTs. LTs represent a family of potent biologically active compounds synthesised by specific cell types and by transcellular biosynthetic mechanisms. Cysteinyl LTs are involved in the pathogenesis of asthma, and recent data indicate that individuals with asthma may have enhanced basal excretion of urinary LTE<sub>4</sub> compared with normal individuals. Tocopherol (vitamin E) and tocopherol acetate strongly inhibit potato 5-LO in an irreversible and noncompetitive way, and, by affecting the redox state of cells possessing 5-LO, they may influence the production of biologically active LTs. It has been reported that normal plasma levels of tocopherol may enhance the lipoxygenation of arachidonic acid, whereas higher tocopherol levels exert a suppressive effect that is consistent with its role as a hydroperoxide scavenger. Receptor-mediated activation of neutrophils in individuals with asthma results in the synthesis of LTs. This activation is inhibited by tocopherol in a concentration-dependent manner. Additional controlled studies are needed to assess the effect of tocopherol on leukotriene production in asthmatic individuals. The results of these studies may be useful in developing new therapeutic approaches in asthmatic/allergic patients.

### **Increase of intestinal Bifidobacterium and suppression of coliform bacteria with short-term yogurt ingestion.**

Chen RM, Wu JJ, Lee SC, Huang AH, Wu HM. Department of Pathology, National Cheng Kung University Medical College, Tainan, Taiwan, Republic of China.

J Dairy Sci 1999 Nov;82(11):2308-2314

To determine whether ingestion of yogurt would alter human intestinal bacterial composition and whether Bifidobacterium numbers would increase in the intestine, 34 healthy volunteers were studied. The experimental period was 26 d, including an initial 8 d without yogurt, 10 d with three bottles (230 ml each) of AB yogurt per day (President Enterprise Corporation, Tainan, Taiwan), and 8 d without yogurt. Stool samples were taken at 3- to 4-d intervals. The bacteria of each fresh stool sample were promptly analyzed by dilution and culture on blood, MacConkey, Center for Disease Control and NNLP agars, the agar contained nalidixic acid, neomycin sulfate, LiCl, and paromomycin sulfate for aerobes, coliforms, anaerobes, and bifidobacteria, respectively. The number of bacteria

was determined as colony-forming units per gram of dried stool. Results indicated that ingestion of AB yogurt increased the counts of anaerobic bacteria, suppressed aerobic bacteria, and significantly elevated the bifidus to coliform ratio. Arbitrarily primed polymerase chain reaction was used to differentiate the identity of bifidobacteria in four volunteers before and after yogurt ingestion and confirmed that *B. bifidum* ingested from the yogurt survived and proliferated in the stool throughout the experiment. However, the elevated bifidus to coliform ratio gradually diminished and disappeared after yogurt consumption was discontinued. In conclusion, ingestion of yogurt increased the numbers of stool bifidobacteria and suppressed coliform bacteria. The ingested bifidobacteria survived for more than 8 d after yogurt consumption was discontinued.

### **Influence of glutamine on cytokine production by human gut in vitro.**

Coeffier M, Miralles-Barrachina O, Le Pessot F, Lalaude O, Daveau M, Lavoinne A, Lerebours E, Dechelotte P. Appareil Digestif Environnement et Nutrition group (ADEN), France.

Cytokine 2001 Feb 7;13(3):148-154

**BACKGROUND:** glutamine modulates cytokine production by immune cells in vitro and protects the gut from experimental enterocolitis, but data on the effect of glutamine on cytokine production in human gut are lacking. **AIM:** to assess the effect of glutamine pre-treatment in vivo and in vitro on cytokine production by intestinal mucosa.

**METHODS:** nine fasted volunteers received either enteral glutamine or saline over 6 h in a cross-over design. Duodenal biopsies were cultured for 24 h with or without glutamine. Cytokine content of culture media was analysed by ELISA, and the expression of cytokine mRNA in biopsies was assessed by semi-quantitative RT-PCR. **Results:** glutamine given in vivo and in vitro significantly decreased IL-6 [1.4 (0.8-8.5) vs 8.9 (1.0-43.9)] and IL-8 production [5.8 (0-51.4) vs. 53.0 (2.5-114.6), pg/mg wet tissue], median (range), both < or =0.01, in comparison to no glutamine experiments. Glutamine did not influence IL-4 production. IL-1beta, IL-10 and TNF-alpha were not detectable in culture media. The expression of any cytokine mRNA was not influenced by glutamine.

**CONCLUSIONS:** glutamine reduces pro-inflammatory cytokine production by human intestinal mucosa, probably by a post-transcriptional pathway. Glutamine could be useful to modulate inflammatory conditions with imbalanced cytokine production. Copyright 2001 Academic Press.

### **Increased nitrosothiols in exhaled breath condensate in inflammatory airway diseases.**

Corradi M, Montuschi P, Donnelly LE, Pesci A, Kharitonov SA, Barnes PJ. Institute of Respiratory Diseases, University of Parma, Italy.

Am J Respir Crit Care Med 2001 Mar;163(4):854-858

Nitrosothiols (RS-NOs) are formed by interaction of nitric oxide (NO) with glutathione and may limit the detrimental effect of NO. Because NO generation is increased in airway inflammation, we have measured RS-NOs in exhaled breath condensate in patients with asthma, cystic fibrosis, or chronic obstructive pulmonary disease (COPD). We also measured exhaled NO and nitrite (NO(2-)) in the same subjects. RS-NOs were detectable in exhaled breath condensate of all subjects. RS-NOs were higher in subjects with severe asthma (0.81 0.06 microM) when compared with normal control subjects (0.11 0.02 microM, < 0.01) and with subjects with mild asthma (0.08 0.01 microM, < 0.01). Elevated RS-NOs values were also found in patients with cystic fibrosis (0.35 0.07 microM, < 0.01), in those with COPD (0.24 0.04 microM, p < 0.01) and in smokers (0.46 0.09 microM, < 0.01). In current smokers there was a correlation (r = 0.8, < 0.05) between RS-NOs values and smoking history (pack/year). We also found elevated concentrations of NO(2-) in patients with severe asthma, cystic fibrosis, or COPD, but not in smokers or patients with mild asthma. This suggests that exhaled NO(2-) is less sensitive than exhaled RS-NOs. This study has shown that RS-NOs are detectable in exhaled breath condensate of healthy subjects and are increased in patients with inflammatory airway diseases. As RS-NOs concentrations in exhaled breath condensate vary in the different airway diseases and increase with the severity of asthma, their measurement may have clinical relevance as a noninvasive biomarker of nitrosative stress.

### **Can immunoregulatory lactic acid bacteria be used as dietary supplements to limit allergies?**

Cross ML, Gill HS. Milk & Health Research Centre, Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand.

Int Arch Allergy Immunol 2001 Jun;125(2):112-119

Studies in gnotobiotic animals have suggested that the intestinal bacterial flora may play an important role in priming the immune system during ontogeny to limit dysfunctional responses, including allergy. Prospective clinical studies have identified a higher incidence of allergy expression in early childhood among children who have low enteric populations of lactic acid bacteria (LAB), such as lactobacilli and bifidobacteria, further supporting a role for gut-colonizing bacteria in regulating immunological atopy. There is some evidence to suggest that supplementing the human diet with probiotic LAB might combat both allergy development and expression of atopy in allergy sufferers; however, definitive information, in the form of controlled intervention trials, remains scant. Recent immunological evidence has indicated that certain strains of LAB can stimulate the production of type I and II interferons and pro-interferon monokines (IL-12 and IL-18), following contact with the immune system; therefore, probiotic forms of immunoregulatory LAB could be used as dietary supplements to modify the gut microflora and provide pro-T helper cell 1 (Th1) STAT-activating signals sufficient to deviate the immune phenotype and correct the Th2-type bias which promotes allergy. This review outlines the clinical and laboratory evidence of a role for LAB in combating allergies, and attempts to explain this phenomenon in

terms of our current understanding of immunoregulatory signals produced by gut-colonizing microbes. Copyright 2001 S. Karger AG, Basel

**Quercetin inhibits anaphylactic contraction of guinea pig ileum smooth muscle.**

Fanning MJ, Macander P, Drzewiecki G, Middleton E Jr.

Int Arch Allergy Appl Immunol 1983;71(4):371-3

Certain flavonoids inhibit antigen-induced release of histamine from mast cells and basophils and also inhibit contraction of guinea pig ileum induced by histamine, acetylcholine, and PGE<sub>2</sub>. We examined the effect of one flavonoid, quercetin, on anaphylactic smooth muscle contraction of ileum from guinea pigs sensitized to egg albumin. Quercetin inhibited both the phasic and tonic components of anaphylactic contraction in a concentration-dependent fashion (IC<sub>50</sub> approximately 10 microM). Whether this is primarily an effect on mast cell mediator release or inhibition of mediator effects on smooth muscle has not been established.

**TPN decreases IL-4 and IL-10 mRNA expression in lipopolysaccharide stimulated intestinal lamina propria cells but glutamine supplementation preserves the expression.**

Fukatsu K, Kudsk KA, Zarzaur BL, Wu Y, Hanna MK, DeWitt RC. The University of Tennessee Health Science Center, Memphis 38163, USA.

Shock 2001 Apr;15(4):318-322

Total parenteral nutrition (TPN) decreases intestinal IgA and levels of Th2 cytokines, interleukin (IL)-4, and IL-10 within the supernatants of intestinal homogenates. These cytokines are known to stimulate IgA production in vitro by cells of the gut-associated lymphoid tissue (GALT). Glutamine (GLN) supplementation of TPN normalizes GALT mass and cytokine levels. Because intestinal homogenates contain mucosa which itself is a source of cytokines, it was unclear whether cytokines change within the GALT itself. This study investigates dietary effects on IL-4 and IL-10 cytokine mRNA expression within isolated GALT lamina propria cells after lipopolysaccharide (LPS) stimulation. Prospective randomized experimental trials were used in this study. Fifty-nine mice were randomized to chow, intravenous TPN (IV-TPN), intragastric TPN (IG-TPN), complex enteral diet (CED), or 2% GLN-supplemented TPN (GLN-TPN). In experiment 1, animals were fed chow, IV-TPN, IG-TPN, or CED for 5 days and received intraperitoneal LPS (100 microg/kg BW), and then were sacrificed 1 h later. Intestine was harvested for GALT lamina propria. Total RNA was extracted from lamina propria cells and cytokine mRNA for IL-4, and IL-10 was measured by reverse transcriptase polymerase chain reaction. IgA levels of intestinal washing were also measured with ELISA. In experiment 2, mRNA for IL-4 and IL-10, and intestinal IgA levels were measured in mice fed chow, IV-TPN, or GLN-TPN as in experiment 1. Both IL-4 and IL-10 mRNA expression

decreased significantly in IV-TPN mice compared to chow or CED feeding. IG-TPN resulted in IL-10 mRNA expression significantly lower than chow or CED but significantly better than IV-TPN. GLN preserved IL-4 and IL-10 mRNA levels, which correlated with intestinal IgA levels. Route and type of nutrition as well as GLN influence message for the Th2 type IgA-stimulating cytokines, IL-4 and IL-10, within the primary site of GALT IgA production, the lamina propria.

**Enrichment of bifidobacteria from human gut contents by oligofructose using continuous culture.**

Gibson GR, Wang X. Medical Research Council, Dunn Clinical Nutrition Centre, Cambridge, UK.

FEMS Microbiol Lett 1994 May 1;118(1-2):121-127

Chemostat cultures of human faecal bacteria were used to determine the bifidogenic effect of oligofructose, a fermentable carbohydrate found in a number of plants. In single stage continuous culture, oligofructose preferentially enriched for bifidobacteria, in comparison to sucrose and inulin. This stimulatory effect was enhanced at a high dilution rate, high substrate concentration and low pH. These parameters are likely to approximate to those that occur in the proximal colon. Studies with a three-stage continuous culture model of the large intestine confirmed the bifidogenic effect of oligofructose. These in vitro data indicate that an increase in the concentration of fructose-based oligosaccharides in the diet may alter the balance of the gut microflora towards bifidobacteria, a purported health-promoting genus.

**Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin.**

Gibson GR, Beatty ER, Wang X, Cummings JH. Medical Research Council, Dunn Clinical Nutrition Centre, Cambridge, England.

Gastroenterology 1995 Apr;108(4):975-982

**BACKGROUND/AIMS:** Oligofructose and inulin are naturally occurring indigestible carbohydrates. In vitro they selectively stimulate the growth of species of Bifidobacterium, a genus of bacteria considered beneficial to health. This study was designed to determine their effects on the large bowel microflora and colonic function in vivo.

**METHODS:** Eight subjects participated in a 45-day study during which they ate controlled diets. For the middle 15 days, 15 g.day<sup>-1</sup> oligofructose was substituted for 15 g.day<sup>-1</sup> sucrose. Four of these subjects went on to a further period with 15 g.day<sup>-1</sup> inulin. Bowel habit, transit time, stool composition, breath H<sub>2</sub> and CH<sub>4</sub>, and the predominant genera of colonic bacteria were measured.

**RESULTS:** Both oligofructose and inulin significantly increased bifidobacteria from 8.8 to 9.5 log<sub>10</sub> g stool<sup>-1</sup> and 9.2 to 10.1 log<sub>10</sub> g stool<sup>-1</sup>, respectively,

whereas bacteroides, clostridia, and fusobacteria decreased when subjects were fed oligofructose, and gram-positive cocci decreased when subjects were fed inulin. Total bacterial counts were unchanged. Fecal wet and dry matter, nitrogen, and energy excretion increased with both substrates, as did breath H<sub>2</sub>. Little change in fecal short-chain fatty acids and breath CH<sub>4</sub> was observed.

**CONCLUSIONS:** A 15-g.day<sup>-1</sup> dietary addition of oligofructose or inulin led to Bifidobacterium becoming the numerically predominant genus in feces. Thus, small changes in diet can alter the balance of colonic bacteria towards a potentially healthier microflora.

**[Role of polyunsaturated fatty acids in diet therapy of children with allergic diseases].** [Article in Russian]

Gorelova ZI, Ladodo KS, Levachev MM, Lupinovich VL, Mamonova LG, Orlova SV, Balabolkin II, Zadkova GF, Arutiunova MB.

Vopr Pitan 1999;68(1):31-35

135 pediatric patients receiving hypoallergic diet were included into the study group. The control group consisted of 20 children. The impact of PUFA omega-3 biologically active supplements (polyen, prima-Oil) was studied in hypoallergic rations. Biochemical indices were simultaneously investigated. The revealed dynamic changes of fatty acid spectrum in plasma and red cell membranes, cellular and humoral immunity status and eicosanoids synthesis were followed by positive clinical changes. Diets enriched with biologically active supplementation (PUFA omega-3) can be recommended for application in pediatric practice.

**Nutritional and pharmacological enhancement of gut-associated lymphoid tissue.**

Hanna MK, Kudsk KA. The University of Tennessee, Memphis, USA.

Can J Gastroenterol 2000 Nov;14:145D-151D

There has been an explosion of research in the field of nutrition over the past quarter century. Clinical studies have demonstrated the effectiveness of providing nutrition by the enteral route in reducing septic morbidity in critically ill patients. These improved outcomes have been substantiated by animal models that show that enteral nutrition decreases gut permeability while maintaining the gut-associated lymphoid tissue (GALT) in mucosal immunity. Evidence points to the important immunological role of the gut in the maintenance of mucosal immunity at both intestinal and extraintestinal sites. The preservation of this mucosal immunity by enteral nutrition is consistent with the lower morbidity seen in severely injured patients who receive nutrition via the gastrointestinal tract. For patients who are unable to be fed by the enteral route and who require parenteral nutrition, several supplements show promise in enhancing the mucosal immune system defenses. The nutritional and pharmacological tactics that may enhance the GALT and thereby maintain mucosal immunity are examined.

## **Diet and childhood asthma in a society in transition: a study in urban and rural Saudi Arabia.**

Hijazi N, Abalkhail B, Seaton A. Department of Community Medicine and Primary Health Care, Faculty of Medicine and Allied Sciences, King Abdulaziz University, Jeddah, Saudi Arabia.

Thorax 2000 Sep;55(9):775-779

**BACKGROUND:** The causes of the worldwide increases in asthma and allergic diseases in childhood, which seem to relate to increasing prosperity, are unknown. We have previously hypothesised that a reduction in the antioxidant component of the diet is an important factor. An investigation was undertaken of dietary and other risk factors for asthma in Saudi Arabia where major lifestyle differences and prevalences of allergic disease are found in different communities.

**METHODS:** From a cross sectional study of 1444 children with a mean age of 12 (SD 1) years in Jeddah and a group of rural Saudi villages, we selected 114 cases with a history of asthma and wheeze in the last 12 months and 202 controls who had never complained of wheeze or asthma, as recorded on the ISAAC questionnaire. Risk factors for asthma and allergies (family history, social class, infections, immunisations, family size, and diet) were ascertained by questionnaire. Atopy was assessed by skin prick testing.

**RESULTS:** In univariate analyses, family history, atopy, and eating at fast food outlets were significant risk factors for wheezy illness, as were the lowest intakes of milk and vegetables and of fibre, vitamin E, calcium, magnesium, sodium, and potassium. These differences were present also in the urban children considered separately. Sex, family size, social class, infections, and parental smoking showed no relationship to risk. In multiple logistic regression analysis, urban residence, positive skin tests, family history of allergic disease, and the lowest intakes of vitamin E, magnesium and sodium related significantly and independently to risk. The lowest tertile of intake of vitamin E was associated with a threefold (95% CI 1.38 to 6.50) increase in risk when adjusted for the other factors. Intake of milk and vegetables both showed inverse linear relationships to being a case.

**CONCLUSIONS:** This study suggests that dietary factors during childhood are an important influence in determining the expression of wheezy illness, after allowing for urban/rural residence, sex, family history, and atopy. The findings are consistent with previous studies in adults and with the hypothesis that change in diet has been a determinant of the worldwide increases in asthma and allergies.

## **Probiotics in primary prevention of atopic disease: a randomized placebo-controlled trial.**

Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Department of Paediatrics, University of Turku and Turku University Hospital, Finland. markal@utu.fi

**BACKGROUND:** Reversal of the progressive increase in frequency of atopic disease would be an important breakthrough for health care and wellbeing in western societies. In the hygiene hypothesis this increase is attributed to reduced microbial exposure in early life. Probiotics are cultures of potentially beneficial bacteria of the healthy gut microflora. We assessed the effect on atopic disease of Lactobacillus GG (which is safe at an early age and effective in treatment of allergic inflammation and food allergy).

**METHODS:** In a double-blind, randomised placebo-controlled trial we gave Lactobacillus GG prenatally to mothers who had at least one first-degree relative (or partner) with atopic eczema, allergic rhinitis, or asthma, and postnatally for 6 months to their infants. Chronic recurring atopic eczema, which is the main sign of atopic disease in the first years of life, was the primary endpoint.

**FINDINGS:** Atopic eczema was diagnosed in 46 of 132 (35%) children aged 2 years. Asthma was diagnosed in six of these children and allergic rhinitis in one. The frequency of atopic eczema in the probiotic group was half that of the placebo group (15/64 [23%] vs 31/68 [46%]; relative risk 0.51 [95% CI 0.32-0.84]). The number needed to treat was 4.5 (95% CI 2.6-15.6).

**INTERPRETATIONS:** Lactobacillus GG was effective in prevention of early atopic disease in children at high risk. Thus, gut microflora might be a hitherto unexplored source of natural immunomodulators and probiotics, for prevention of atopic disease.

### **Dietary fatty acids and allergy.**

Kankaanpaa P, Sutas Y, Salminen S, Lichtenstein A, Isolauri E. Department of Biochemistry and Food Chemistry, University of Turku, Finland.  
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Ann Med 1999 Aug;31(4):282-287

The increase in the prevalence of atopic diseases has recently been linked to altered consumption of polyunsaturated fatty acids (PUFAs). As typical Western diets contain almost 10 times more linoleic acid (18:2 omega-6) than alpha-linolenic acid (18:3 omega-3), it is the metabolism of the former that predominates. Subsequently produced arachidonic acid-derived eicosanoids alter the balance of T-helper cells type 1 and type 2 thus favouring the production of immunoglobulin (Ig)E. In atopic subjects, the impact of this excessive eicosanoid production may be further strengthened as a result of changes in cyclic nucleotide metabolism exacerbated by substrate availability. Dietary omega-3 fatty acids can have marked influence on both specific and nonspecific immune responses in modifying eicosanoid production and replacing omega-6 fatty acids in cell membranes. Therefore, it is concluded that careful manipulation of dietary PUFAs may play a key role in the successful management of inflammation associated with atopic diseases.

**Polyunsaturated fatty acids in maternal diet, breast milk, and serum lipid fatty acids of infants in relation to atopy.**

Kankaanpaa P, Nurmela K, Erkkila A, Kalliomaki M, Holmberg-Marttila D, Salminen S, Isolauri E. Departments of Biochemistry and Food Chemistry, and Pediatrics, University of Turku, Turku, Finland.

Allergy 2001 Jul;56(7):633-638

**BACKGROUND:** The increased consumption of n-6 polyunsaturated fatty acids (PUFA) has been shown to coincide with the increased prevalence of atopic diseases. We aimed to investigate whether maternal diet and atopic status influence the PUFA composition of breast milk and the serum lipid fatty acids of infants.

**METHODS:** Maternal diet was assessed by a food questionnaire. The PUFA composition of breast milk obtained at 3 months from 20 allergic and 20 healthy mothers and of their infants' (10 atopic and 10 nonatopic/group of mothers) serum lipids was analyzed.

**RESULTS:** Although no differences in maternal PUFA intake were observed, the breast milk of allergic mothers contained less gamma-linolenic acid (18:3 n-6) than that of healthy mothers. Similarly, atopic infants had less gamma-linolenic acid in phospholipids than healthy infants, although n-6 PUFA were elevated in other serum lipid fractions in atopic infants. The serum lipid fatty acids in atopic infants did not correlate with those in maternal breast milk.

**CONCLUSION:** Our results suggest that dietary n-6 PUFA are not as readily transferred into breast milk or incorporated into serum phospholipids, but may be utilized for other purposes, such as eicosanoid precursors, in allergic/atopic individuals. Subsequently, high dietary proportions of n-6 PUFA, or reduced proportions of regulatory PUFA, such as gamma-linolenic acid and n-3 PUFA, may be a risk factor for the development of atopic disease.

**Aloe vera.**

Klein AD, Penneys NS. Department of Dermatology, University of Miami School of Medicine, FL.

J Am Acad Dermatol 1988 Apr;18(4 Pt 1):714-720

We review the scientific literature regarding the aloe vera plant and its products. Aloe vera is known to contain several pharmacologically active ingredients, including a carboxypeptidase that inactivates bradykinin in vitro, salicylates, and a substance(s) that inhibits thromboxane formation in vivo. Scientific studies exist that support an antibacterial and antifungal effect for substance(s) in aloe vera. Studies and case reports provide support for the use of aloe vera in the treatment of radiation ulcers and stasis ulcers in man and burn and frostbite injuries in animals. The evidence for a potential beneficial effect associated with the use of

aloe vera is sufficient to warrant the design and implementation of well-controlled clinical trials.

**Glutamine-enriched total parenteral nutrition maintains intestinal interleukin-4 and mucosal immunoglobulin A levels.**

Kudsk KA, Wu Y, Fukatsu K, Zarzaur BL, Johnson CD, Wang R, Hanna MK. University of Tennessee, Memphis, USA.

JPEN J Parenter Enteral Nutr 2000 Sep;24(5):270-274

**BACKGROUND:** Total parenteral nutrition (TPN) prevents progressive malnutrition but fails to maintain intestinal gut-associated lymphoid tissue (GALT) or established respiratory antiviral or antibacterial mucosal immunity. Our previous work demonstrated that decreases in intestinal immunoglobulin A (IgA) were associated with decreases in Th2-type IgA-stimulating cytokines, interleukin (IL)-4 and IL-10. Because glutamine supplementation of TPN partially preserves respiratory defenses and normalizes GALT, we investigated the ability of parenteral glutamine to normalize respiratory and intestinal IgA levels and measured Th2 cytokines in intestinal homogenates.

**METHODS:** Animals were cannulated and randomly assigned to receive chow (n = 17), TPN (n = 18), or an isonitrogenous, isocaloric TPN solution formulated by removing the appropriate amount of amino acids and replacing them with 2% glutamine (n = 18) for 5 days. Respiratory tract and intestinal washings were obtained for IgA and the intestine homogenized and analyzed for IL-4 and IL-10.

**RESULTS:** TPN decreased intestinal and respiratory IgA in association with decreases in intestinal IL-4 and IL-10 compared with chow-fed animals. Glutamine significantly improved respiratory and intestinal IgA levels, significantly improved IL-4 compared with TPN animals, and maintained IL-10 levels midway between chow-fed and TPN animals.

**CONCLUSIONS:** Glutamine-enriched TPN preserved both extraintestinal and intestinal IgA levels and had a normalizing effect on Th2-type IgA-stimulating cytokines.

**Oligosaccharides in human milk: structural, functional, and metabolic aspects.**

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Annu Rev Nutr 2000;20:699-722

Research on human milk oligosaccharides (HMOs) has received much attention in recent years. However, it started about a century ago with the observation that oligosaccharides might be growth factors for a so-called bifidus flora in breast-fed

infants and extends to the recent finding of cell adhesion molecules in human milk. The latter are involved in inflammatory events recognizing carbohydrate sequences that also can be found in human milk. The similarities between epithelial cell surface carbohydrates and oligosaccharides in human milk strengthen the idea that specific interactions of those oligosaccharides with pathogenic microorganisms do occur preventing the attachment of microbes to epithelial cells. HMOs may act as soluble receptors for different pathogens, thus increasing the resistance of breast-fed infants. However, we need to know more about the metabolism of oligosaccharides in the gastrointestinal tract. How far are oligosaccharides degraded by intestinal enzymes and does oligosaccharide processing (e.g. degradation, synthesis, and elongation of core structures) occur in intestinal epithelial cells? Further research on HMOs is certainly needed to increase our knowledge of infant nutrition as it is affected by complex oligosaccharides.

**[Effects of ginkgo leave concentrated oral liquor in treating asthma].** [Article in Chinese]

Li MH, Zhang HL, Yang BY. Qingdao Hospital of Integrated Traditional and Western Medicine, Shandong.

Zhongguo Zhong Xi Yi Jie He Za Zhi 1997 Apr;17(4):216-218

**OBJECTIVE:** To determine the effects of Ginkgo leave concentrated oral liquor (GLC) on airway inflammation.

**METHODS:** Airway hyperreactivity and clinical symptoms and pulmonary functions of asthma patients were determined.

**RESULTS:** In contrast to placebo group, GLC significantly reduced airway hyperreactivity ( $< 0.05$ ) and improved clinical symptoms ( $< 0.05$ ), pulmonary functions ( $< 0.05$ ) of the asthmatic patients.

**CONCLUSION:** GLC is an effective drug of anti airway inflammation.

**In vitro effects of Ginkgolide B on lymphocyte activation in atopic asthma: comparison with cyclosporin A.**

Mahmoud F, Abul H, Onadeko B, Khadadah M, Haines D, Morgan G. Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences and Nursing, Kuwait University, Sulaibekhat.

Jpn J Pharmacol 2000 Jul;83(3):241-245

The effects of Ginkgolide B (BN52021) on in vitro activation responses of human peripheral blood mononuclear cells (PBMC) from asthmatic patients was measured using 2-channel flow cytometric analysis of activation-associated cell surface antigens or ELISA assays for cytokines known to be expressed by PBMC during T1 or T2 immunological activation. BN52021 is an anti-inflammatory

extract of Ginkgo biloba and has been used therapeutically. It is a known inhibitor of platelet activating factor (PAF), which is important in the pathogenesis of asthma, and may synergise with cyclosporin A (CyA) to inhibit pathogenic immune activation in asthmatics. We compared the inhibitory effects of BN52021 and CyA (1 microM each) on activation of PBMC of asthmatic patients stimulated by phorbol myristate acetate and calcium ionophore. Inhibition of production of the cytokines IL-4 and IL-5 by BN52021 was insignificant compared to CyA. However, BN52021 significantly reversed the increase in activation-associated CD45RA expression, with a trend towards decreased expression of HLA-DR. Lymphocyte activation markers were not significantly altered by CyA. Since they appear to have differing effects on activated cells, the anti-inflammatory effects of CyA and BN52021 in atopic asthma is potentially additive. The present approach may be useful for preliminary evaluation of novel therapeutic modalities for asthma treatment.

### **Study of the effect of Lactobacillus paracasei and fructooligosaccharides on the faecal microflora in weanling piglets.**

Nemcova R, Bomba A, Gancarcikova S, Herich R, Guba P Research Institute of Veterinary Medicine, Kosice, Slovak Republic.

Berl Munch Tierarztl Wochenschr 1999 Jun-Jul;112(6-7):225-8

The influence of administration of Lactobacillus paracasei alone and mixture of Lactobacillus paracasei and fructooligosaccharide on faecal bacteria counts in the weanling pigs was investigated. The administration of Lactobacillus paracasei alone significantly decreased Clostridium (< 0.05) and Enterobacteriaceae (< 0.05) counts as compared to the control. Lactobacillus paracasei administered in combination with fructooligosaccharide significantly increased Lactobacillus (< 0.01-< 0.05), Bifidobacterium (< 0.05), total anaerobes (< 0.05), and total aerobes (< 0.05) counts compared to control group as well as Lactobacillus paracasei group and significantly decreased Clostridium (< 0.05) and Enterobacteriaceae (< 0.01) counts compared to control group. The results obtained point out to a synergic effect of the combination of Lactobacillus paracasei and fructooligosaccharide on numbers of bacterial populations observed in the faeces of the weanling pigs.

### **Clinical applications of probiotic agents.**

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Am J Clin Nutr 2001 Jun;73(6):1147S-1151S

In the past century the beneficial roles of nonpathogenic bacteria in the intestinal lumen were described. In the past decade there has been a dramatic increase in scientific work supporting the concept that there are clinical benefits to ingesting specific nonpathogenic organisms (probiotics). The potential benefits of modifying the intestinal flora composition of certain high-risk groups, eg,

premature infants, travelers, and children receiving antibiotics, are emerging in the literature. Studies documenting prophylactic and therapeutic benefits in acute viral gastroenteritis and in atopic disease point not only to the potential applications, but also to the fact that the mechanisms of action of these agents may be due to their interaction with the gut as an immunologic organ. The benefits documented thus far are of varying degree and are most likely dependent on the number of agents, the dose, the dosing patterns, and the characteristics of the host and its underlying luminal microbial environment. Consequently, the safety and specification of a particular probiotic agent and methods of delivery to a particular population for a particular purpose should be carefully documented before making broad recommendations. The cost-benefit assessment of adding probiotics to our diet for prophylactic or therapeutic purposes, as well as better regulation of these agents as commercial products, is also needed.

### **Consequences of magnesium deficiency on the enhancement of stress reactions; Preventive and therapeutic implications (A review)**

Seelig M.S. Dept of Nutrition, School of Public Health/Medicine, Univ of North Carolina, Chapel Hill, NC

J. Am. Coll. Nutr. (USA), 1994, 13/5 (429-446)

Stress intensifies release of catecholamines and corticosteroids that increase survival of normal animals when their lives are threatened. When magnesium (Mg) deficiency exists, stress paradoxically increases risk of cardiovascular damage including hypertension, cerebrovascular and coronary constriction and occlusion, arrhythmias and sudden cardiac death (SCD). In affluent societies, severe dietary Mg deficiency is uncommon, but dietary imbalances such as high intakes of fat and/or calcium (Ca) can intensify Mg inadequacy, especially under conditions of stress. Adrenergic stimulation of lipolysis can intensify its deficiency by complexing Mg with liberated fatty acids (FA). A low Mg/Ca ratio increases release of catecholamines, which lowers tissue (i.e. myocardial) Mg levels. It also favors excess release or formation of factors (derived both from FA metabolism and the endothelium), that are vasoconstrictive and platelet aggregating; a high Ca/Mg ratio also directly favors blood coagulation, which is also favored by excess fat and its mobilization during adrenergic lipolysis. Auto-oxidation of catecholamines yields free radicals, which explains the enhancement of the protective effect of Mg by anti-oxidant nutrients against cardiac damage caused by beta-catecholamines. Thus, stress, whether physical (i.e. exertion, heat, cold, trauma-accidental or surgical, burns), or emotional (i.e. pain, anxiety, excitement or depression) and dyspnea as in asthma increases need for Mg. Genetic differences in Mg utilization may account for differences in vulnerability to Mg deficiency and differences in body responses to stress.

### **Continuous culture selection of bifidobacteria and lactobacilli from human faecal samples using fructooligosaccharide as selective substrate.**

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The human large intestine contains a large and diverse population of bacteria. Certain genera, namely *Bifidobacterium* and *Lactobacillus*, are thought to exert health-promoting effects. Prebiotics such as fructooligosaccharides (FOS) have been shown to stimulate the growth of endogenous bifidobacteria. In this study, changes of lactic acid producing bacteria in continuous culture fermentors (semi-defined, anaerobic medium containing 5 g l(-1) FOS, dilution rate of 0.1 h<sup>-1</sup>, pH 5.5) were followed over a 21 d period after inoculation with blended human faeces from four healthy adults. Samples were also taken every 3 d for influent/effluent FOS, short chain fatty acid (SCFA), lactate and microbiological analyses. Results showed that SCFA concentrations decreased abruptly 1 d after inoculation while lactate concentrations increased. Classical methods of enumeration using selective media showed that the proportion of total culturable count represented by bifidobacteria and lactobacilli increased from 11.9% on day 1 to 98.1% on day 21. However, molecular methods using genus-specific 16S rRNA oligonucleotide probes indicated that the bifidobacterial population maintained a level between 10 and 20% of total 16S rRNA during the first 6 d and disappeared rapidly when the maximum concentration of lactate was reached. Lactobacilli, which were initially present in low numbers, increased until day 9 and remained at high levels (20-42% of total 16S rRNA) to day 21, with the exception of day 18. Although FOS has usually been regarded as a selective substrate for bifidobacteria, these observations suggest that: (1) lactobacilli are also able to use FOS, (2) lactobacilli can out-compete bifidobacteria in continuous culture at pH 5.2-5.4 when FOS is the primary carbon and energy source, and (3) bifidobacteria can grow faster on FOS than lactobacilli under controlled conditions.

**Protective effect of bifidus milk on the experimental infection with *Salmonella enteritidis* subsp. *typhimurium* in conventional and gnotobiotic mice.**

Silva AM, Bambirra EA, Oliveira AL, Souza PP, Gomes DA, Vieira EC, Nicoli JR. Departamento de Microbiologia, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.

The ability of *Bifidobacterium bifidum* from a commercial bifidus milk to antagonize *Salmonella enteritidis* subsp. *typhimurium* in vivo, and to reduce the pathological consequences for the host, was determined using conventional and gnotobiotic mice. Conventional animals received daily, by gavage, 0.1 ml bifidus milk containing about 10<sup>9</sup> cfu *B. bifidum* and germ-free animals received a single 0.1 ml dose. The conventional and gnotobiotic groups were challenged orally with 10<sup>2</sup> cfu of the pathogenic bacteria 5 and/or 10 d after the beginning of treatment. Control groups were treated with milk. Bifidus milk protected both animal models against the challenge with the pathogenic bacteria, as demonstrated by survival and histopathological data. However, to obtain the protective effect in gnotobiotic animals, the treatment had to be initiated 10 d

before the challenge. In experimental and control gnotobiotic mice, *Salm. enteritidis* subsp. *typhimurium* became similarly established at levels ranging from  $10^8$  to  $10^9$  viable cells g<sup>-1</sup> of faeces and remained at these high levels until the animals died or were sacrificed. It was concluded that the protection against *Salm. enteritidis* subsp. *typhimurium* observed in conventional and gnotobiotic mice treated with bifidus milk was not due to the reduction of the intestinal populations of the pathogenic bacteria.

**[Effects of oral administration of bifidobacteria on intestinal microflora in premature and newborn infants]. [Article in German]**

Uhlemann M, Heine W, Mohr C, Plath C, Pap S. Kinder- und Jugendklinik der Universität Rostock.

Z Geburtshilfe Neonatol 1999 Sep;203(5):213-217

In a prospective, randomised study the effects of orally administered bifidobacteria on the intestinal microflora were investigated in 100 preterm and term neonates under intensive care conditions during the first 21 days of life. The 50 infants (group with bifidobacteria) received lyophilized bifidobacteria (Topfer Bifidus) via nasogastral tube with an initial dosage of 3 times daily  $1.25 \times 10^8$  bifidobacteria on day 2 of life and a daily dosage of 6 times  $1.25 \times 10^8$  bifidobacteria on day 3 until day 21 of life. The other 50 infants (control group) did not receive bifidobacteria. The preterm and term neonates were fed either with pasteurized mother's milk or milk from healthy female donors ( $n = 79$ ) or with an infant formula (Alfare,  $n = 13$ ) or initially with Alfare and thereafter with mother's milk ( $n = 8$ ). The intestinal microflora of preterm and term neonates under intensive care conditions could be influenced by the oral administration of bifidobacteria. The administration of bifidobacteria resulted in the group of inoculated infants in a significantly earlier colonization of bifidobacteria (8.1  $\pm$  3.9 days of life) than in the control group (11.3  $\pm$  4.7 days of life). On day 7 a bifidobacterial dominance ( $< 90\%$  of the intestinal microflora) could be found in 26% of infants with inoculation of bifidobacteria and only in 2% of the control group ( $< 0.001$ ). These significant differences could be shown until day 21 of life. A difference in septicemia frequency between the two groups could not be demonstrated. At the beginning of the infection a bifidobacterial dominance was found in only one of 23 cases of septicemia.

**The effect of a newly developed ointment containing eicosapentaenoic acid and docosahexaenoic acid in the treatment of atopic dermatitis.**

Watanabe T, Kuroda Y. Department of Pediatrics, Kagawa Prefectural Tsuda Hospital, Japan.

J Med Invest 1999 Aug;46(3-4):173-177

While various therapeutic modalities have been tried for atopic dermatitis (AD), numerous obstinate cases exist in which sufficient effects cannot be obtained. Therefore, we developed and prepared an ointment containing docosahexaenoic

acid and eicosapentaenoic acid as a topical therapeutics for AD. We applied this ointment to 64 patients with AD (aged between 2 months and 29 years) who showed poor responses to conventional therapies and obtained satisfactory results. This ointment is considered a new topical preparation for AD.

### **Immune senescence and adrenal steroids: Immune dysregulation and the action of dehydroepiandrosterone (DHEA) in old animals**

Weksler M.E. Department of Medicine, Cornell University Medical College, New York, NY 10021 USA

Eur J Clin Pharmacol 1993;45 Suppl 1:S21-3; discussion S43-4

Immune senescence is characterized by dysregulation of the immune system. The disorder occurs during old age and is manifested by an increased production of autoantibodies and a decreased production of antibodies to most foreign antigens. These events seem to reflect an altered ratio of activity between the CD5+ and CD5- B cell subsets. Likewise, there is dysregulation of cytokine production with an increased production of IL-4, IL-5 and IL-6 associated with a decreased production of IL-2. This appears to reflect an altered ratio of activity between the Th1 and Th2 cell subsets. Dehydroepiandrosterone (DHEA) is one of the three principal adrenal steroids; its serum concentration declines with age. Recent results suggest that in vitro culture of lymphocytes, from aged donors, with DHEA or in vivo treatment of old mice with DHEA sulphate results in the augmentation of the antibody response to foreign antigens and a reversal in the dysregulated cytokine production by T cells. Thus, a decline in one of the three principal adrenal steroids is associated with age-associated changes in the immune system. Some of these changes can be reversed by exposure to DHEA.

### **Reduced levels of glutathione S-transferases in patch test reactions to dithranol and sodium lauryl sulphate as demonstrated by quantitative immunocytochemistry: evidence for oxidative stress in acute irritant contact dermatitis.**

Willis CM, Britton LE, Reiche L, Wilkinson JD. Department of Dermatology, Amersham Hospital, Whielden Street, Amersham, Bucks, HP7 0JD, UK. carolynwillis@sbnhst.ftech.co.uk

Eur J Dermatol 2001 Mar;11(2):99-104

There is increasing evidence that oxidative stress plays a role in the pathogenesis of acute irritant contact dermatitis. As part of on-going studies into the effect of irritant chemicals on the anti-oxidant enzyme systems in the skin, we have examined the changing levels of two classes of glutathione S-transferase in patch test reactions to dithranol and sodium lauryl sulphate, using quantitative immunocytochemistry. Although no changes were evident after 6 hrs, significant reductions in the density of staining for glutathione S-transferase alpha were seen with both irritants after 48 hrs and 96 hrs. Glutathione S-transferase pi levels were reduced to a lesser degree, reaching significance for dithranol at the 96 hrs time

point only, and for sodium lauryl sulphate at 48 hrs only. The results support the hypothesis that oxidative stress plays a role in chemically-induced inflammation, not only in the case of irritants such as dithranol which are known to directly generate reactive oxygen species, but also with chemicals not generally associated with free radical generation.

**Metabolic support of the gastrointestinal tract: potential gut protection during intensive cytotoxic therapy.**

Wilmore DW. Department of Surgery, Harvard Medical School, Brigham and Women's Hospital, Boston, Massachusetts 02115, USA.

Cancer 1997 May 1;79(9):1794-1803

**BACKGROUND:** Potentially curative options involving cytoablative therapies are now available for the treatment of almost all human tumors, but major toxicities represent the rate-limiting step in achieving a cure with these therapies. With successful hematoprotective strategies now in use, it is apparent that the gastrointestinal tract will be the rate-limiting organ system that prevents further dose escalation in many cancer patients.

**METHODS:** A review of the English language literature was conducted. Paperchase, a computer-based application that reviews the data bases of the National Library of Medicine and the National Cancer Institute, was used to obtain pertinent literature.

**RESULTS:** A variety of gut-protective nutrients and growth factors were identified. These substances may be useful in preventing dose-limiting gastrointestinal symptoms. Animal studies and some patient data suggest that the amino acid glutamine stimulates mucosal growth and promotes gut health. When nutrient administration is coupled with growth factors, such as growth hormone, insulin-like growth factor-1, glucagon-like peptide-2, and interleukin-11, a high level of bowel protection should be attained.

**CONCLUSIONS:** Therapy is evolving that may be useful in protecting the intestinal mucosa and preventing dose-limiting gastrointestinal symptoms.

**A modified determination of coenzyme Q10 in human blood and CoQ10 blood levels in diverse patients with allergies.**

Ye CQ, Folkers K, Tamagawa H, Pfeiffer C Institute for Biomedical Research, University of Texas, Austin.

Biofactors 1988 Dec;1(4):303-6

Two situations required a modified determination of coenzyme Q10 (CoQ10) in human blood and organ tissue. Blood from patients with AIDS and cancer raised apprehensions about safety to an analyst, and the number of specimens for analysis is increasing enormously. A modified determination replaces silica gel-

TLC with disposable Florisil columns, and steps were simplified to allow more analyses per unit time. Data from the modified determination are quantitatively compatible with data from older and tedious procedures. This determination was used for blood from 36 diverse patients with allergies. The mean CoQ10 blood level of these patients is not different from the mean level of so-called normal individuals, but approximately 40% (14/36) of these allergic patients had levels up to 0.65 micrograms/ml, which is the level of dying class IV cardiac patients. The biosynthesis of CoQ10 in human tissues is a complex process that requires several vitamins and micronutrients, so that countless vitamin-unsupplemented Americans may be deficient in CoQ10. The relationship of allergies to autoimmune mechanisms and immunity, and the established relationship of CoQ10 to immune states, may be a rationale for therapeutic trials of administering CoQ10 to patients with allergies who have low CoQ10 blood levels and are very likely deficient.

### 3. Alzheimers Disease

Preventative and curative options include:

Acetylcholine, Ginkgo biloba, Vitamin E, Vitamin C, N-acetyl cysteine, Essential fatty acids, Curcumin, Vitamin B12, Vitamin B6, Folic acid, SAME, Methylcobalamin, Phosphatidylserine, Acetyl-L-carnitine, Melatonin, Carnosine, DHEA, Vitamin K.

#### **The possible role of vitamin K deficiency in the pathogenesis of Alzheimer's disease and in augmenting brain damage associated with cardiovascular disease.**

Allison AC. SurroMed Corporation, Mountain View, California 94043, USA.

Med Hypotheses 2001 Aug;57(2):151-5

The incidence of Alzheimer's disease (AD) increases with age and in carriers of the apolipoprotein E4 genotype. A relative deficiency of vitamin K, affecting the extrahepatic functions of the vitamin, is common in ageing men and women. The concentration of vitamin K is lower in the circulating blood of APOE4 carriers than in that of persons with other APOE genotypes. Evidence is accumulating that vitamin K has important functions in the brain, including the regulation of sulfotransferase activity and the activity of a growth factor/tyrosine kinase receptor (Gas 6/Axl). The hypothesis is now proposed that vitamin K deficiency contributes to the pathogenesis of AD and that vitamin K supplementation may have a beneficial effect in preventing or treating the disease. Vitamin K may also reduce neuronal damage associated with cardiovascular disease. Copyright 2001 Harcourt Publishers Ltd.

#### **[A new interventional strategy for Alzheimer's disease by Japanese herbal medicine]. [Article in Japanese]**

Arai H, Suzuki T, Sasaki H, Hanawa T, Toriizuka K, Yamada H Department of Geriatric Medicine, Tohoku University School of Medicine.

Nippon Ronen Igakkai Zasshi 2000 Mar;37(3):212-5

A Japanese herbal medicine termed "Kami-Umtan-To (KUT)" was first described in Japanese literature in 1626, KUT consists of 13 different herbs, and it has been used for a long time in the treatment of a variety of neuropsychiatric problems including neurosis and insomnia. Recently, Yabe et al. have demonstrated that KUT increased both choline acetyltransferase (ChAT) and nerve growth factor at the protein and mRNA levels in cultured rat brain cells. Moreover, the same research group has reported that KUT improved mean latency on passive avoidance test in both basal forbrain lesioned and aged rats. KUT significantly

improved the survival rate, and increased the number of ChAT-positive neurons in aged rats. Here, we report a 12-month open clinical trial of KUT and combination of estrogen, vitamin E and NSAID to aim at slowing down the progression of Alzheimer's disease (AD). Twenty AD patients (MMSE score: 18.6 +/- 5.8) received extracts from original KUT herbs, and 7AD patients (MMSE score: 21.3 +/- 2.8) were placed on the combination therapy. Rate of cognitive decline as measured by change in MMSE score per year was significantly slower ( $p = 0.04$ , ANOVA) in the KUT group (1.4 points) and the combination group (0.4 points) as compared to 4.1 points in 32 control AD patients (MMSE score: 20.8 +/- 5.6) who received no medicines for AD. Any of CSF measures including tau, and A beta 1-42 did not differ significantly after KUT therapy. The efficacy of the KUT therapy was most obvious at 3 months. Our results suggest that traditional Japanese herbal medicine(s) may serve a new interventional strategy for AD.

### **Inositol treatment of Alzheimer's disease: a double blind, cross-over placebo controlled trial.**

Barak Y, Levine J, Glasman A, Elizur A, Belmaker RH. Abarbanel Mental Health Center, Bat Yam, Israel.

Prog Neuropsychopharmacol Biol Psychiatry 1996 May;20(4):729-35

1. A double-blind controlled crossover trial of 6 gm of inositol daily vs glucose for one month each was carried out in 11 Alzheimer patients. 2. Overall CAMCOG scores showed a trend for greater improvement with inositol that was not significant. 3. Language and orientation improved significantly more on inositol than on placebo. There were no serious side effects. 4. Higher doses of inositol should be studied in Alzheimer's Disease for longer periods.

### **The Ginkgo biloba extract (EGb 761) protects hippocampal neurons against cell death induced by beta-amyloid.**

Bastianetto S, Ramassamy C, Dore S, Christen Y, Poirier J, Quirion R Douglas Hospital Research Centre, Department of Psychiatry, McGill University, 6875 Bld LaSalle, Verdun, Quebec, Canada.

Eur J Neurosci 2000 Jun;12(6):1882-90

Substantial evidence suggests that the accumulation of beta-amyloid (A $\beta$ )-derived peptides, and to a lesser extent free radicals, may contribute to the aetiology and/or progression of Alzheimer's disease (AD). Ginkgo biloba extract (EGb 761) is a well-defined plant extract containing two major groups of constituents, i.e. flavonoids and terpenoids. It is viewed as a polyvalent agent with a possible therapeutic use in the treatment of neurodegenerative diseases of multifactorial origin, e.g. AD. We have investigated here the potential effectiveness of EGb 761 against toxicity induced by (A $\beta$ )-derived peptides (A $\beta$ <sub>25-35</sub>, A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>1-42</sub>) on hippocampal primary cultured cells, this area being severely affected in AD. A co-treatment with EGb 761

concentration-dependently (10-100 microg/mL) protected hippocampal neurons against toxicity induced by Abeta fragments, with a maximal and complete protection at the highest concentration tested. Similar, albeit less potent protective effects were seen with the flavonoid fraction of the extract (CP 205), while the terpenes were ineffective. Most interestingly, EGb 761 (100 microg/mL) was even able to protect (up to 8 h) hippocampal cells from a pre-exposure to Abeta25-35 and Abeta1-40. EGb 761 was also able to both protect and rescue hippocampal cells from toxicity induced by H<sub>2</sub>O<sub>2</sub> (50-150 microM), a major peroxide possibly involved in mediating Abeta toxicity. Moreover, EGb 761 (10-100 microg/mL), and to a lesser extent CP 205 (10-50 microg/mL), completely blocked Abeta-induced events, e.g. reactive oxygen species accumulation and apoptosis. These results suggest that the neuroprotective effects of EGb 761 are partly associated with its antioxidant properties and highlight its possible effectiveness in neurodegenerative diseases, e.g. AD via the inhibition of Abeta-induced toxicity and cell death.

### **Mitochondria, NO and neurodegeneration.**

Beal MF Neurochemistry Laboratory, Neurology Service/WRN 408,  
Massachusetts General Hospital, Boston, USA.

Biochem Soc Symp 1999;66:43-54

A role for mitochondrial dysfunction in neurodegenerative disease is gaining increasing support. Mitochondrial dysfunction may be linked to neurodegenerative diseases through a variety of different pathways, including free-radical generation, impaired calcium buffering and the mitochondrial permeability transition. This can lead to both apoptotic and necrotic cell death. Recent evidence has shown that there is a mitochondrial defect in Friedreich's ataxia, which leads to increased mitochondrial iron content, that appears to be linked to increased free-radical generation. There is evidence that the point mutations in superoxide dismutase which are associated with amyotrophic lateral sclerosis may contribute to mitochondrial dysfunction. There is also evidence for bioenergetic defects in Huntington's disease. Studies of cybrid cell lines have implicated mitochondrial defects in both Parkinson's disease and Alzheimer's disease. If mitochondrial dysfunction plays a role in neurodegenerative diseases then therapeutic strategies such as coenzyme Q10 and creatine may be useful in attempting to slow the disease process.

### **Vitamin E protects neurons against oxidative cell death in vitro more effectively than 17-beta estradiol and induces the activity of the transcription factor NF-kappaB.**

Behl C. Independent Research Group Neurodegeneration, Max Planck Institute of Psychiatry, Munich, Federal Republic of Germany. [chris@mpipsykl.mpg.de](mailto:chris@mpipsykl.mpg.de)

J Neural Transm 2000;107(4):393-407

Antioxidants can function as powerful protectants for neurons *in vitro*. Here, the neuroprotective activity of lipophilic free radical scavengers synthetic (+/-) alpha-tocopherol (synthetic vitamin E) and natural (+) alpha-tocopherol (natural vitamin E) against oxidative stress was investigated and compared to the neuroprotective effect of the female sex hormone estradiol. Employing mouse clonal hippocampal HT22 cells and rat cerebellar granule neurons, we found that both types of alpha-tocopherol exerted a higher neuroprotective antioxidant activity than 17-beta estradiol. At concentrations as low as 100 nM, synthetic (+/-) alpha-tocopherol and natural (+) alpha-tocopherol protected neurons effectively against the oxidative cell death caused by the Alzheimer's disease-associated amyloid beta protein, hydrogen peroxide, and the excitatory amino acid glutamate. Moreover, vitamin E induced the activity of the redox-sensitive transcription factor NF-kappaB, which is involved in the control of nerve cell survival and, therefore, may play also a role in vitamin E-induced neuroprotection. These results may have implications regarding the prevention and treatment of oxidative stress-related degenerative disorders such as Alzheimer's disease.

**Thiamine pyrophosphate and pyridoxamine inhibit the formation of antigenic advanced glycation end-products: comparison with aminoguanidine.**

Booth AA, Khalifah RG, Hudson BG. Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City 66160-7421, USA.

Biochem Biophys Res Commun 1996 Mar 7;220(1):113-9

Nonenzymatic glycation of proteins by glucose leading to the formation of toxic and immunogenic advanced glycation end products (AGEs) may be a major contributor to the pathological manifestations of diabetes mellitus, aging, and, possibly, neurodegenerative diseases such as Alzheimer's. We tested the *in vitro* inhibition of antigenic AGE formation on bovine serum albumin, ribonuclease A, and human hemoglobin by various vitamin B1 and B6 derivatives. Among the inhibitors, pyridoxamine and thiamine pyrophosphate potently inhibited AGE formation and were more effective than aminoguanidine, suggesting that these two compounds may have novel therapeutic potential in preventing vascular complications of diabetes. An unexpected finding was that aminoguanidine inhibited the late kinetic stages of glycation much more weakly than the early phase.

**In vitro kinetic studies of formation of antigenic advanced glycation end products (AGEs). Novel inhibition of post-Amadori glycation pathways**

Booth A.A.; Khalifah R.G.; Todd P.; Hudson B.G. USA

Journal of Biological Chemistry (USA), 1997, 272/9 (5430-5437)

Nonenzymatic protein glycation (Maillard reaction) leads to heterogeneous, toxic, and antigenic advanced glycation end products ('AGEs') and reactive precursors

that have been implicated in the pathogenesis of diabetes, Alzheimer's disease, and normal aging. In vitro inhibition studies of AGE formation in the presence of high sugar concentrations are difficult to interpret, since AGE-forming intermediates may oxidatively arise from free sugar or from Schiff base condensation products with protein amino groups, rather than from just their classical Amadori rearrangement products. We recently succeeded in isolating an Amadori intermediate in the reaction of ribonuclease A (RNase) with ribose (Khalifah, R. G., Todd, P., Booth, A. A., Yang, S. X., Mott, J. D., and Hudson, B. G. (1996) *Biochemistry* 35, 4645- 4654) for rapid studies of post-Amadori AGE formation in absence of free sugar or reversibly formed Schiff base precursors to Amadori products. This provides a new strategy for a better understanding of the mechanism of AGE inhibition by established inhibitors, such as aminoguanidine, and for searching for novel inhibitors specifically acting on post-Amadori pathways of AGE formation. Aminoguanidine shows little inhibition of post-Amadori AGE formation in RNase and bovine serum albumin, in contrast to its apparently effective inhibition of initial (although not late) stages of glycation in the presence of high concentrations of sugar. Of several derivatives of vitamins B1 and B6 recently studied for possible AGE inhibition in the presence of glucose (Booth, A. A., Khalifah, R. G., and Hudson, B. G. (1996) *Biochem. Biophys. Res. Commun.* 220, 113-119), pyridoxamine and, to a lesser extent, thiamine pyrophosphate proved to be novel and effective post-Amadori inhibitors that decrease the final levels of AGEs formed. Our mechanism- based approach to the study of AGE inhibition appears promising for the design and discovery of novel post-Amadori AGE inhibitors of therapeutic potential that may complement others, such as aminoguanidine, known to either prevent initial sugar attachment or to scavenge highly reactive dicarbonyl intermediates.

**[Vitamin B12 deficiency in geriatrics]. [Article in German]**

Bopp-Kistler I, Ruegger-Frey B, Grob D, Six P Klinik fur Geriatrie und Rehabilitation, Stadtsptal Waid, Zurich. irene.bopp@waid.stzh.ch

*Schweiz Rundsch Med Prax* 1999 Nov 4;88(45):1867-75

Cobalamin deficiency increases with advancing age. The cut-off point of serum concentration should be raised, because many elderly people with "normal" serum vitamin B12 concentrations are metabolically deficient in cobalamin. The measurement of the metabolites homocysteine and/or methylmalonic acid is recommended. Cobalamin deficiency may result in a variety of atypical symptoms. Hematological changes typical of megaloblastic anemia are absent in a majority of patients with neuropsychiatric disorders. Generally underlying pernicious anemia is not the main cause of cobalamin deficiency in the elderly. Protein-bound cobalamin malabsorption due to atrophic gastritis with hypo- or achlorhydria is a common cause of cobalamin deficiency in elderly people. An important manifestation of cobalamin deficiency is cognitive impairment. Much controversy exists on the subject of the association of dementia of the Alzheimer type with cobalamin deficiency. In several studies dementia has been related to low serum cobalamin levels. Physicians should be liberal of cobalamin therapy. The window of opportunity for effective intervention may be as short as one year

from the onset of medical symptoms. At last a compilation of recommendations is given.

### **S-adenosylmethionine levels in psychiatric and neurological disorders: a review.**

Bottiglieri T, Hyland K. Metabolic Disease Center, Baylor Research Institute, Dallas, TX 75226.

Acta Neurol Scand Suppl 1994;154:19-26

**INTRODUCTION**--S-adenosylmethionine (SAME) is an important methyl donor in over 35 methylation reactions involving DNA, proteins, phospholipids and catechol- and indole-amines.

**MATERIAL AND METHODS**--This article reviews the studies that have examined brain and blood levels of SAME in several psychological, neurological and metabolic disorders.

**RESULTS**--Although studies have found no consistent changes in whole blood SAME levels in psychiatric patients, other investigators have found low cerebrospinal fluid (CSF) SAME levels in patients with neurological disorders such as Alzheimer's dementia, subacute combined degeneration of the spinal cord (SACD), and HIV-related neuropathies, as well as in patients with metabolic disorders such as 5, 10-CH<sub>2</sub>-H<sub>4</sub> folate reductase deficiency.

**CONCLUSION**--Intravenous or oral administration of SAME thus represents a possible treatment for these neurological and metabolic disorders.

### **Effect of melatonin in selected populations of sleep-disturbed patients.**

Brusco LI, Fainstein I, Marquez M, Cardinali DP. Departamento de Fisiologia, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina.

Biol Signals Recept 1999 Jan-Apr;8(1-2):126-31

In an open pilot study on the efficacy of melatonin in the treatment of sleep disorders, patients with sleep disturbances alone, patients with sleep disturbances and signs of depression and patients with sleep disorders and dementia received 3 mg melatonin p.o. for 21 days, at bed time. After 2-3 days of treatment, melatonin significantly augmented sleep quality and decreased the number of awakening episodes in patients with sleep disturbances associated or not with depression. Estimates of next-day alertness improved significantly only in patients with primary insomnia. Agitated behavior at night (sundowning) decreased significantly in dementia patients. In a second retrospective study, 14 Alzheimer's disease (AD) patients received 9 mg melatonin daily for 22-35 months. A significant improvement of sleep quality was found, while there were no significant differences between initial and final neuropsychological evaluation (Functional Assessment Tool for AD, Mini-Mental). The results indicate that

melatonin can be useful to treat sleep disturbances in elderly insomniacs and AD patients.

**Melatonin treatment stabilizes chronobiologic and cognitive symptoms in Alzheimer's disease.**

Brusco LI, Marquez M, Cardinali DP. Departamento de Fisiologia, Facultad de Medicina, Universidad de Buenos Aires, Argentina.

Neuroendocrinol Lett 2000;21(1):39-42

**OBJECTIVES:** A retrospective study on the efficacy of melatonin in treatment of sleep and cognitive disorders of Alzheimer's disease was conducted.

**METHODS:** Fourteen patients (8 females, 6 males), mean +/- S.D. age 72 +/- 9 years were included. All patients received 9 mg gelatin melatonin capsules p.o. daily at bedtime for 22 to 35 months. Overall quality of sleep was assessed from sleep logs filled in by the patients or their caretakers. Neuropsychological evaluation was performed by Functional Assessment Tool For Alzheimer's Disease (FAST), Mini-Mental, Alzheimer's Disease Assessment Scale (ADAS), and Mattis' and Blessed's scales. At diagnosis, all patients had cognitive and neuroimaging alterations (cortical and bitemporal atrophy) compatible with different evolutionary stages of the disease.

**RESULTS:** At the time of assessment, a significant improvement of sleep quality was found in all cases examined. There were no significant differences between initial and present evaluation in scores of FAST, Mini-Mental, and ADAS, and of Mattis' and Blessed's scales. Clinically, the patients exhibited lack of progression of the cognitive and behavioral signs of the disease during the time they received melatonin. Sundowning was no longer detectable in 12 patients and persisted, although attenuated, in 2 patients.

**CONCLUSION.** The results suggest that melatonin can be useful for treatment of Alzheimer's disease.

**Evidence for forebrain cholinergic neuronal loss in congenital ornithine transcarbamylase deficiency.**

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Metab Brain Dis 2000 Mar;15(1):83-91

Congenital ornithine transcarbamylase (OTC) deficiency in humans results in failure to thrive, hypotonia, seizures and mental retardation. Neuropathologic evaluation reveals significant cerebral cortical atrophy, delayed myelination and Alzheimer type II astrocytosis. Using an animal model of congenital OTC deficiency, the sparse fur (spf) mouse, studies reveal convincing evidence of a loss of forebrain cholinergic neurons in this condition. Evidence includes (i)

reduced activities of the cholinergic nerve terminal enzyme choline acetyltransferase (ChAT), (ii) a 25% loss of ChAT immunostaining, (iii) reduced high affinity transport of [3H]choline by cortical synaptosomes and (iv) a selective reduction in densities of presynaptic muscarinic M2 binding sites, in spf mouse brain compared to controls. A partial correction of the cholinergic deficit was observed following treatment with acetyl-L-carnitine. Possible mechanisms responsible for cholinergic neuronal loss in congenital OTC deficiency include decreased synthesis of the ChAT substrate acetyl CoA, impaired cerebral energy metabolism and NMDA receptor-mediated excitotoxicity. Loss of forebrain cholinergic neurons is consistent with the severe cognitive impairment characteristic of congenital OTC deficiency.

### **Relationships between dehydroepiandrosterone sulfate (DHEAS) and cortisol (CRT) plasma levels and everyday memory in Alzheimer's disease patients compared to healthy controls.**

Carlson LE, Sherwin BB, Chertkow HM Department of Psychology, McGill University, Montreal, Canada.

Horm Behav 1999 Jun;35(3):254-63

Fifty-two age-matched Alzheimer's disease (AD) patients (26 men, 26 women), mean age 76.2 years, were assessed with the Rivermead Behavioural Memory Test, a test of everyday memory, coincident with the measurement of plasma cortisol (CRT) and dehydroepiandrosterone sulfate (DHEAS) via radioimmunoassay. The AD patients were compared to a control group of age- and gender-matched healthy elderly men and women. No differences were found between the AD patients and the controls in DHEAS or CRT levels, or in the DHEAS/CRT ratio. There were no gender differences in DHEAS or CRT levels, or in the DHEAS/CRT ratio in subjects with AD. However, AD patients with higher levels of DHEAS scored better than those with lower levels on the subtests of Remembering a Name associated with a picture, Digit Span Total and Forward, and the Mini Mental Status Exam. AD patients with higher CRT levels performed worse on Delayed Route Recall than those with lower levels. These findings suggest that AD patients with higher endogenous levels of DHEAS may perform better on some memory tasks than those with lower levels, while AD patients with lower levels of CRT may perform better than those with higher CRT.

### **Oxidative stress and Alzheimer disease.**

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Am J Clin Nutr 2000 Feb;71(2):621S-629S

Research in the field of molecular biology has helped to provide a better understanding of both the cascade of biochemical events that occurs with Alzheimer disease (AD) and the heterogeneous nature of the disease. One hypothesis that accounts for both the heterogeneous nature of AD and the fact that

aging is the most obvious risk factor is that free radicals are involved. The probability of this involvement is supported by the fact that neurons are extremely sensitive to attacks by destructive free radicals. Furthermore, lesions are present in the brains of AD patients that are typically associated with attacks by free radicals (eg, damage to DNA, protein oxidation, lipid peroxidation, and advanced glycosylation end products), and metals (eg, iron, copper, zinc, and aluminum) are present that have catalytic activity that produce free radicals. beta-Amyloid is aggregated and produces more free radicals in the presence of free radicals; beta-amyloid toxicity is eliminated by free radical scavengers. Apolipoprotein E is subject to attacks by free radicals, and apolipoprotein E peroxidation has been correlated with AD. In contrast, apolipoprotein E can act as a free radical scavenger and this behavior is isoform dependent. AD has been linked to mitochondrial anomalies affecting cytochrome-c oxidase, and these anomalies may contribute to the abnormal production of free radicals. Finally, many free radical scavengers (eg, vitamin E, selegiline, and Ginkgo biloba extract EGb 761) have produced promising results in relation to AD, as has desferrioxamine-an iron-chelating agent-and antiinflammatory drugs and estrogens, which also have an antioxidant effect.

### **Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer disease.**

Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L, Ueland PM Clinical Trial Service Unit, Nuffield Department of Clinical Medicine, Oxford, England.

Arch Neurol 1998 Nov;55(11):1449-55

**BACKGROUND:** Recent studies suggest that vascular disease may contribute to the cause of Alzheimer disease (AD). Since elevated plasma total homocysteine (tHcy) level is a risk factor for vascular disease, it may also be relevant to AD.

**OBJECTIVE:** To examine the association of AD with blood levels of tHcy, and its biological determinants folate and vitamin B12. **DESIGN:** Case-control study of 164 patients, aged 55 years or older, with a clinical diagnosis of dementia of Alzheimer type (DAT), including 76 patients with histologically confirmed AD and 108 control subjects. **SETTING:** Referral population to a hospital clinic between July 1988 and April 1996.

**MAIN OUTCOME MEASURES:** Serum tHcy, folate, and vitamin B12 levels in patients and controls at entry; the odds ratio of DAT or confirmed AD with elevated tHcy or low vitamin levels; and the rate of disease progression in relation to tHcy levels at entry. **RESULTS:** Serum tHcy levels were significantly higher and serum folate and vitamin B12 levels were lower in patients with DAT and patients with histologically confirmed AD than in controls. The odds ratio of confirmed AD associated with a tHcy level in the top third ( $< \text{or} = 14$  micromol/L) compared with the bottom third ( $< \text{or} = 11$  micromol/L) of the control distribution was 4.5 (95% confidence interval, 2.2-9.2), after adjustment for age, sex, social class, cigarette smoking, and apolipoprotein E epsilon4. The corresponding odds ratio for the lower third compared with the upper third of

serum folate distribution was 3.3 (95% confidence interval, 1.8-6.3) and of vitamin B12 distribution was 4.3 (95% confidence interval, 2.1-8.8). The mean tHcy levels were unaltered by duration of symptoms before enrollment and were stable for several years afterward. In a 3-year follow-up of patients with DAT, radiological evidence of disease progression was greater among those with higher tHcy levels at entry.

**CONCLUSIONS:** Low blood levels of folate and vitamin B12, and elevated tHcy levels were associated with AD. The stability of tHcy levels over time and lack of relationship with duration of symptoms argue against these findings being a consequence of disease and warrant further studies to assess the clinical relevance of these associations for AD.

### **Essential fatty acids in Alzheimer's disease.**

Corrigan FM, Van Rhijn A, Horrobin DF. Argyll and Bute Hospital, Lochgilphead, Scotland.

Ann N Y Acad Sci 1991;640:250-2

Concentrations of essential fatty acids (EFAs) in plasma and red blood cell phospholipids were found to be abnormal in patients with Alzheimer's disease. A double-blind, placebo-controlled trial of treatment with EFAs plus appropriate antioxidants was carried out in 36 patients with Alzheimer's disease. After 20 weeks both the EFA and placebo groups had improved, but the degree of improvement was consistently greater in the EFA group.

### **Alzheimer's disease risk factors as related to cerebral blood flow: additional evidence.**

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Med Hypotheses 1998 Jan;50(1):25-36

In a previous report, Alzheimer's disease risk factors, including alcohol abuse, depression, Down's syndrome, cerebral glucose metabolism defect, head trauma, old age, Parkinson's disease, sleep disturbance, and underactivity, were shown to have an association with reduced cerebral blood flow. In this report an attempt is made to strengthen a hypothesis that reduced cerebral blood flow may be a required cofactor in the cause of Alzheimer's disease with examples of additional putative risks, including aluminum, ApoE 4 alleles, estrogen deficiency, family history of dementia, low education-attainment, olfactory deficit, and underactivity coupled with gender, considered to have a relationship or potential relationship with reduced cerebral blood flow. Factors, believed to ameliorate Alzheimer's disease, associated with improved or stabilized cerebral blood flow are tabulated. A tentative cerebral blood flow nomogram is shown as a potential model to possibly help predict Alzheimer's disease susceptibility.

### **Alzheimer's disease risk factors as related to cerebral blood flow.**

Crawford JG. Indiana University School of Medicine, Terre Haute Center for Medical Science, IN 47809, USA.

Med Hypotheses 1996 Apr;46(4):367-77

Inconsistencies within results of case-control studies on Alzheimer's disease risk factors led to a search of the literature for a potential cofactor. Reduced cerebral blood flow was selected and literature was surveyed for evidence of a cerebral blood flow linkage with the more than 40 putative risks. Alcohol abuse, depression, head trauma, underactivity, old age, sleep disturbance, glucose utilization, Down's syndrome, and Parkinson's disease are risk factors where an association with reduced cerebral blood flow is documented. Studies were cited showing that improved cerebral blood flow is associated with factors thought to be helpful in Alzheimer's disease, such as education or occupational attainment, exercise, headache, smoking, and arthritis/anti-inflammatory drugs to the extent that aspirin is used. Sugar consumption is identified as a potential risk factor with glucose management in Alzheimer's disease also shown to involve reduced cerebral blood flow. An hypothesis is developed showing how compromised regional cerebral blood flow could fit as a cofactor for genetic, autoimmune, and neurotoxic aspects of Alzheimer's disease.

### **Effects of phosphatidylserine in Alzheimer's disease**

Crook T, Petrie W, Wells C, Massari DC Memory Assessment Clinics, Inc., Bethesda, MD 20814. USA

Psychopharmacol. Bull. (USA), 1992, 28/1 (61-66)

We studied 51 patients meeting clinical criteria for probable Alzheimer's disease (AD). Patients were treated for 12 weeks with a formulation of bovine cortex phosphatidylserine (BC-PS; 100 mg t.i.d.) or placebo, and those treated with the drug improved on several cognitive measures relative to those administered placebo. Differences between treatment groups were most apparent among patients with less severe cognitive impairment. Results suggest that phosphatidylserine may be a promising candidate for study in the early stages of AD.

### **Treatment of Alzheimer's disease.**

Cummings JL. UCLA Alzheimer's Disease Center, UCLA School of Medicine, Los Angeles, California, USA.

Clin Cornerstone 2001;3(4):27-39

A growing consensus indicates that Alzheimer's disease (AD) results from an increase in the production or accumulation of beta-amyloid protein (A beta) leading to nerve cell death. Mechanisms by which A beta accumulation leads to

neuronal death include oxidative damage and inflammation. This article discusses the management of AD patients with antioxidants, cholinesterase inhibitors, and psychotropic agents. Studies show that these agents can slow the progression of the disease, improve cognition, and reduce behavioral disturbances. A therapeutic alliance between physician and caregiver is an essential element in successfully managing the AD patient. The 3Rs--repeat, reassure, and redirect--can help caregivers reduce behavioral disturbances in patients with AD and limit the need for pharmacologic management.

**Impaired cerebrovascular perfusion. Summary of evidence in support of its causality in Alzheimer's disease.**

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Ann N Y Acad Sci 2000;924:136-52

After nearly a century of inquiry, the cause of Alzheimer's disease (AD) remains to be found. In this review, basic and clinical evidence is presented that assembles and hypothetically explains most of the key pathologic events associated with the development of AD. These pathologic events are triggered in AD by impaired cerebral perfusion originating in the microvasculature that affects the optimal delivery of glucose and oxygen and results in an energy metabolic breakdown of brain cell biosynthetic and synaptic pathways. We propose that two factors must be present before cognitive dysfunction and neurodegeneration is expressed in the AD brain: (1) advanced aging, (2) presence of a condition that lowers cerebral perfusion, such as a vascular risk factor. The first factor introduces a normal but potentially menacing process that lowers cerebral blood flow in proportion to increased aging, while the second factor adds a crucial burden that further lowers brain perfusion and places vulnerable neurons in a state of metabolic compromise leading to a death pathway. These two factors will lead to a critically attained threshold of cerebral hypoperfusion (CATCH). CATCH is a self-sustaining and progressive circulatory insufficiency that will destabilize neurons, synapses, neurotransmission, and cognitive function, creating in its wake a neurodegenerative process characterized by the formation of senile plaques, neurofibrillary tangles, amyloid angiopathy, and, in some cases, Lewy bodies. Since any of a considerable number of vessel-related conditions must be present in the aging individual for cognition to be affected, CATCH supports the heterogeneous disease profile assumed to be characteristic of the AD syndrome. A brief discussion of target therapy based on the proposed pathogenesis of AD is also reviewed.

**Double-blind randomized controlled study of phosphatidylserine in senile demented patients.**

Delwaide PJ, Gyselynck-Mambourg AM, Hurllet A, Ylieff M.

Acta Neurol Scand 1986 Feb;73(2):136-40

A double-blind randomized controlled study was conducted in 42 hospitalized demented patients to evaluate the therapeutic effect of phosphatidylserine (BS-PS). Half of the patients received 3 X 100 mg of this product, and the other half a placebo of the same appearance. After a wash-out period, prescription lasted for six weeks. To evaluate the patients, two distinct rating scales were used: the Crichton Scale and an original one (Peri Scale) designed in our geriatric unit (see Appendix). A circle crossing test was added. Out of the 35 patients who completed the trial, 18 had received placebo and 17 BC-PS. The results indicated a trend toward improvement in the BC-PS treated patients and an analysis of covariance showed a significant ( $p$  less than 0.05) treatment effect on the Peri Scale. The results at the end of the treatment period were compared with those obtained three weeks later. Here again there was a statistically significant difference in the Peri Scale results, indicating that modifications are drug-related. The behavioral improvement shown in this study is in agreement with experimental studies on aged animals.

### **Ginkgo biloba extract: mechanisms and clinical indications.**

Diamond BJ, Shiflett SC, Feiwel N, Matheis RJ, Noskin O, Richards JA, Schoenberger NE Department of Research, Center for Research in Complementary and Alternative Medicine, Kessler Medical Rehabilitation Research and Education Corporation, West Orange, NJ 07052, USA.

Arch Phys Med Rehabil 2000 May;81(5):668-78

**OBJECTIVE:** Ginkgo biloba may have a role in treating impairments in memory, cognitive speed, activities of daily living (ADL), edema, inflammation, and free-radical toxicity associated with traumatic brain injury (TBI), Alzheimer's dementia, stroke, vasoocclusive disorders, and aging. The purpose of this review is to provide a synthesis of the mechanisms of action, clinical indications, and safety of Ginkgo biloba extract.

**DATA SOURCES:** Empirical studies, reviews, chapters, and conference proceedings were identified in the following databases: Medline, the Research Council for Complementary Medicine based on the British Library database, and PsychInfo. Ginkgo biloba, EGb 761, Tanakan, Tebonin, Rokan, and LI 1370 were the principal index terms.

**STUDY SELECTION AND DATA EXTRACTION:** Controlled clinical studies with both positive and negative findings are included, in addition to animals studies illustrating mechanisms of activity.

**DATA SYNTHESIS:** Ginkgo has shown activity centrally and peripherally, affecting electrochemical, physiologic, neurologic, and vascular systems in animals and humans with few adverse side effects or drug interactions. Ginkgo shows promise in patients with dementia, normal aging, and cerebrovascular-related disorders. Clinical indications include memory, information processing, and ADL.

**CONCLUSIONS:** Ginkgo shows promise in treating some of the neurologic sequelae associated with Alzheimer's disease, TBI, stroke, normal aging, edema, tinnitus, and macular degeneration. Mechanisms of action may include antioxidant, neurotransmitter/receptor modulatory, and antiplatelet activating factor properties. While safe, caution is advised when recommending ginkgo to patients taking anticoagulants. Future studies should examine dose effects, component activity, mechanisms, and clinical applications.

**Vitamin B12 deficiency in dementia and cognitive impairment: the effects of treatment on neuropsychological function.**

Eastley R, Wilcock GK, Bucks RS. Avon and Western Wiltshire Mental Health Care NHS Trust, Southmead Hospital, Bristol, UK.

Int J Geriatr Psychiatry 2000 Mar;15(3):226-33

**BACKGROUND:** Vitamin B12 assay is part of the routine investigation of dementia, although few studies have investigated the effects of treatment on cognition. We examined the effects of B12 treatment on neuropsychological function and disease progression in patients presenting with dementia or cognitive impairment.

**METHODS:** From 1432 patients who were assessed at the Bristol Memory Disorders Clinic, 125 patients with low serum B12 were identified. Sixty-six patients presenting with dementia, and 22 with cognitive impairment were seen for a second assessment after treatment. Changes in neuropsychological test scores were compared with those of patients with normal serum B12, matched by age and diagnosis.

**RESULTS:** The majority of patients with low serum B12 had normal Hb and MCV values. We found no cases of reversible B12 deficiency dementia. The B12 treatment patients who presented with dementia showed no significant improvement, and no less deterioration, in their neuropsychological function than their matched group. However, a treatment effect was demonstrated among the patients presenting with cognitive impairment. These improved significantly compared to matched patients on the verbal fluency test ( $p < 0.01$ ).

**CONCLUSION:** All patients with cognitive impairment should be investigated for B12 deficiency. Vitamin B12 treatment may improve frontal lobe and language function in patients with cognitive impairment, but rarely reverses dementia. Copyright 2000 John Wiley & Sons, Ltd.

**Immunological mechanisms and the spectrum of psychiatric syndromes in Alzheimer's disease.**

Eikelenboom P, Hoogendijk WJ, Jonker C, van Tilburg W. Graduate School Neuroscience, Amsterdam, The Netherlands

J Psychiatr Res 2002 Sep-Oct;36(5):269-80

Pathological, genetic and epidemiological studies support the opinion that inflammatory mechanisms are involved in the pathogenesis of Alzheimer's disease (AD). Recent pathological and neuroradiological (PET) data show that activation of microglia is an early pathogenic event that precedes the process of severe neuropil destruction in AD brains. In this paper we review the evidence that inflammatory mediators can play a pathogenic role in some behavioural disorders frequently encountered during the clinical course in AD patients. Motivational disturbances are the most striking of the depressive symptoms in AD and can be present in a preclinical stage of the disease. Experimental animal studies and clinical trials in humans have shown that cytokines can induce similar symptoms which were described as 'sickness behaviour' or 'depressive-like' state. Delirious states are frequently observed in more advanced stages of dementia. Delirium is generally considered the result of an imbalance in neurotransmitter systems with severe deficits of the cholinergic systems. Animal studies show that pro-inflammatory cytokines, such as interleukin-1, induce a reduced activity of the cholinergic system. In AD, the release of cytokines would exacerbate any already existing disturbances in the cholinergic neurotransmission. This could explain the susceptibility of demented patients to delirium provoked by a wide variety of trivial incidents that are accompanied by an acute phase response. The data reviewed in this paper suggest that it could be worthwhile employing a neuroimmunological approach to study at molecular level the pathogenesis of a broad spectrum of behavioural disturbances common in the clinical course of AD patients.

**Double-blind cross-over study of phosphatidylserine vs. placebo in patients with early dementia of the Alzheimer type .** Engel RR, Satzger W, Gunther W, Kathmann N, Bove D, Gerke S, Munch U, Hippus H. Psychiatric Hospital, University of Munich, Germany.

Eur Neuropsychopharmacol 1992 Jun;2(2):149-55

Thirty-three patients with mild primary degenerative dementia according to DSM-III (MMS between 15 and 27) took part in a double-blind cross-over study of phosphatidylserine (Fidia, 300 mg/d) versus placebo. Both treatment phases lasted for 8 weeks with an 8 week washout phase in between and a 4 week washout phase before treatment phase one. Clinical global improvement ratings showed significantly more patients improving under BC-PS than under placebo during treatment phase one. The improvement carried over to the following wash-out and treatment phases. There were no significant improvements in GDS dementia rating scale, psychometric tests or P300-latency. 16-channel EEG mapping findings indicated that the patients initially showed higher power values in all frequency bands (except alpha), when compared to a younger, healthy control group. BC-PS reduced the higher power values compared to placebo, shifting EEG power more towards the normal level.

**Treatment of Alzheimer's disease with short- and long-term oral THA and lecithin: a double-blind study.**

Fitten LJ, Perryman KM, Gross PL, Fine H, Cummins J, Marshall C. VA Medical Center, Sepulveda, Calif 91343.

Am J Psychiatry 1990 Feb;147(2):239-42

Ten Alzheimer's disease patients underwent a trial of oral tetrahydroaminoacridine (THA) and lecithin. After 3 inpatient weeks there was no clear therapeutic effect. Three of six patients able to continue in long-term treatment showed measurable cognitive improvement, but only one displayed clinically obvious improvement.

**The incidence of dementia and intake of animal products: preliminary findings from the Adventist Health Study.**

Giem P, Beeson WL, Fraser GE Department of Preventive Medicine, School of Medicine, Loma Linda University, CA 92350.

Neuroepidemiology 1993;12(1):28-36

We investigated the relationship between animal product consumption and evidence of dementia in two cohort substudies. The first enrolled 272 California residents matched for age, sex, and zip code (1 vegan, 1 lacto-ovo-vegetarian, and 2 'heavy' meat eaters in each of 68 quartets). This design ensured a wide range of dietary exposure. The second included 2,984 unmatched subjects who resided within the Loma Linda, California area. All subjects were enrolled in the Adventist Health Study. The matched subjects who ate meat (including poultry and fish) were more than twice as likely to become demented as their vegetarian counterparts (relative risk 2.18,  $p = 0.065$ ) and the discrepancy was further widened (relative risk 2.99,  $p = 0.048$ ) when past meat consumption was taken into account. There was no significant difference in the incidence of dementia in the vegetarian versus meat-eating unmatched subjects. There was no obvious explanation for the difference between the two substudies, although the power of the unmatched sub-study to detect an effect of 'heavy' meat consumption was unexpectedly limited. There was a trend towards delayed onset of dementia in vegetarians in both substudies.

**Early diagnosis of cognitive impairment in the elderly with the focus on Alzheimer's disease.**

Gottfries CG, Lehmann W, Regland B. Department of Psychiatry and Neurochemistry, Institute of Clinical Neuroscience, Goteborg University, Molndal, Sweden.

J Neural Transm 1998;105(8-9):773-86

In dementia disorders, it can be assumed that the pathological process in the brain has been present for a long time. It is therefore of importance to have a preclinical or an early clinical diagnosis. Obviously, vulnerability genes, such as ApoE-4, can be diagnosed preclinically. As we have no treatment to offer patients with

genetic risk factors, genotyping for ApoE-4 is at present of no clinical use. Trained neuropsychologists have today access to sensitive tests which reveal cognitive impairment before the disturbances reach the level of dementia. Laboratory investigations of cerebrospinal fluid have so far yielded no great results. Tau protein appears to be the most sensitive marker, but it is unspecific. Chromogranin A separates early onset from late onset Alzheimer's disease and seems to be a marker for synaptic degeneration. Synaptotagmin was also found to be reduced in patients with early onset Alzheimer's disease. Still we do not know, however, whether these proteins are early markers for degenerative processes in the brain. Laboratory investigations of blood have not yielded markers of use in early or differential diagnosis of dementia disorders. In a study at our own institute, however, we found serum-homocysteine (S-HCY) to be an early and sensitive marker for cognitive impairment. In patients with dysmentia (mild cognitive impairment), no less than 39% had pathological S-HCY levels, indicating insufficient 1-carbon metabolism.

### **Vitamin E and Alzheimer disease: the basis for additional clinical trials.**

Grundman M. Alzheimer's Disease Cooperative Study, 9500 Gilman Drive 0949, La Jolla, CA, 92093-0949, USA. mgrundman@ucsd.edu.

Am J Clin Nutr 2000 Feb;71(2):630S-636S

Many lines of evidence suggest that oxidative stress is important in the pathogenesis of Alzheimer disease. In particular, beta-amyloid, which is found abundantly in the brains of Alzheimer disease patients, is toxic in neuronal cell cultures through a mechanism involving free radicals. Vitamin E prevents the oxidative damage induced by beta-amyloid in cell culture and delays memory deficits in animal models. A placebo-controlled, clinical trial of vitamin E in patients with moderately advanced Alzheimer disease was conducted by the Alzheimer's Disease Cooperative Study. Subjects in the vitamin E group were treated with 2000 IU (1342 alpha-tocopherol equivalents) vitamin E/d. The results indicated that vitamin E may slow functional deterioration leading to nursing home placement. A new clinical trial is planned that will examine whether vitamin E can delay or prevent a clinical diagnosis of Alzheimer disease in elderly persons with mild cognitive impairment.

### **The effect of tetrahydrofolate on tetrahydrobiopterin metabolism.**

Hamon CG, Blair JA, Barford PA.

J Ment Defic Res 1986 Jun;30 ( Pt 2):179-83

5-Methyltetrahydrofolate and vitamin B12 appear to be required for the biosynthesis of tetrahydrobiopterin. A deficiency of either could be sufficient to bring about neurological change which can be corrected by reversing the deficiency. Patients with senile dementia could possibly be benefited by the administration of 5-methyltetrahydrofolate.

**Long-term effects of phosphatidylserine, pyritinol, and cognitive training in Alzheimer's disease. A neuropsychological, EEG, and PET investigation**

Heiss WD, Kessler J, Mielke R, Szelies B, Herholz K Max-Planck-Institut für neurologische Forschung.

Dementia 1994 Mar-Apr;5(2):88-98

70 patients with probable Alzheimer's disease were randomly allocated to four groups: 17 patients received only social support. 18 cognitive training twice a week, in 17 cognitive training was combined with pyritinol 2 x 600 mg/day and in 18 cognitive training was combined with phosphatidylserine 2 x 200 mg/day. Treatment duration was 6 months. Before and after treatment, the patients underwent neuropsychological testing as well as measurement of the regional cerebral metabolic rate for glucose using positron emission tomography and 18F-2-fluoro-2-deoxy-D-glucose. Before treatment the groups were comparable in respect to resting and activated glucose pattern achieved by a visual recognition task. Electrophysiological changes were assessed as EEG power, globally and in 4 frequency bands. This 6-month study in four groups of patients with Alzheimer's disease indicated that phosphatidylserine treatment has an effect on different measures of brain function. Since neuropsychological improvements were best documented after 8 and 16 weeks and faded towards the end of the treatment period, it must be concluded that this symptomatic therapy is mainly of short-term benefit and was overcome by the progressive pathological changes at the end of the treatment period.

**JC virus infection and Alzheimer's disease: reappraisal of an in situ hybridization approach.**

Heinonen O, Syrjanen S, Mantyjarvi R, Syrjanen K, Riekkinen P. Department of Neurology, University of Kuopio, Finland.

Ann Neurol 1992 Apr;31(4):439-41

To assess the validity of the recently reported data on frequent occurrence of latent JC virus (JCV) infections in the brains of patients with Alzheimer's disease, we used in situ hybridization with biotinylated whole genomic JCV probes and the streptavidin-biotinylated alkaline phosphatase method to examine brain sections of such patients. We did not find any signs of JCV either in the brains of the patients with Alzheimer's disease or in those of nondemented, elderly control patients. Non-specific staining of corpora amylacea-like bodies, however, was invariably detected with in situ hybridization using JCV probes.

**DHEA-S plasma levels and incidence of Alzheimer's disease.**

Hillen T, Lun A, Reischies FM, Borchelt M, Steinhagen-Thiessen E, Schaub RT Department of Internal Medicine-Geriatrics, Medical Faculty, Humboldt University Berlin/Charite, Germany.

Biol Psychiatry 2000 Jan 15;47(2):161-3

**BACKGROUND:** Cross-sectional studies controlling for age and gender reported a relationship between Alzheimer's disease and low dehydroepiandrosterone sulphate (DHEA-S) plasma levels. Prospective data with sufficient control for confounding factors are lacking.

**METHODS:** A nested case-control study examined baseline DHEA-S in participants of the Berlin Aging Study. Cases (n = 14) developed dementia of the Alzheimer type within 3 years. Control group A (n = 14) was matched for gender, age, multimorbidity, and immobility. Control group B (n = 13) was matched for gender and age and comprised participants free from multimorbidity, immobility, multimедication, need of help, incontinence, visual impairment, hearing impairment, and depression.

**RESULTS:** The mean plasma DHEA-S concentration of case subjects was 1.02 0.61  $\mu\text{mol/L}$ . Both control groups had higher mean DEHA-S levels, in control group A, it was 1.89 1.24  $\mu\text{mol/L}$  (p = .012) and in control group B 1.70 1.38  $\mu\text{mol/L}$  (p = .093).

**CONCLUSIONS:** This population-based prospective study supports the role of DHEA-S as a risk factor for Alzheimer's disease.

### **Carnosine, a protective, anti-ageing peptide?**

Hipkiss AR. Molecular Biology and Biophysics Group, King's College London, Strand, UK.

Int J Biochem Cell Biol 1998 Aug;30(8):863-8

Carnosine (beta-alanyl-L-histidine) has protective functions additional to anti-oxidant and free-radical scavenging roles. It extends cultured human fibroblast life-span, kills transformed cells, protects cells against aldehydes and an amyloid peptide fragment and inhibits, in vitro, protein glycation (formation of cross-links, carbonyl groups and AGEs) and DNA/protein cross-linking. Carnosine is an aldehyde scavenger, a likely lipofuscin (age pigment) precursor and possible modulator of diabetic complications, atherosclerosis and Alzheimer's disease.

### **Pluripotent protective effects of carnosine, a naturally occurring dipeptide.**

Hipkiss AR, Preston JE, Himsworth DT, Worthington VC, Keown M, Michaelis J, Lawrence J, Mateen A, Allende L, Eagles PA, Abbott NJ. Molecular Biology and Biophysics Group, King's College London, Strand, United Kingdom.  
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Ann N Y Acad Sci 1998 Nov 20;854:37-53

Carnosine is a naturally occurring dipeptide (beta-alanyl-L-histidine) found in brain, innervated tissues, and the lens at concentrations up to 20 mM in humans.

In 1994 it was shown that carnosine could delay senescence of cultured human fibroblasts. Evidence will be presented to suggest that carnosine, in addition to antioxidant and oxygen free-radical scavenging activities, also reacts with deleterious aldehydes to protect susceptible macromolecules. Our studies show that, in vitro, carnosine inhibits nonenzymic glycosylation and cross-linking of proteins induced by reactive aldehydes (aldose and ketose sugars, certain triose glycolytic intermediates and malondialdehyde (MDA), a lipid peroxidation product). Additionally we show that carnosine inhibits formation of MDA-induced protein-associated advanced glycosylation end products (AGEs) and formation of DNA-protein cross-links induced by acetaldehyde and formaldehyde. At the cellular level 20 mM carnosine protected cultured human fibroblasts and lymphocytes, CHO cells, and cultured rat brain endothelial cells against the toxic effects of formaldehyde, acetaldehyde and MDA, and AGEs formed by a lysine/deoxyribose mixture. Interestingly, carnosine protected cultured rat brain endothelial cells against amyloid peptide toxicity. We propose that carnosine (which is remarkably nontoxic) or related structures should be explored for possible intervention in pathologies that involve deleterious aldehydes, for example, secondary diabetic complications, inflammatory phenomena, alcoholic liver disease, and possibly Alzheimer's disease.

#### **Endogenous mechanisms of neuroprotection: role of zinc, copper, and carnosine.**

Horning MS, Blakemore LJ, Trombley PQ. Biomedical Research Facility, Department of Biological Science, Florida State University, Tallahassee 32306-4340, USA. horning@neuro.fsu.edu

Brain Res 2000 Jan 3;852(1):56-61

Zinc and copper are endogenous transition metals that can be synaptically released during neuronal activity. Synaptically released zinc and copper probably function to modulate neuronal excitability under normal conditions. However, zinc and copper also can be neurotoxic, and it has been proposed that they may contribute to the neuropathology associated with a variety of conditions, such as Alzheimer's disease, stroke, and seizures. Recently, we demonstrated that carnosine, a dipeptide expressed in glial cells throughout the brain as well as in neuronal pathways of the visual and olfactory systems, can modulate the effects of zinc and copper on neuronal excitability. This result led us to hypothesize that carnosine may modulate the neurotoxic effects of zinc and copper as well. Our results demonstrate that carnosine can rescue neurons from zinc- and copper-mediated neurotoxicity and suggest that one function of carnosine may be as an endogenous neuroprotective agent.

#### **Health benefits of docosahexaenoic acid.**

Horrocks LA, Yeo YK Docosa Foods Ltd, 1275 Kinnear Road, Columbus, OH 43212-1155, USA,

Pharmacol Res 1999 Sep;40(3):211-25

Docosahexaenoic acid (DHA) is essential for the growth and functional development of the brain in infants. DHA is also required for maintenance of normal brain function in adults. The inclusion of plentiful DHA in the diet improves learning ability, whereas deficiencies of DHA are associated with deficits in learning. DHA is taken up by the brain in preference to other fatty acids. The turnover of DHA in the brain is very fast, more so than is generally realized. The visual acuity of healthy, full-term, formula-fed infants is increased when their formula includes DHA. During the last 50 years, many infants have been fed formula diets lacking DHA and other omega-3 fatty acids. DHA deficiencies are associated with foetal alcohol syndrome, attention deficit hyperactivity disorder, cystic fibrosis, phenylketonuria, unipolar depression, aggressive hostility, and adrenoleukodystrophy. Decreases in DHA in the brain are associated with cognitive decline during aging and with onset of sporadic Alzheimer disease. The leading cause of death in western nations is cardiovascular disease. Epidemiological studies have shown a strong correlation between fish consumption and reduction in sudden death from myocardial infarction. The reduction is approximately 50% with 200 mg day<sup>-1</sup> of DHA from fish. DHA is the active component in fish. Not only does fish oil reduce triglycerides in the blood and decrease thrombosis, but it also prevents cardiac arrhythmias. The association of DHA deficiency with depression is the reason for the robust positive correlation between depression and myocardial infarction. Patients with cardiovascular disease or Type II diabetes are often advised to adopt a low-fat diet with a high proportion of carbohydrate. A study with women shows that this type of diet increases plasma triglycerides and the severity of Type II diabetes and coronary heart disease. DHA is present in fatty fish (salmon, tuna, mackerel) and mother's milk. DHA is present at low levels in meat and eggs, but is not usually present in infant formulas. EPA, another long-chain n-3 fatty acid, is also present in fatty fish. The shorter chain n-3 fatty acid, alpha-linolenic acid, is not converted very well to DHA in man. These longchain n-3 fatty acids (also known as omega-3 fatty acids) are now becoming available in some foods, especially infant formula and eggs in Europe and Japan. Fish oil decreases the proliferation of tumour cells, whereas arachidonic acid, a longchain n-6 fatty acid, increases their proliferation. These opposite effects are also seen with inflammation, particularly with rheumatoid arthritis, and with asthma. DHA has a positive effect on diseases such as hypertension, arthritis, atherosclerosis, depression, adult-onset diabetes mellitus, myocardial infarction, thrombosis, and some cancers. Copyright 1999 Academic Press.

### **High brain myo-inositol levels in the prodementia phase of Alzheimer's disease in adults with Down's syndrome: a 1H MRS study.**

Huang W, Alexander GE, Daly EM, Shetty HU, Krasuski JS, Rapoport SI, Schapiro MB Laboratory of Neurosciences, National Institute on Aging, Clinical Center, NIH, Bethesda, MD, USA. whuang@clio.rad.sunysb.edu

Am J Psychiatry 1999 Dec;156(12):1879-86

OBJECTIVE: An extra portion of chromosome 21 in Down's syndrome leads to a dementia in later life that is phenotypically similar to Alzheimer's disease. Down's

syndrome therefore represents a model for studying preclinical stages of Alzheimer's disease. Markers that have been investigated in symptomatic Alzheimer's disease are myoinositol and N-acetyl-aspartate. The authors investigated whether abnormal brain levels of myo-inositol and other metabolites occur in the preclinical stages of Alzheimer's disease associated with Down's syndrome.

**METHOD:** The authors used 1H magnetic resonance spectroscopy (MRS) with external standards to measure absolute brain metabolite concentrations in 19 nondemented adults with Down's syndrome and 17 age- and sex-matched healthy comparison subjects.

**RESULTS:** Concentrations of myoinositol and choline-containing compounds were significantly higher in the occipital and parietal regions of the adults with Down's syndrome than in the comparison subjects. Within the Down's syndrome group, older subjects (42-62 years, N = 11) had higher myo-inositol levels than younger subjects (28-39 years, N = 8). Older subjects in both groups had lower N-acetylaspartate levels than the respective younger subjects, although this old-young difference was not greater in the Down's syndrome group.

**CONCLUSIONS:** The approximately 50% higher level of myo-inositol in Down's syndrome suggests a gene dose effect of the extra chromosome 21, where the human osmoregulatory sodium/myo-inositol cotransporter gene is located. The even higher myoinositol level in older adults with Down's syndrome extends to the predementia phase earlier findings of high myoinositol levels in symptomatic Alzheimer's disease.

### **Inflammatory mechanisms in Alzheimer's disease**

Hull M.; Strauss S.; Berger M.; Volk B.; Bauer J. Department of Psychiatry, Freiburg University Medical School, D-79104 Freiburg Germany

European Archives of Psychiatry and Clinical Neuroscience (Germany) 1996, 246/3 (124-128)

In recent years many studies have indicated an involvement of inflammatory mechanisms in Alzheimer's disease (AD). Acute-phase proteins such as alpha1-antichymotrypsin and c-reactive protein, elements of the complement system, and activated microglial and astroglial cells are consistently found in brains of AD patients. Most importantly, also cytokines such as interleukin-6 (IL-6) have been detected in the cortices of AD patients, indicating a local activation of components of the unspecific inflammatory system. Up to now it has remained unclear whether inflammatory mechanisms represent a primary event or only an unspecific reaction to brain tissue damage. Therefore, we investigated whether IL-6 immunoreactivity could be found in plaques prior to the onset of neuritic changes, or whether the presence of this cytokine is restricted to later stages of plaque pathology. We confirmed our previous observation that IL-6 is detectable in a significant proportion of plaques in the brains of demented patients. In AD patients IL-6 was found in diffuse plaques in a significant higher ratio as would

have been expected from a random distribution of IL-6 among all plaque types. This observation suggests that IL-6 may precede neuritic changes, and that immunological mechanism may be involved both in the transformation from diffuse to neuritic plaques in AD and in the development of dementia.

### **Cerebrospinal fluid levels of alpha-tocopherol (vitamin E) in Alzheimer's disease.**

Jimenez-Jimenez FJ; de Bustos F; Molina JA; Benito-Leon J; Tallon-Barranco A; Gasalla T; Orti-Pareja M; Guillamon F; Rubio JC; Arenas J; Enriquez-de-Salamanca R. Department of Neurology, Hospital Universitario Principe de Asturias, Alcala de Henares, Spain.

J Neural Transm (Austria) 1997, 104 (6-7) p703-10

We compared CSF and serum levels, and the CSF/serum ratio of alpha-tocopherol (vitamin E), measured by HPLC, in 44 apparently well-nourished patients with Alzheimer's disease (AD) and 37 matched controls. CSF and serum vitamin E levels were correlated, both in AD patients and in controls. The mean CSF and serum vitamin E levels were significantly lower in AD patients, and the CSF/serum ratio of AD patients did not differ significantly between the 2 study groups. CSF vitamin E levels did not correlate with age, age at onset, duration of the disease and score of the Minimal State Examination in the AD group. Weight and body mass index were significantly lower in AD patients than in controls. These results suggest that low CSF and serum vitamin E concentrations in AD patients could be related with a deficiency of dietary intake of vitamin E.

### **Serum levels of beta-carotene, alpha-carotene and vitamin A in patients with Alzheimer's disease.**

Jimenez-Jimenez FJ, Molina JA, de Bustos F, Orti-Pareja M, Benito-Leon J, Tallon-Barranco A, Gasalla T, Porta J, Arenas J. Department of Neurology of Hospital 'Principe de Asturias', University of Alcala de Henares, Madrid, Spain.

Eur J Neurol 1999 Jul;6(4):495-7

To elucidate the possible role of carotenoids and vitamin A as risk factors for Alzheimer's disease (AD), we compared serum levels of beta-carotene and alpha-carotene, and vitamin A, measured by isocratic high performance liquid chromatography, of 38 AD patients and 42 controls. The serum levels of alpha-carotene did not differ significantly between AD patients and control groups. However, the serum levels of beta-carotene and vitamin A were significantly lower in the AD-patient group. These values did not correlate to age, age at onset or score on the MiniMental State Examination. Weight and body mass index were significantly lower in AD patients than in controls. These results suggest that low serum beta-carotene concentrations in AD patients could be related to a deficiency in dietary intake of this provitamin, although its possible relationship with risk for AD could not be excluded. Copyright 1999 Lippincott Williams & Wilkins

### **Effect of curcumin and capsaicin on arachidonic acid metabolism and lysosomal enzyme secretion by rat peritoneal macrophages**

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Lipids (United States) 1997, 32/11 (1173-1180)

The inflammatory mediators secreted by macrophages play an important role in autoimmune diseases. Spice components, such as curcumin from turmeric and capsaicin from red pepper, are shown to exhibit antiinflammatory properties. The influence of these spice components on arachidonic acid metabolism and secretion of lysosomal enzymes by macrophages was investigated. Rat peritoneal macrophages preincubated with 10  $\mu$ M curcumin or capsaicin for 1 h inhibited the incorporation of arachidonic acid into membrane lipids by 82 and 76%: prostaglandin E<sub>2</sub> by 45 and 48%; leukotriene B<sub>4</sub> by 61 and 46%, and leukotriene C<sub>4</sub> by 34 and 48%, respectively, but did not affect the release of arachidonic acid from macrophages stimulated by phorbol myristate acetate. However, the secretion of 6-keto PG F<sub>1</sub>( $\alpha$ ) was enhanced by 40 and 29% from macrophages preincubated with 10  $\mu$ M curcumin or capsaicin, respectively, as compared to those produced by control cells. Curcumin and capsaicin also inhibited the secretion of collagenase, elastase, and hyaluronidase to the maximum extent of 57, 61, 66%, and 46, 69, 67%, respectively. These results demonstrated that curcumin and capsaicin can control the release of inflammatory mediators such as eicosanoids and hydrolytic enzymes secreted by macrophages and thereby may exhibit antiinflammatory properties.

### **Is metabolic evidence for vitamin B-12 and folate deficiency more frequent in elderly patients with Alzheimer's disease?**

Joosten E; Lesaffre E; Riezler R; Ghekiere V; Dereymaeker L; Pelemans W; Dejaeger E Department of Pathophysiology, University Hospitals K. U. Leuven, Belgium.

J Gerontol A Biol Sci Med Sci (United States) Mar 1997, 52 (2) M76-9.

**BACKGROUND:** It is still unclear whether there is an association between Alzheimer's disease and vitamin B-12 or folate deficiency. This study was designed to investigate whether patients with Alzheimer's disease are particularly prone to metabolically significant cobalamin or folate deficiency as compared to nondemented hospitalized controls and healthy elderly controls living at home.

**METHODS:** Evaluation for the diagnosis of Alzheimer's disease, routine laboratory tests, serum folate and vitamin B-12, serum methylmalonic acid (MMA), total homocysteine (tHcy), and radiological tests was performed in 52 patients with Alzheimer's disease (AD), 50 nondemented hospitalized controls, and 49 healthy elderly subjects living at home.

**RESULTS:** Serum vitamin B-12 and folate levels are comparable between patients with AD, hospitalized control patients, and subjects living at home. Patients with AD have the highest serum MMA and tHcy levels. The MMA levels of patients with AD and hospitalized controls are not different, but the mean tHcy level is significantly higher in patients with AD as compared to nondemented patients or subjects living at home.

**CONCLUSION:** The interpretation of the vitamin B-12 and folate status in patients with AD depends largely on the methodology (i.e., serum vitamin vs metabolite levels) and the selection of the control group. Although patients with AD have the highest tHcy and MMA levels, metabolically significant vitamin B-12 and folate deficiency is also a substantial problem in nondemented elderly patients.

### **Alzheimer's disease: risk and protection.**

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Med J Aust 1997 Oct 20;167(8):443-6

Only four risk factors for Alzheimer's disease can be regarded as confirmed--old age, family history of dementia, apo-E genotype and Down syndrome. Other disputed risk factors with some supporting evidence include ethnic group, head trauma and aluminium in drinking water. Possible protection factors, such as anti-inflammatory drugs, oestrogen replacement therapy and a high education level, are of great interest because they suggest possible preventive action.

### **A review of nutrients and botanicals in the integrative management of cognitive dysfunction.**

Kidd PM

Altern Med Rev 1999 Jun;4(3):144-61

Dementias and other severe cognitive dysfunction states pose a daunting challenge to existing medical management strategies. An integrative, early intervention approach seems warranted. Whereas, allopathic treatment options are highly limited, nutritional and botanical therapies are available which have proven degrees of efficacy and generally favorable benefit-to-risk profiles. This review covers five such therapies: phosphatidylserine (PS), acetyl-l-carnitine (ALC), vinpocetine, Ginkgo biloba extract (GbE), and Bacopa monniera (Bacopa). PS is a phospholipid enriched in the brain, validated through double-blind trials for improving memory, learning, concentration, word recall, and mood in middle-aged and elderly subjects with dementia or age-related cognitive decline. PS has an excellent benefit-to-risk profile. ALC is an energizer and metabolic cofactor which also benefits various cognitive functions in the middle-aged and elderly, but with a slightly less favorable benefit-to-risk profile. Vinpocetine, found in the

lesser periwinkle *Vinca minor*, is an excellent vasodilator and cerebral metabolic enhancer with proven benefits for vascular-based cognitive dysfunction. Two meta-analyses of GbE demonstrate the best preparations offer limited benefits for vascular insufficiencies and even more limited benefits for Alzheimer's, while "commodity" GbE products offer little benefit, if any at all. GbE (and probably also vinpocetine) is incompatible with blood-thinning drugs. Bacopa is an Ayurvedic botanical with apparent anti-anxiety, anti-fatigue, and memory-strengthening effects. These five substances offer interesting contributions to a personalized approach for restoring cognitive function, perhaps eventually in conjunction with the judicious application of growth factors.

### **Do raised brain aluminium levels in Alzheimer's dementia contribute to cholinergic neuronal deficits?**

King RG.

Med Hypotheses 1984 Jul;14(3):301-6

Raised aluminium levels have been found in brains of patients with Alzheimer's dementia (1,2), a disease in which reductions have been reported in various parameters of presynaptic cholinergic nerve function, including choline uptake, acetylcholine synthesis and choline acetyltransferase activity (3). Aluminium has been found to inhibit choline transport by isolated rat brain nerve endings (4) and human erythrocytes (5), and also to cause an encephalopathy in rabbits with neurofibrillary tangles and reduced neuronal choline acetyltransferase activity (6). It is therefore hypothesised that raised brain aluminium levels in Alzheimer's dementia may contribute to the cholinergic neuronal deficits in this disease. If this is the case, then aluminium chelating agents may be of value in its treatment.

### **Influence of vitamin E and C supplementation on lipoprotein oxidation in patients with Alzheimer's disease.**

Kontush A, Mann U, Arlt S, Ujeyl A, Luhrs C, Muller-Thomsen T, Beisiegel U. Clinic of Internal Medicine, University Hospital Eppendorf, Martinistrasse 52, 20246 Hamburg, Germany.

Free Radic Biol Med 2001 Aug 1;31(3):345-54

Because increased oxidation is an important feature of Alzheimer's disease (AD) and low concentrations of antioxidant vitamins C and E have been observed in cerebrospinal fluid (CSF) of AD patients, supplementation with these antioxidants might delay the development of AD. Major targets for oxidation in brain are lipids and lipoproteins. We studied whether supplementation with antioxidative vitamins E and C can increase their concentrations not only in plasma but also in CSF, and as a consequence decrease the susceptibility of lipoproteins to in vitro oxidation. Two groups, each consisting of 10 patients with AD, were for 1 month supplemented daily with either a combination of 400 IU vitamin E and 1000 mg vitamin C, or 400 IU vitamin E alone. We found that supplementation with vitamin E and C significantly increased the concentrations of both vitamins in

plasma and CSF. Importantly, the abnormally low concentrations of vitamin C were returned to normal level following treatment. As a consequence, susceptibility of CSF and plasma lipoproteins to in vitro oxidation was significantly decreased. In contrast, the supplementation with vitamin E alone significantly increased its CSF and plasma concentrations, but was unable to decrease the lipoprotein oxidizability. These findings document a superiority of a combined vitamin E + C supplementation over a vitamin E supplementation alone in AD and provide a biochemical basis for its use.

**Music therapy increases serum melatonin levels in patients with Alzheimer's disease.**

Kumar AM, Tims F, Cruess DG, Mintzer MJ, Ironson G, Loewenstein D, Cattan R, Fernandez JB, Eisdorfer C, Kumar M. Department of Psychiatry and Behavioral Sciences, University of Miami School of Medicine, FL 33101, USA. akumar@med.miami.edu

Altern Ther Health Med 1999 Nov;5(6):49-57

**CONTEXT:** Music therapy is known to have healing and relaxing effects. Although these effects appear to be mediated by release of neurotransmitters and neurohormones, the specific neurohormonal systems involved have not been fully investigated.

**OBJECTIVE:** To assess the effects of a music therapy intervention on concentrations of melatonin, norepinephrine, epinephrine, serotonin, and prolactin in the blood of a group of patients with Alzheimer's disease.

**DESIGN:** Blood samples were obtained before initiating the therapy, immediately at the end of 4 weeks of music therapy sessions, and at 6 weeks follow-up after cessation of the sessions.

**SETTING:** Miami Veterans Administration Medical Center, Miami, Fla.

**PATIENTS:** 20 male inpatients with Alzheimer's disease.

**INTERVENTION:** 30- to 40-minute morning sessions of music therapy 5 times per week for 4 weeks.

**MAIN OUTCOME MEASURES:** Changes in melatonin, norepinephrine, epinephrine, serotonin, and prolactin following music therapy.

**RESULTS:** Melatonin concentration in serum increased significantly after music therapy and was found to increase further at 6 weeks follow-up. A significant increase was found between baseline values and data recorded after the music therapy sessions as well as at 6 weeks follow-up. Norepinephrine and epinephrine levels increased significantly after 4 weeks of music therapy, but returned to pretherapy levels at 6 weeks follow-up. Serum concentration of prolactin and

platelet serotonin levels remained unchanged after 4 weeks of music therapy and at 6 weeks follow-up.

**CONCLUSION:** Increased levels of melatonin following music therapy may have contributed to patients' relaxed and calm mood.

### **Melatonin affects the metabolism of the beta-amyloid precursor protein in different cell types.**

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J Pineal Res 1999 Apr;26(3):137-46

Melatonin is released in mammals during the dark phase of the circadian cycle, and its production declines with age in animals and humans. Since supplemental administration of melatonin may be beneficial in delaying age-related degenerative conditions, it is necessary to study its effect on neuronal differentiation and the processing of key neuronal proteins, such as beta-amyloid precursor protein (beta APP) and synaptophysin. One of the important pathological hallmarks of Alzheimer's disease (AD) is the cerebrovascular deposition of amyloid plaques. The amyloid in senile plaques is mainly composed of the amyloid beta-peptide (A beta) of 39-43 amino acids derived from a larger beta APP. The proteolytic cleavage by 'alpha-secretase' generate soluble derivatives of beta APP (sAPP), lacking the cytoplasmic tail, transmembrane domain, and a small portion of the extracellular domain. Here levels of sAPP and beta APP were analyzed in cell lines of different origins by Western immunoblot of samples from conditioned media and cell lysates, respectively. Normal levels of secretion of sAPP into conditioned media were severely inhibited by treating different cell lines with a high dose of melatonin. In PC12 cells, levels of the fully matured beta APP forms of the post-Golgi compartment were more drastically decreased than the unglycosylated beta APP of the endoplasmic-reticulum (ER) forms. In other cell types, the unglycosylated ER-bound beta APP derivatives are predominant forms that were marginally affected by melatonin treatment. When the treatment of cells with melatonin was withdrawn, the normal level of secretion of sAPP was restored. Melatonin reduces the secretion of soluble A beta. Melatonin also inhibits the secretion of synaptophysin in PC12 cells. Taken together, these data suggest that melatonin probably affects the secretion of sAPP in the conditioned medium by interfering with its full maturation, and melatonin also affects the presynaptic terminal marker.

### **Interactions between melatonin, reactive oxygen species, and nitric oxide.**

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Ann N Y Acad Sci 1999;893:325-30

Accumulation of reactive oxygen species is critical for the neuropathology of Alzheimer's disease. Melatonin hormone, an antioxidant, could play a key role in aging and senescence. Nitric oxide, a biologically active unstable radical, is synthesized by nitric oxide synthase when converting L-arginine to L-citrulline. We have investigated whether the treatment of cultured cells with melatonin could possibly reduce the release of free radicals and other ROS. We assayed NO indirectly by measuring the level of its stable end products, nitrite/nitrate (NO<sub>x</sub>), using the Griess reagent. When the neuroblastoma cells such as N1E-115 were treated with a NO donor such as sodium nitroprusside (SNP), a significant level of NO<sub>x</sub> was detected in a time- and dose-dependent manner in the conditioned medium compared to the untreated cells or SNP-containing media. In neuroblastoma cells, the release of NO<sub>x</sub> as mediated by SNP was significantly inhibited by treatment with (i) carboxy-PTIO, a NO scavenger; (ii) SOD-1, superoxide dismutase; and (iii) melatonin. In these cells SNP-mediated NO<sub>x</sub> release was mediated by superoxide ions and/or free radicals that can be inhibited by melatonin. The ROS-scavenging function of melatonin along with its neuroprotective and neurodifferentiating role can be utilized for the prevention of neurodegenerative disorders such as AD.

**A placebo-controlled, double-blind, randomized trial of an extract of Ginkgo biloba for dementia. North American EGb Study Group.**

Le Bars PL, Katz MM, Berman N, Itil TM, Freedman AM, Schatzberg AF. New York Institute for Medical Research, Tarrytown 10591, USA. NYI@HZI.com

JAMA 1997 Oct 22-29;278(16):1327-32

**CONTEXT:** EGb 761 is a particular extract of Ginkgo biloba used in Europe to alleviate symptoms associated with numerous cognitive disorders. Its use in dementias is based on positive results from only a few controlled clinical trials, most of which did not include standard assessments of cognition and behavior.

**OBJECTIVE:** To assess the efficacy and safety of EGb in Alzheimer disease and multi-infarct dementia.

**DESIGN:** A 52-week, randomized double-blind, placebo-controlled, parallel-group, multicenter study.

**PATIENTS:** Mildly to severely demented outpatients with Alzheimer disease or multi-infarct dementia, without other significant medical conditions.

**INTERVENTION:** Patients assigned randomly to treatment with EGb (120 mg/d) or placebo. Safety, compliance, and drug dispensation were monitored every 3 months with complete outcome evaluation at 12, 26, and 52 weeks.

**PRIMARY OUTCOME MEASURES:** Alzheimer's Disease Assessment Scale-Cognitive subscale (ADAS-Cog), Geriatric Evaluation by Relative's Rating Instrument (GERRI), and Clinical Global Impression of Change (CGIC).

**RESULTS:** From 309 patients included in an intent-to-treat analysis, 202 provided evaluable data for the 52-week end point analysis. In the intent-to-treat analysis, the EGb group had an ADAS-Cog score 1.4 points better than the placebo group ( $P=.04$ ) and a GERRI score 0.14 points better than the placebo group ( $P=.004$ ). The same patterns were observed with the evaluable data set in which 27% of patients treated with EGb achieved at least a 4-point improvement on the ADAS-Cog, compared with 14% taking placebo ( $P=.005$ ); on the GERRI, 37% were considered improved with EGb, compared with 23% taking placebo ( $P=.003$ ). No difference was seen in the CGIC. Regarding the safety profile of EGb, no significant differences compared with placebo were observed in the number of patients reporting adverse events or in the incidence and severity of these events.

**CONCLUSIONS:** EGb was safe and appears capable of stabilizing and, in a substantial number of cases, improving the cognitive performance and the social functioning of demented patients for 6 months to 1 year. Although modest, the changes induced by EGb were objectively measured by the ADAS-Cog and were of sufficient magnitude to be recognized by the caregivers in the GERRI.

**A 26-week analysis of a double-blind, placebo-controlled trial of the ginkgo biloba extract EGb 761 in dementia.**

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Dement Geriatr Cogn Disord 2000 Jul-Aug;11(4):230-7

This intent-to-treat (ITT) analysis was performed to provide a realistic image of the efficacy that could be expected after 26 weeks treatment with a 120-mg dose (40 mg t.i.d.) of EGb 761 (EGb). The data were collected during a 52-week, double-blind, placebo-controlled, fixed dose, parallel-group, multicenter study. Patients were mildly to severely impaired and diagnosed with uncomplicated Alzheimer's disease or multi-infarct dementia according to ICD-10 and DSM-III-R criteria. The primary outcome measures included the Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog), Geriatric Evaluation by Relative's Rating Instrument (GERRI) and Clinical Global Impression of Change. From 309 patients included in the ITT analysis, 244 patients (76% for placebo and 73% for EGb) actually reached the 26th week visit. In comparison to the baseline values, the placebo group showed a statistically significant worsening in all domains of assessment, while the group receiving EGb was considered slightly improved on the cognitive assessment and the daily living and social behavior. Mean treatment differences favored EGb with 1.3 and 0.12 points, respectively, on the ADAS-Cog ( $p = 0.04$ ) and the GERRI ( $p = 0.007$ ). In the group receiving EGb, 26% of the patients achieved at least a 4-point improvement on the ADAS-Cog, compared to 17% with placebo ( $p = 0.04$ ). On the GERRI, 30% of the EGb group improved and 17% worsened, while the placebo group showed an opposite trend with 37% of patients worsening for 25% improved ( $p = 0.006$ ). Regarding safety, no differences between EGb and placebo were observed.

### **Hyperhomocysteinemia in dementia.**

Leblhuber F, Walli J, Artner-Dworzak E, Vrecko K, Widner B, Reibnegger G, Fuchs D. Department of Gerontology, Landesnervenklinik Wagner Jauregg, Linz, Austria.

J Neural Transm 2000;107(12):1469-74

Hyperhomocysteinemia is a strong risk factor for atherosclerotic vascular disease, and elevated serum homocysteine is correlated with vitamin B deficiency. In this pilot study, significantly elevated homocysteine levels were found in patients with Alzheimer's disease as well as in patients with vascular dementia, probably indicating similar pathophysiological pathways. We found significant correlations between low folic acid concentrations as well as high homocysteine concentrations and cognitive decline. Supplementation with folic acid may be an inexpensive way to reduce elevated homocysteine levels in demented patients.

### **Identification of cognitive impairment in the elderly: homocysteine is an early marker.**

Lehmann M, Gottfries CG, Regland B. Goteborg University, Institute of Clinical Neuroscience, Department of Psychiatry and Neurochemistry, Molndal, Sweden.

Dement Geriatr Cogn Disord 1999 Jan-Feb;10(1):12-20

In 336 consecutive patients attending a university-affiliated memory unit, clinical and psychological findings, neuroimaging and laboratory tests were analyzed. The patients were diagnosed with early Alzheimer's disease 3%, senile dementia (SDAT) 16%, vascular dementia (VAD) 20%, other dementias 9%, minor cognitive impairment (dysmentia) 32% and subjective symptoms only 21%. Increases in vascular risk factors, serum homocysteine, ApoE4 load and neuroimaging pathology were found in dementia but also in dysmentia and in patients with subjective symptoms only. The homocysteine levels correlated inversely with cognitive performance. The increases in serum homocysteine, which were pathological in VAD, Dysmentia and SDAT, may be indicative of a disturbed cerebral one-carbon metabolism and signal-accelerated development of cognitive disease.

### **A double-blind, placebo controlled trial of high-dose lecithin in Alzheimer's disease.**

Little A, Levy R, Chuaqui-Kidd P, Hand D.

J Neurol Neurosurg Psychiatry 1985 Aug;48(8):736-42

The first long-term double-blind placebo controlled trial of high dose lecithin in senile dementia of the Alzheimer type is reported. Fifty one subjects were given 20-25 g/day of purified soya lecithin (containing 90% phosphatidyl plus lysophosphatidyl choline) for six months and followed up for at least a further six

months. Plasma choline levels were monitored throughout the treatment period. There were no differences between the placebo group and the lecithin group but there was an improvement in a subgroup of relatively poor compliers. These were older and had intermediate levels of plasma choline. It is suggested that the effects of lecithin are complex but that there may be a "therapeutic window" for the effects of lecithin in the condition and that this may be more evident in older patients.

**Decreased melatonin levels in postmortem cerebrospinal fluid in relation to aging, Alzheimer's disease, and apolipoprotein E-epsilon4/4 genotype.**

Liu RY, Zhou JN, van Heerikhuizen J, Hofman MA, Swaab DF. Netherlands Institute for Brain Research, Amsterdam.

J Clin Endocrinol Metab 1999 Jan;84(1):323-7

Sleep disruption, nightly restlessness, sundowning, and other circadian disturbances are frequently seen in Alzheimer's disease (AD) patients. Changes in the suprachiasmatic nucleus and pineal gland are thought to be the biological basis for these behavioral disturbances. Melatonin is the main endocrine message for circadian rhythmicity from the pineal. To determine whether melatonin production was affected in AD, melatonin levels were determined in the cerebrospinal fluid (CSF) of 85 patients with AD (mean age, 75 +/- 1.1 yr) and in 82 age-matched controls (mean age, 76 +/- 1.4 yr). Ventricular postmortem CSF was collected from clinically and neuropathologically well defined AD patients and from control subjects without primary neurological or psychiatric disease. In old control subjects (>80 yr of age), CSF melatonin levels were half of those in control subjects of 41-80 yr of age [176 +/- 58 (n = 29) and 330 +/- 66 (n = 53) pg/mL, respectively; P = 0.016]. We did not find a diurnal rhythm in CSF melatonin levels in control subjects. In AD patients the CSF melatonin levels were only one fifth (55 +/- 7 pg/mL) of those in control subjects (273 +/- 47 pg/mL; P = 0.0001). There was no difference in the CSF melatonin levels between the presenile (42 +/- 11 pg/mL; n = 21) and the senile (59 +/- 8 pg/mL; n = 64; P = 0.35) AD patients. The melatonin level in AD patients expressing apolipoprotein E-epsilon3/4 (71 +/- 11 pg/mL) was significantly higher than that in patients expressing apolipoprotein E-epsilon4/4 (32 +/- 8 pg/ml; P = 0.02). In the AD patients no significant correlation was observed between age of onset or duration of AD and CSF melatonin levels. In the present study, a dramatic decrease in the CSF melatonin levels was found in old control subjects and even more so in AD patients. Whether supplementation of melatonin may indeed improve behavioral disturbances in AD patients should be investigated.

**Association between changes in adrenal secretion and cerebral morphometric correlates in normal aging and senile dementia.**

Magri F, Terenzi F, Ricciardi T, Fioravanti M, Solerte SB, Stabile M, Balza G, Gandini C, Villa M, Ferrari E Department of Internal Medicine and Medical Therapy, Chair of Geriatrics, University of Pavia, Italy. ferrari@ipv36.unipv.it

Dement Geriatr Cogn Disord 2000 Mar-Apr;11(2):90-9

The circadian organization of adrenal secretion was studied in 23 healthy elderly subjects, 23 elderly demented patients and 10 healthy young subjects, in order to investigate the relationships between the hypothalamic-pituitary-adrenal axis and some cerebral morphometric parameters. The cerebral morphometric analysis was performed in some subjects of the three groups by MRI. A significant increase in cortisol levels during evening and nighttime was found in both groups of the aged subjects. In elderly subjects, particularly if demented, the mean serum dehydroepiandrosterone sulfate (DHEAs) levels throughout the 24-hour cycle were significantly lower than in young controls. A significant reduction of the hippocampal and temporal volume and an enlargement of the lateral ventricles were found in aged subjects, these changes being significantly related to subjects' age. Moreover, the hippocampal volume was positively correlated with the circadian mesor of DHEAs (i.e., the circadian rhythm adjusted mean) and with the cortisol nocturnal increase. Our data may suggest the existence of a link between the selective impairment of cortisol secretion and DHEAs levels, and the progression of hippocampal degeneration.

**The therapeutic potential for tryptophan and melatonin: possible roles in depression, sleep, Alzheimer's disease and abnormal aging.**

Maurizi CP.

Med Hypotheses 1990 Mar;31(3):233-42

Evidence suggests that stress and/or a dietary lack of tryptophan may make deficiencies of serotonin and melatonin common. In addition, older animals and human beings have a reduced ability to synthesize melatonin. Disorders of melatonin levels and rhythms are suggested to be a cause of affective disease, abnormal sleep, Alzheimer's disease, and some age related disorders. If these ideas prove to be true, then preventive measures are possible.

**Analogues, ageing and aberrant assimilation of vitamin B12 in Alzheimer's disease.**

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Dement Geriatr Cogn Disord 2001 Mar-Apr;12(2):133-7

Vitamin B12 assimilation might be disrupted in patients with Alzheimer's disease. We therefore measured B12 carrier protein saturation and inactive B12 'analogues' in patients compared with healthy elderly individuals in a prospective case-controlled survey. Twenty-three patients, aged 60 or over, with features compatible with DSM-IV criteria for primary degenerative dementia of the Alzheimer type were recruited together with 18 cognitively intact age-matched control subjects. Total vitamin B12 (active corrinoids), holo- and apo-haptocorrin

and transcobalamin were measured in serum. B12 analogues (inactive corrinoids) were estimated from the difference between R-binder-determined corrinoids and an intrinsic factor based B12 assay. Alzheimer patients had significantly lower active corrinoid than control subjects and the analogue/corrinoid ratio was significantly higher in the Alzheimer group. The inter-relationship between age, analogues and transcobalamin polarised patients into two distinct groups. Two disparate mechanisms might exist for the development of cerebral B12 deficiency in Alzheimer's disease, although both imply a disruption of selective B12 assimilation and analogue elimination in such patients. Copyright 2001 S. Karger AG, Basel

### **Total serum homocysteine in senile dementia of Alzheimer type.**

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Int J Geriatr Psychiatry 1998 Apr;13(4):235-9

**OBJECTIVE:** The main hypothesis was that subtle vitamin B12 deficiencies occur more commonly in senile dementia of Alzheimer type (SDAT) than in healthy elderly individuals, and may be revealed by elevated total serum homocysteine (tHcy). A subsidiary hypothesis was that such deficiencies would be nutritionally independent as determined by retinol binding protein (RBP).

**DESIGN:** A prospective case-controlled survey.

**SETTING:** A Welsh urban psychogeriatric assessment centre and local general practice.

**PATIENTS:** Thirty patients, aged 65 or over, seen consecutively in 1994 with features compatible with DSM-III-R criteria for primary degenerative dementia of Alzheimer type and 30 cognitively intact age-matched control subjects.

**MEASURES:** Diagnosis was assessed using the CAMDEX. Cognitive scores were evaluated with the CAMCOG scale for patients and MMSE scores for control subjects. tHcy was measured using high performance liquid chromatography (HPLC), and RBP assayed by a radial immunodiffusion method.

**RESULTS:** Patients had a highly significant elevation of tHcy compared with control ( $p < 0.0001$ ). Multiple regression highlighted the interrelated effects of tHcy and total serum cobalamin on cognitive scores. RBP did not differ between groups. Macrocytosis was absent, and neutrophil hypersegmentation uncommon, in hyperhomocysteinaemic patients.

**CONCLUSIONS:** SDAT patients have significantly elevated tHcy. This is independent of RBP determined nutritional status. 'Classical' haematological changes of cobalamin or folate deficiency are poor predictors of tHcy in these patients. Aberrant cobalamin tissue delivery appears to contribute to SDAT

cognitive decline. Relative contributions of other tHcy determinants require further investigation.

### **Homocysteine and cognitive decline in healthy elderly.**

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Dement Geriatr Cogn Disord 2001 Sep-Oct;12(5):309-13

Serum homocysteine is increased, and correlates inversely with cognitive scores, in Alzheimer's disease (AD), vascular dementia and "age-associated memory impairment". Elevated levels might signal accelerated cognitive decline, although this remains to be established. We therefore repeated Mini-Mental State Examinations, together with additional ADAS-Cog assessments, in 32 healthy elderly individuals to determine whether prior homocysteine levels predicted cognitive changes over a 5-year period. Homocysteine predicted follow-up cognitive scores and rate of decline in cognitive performance independently of age, sex, education, renal function, vitamin B status, smoking and hypertension ( $p < 0.001$ ). Homocysteine predicted word recall ( $p = 0.01$ ), orientation ( $p = 0.02$ ) and constructional praxis scores ( $p < 0.0001$ ). One subject, with the second highest initial homocysteine, had developed probable AD at follow-up. Fasting total serum homocysteine appears to be an independent predictor of cognitive decline in healthy elderly and exerts a maximal effect on spatial copying skills. Copyright 2001 S. Karger AG, Basel

### **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 Nutrition 21/AMBI, San Diego, CA, USA.

Med Hypotheses 1999 Nov;53(5):369-74

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.

### **Subnormal serum vitamin B12 and behavioural and psychological symptoms in Alzheimer's disease.**

Meins W, Muller-Thomsen T, Meier-Baumgartner HP Memory-Clinic, Department of Geriatric Medicine, Albertinen Hospital, Hamburg, Germany.

Int J Geriatr Psychiatry 2000 May;15(5):415-8

The objective of this study was to examine whether patients with Alzheimer's disease (AD) with subnormal vitamin B12 levels show more frequent behavioural and psychological symptoms of dementia (BPSD) than AD patients with normal vitamin B12 levels. The design was a prospective case-control study. The study took place at a memory-clinic of a department of geriatric medicine in a teaching hospital. There were seventy-three consecutive outpatients with probable AD, including 61 patients with normal and 12 patients with subnormal (<200 pg/ml) vitamin B12. BPSD were measured using the subscales disturbed behaviour and mood of the Nurses' Observation Scale for Geriatric Patients (NOSGER), the Cornell Scale for Depression and the four criteria for personality change in dementia from the International Classification of Diseases (ICD-10). Controlling for dementia duration and degree of severity of the cognitive deficits, there were significant inverse associations between vitamin B12 status and ICD-10 irritability ( $p=0.045$ ) and NOSGER subscale disturbed behaviour ( $p=0.015$ ). Low vitamin B12 serum levels are associated with BPSD in AD. Vitamin B12 could play a role in the pathogenesis of behavioural changes in AD.

### **Homocysteine and Alzheimer's disease.**

Miller JW. University of California-Davis Medical Center, Department of Medical Pathology, Sacramento 95817, USA.

Nutr Rev 1999 Apr;57(4):126-9

In a recent case-control study of 164 patients with clinically diagnosed Alzheimer's disease (AD), including 76 patients with the AD diagnosis confirmed postmortem, mean total serum homocysteine concentration was found to be significantly higher than that of a control group of elderly individuals with no evidence of cognitive impairment. Because homocysteine is considered an independent risk factor for vascular disease, this finding is consistent with the

emerging hypothesis that vascular disease is a contributing factor in the pathogenesis of AD.

### **Vitamin E and vitamin C supplement use and risk of incident Alzheimer disease.**

Morris MC, Beckett LA, Scherr PA, Hebert LE, Bennett DA, Field TS, Evans DA. Rush Institute for Healthy Aging and Rush Alzheimer's Disease Center, Rush University, Chicago, Illinois, USA.

Alzheimer Dis Assoc Disord 1998 Sep;12(3):121-6

Oxidative stress may play a role in neurologic disease. The present study examined the relation between use of vitamin E and vitamin C and incident Alzheimer disease in a prospective study of 633 persons 65 years and older. A stratified random sample was selected from a disease-free population. At baseline, all vitamin supplements taken in the previous 2 weeks were identified by direct inspection. After an average follow-up period of 4.3 years, 91 of the sample participants with vitamin information met accepted criteria for the clinical diagnosis of Alzheimer disease. None of the 27 vitamin E supplement users had Alzheimer disease compared with 3.9 predicted based on the crude observed incidence among nonusers ( $p = 0.04$ ) and 2.5 predicted based on age, sex, years of education, and length of follow-up interval ( $p = 0.23$ ). None of the 23 vitamin C supplement users had Alzheimer disease compared with 3.3 predicted based on the crude observed incidence among nonusers ( $p = 0.10$ ) and 3.2 predicted adjusted for age, sex, education, and follow-up interval ( $p = 0.04$ ). There was no relation between Alzheimer disease and use of multivitamins. These data suggest that use of the higher-dose vitamin E and vitamin C supplements may lower the risk of Alzheimer disease.

### **Hippocampal perfusion and pituitary-adrenal axis in Alzheimer's disease.**

Murialdo G, Nobili F, Rollero A, Gianelli MV, Copello F, Rodriguez G, Polleri A Department of Endocrinological and Metabolic Sciences, Epidemiology Service, University of Genova, Italy. disem@unige.it

Neuropsychobiology 2000;42(2):51-7

The hippocampus is involved in Alzheimer's disease (AD) and regulates the hypothalamus-pituitary-adrenal axis (HPAA). Enhanced cortisol secretion has been reported in AD. Increased cortisol levels affect hippocampal neuron survival and potentiate beta-amyloid toxicity. Conversely, dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) are believed to antagonize noxious glucocorticoid effects and exert a neuroprotective activity. The present study was aimed at investigating possible correlations between hippocampus perfusion - evaluated by SPECT - and HPAA function in AD. Fourteen patients with AD and 12 healthy age-matched controls were studied by (99m)Tc-HMPAO high-resolution brain SPECT. Plasma adrenocorticotropin, cortisol, and DHEAS levels were determined at 2.00, 8.00, 14.00, 20.00 h in all subjects and their mean values

were computed. Cortisol/DHEAS ratios (C/Dr) were also calculated. Bilateral impairment of SPECT hippocampal perfusion was observed in AD patients as compared to controls. Mean cortisol levels were significantly increased and DHEAS titers were lowered in patients with AD, as compared with controls. C/Dr was also significantly higher in patients. Using a stepwise procedure for dependent SPECT variables, the variance of hippocampal perfusional data was accounted for by mean basal DHEAS levels. Moreover, hippocampal SPECT data correlated directly with mean DHEAS levels, and inversely with C/Dr. These data show a relationship between hippocampal perfusion and HPA function in AD. Decreased DHEAS, rather than enhanced cortisol levels, appears to be correlated with changes of hippocampal perfusion in dementia.

### **Congeners of N(alpha)-acetyl-L-cysteine but not aminoguanidine act as neuroprotectants from the lipid peroxidation product 4-hydroxy-2-nonenal.**

Neely MD, Zimmerman L, Picklo MJ, Ou JJ, Morales CR, Montine KS, Amaranth V, Montine TJ. Departments of Pathology and Pharmacology, and the Center for Molecular Neurosciences, Vanderbilt University Medical Center, Nashville, TN 37232, USA.

Free Radic Biol Med 2000 Nov 15;29(10):1028-36

Increased generation of neurotoxic lipid peroxidation products is proposed to contribute to the pathogenesis of Alzheimer's disease (AD). Current antioxidant therapies are directed at limiting propagation of brain lipid peroxidation. Another approach would be to scavenge the reactive aldehyde products of lipid peroxidation. N(alpha)-acetyl-L-cysteine (NAC) and aminoguanidine (AG) react rapidly and irreversibly with 4-hydroxy-2-nonenal (HNE) in vitro, and both have been proposed as potential scavengers of HNE in biological systems. We have compared NAC, AG, and a series of congeners as scavengers of HNE and as neuroprotectants from HNE. Our results showed that while both NAC and AG had comparable chemical reactivity with HNE, only NAC and its congeners were able to block HNE-protein adduct formation in vitro and in neuronal cultures. Moreover, NAC and its congeners, but not AG, effectively protected brain mitochondrial respiration and neuronal microtubule structure from the toxic effects of HNE. We conclude that NAC and its congeners, but not AG, may act as neuroprotectants from HNE.

### **Could diet be one of the causal factors of Alzheimer's disease?**

Newman PE.

Med Hypotheses 1992 Oct;39(2):123-6

Recent developments show that the brains of persons who have died from Alzheimer's Disease (AD) have a deficiency of Essential Fatty acids in one of the principal classes of phospholipids. It is hypothesized that faulty brain cell membranes resulting from this deficiency may allow passage of an enzyme into the bilayer membrane space which cuts beta amyloid precursor proteins attached

to such cells at a critical intramembrane position releasing a complete sequence of beta amyloid protein into the extracellular space. Beta amyloid protein appears to be the principal active constituent of senile plaques thought to be a probable cause of brain damage resulting in AD. Treatment of persons suffering from AD with desferrioxamine, a trivalent ion chelator to remove aluminium has shown results in slowing the progression of this disease, implicating aluminium and/or other chelated substances in its etiology. Both EFA deficiency and aluminium build-up may be prevented by dietary precautions.

### **Alzheimer's disease revisited.**

Newman PE. Paris, France.

Med Hypotheses 2000 May;54(5):774-6

In a previous paper, it was suggested that a relative deficiency of essential fatty acids might play a role in the etiology of sporadic or non-familial Alzheimer's disease. A recent article regarding dementia in the Rotterdam Study reinforces this suggestion. It is also hypothesized that this relative deficiency could facilitate passage of aluminum into the brain, aluminum being increasingly suggested as one of the possible pathogenic factors in AD. It is further suggested that hypomethylation caused by a deficiency of S-adenosylmethionine might also play a role in the etiology of this disease and perhaps even of Parkinson's disease. Copyright 2000 Harcourt Publishers Ltd.

### **[Analysis of dietary factors in Alzheimer's disease: clinical use of nutritional intervention for prevention and treatment of dementia] [Article in Japanese]**

Otsuka M. Department of Neurology, Jichi Medical School, Omiya Medical Center.

Nippon Ronen Igakkai Zasshi 2000 Dec;37(12):970-3

To determine dietary factors involved in the pathological process of Alzheimer's disease (AD), we analyzed food consumption and intake of nutrients using Self-administered Diet History Questionnaire (DHQ) developed for Japanese. Sixty four AD patients and 80 age-matched healthy subjects were enrolled in this study. AD was diagnosed according to the criteria of DSM-IV. Dietary behaviors of AD patients was markedly deviated from those of age-matched healthy elderly. AD patients disliked fish and green-yellow vegetables and took more meats than controls. Energy-adjusted analysis of nutrients revealed that AD patients took less vitamin C and carotene. Most conspicuously, AD patients took significantly smaller amount of n-3 polyunsaturated fatty acid (PUFA) reflecting low consumption of fish, and their n-6/n-3 ratio was significantly increased. These habits started from 3 months to 44 years before the onset of dementia, suggesting these dietary abnormalities are not merely the consequence of dementia. Rather, it implies that AD might be a life style-related disease such as coronary heart disease, western style diet-associated cancer and hyperallergy. To see if cognitive function was improved by correcting the n-6/n-3 ratio, we prescribed

eicospentaenoic acid (EPA), one type of n-3 PUFA, for AD patients. Cognitive function was evaluated using MMSE. Administration of EPA (900 mg/day) improved MMSE significantly with maximal effects at 3 months and the effects lasted 6 months. However, the score of MMSE decreased after 6 months. The present study showed that nutritional intervention is useful for the prevention of AD, and also for the therapy of dementia, though it has some limitation.

### **Alzheimer beta protein mediated oxidative damage of mitochondrial DNA: prevention by melatonin.**

Pappolla MA, Chyan YJ, Poeggeler B, Bozner P, Ghiso J, LeDoux SP, Wilson GL. University of South Alabama Medical Center, Department of Pathology and Neurology, Mobile 36617, USA. mpappoll@usmail.usouthal.edu

J Pineal Res 1999 Nov;27(4):226-9

Most contemporary progress in Alzheimer's disease (AD) stems from the study of a 42 43 amino acid peptide. called the amyloid beta protein (Abeta), as the main neuropathologic marker of the disorder. It has been demonstrated that Abeta has neurotoxic properties and that such effects are mediated by free-radicals. Exposure of neuronal cells to Abeta results in a spectrum of oxidative lesions that are profoundly harmful to neuronal homeostasis. We had previously shown that Abeta25-35 induces oxidative damage to mitochondrial DNA (mtDNA) and that this modality of injury is prevented by melatonin. Because Abeta25 35 does not occur in AD and because the mode of toxicity by Abeta25-35 may be different from that of Abeta1-42 (the physiologically relevant form of Abeta), we extended our initial observations to determine whether oxidative damage to mtDNA could also be induced by Abeta1-42 and whether this type of injury is prevented by melatonin. Exposure of human neuroblastoma cells to Abeta1-42 resulted in marked oxidative damage to mtDNA as determined by a quantitative polymerase chain reaction method. Addition of melatonin to cell cultures along with Abeta completely prevented the damage. This study supports previous findings with Abeta25-35, including a causative role for Abeta in the mitochondrial oxidative lesions present in AD brains. Most important, the data confirms the neuroprotective role of melatonin in Abeta-mediated oxidative injury. Because melatonin also inhibits amyloid aggregation, lacks toxicity, and efficiently crosses the blood-brain barrier, this hormone appears superior to other available antioxidants as a candidate for pharmacologic intervention in AD.

### **Acetylcholine in mind: a neurotransmitter correlate of consciousness?**

Perry E, Walker M, Grace J, Perry R. MRC Neurochemical Pathology Unit, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne, UK NE4 6BE.

Trends Neurosci 1999 Jun;22(6):273-80

The cholinergic system is one of the most important modulatory neurotransmitter systems in the brain and controls activities that depend on selective attention,

which are an essential component of conscious awareness. Psychopharmacological and pathological evidence supports the concept of a 'cholinergic component' of conscious awareness. Drugs that antagonize muscarinic receptors induce hallucinations and reduce the level of consciousness, while the nicotinic receptor is implicated as being involved in the mechanism of action of general (inhalational) anaesthetics. In degenerative diseases of the brain, alterations in consciousness are associated with regional deficits in the cholinergic system. In Alzheimer's disease (AD), there is a loss of explicit (more than implicit) memory and hypoactivity of cholinergic projections to the hippocampus and cortex, while the visual hallucinations experienced by subjects with Dementia with Lewy bodies (DLB) are associated with reductions in neocortical ACh-related activity. In Parkinson's disease, the additional loss of pedunculopontine cholinergic neurones, which control REM (rapid eye movement) sleep or dreaming, is likely to contribute to REM abnormalities, which also occur in DLB. Widespread basal-forebrain and rostral brainstem cholinergic pathways, which include converging projections to the thalamus, appear to be located strategically for generating and integrating conscious awareness. Alleviation of a range of cognitive and non-cognitive symptoms by drugs that modulate the cholinergic system, which are being developed for the treatment of AD and related disorders, could be caused by changes in consciousness.

#### **Effects of physostigmine and lecithin on memory in Alzheimer disease.**

Peters BH, Levin HS.

Ann Neurol 1979 Sep;6(3):219-21

Because there is evidence that central cholinergic mechanisms are depleted in dementia, we studied the effects of central cholinergic augmentation on the memory of 5 patients with Alzheimer disease. Patients received placebo, lecithin, physostigmine, or lecithin plus physostigmine in a double-blind study using titrated doses of the acetylcholinesterase inhibitor physostigmine. Memory was evaluated with alternate forms of the selective reminding procedure. Compared with lecithin alone, the combination of physostigmine and lecithin consistently enhanced memory storage and retrieval; physostigmine without lecithin produced no memory facilitation. The strategy of combining a cholinergic agonist and precursor holds promise, although a larger clinical trial is needed.

#### **Medicinal plants and Alzheimer's disease: from ethnobotany to phytotherapy.**

Perry EK, Pickering AT, Wang WW, Houghton PJ, Perry NS Medical Research Council, Newcastle General Hospital, Newcastle upon Tyne. e.k.perry@ncl.ac.uk

J Pharm Pharmacol 1999 May;51(5):527-34

The use of complementary medicines, such as plant extracts, in dementia therapy varies according to the different cultural traditions. In orthodox Western medicine, contrasting with that in China and the Far East for example,

pharmacological properties of traditional cognitive- or memory-enhancing plants have not been widely investigated in the context of current models of Alzheimer's disease. An exception is *Ginkgo biloba* in which the ginkgolides have antioxidant, neuroprotective and cholinergic activities relevant to Alzheimer's disease mechanisms. The therapeutic efficacy of Ginkgo extracts in Alzheimer's disease in placebo controlled clinical trials is reportedly similar to currently prescribed drugs such as tacrine or donepezil and, importantly, undesirable side effects of Ginkgo are minimal. Old European reference books, such as those on medicinal herbs, document a variety of other plants such as *Salvia officinalis* (sage) and *Melissa officinalis* (balm) with memory-improving properties, and cholinergic activities have recently been identified in extracts of these plants. Precedents for modern discovery of clinically relevant pharmacological activity in plants with long-established medicinal use include, for example, the interaction of alkaloid opioids in *Papaver somniferum* (opium poppy) with endogenous opiate receptors in the brain. With recent major advances in understanding the neurobiology of Alzheimer's disease, and as yet limited efficacy of so-called rationally designed therapies, it may be timely to re-explore historical archives for new directions in drug development. This article considers not only the value of an integrative traditional and modern scientific approach to developing new treatments for dementia, but also in the understanding of disease mechanisms. Long before the current biologically-based hypothesis of cholinergic derangement in Alzheimer's disease emerged, plants now known to contain cholinergic antagonists were recorded for their amnesia- and dementia-inducing properties.

**Acetyl-L-carnitine physical-chemical, metabolic, and therapeutic properties: relevance for its mode of action in Alzheimer's disease and geriatric depression.**

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Mol Psychiatry 2000 Nov;5(6):616-32

Acetyl-L-carnitine (ALCAR) contains carnitine and acetyl moieties, both of which have neurobiological properties. Carnitine is important in the beta-oxidation of fatty acids and the acetyl moiety can be used to maintain acetyl-CoA levels. Other reported neurobiological effects of ALCAR include modulation of: (1) brain energy and phospholipid metabolism; (2) cellular macromolecules, including neurotrophic factors and neurohormones; (3) synaptic morphology; and (4) synaptic transmission of multiple neurotransmitters. Potential molecular mechanisms of ALCAR activity include: (1) acetylation of -NH<sub>2</sub> and -OH functional groups in amino acids and N terminal amino acids in peptides and proteins resulting in modification of their structure, dynamics, function and turnover; and (2) acting as a molecular chaperone to larger molecules resulting in a change in the structure, molecular dynamics, and function of the larger molecule. ALCAR is reported in double-blind controlled studies to have beneficial effects in major depressive disorders and Alzheimer's disease (AD), both of which are highly prevalent in the geriatric population.

### **In-vivo glutathione elevation protects against hydroxyl free radical-induced protein oxidation in rat brain.**

Pocernich CB, La Fontaine M, Butterfield DA. Department of Chemistry, University of Kentucky, Lexington 40506, USA.

Neurochem Int 2000 Mar;36(3):185-91

Glutathione deficiency has been associated with a number of neurodegenerative diseases including Lou Gehrig's disease, Parkinson's disease, and HIV. A crucial role for glutathione is as a free radical scavenger. Alzheimer's disease (AD) brain is characterized by oxidative stress, manifested by protein oxidation, lipid oxidation, oxidized glutathione, and decreased activity of glutathione S-transferase, among others. Reasoning that elevated levels of endogenous glutathione would offer protection against free radical-induced oxidative stress, rodents were given in vivo injections of N-acetylcysteine (NAC), a known precursor of glutathione, to study the vulnerability of isolated synaptosomal membranes treated with Fe<sup>2+</sup>/H<sub>2</sub>O<sub>2</sub>, a known hydroxyl free radical producer. Protein carbonyls, a marker of protein oxidation, were measured. NAC significantly increased endogenous glutathione levels in cortical synaptosome cytosol (P < 0.01). As reported previously, protein carbonyl levels of the Fe<sup>2+</sup>/H<sub>2</sub>O<sub>2</sub>-treated synaptosomes were significantly higher compared to that of non-treated controls (P < 0.01), consistent with increased oxidative stress. In contrast, protein carbonyl levels in Fe<sup>2+</sup>/H<sub>2</sub>O<sub>2</sub>-treated synaptosomes isolated from NAC-injected animals were not significantly different from saline-injected non-treated controls, demonstrating protection against hydroxyl radical induced oxidative stress. These results are consistent with the notion that methods to increase endogenous glutathione levels in neurodegenerative diseases associated with oxidative stress, including AD, may be promising.

### **Cognitive deficit induced by acute tryptophan depletion in patients with Alzheimer's disease.**

Porter RJ, Lunn BS, Walker LL, Gray JM, Ballard CG, O'Brien JT. Academic Department of Psychiatry, University of Newcastle upon Tyne, England.

Am J Psychiatry 2000 Apr;157(4):638-40

**OBJECTIVE:** The study assessed the effects on global cognitive function and mood of a reduction of brain serotonin by means of acute tryptophan depletion in 16 patients with dementia of the Alzheimer type and in 16 cognitively intact comparison subjects.

**METHOD:** In a double-blind, crossover design, subjects received a tryptophan-free amino acid drink to induce acute tryptophan depletion and, on a separate occasion, a placebo drink containing a balanced mixture of amino acids. On each occasion, ratings of depressed mood were made at baseline and 4 and 7 hours later, and the Modified Mini-Mental State was administered at baseline and 4 hours later.

**RESULTS:** Patients with dementia of the Alzheimer type had a significantly lower mean score on the Modified Mini-Mental State after acute tryptophan depletion than after receiving placebo. The comparison group showed no difference in mean score on the Modified Mini-Mental State after acute tryptophan depletion and after receiving placebo. No significant changes in mood were found in either group.

**CONCLUSIONS:** Acute tryptophan depletion significantly impaired cognitive function in patients with dementia of the Alzheimer type. Compromised serotonergic function, in combination with cholinergic deficit, may make an important contribution to cognitive decline in dementia of the Alzheimer type.

**Toxic effects of beta-amyloid(25-35) on immortalised rat brain endothelial cell: protection by carnosine, homocarnosine and beta-alanine.**

Preston JE, Hipkiss AR, Himsworth DT, Romero IA, Abbott JN. Institute of Gerontology, King's College London, UK. j.preston@kcl.ac.uk

Neurosci Lett 1998 Feb 13;242(2):105-8

The effect of a truncated form of the neurotoxin beta-amyloid peptide (A beta25-35) on rat brain vascular endothelial cells (RBE4 cells) was studied in cell culture. Toxic effects of the peptide were seen at 200 microg/ml A beta using a mitochondrial dehydrogenase activity (MTT) reduction assay, lactate dehydrogenase release and glucose consumption. Cell damage could be prevented completely at 200 microg/ml A beta and partially at 300 microg/ml A beta, by the dipeptide carnosine. Carnosine is a naturally occurring dipeptide found at high levels in brain tissue and innervated muscle of mammals including humans. Agents which share properties similar to carnosine, such as beta-alanine, homocarnosine, the anti-glycating agent aminoguanidine, and the antioxidant superoxide dismutase (SOD), also partially rescued cells, although not as effectively as carnosine. We postulate that the mechanism of carnosine protection lies in its anti-glycating and antioxidant activities, both of which are implicated in neuronal and endothelial cell damage during Alzheimer's disease. Carnosine may therefore be a useful therapeutic agent.

**Melatonin as a pharmacological agent against neuronal loss in experimental models of Huntington's disease, Alzheimer's disease and parkinsonism.**

Reiter RJ, Cabrera J, Sainz RM, Mayo JC, Manchester LC, Tan DX. Department of Cellular and Structural Biology, University of Texas Health Science Center, San Antonio 78229-3900, USA. Reiter@uthscsa.edu

Ann N Y Acad Sci 1999;890:471-85

This review summarizes the experimental findings related to the neuroprotective role of melatonin. In particular, it focuses on research directed at models of Huntington's disease, Alzheimer's disease and Parkinsonism. Melatonin has been shown to be highly effective in reducing oxidative damage in the central nervous

system; this efficacy derives from its ability to directly scavenge a number of free radicals and to function as an indirect antioxidant. In particular, melatonin detoxifies the highly toxic hydroxyl radical as well as the peroxy radical, peroxynitrite anion, nitric oxide, and singlet oxygen, all of which can damage macromolecules in brain cells. Additionally, melatonin stimulates a variety of antioxidative enzymes including superoxide dismutase, glutathione peroxidase and glutathione reductase. One additional advantage melatonin has in reducing oxidative damage in the central nervous system is the ease with which it crosses the blood-brain barrier. This combination of actions makes melatonin a highly effective pharmacological agent against free radical damage. The role of physiological levels of melatonin in forestalling oxidative damage in the brain is currently being tested.

### **Nutritional status of free-living Alzheimer's patients.**

Renvall MJ, Spindler AA, Ramsdell JW, Paskvan M. Department of Medicine, University of California Medical Center, San Diego 92103-1990.

Am J Med Sci 1989 Jul;298(1):20-7

Self-reported, dietary intake and biochemical estimates of thiamine, riboflavin, folate, vitamin B-12, protein, and iron were compared in 22, free-living elders by individuals who had senile dementia of the Alzheimer's type (SDAT) and in 41 who were cognitively normal (CN). The two groups did not differ significantly in their intake of these nutrients or the number of deficiency states for intake (less than 67% RDA). Low serum transketolase (thiamin;  $p$  less than 0.055), red blood cell (RBC) folate ( $p$  less than 0.06), and serum vitamin B-12 ( $p$  less than 0.05) levels occurred more often in SDAT patients than in CN subjects. Individuals in both groups who used multivitamin supplements had significantly higher biochemical values for thiamine ( $p$  less than 0.03), riboflavin ( $p$  less than 0.01), and vitamin B-12 ( $p$  less than 0.003) than nonsupplement users. Because of the differences in vitamin B-12 and RBC folate levels between groups, a retrospective analysis was performed on a larger group of subjects drawn from a geriatric assessment clinic. Patients with SDAT had significantly lower serum vitamin B-12 ( $p$  less than 0.01) and lower RBC folate ( $p$  less than 0.03) values than CN subjects. Which mean values for vitamin B-12 and RBC folate were grouped by degree of impairment in SDAT subjects, vitamin B-12 was significantly lower in mildly and moderately impaired subjects than in those with normal cognition. Mean values for both nutrients did not differ significantly between severely impaired and CN subjects. There was a significant quadratic relationship between cognitive impairment and biochemical values for vitamin B-12. (ABSTRACT TRUNCATED AT 250 WORDS)

### **Low plasma vitamin C in Alzheimer patients despite an adequate diet.**

Riviere S, Birlouez-Aragon I, Nourhashemi F, Vellas B. Hopital La Grave-Casselard, Toulouse, France.

Int J Geriatr Psychiatry 1998 Nov;13(11):749-54

**OBJECTIVE:** To compare the vitamin C and E plasma levels in patients with Alzheimer's disease (AD) and to assess the vitamin C intake and nutritional status.

**DESIGN:** Case-control study. Four groups of sex- and age-matched subjects were compared: severe AD and moderate AD, in patients with moderate AD and controls.

**SETTING:** Community and hospitalized patients in the region of Toulouse, France.

**PARTICIPANTS:** Patients with dementia who fulfilled criteria for Alzheimer's disease: severe Alzheimer group (N = 20), Mini-Mental State Examination (MMSE) score range 0-9; moderate Alzheimer group (N = 24), MMSE 10-23; hospitalized Alzheimer group (N = 9), MMSE 10-23. Control group (N = 19), MMSE 24-30.

**MEASURES:** Plasma vitamin E and C were quantified by HPLC-fluorescence. Consumption of raw and cooked fruit and vegetables was evaluated in order to determine the mean vitamin C intakes. Mini Nutritional Assessment (MNA) and plasma albumin were used to measure nutritional status.

**RESULTS:** Institutionalized and community subjects were analysed separately. MNA scores were normal in home-living Alzheimer subjects with moderate dementia and significantly lower in those with severe disease, despite normal plasma albumin levels. In the home-living Alzheimer subjects, vitamin C plasma levels decreased in proportion to the severity of the cognitive impairment despite similar vitamin C intakes, whereas vitamin E remained stable. The hospitalized Alzheimer subjects had lower MNA scores and albumin levels but normal vitamin C intakes, but their plasma vitamin C was lower than that of community-living subjects. Institutionalized Alzheimer subjects had significantly lower MNA scores but normal vitamin C and albumin levels and vitamin C intakes compared with community-dwelling subjects of similar degree of cognitive impairment.

**CONCLUSION:** Plasma vitamin C is lower in AD in proportion to the degree of cognitive impairment and is not explained by lower vitamin C intake. These results support the hypothesis that oxygen-free radicals may cause damage.

**A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer ' s disease. The Alzheimer ' s Disease Cooperative Study**

Sano M; Ernesto C; Thomas RG; Klauber MR; Schafer K; Grundman M; Woodbury P; Growdon J; Cotman CW; Pfeiffer E; Schneider LS; Thal LJ  
Department of Neurology, Columbia University College of Physicians and Surgeons, New York, USA.

N Engl J Med (United States) Apr 24 1997, 336 (17) p1216-22

**BACKGROUND:** There is evidence that medications or vitamins that increase the levels of brain catecholamines and protect against oxidative damage may reduce the neuronal damage and slow the progression of Alzheimer's disease.

**METHODS:** We conducted a double-blind, placebo-controlled, randomized, multicenter trial in patients with Alzheimer's disease of moderate severity. A total of 341 patients received the selective monoamine oxidase inhibitor selegiline (10 mg a day), alpha-tocopherol (vitamin E, 2000 IU a day), both selegiline and alpha-tocopherol, or placebo for two years. The primary outcome was the time to the occurrence of any of the following: death, institutionalization, loss of the ability to perform basic activities of daily living, or severe dementia (defined as a Clinical Dementia Rating of 3).

**RESULTS:** Despite random assignment, the baseline score on the Mini-Mental State Examination was higher in the placebo group than in the other three groups, and this variable was highly predictive of the primary outcome ( $P < 0.001$ ). In the unadjusted analyses, there was no statistically significant difference in the outcomes among the four groups. In analyses that included the base-line score on the Mini-Mental State Examination as a covariate, there were significant delays in the time to the primary outcome for the patients treated with selegiline (median time, 655 days;  $P=0.012$ ), alpha-tocopherol (670 days,  $P=0.001$ ) or combination therapy (585 days,  $P=0.049$ ), as compared with the placebo group (440 days).

**CONCLUSIONS:** In patients with moderately severe impairment from Alzheimer's disease, treatment with selegiline or alpha-tocopherol slows the progression of disease.

**Tyrosine hydroxylase, tryptophan hydroxylase, biopterin, and neopterin in the brains of normal controls and patients with senile dementia of Alzheimer type.**

Sawada M, Hirata Y, Arai H, Iizuka R, Nagatsu T.

J Neurochem 1987 Mar;48(3):760-4

The activities of tyrosine hydroxylase and tryptophan hydroxylase, and the concentrations of the biopterin cofactor and the precursor neopterin were measured in 14 regions of postmortem brains from four histologically verified patients of senile dementia of the Alzheimer type (SDAT) and eight histologically normal controls. Neopterin concentrations were measured in the human brain for the first time. The activities of tyrosine hydroxylase and tryptophan hydroxylase in the brains of patients with SDAT were significantly reduced in the substantia nigra and in the lateral segment of the globus pallidus, locus ceruleus, and substantia nigra, respectively. The concentrations of total biopterin in the brains of patients with SDAT were significantly reduced in the putamen and substantia nigra, but the total neopterin concentrations did not change significantly. These results suggest that the reduction in biogenic amines in SDAT might be related to reductions in biosynthetic enzymes associated with biogenic amines, due to destruction of monoaminergic neurons.

### **Phenolic antioxidants attenuate neuronal cell death following uptake of oxidized low-density lipoprotein.**

Schroeter H, Williams RJ, Matin R, Iversen L, Rice-Evans CA. Wolfson Centre for Age-Related Diseases, Guy's, King's, and St. Thomas's School of Biomedical Sciences, King's College, Guy's Campus, London, England.

Free Radic Biol Med 2000 Dec 15;29(12):1222-33

Oxidative stress is implicated in neuronal loss associated with neurodegeneration such as in Parkinson's disease, Alzheimer's disease and age-related cognitive decline. Recent reports indicate that the consumption of flavonoid-rich fruits partly reverses the age-related neuronal and cognitive decline. In this study, cultured striatal neurons were exposed to oxidized lipids in the form of low-density lipoprotein (oxLDL) as a model for the induction of oxidative injury, and the abilities of phenolic antioxidants, flavonoids and hydroxycinnamic acid derivatives, to attenuate this neuronal damage were examined. OxLDL was demonstrated to enter neuronal cells and to be capable of eliciting neurotoxicity in a dose- and time-dependent manner, inducing DNA fragmentation and cell lysis. Flavonoids exert protective effects, which appear to be related to specific structural characteristics, particularly relevant being those defining their reduction potentials and partition coefficients. In summary, these data suggest a possible role for flavonoids in reducing neurodegeneration associated with chronic disorders in which oxidative stress is implicated.

### **CSF-folate levels are decreased in late-onset AD patients.**

Serot JM, Christmann D, Dubost T, Bene MC, Faure GC. Laboratoire d'Immunologie, GRIP, JE DRED 251, Faculte de Medecine, UHP, Nancy, France. faure@grip.u-nancy.fr

J Neural Transm 2001;108(1):93-9

Folates are involved in the cerebral metabolism of cobalamine, methionine, L-tyrosine and acetylcholine. Remarkably CSF-folate levels are 3 to 4 times higher than blood-folate levels. To reach the brain, folates are actively transported by choroid plexus (CP) as well as vitamins B6, B12, C and E. Epithelial atrophy having been reported in aging and in Alzheimer's disease (AD), we measured the CSF folate-levels of 126 patients, including 30 AD consecutive patients to evaluate whether CP functions of folate-transport were impaired. CSF-folate concentrations did not vary with age (10.47 +/- 1.93ng/ml between 20 and 60 years; 9.96 +/- 2.01 ng/ml in elderly control patients older than 60 years of age,  $p > 0.05$ ) while late-onset AD patients had significantly lower CSF-folate levels (8.26 +/- 1.82 ng/ml,  $p < 0.001$ ). These data support a specific alteration of CP transport function in AD patients.

### **The pathogenesis of Alzheimer's disease.**

Small GW. Department of Psychiatry and Biobehavioral Sciences, University of California at Los Angeles School of Medicine, and the Veterans Affairs Medical Center, USA.

J Clin Psychiatry 1998;59 Suppl 9:7-14

Despite consensus on clinical and neuropathologic definitions of Alzheimer's disease, limited information is available on its causes and pathogenesis. Current data suggest interactions among the various possible biological and environmental influences that result in a common pathway leading to the disease. Biological influences include genetic mutations causing the disease phenotype and polymorphisms contributing to disease risk. Alterations in immune or inflammatory responses may also represent biological influences. Various environmental influences that may interact with endogenous biological factors include education, traumatic injury, oxidative stress, drugs, and hormone replacement. The author describes some recent findings that suggest possible pathogenic mechanisms, which may eventually have important treatment implications.

#### **Vitamin E for Alzheimer's disease.**

Tabet N, Birks J, Grimley Evans J. Old Age Psychiatry, The Maudsley Hospital, Denmark Hill, London, UK, SE5 8AZ. N.Tabet@iop.kcl.ac.uk

Cochrane Database Syst Rev 2000;(4):CD002854

**BACKGROUND:** Vitamin E is a dietary compound that functions as an antioxidant scavenging toxic free radicals. Evidence that free radicals may contribute to the pathological processes in Alzheimer's disease has led to interest in the use of vitamin E in the treatment of this disorder.

**OBJECTIVES:** To examine the effects of vitamin E treatment for people with Alzheimer's disease.

**SEARCH STRATEGY:** The Cochrane Dementia Group Register of Clinical Trials was searched with the following terms: vitamin E, Alzheimer's disease, dementia, alpha-tocopherol, cognitive impairment, cognitive function and controlled trials. The latest search was carried out in July 2000.

**SELECTION CRITERIA:** All unconfounded, double blind, randomized trials in which treatment with vitamin E at any dose was compared with placebo for patients with Alzheimer's disease.

**DATA COLLECTION AND ANALYSIS:** Two reviewers independently applied the selection criteria and assessed study quality. One reviewer extracted and analysed the data. For each outcome measure data were sought on every patient randomized. Where such data were not available an analysis of patients who completed treatment was conducted.

**MAIN RESULTS:** Only one study was identified which met the inclusion criteria (Sano 1997). The primary outcome used in this study of 341 participants was survival time to the first of 4 endpoints, death, institutionalisation, loss of 2 out of 3 basic activities of daily living, or severe dementia, defined as a global Clinical Dementia Rating of 3. The investigators reported the total numbers in each group who reached the primary endpoint within two years for participants completing the study ("completers"). There appeared to be some benefit from vitamin E with fewer participants reaching endpoint - 58% (45/77) of completers compared with 74% (58/78) - a Peto odds ratio of 0.49, 95% confidence interval 0.25 to 0.96. However, more participants taking vitamin E suffered a fall (12/77 compared with 4/78; odds ratio 3.07, 95% CI 1.09 to 8.62). It was not possible to interpret the reported results for specific endpoints or for secondary outcomes of cognition, dependence, behavioural disturbance and activities of daily living.

**REVIEWER'S CONCLUSIONS:** There is insufficient evidence of efficacy of vitamin E in the treatment of people with with Alzheimer's disease. The one published trial of acceptable methodology (Sano 1997) was restricted to patients with moderate disease, and the published results are difficult to interpret. There is sufficient evidence of possible benefit to justify further studies. There was an excess of falls in the vitamin E group compared with placebo which requires further evaluation.

**Huperzine A (shuangyiping): a promising drug for Alzheimer's disease.**

Tang XC. Department of Pharmacology, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, China.

Zhongguo Yao Li Xue Bao 1996 Nov;17(6):481-4

Hup A, a novel alkaloid isolated from Chinese herb *Huperzia serrata*, is a potent and selective inhibitor of AChE, with a rapid absorption and penetration into the brain in experimental animals. The inhibition is reversible with a longer duration of action. Hup A exhibited memory-enhancing activities in a broad range of animal cognitive model. Compared to Phy, Tac, and Gal, Hup A has better therapeutic indices, and peripheral cholinergic side effects are minimal at therapeutic doses. These findings suggest that Hup A is a promising candidate for clinical development as a symptomatic treatment for AD.

**A 1-year controlled trial of acetyl-l-carnitine in early-onset AD.**

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Neurology 2000 Sep 26;55(6):805-10

**OBJECTIVE:** To determine the efficacy of acetyl-l-carnitine (ALCAR) on the rate of decline in early-onset AD patients.

**METHODS:** A 1-year, multicenter, double-blind, placebo-controlled, randomized trial was conducted. Subjects were 45 to 65 years old, with a diagnosis of probable AD according to National Institute of Neurological Communicative Disorders-Alzheimer's Disease and Related Disorders Association criteria and had a Mini-Mental State Examination (MMSE) score between 12 and 26. They were treated with ALCAR (1 g tid) or placebo. Primary outcome measures were the Alzheimer's Disease Assessment Scale-Cognitive Component and the Clinical Dementia Rating Scale. Secondary measures included the ADAS Non-Cognitive Subscale, the MMSE, an Activities of Daily Living Scale (ADL), and a Clinician-Based Impression of Change (CIBIC).

**RESULTS:** Two-hundred twenty-nine patients were enrolled and randomized to drug treatment, with 117 taking placebo and 112 taking ALCAR. There were no significant differences between the two groups at baseline. For the primary outcome measures, there were no significant differences between the treatment groups on the change from baseline to endpoint in the intent-to-treat analysis. In the completer sample only, there was less deterioration in the MMSE for the ALCAR-treated subjects. There was no difference in rate of decline on the CIBIC and the ADL scale. There were no significant differences in the incidence of adverse events by treatment arm.

**CONCLUSION:** Overall, in a prospectively performed study in young-onset AD patients, ALCAR failed to slow decline. Less decline was seen on the MMSE in the completer sample only, with the difference being mediated by reducing decline in attention. A combination of ALCAR and a cholinesterase inhibitor should be tested for additivity.

### **Oral physostigmine and lecithin improve memory in Alzheimer disease.**

Thal LJ, Fuld PA, Masur DM, Sharpless NS.

Ann Neurol 1983 May;13(5):491-6

Eight patients with early Alzheimer disease were treated with gradually increasing multiple daily doses of oral physostigmine and supplemental lecithin. Six individuals showed improvement in total recall and retrieval from long-term storage (LTR), with a decrease in intrusions (a measure of inaccurate recall). The optimal individual dose was either 2.0 or 2.5 mg of physostigmine for each responding patient. Results of this open trial were subsequently replicated during a double-blind crossover trial comparing physostigmine treatment to placebo. All six patients again demonstrated improvement in total recall and LTR, with a decrease in intrusions. The decrease in intrusions was strongly correlated with increasing inhibition of cholinesterase activity in cerebrospinal fluid, suggesting that the degree of improvement in the patient's memory was related to the amount of physostigmine that reached the brain. Other neurotransmitters and metabolites in cerebrospinal fluid were unaffected by the physostigmine therapy, suggesting a specific effect of physostigmine on the cholinergic system. The results suggest that small oral doses of physostigmine combined with lecithin ingestion have therapeutic benefit for some patients with Alzheimer disease.

### **Interactions between carnosine and zinc and copper: implications for neuromodulation and neuroprotection.**

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Biochemistry (Mosc) 2000 Jul;65(7):807-16

This review examines interactions in the mammalian central nervous system (CNS) between carnosine and the endogenous transition metals zinc and copper. Although the relationship between these substances may be applicable to other brain regions, the focus is on the olfactory system where these substances may have special significance. Carnosine is not only highly concentrated in the olfactory system, but it is also contained in neurons (in contrast to glia cells in most of the brain) and has many features of a neurotransmitter. Whereas the function of carnosine in the CNS is not well understood, we review evidence that suggests that it may act as both a neuromodulator and a neuroprotective agent. Although zinc and/or copper are found in many neuronal pathways in the brain, the concentrations of zinc and copper in the olfactory bulb (the target of afferent input from sensory neurons in the nose) are among the highest in the CNS. Included in the multitude of physiological roles that zinc and copper play in the CNS is modulation of neuronal excitability. However, zinc and copper also have been implicated in a variety of neurologic conditions including Alzheimer's disease, Parkinson's disease, stroke, and seizures. Here we review the modulatory effects that carnosine can have on zinc and copper's abilities to influence neuronal excitability and to exert neurotoxic effects in the olfactory system. Other aspects of carnosine in the CNS are reviewed elsewhere in this issue.

### **Vitamin E supplementation prevents spatial learning deficits and dendritic alterations in aged apolipoprotein E-deficient mice.**

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Eur J Neurosci 2000 Dec;12(12):4541-6

Recent studies have suggested that altered function of apolipoprotein E might lead to Alzheimer's disease via oxidative stress. In this context, the objective of this study was to determine if antioxidative treatment with vitamin E was neuroprotective in apolipoprotein E-deficient mice. For this purpose, 1-month-old control and apolipoprotein E-deficient mice received dietary vitamin E for 12 months. We showed that, compared to apolipoprotein E-deficient mice who received a regular diet, mice treated with vitamin E displayed a significantly improved behavioural performance in the Morris water maze. This improved performance was associated with preservation of the dendritic structure in vitamin E-treated apolipoprotein E-deficient mice. In addition, whilst untreated apolipoprotein E-deficient mice displayed increased levels of lipid peroxidation

and glutathione, vitamin E-treated mice showed near normal levels of both lipid peroxidation and glutathione. These results support the contention that vitamin E prevents the age-related neurodegenerative alterations in apolipoprotein E-deficient mice.

**The action of acetyl-L-carnitine on the neurotoxicity evoked by amyloid fragments and peroxide on primary rat cortical neurones.**

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Ann N Y Acad Sci 2001 Jun;939:162-78

The amyloid beta-peptides have been implicated in the excitotoxic mechanism of neuronal injury in the pathogenesis of Alzheimer's disease. In this paper we examine the effect of different amyloid fragments (beta A1-40, A1-28, and A25-35), as well as potential neuroprotective compounds on rat cortical neuron viability. Exposure of neurones to beta A25-35 or A1-40 at concentrations as low as 1 microgram/ml inhibited, significantly, the MTT response and this level of inhibition was similar after 24-h or three-day exposure. Furthermore, the level of inhibition was not affected by the presence or absence of 5% horse serum in the medium. Preexposure (10 min) of neurones to ALC at concentrations of 0.1, 1, 5, and 10 mM attenuated the inhibition of the MTT response caused by beta A25-35 (50 micrograms/ml) in serum free medium for 24 h. The treatment of cells with vitamin E (100 microM), catalase (4 mg/ml), NGF (0.1 and 10 ng/ml), or cycloheximide (0.1 microgram/ml) significantly restored the MTT response that was inhibited by beta A25-35. The mechanism for the protective actions of these compounds against beta A25-35 toxicity is not clear but may involve free radical scavenger action and preservation of energy production, although other mechanisms, especially for ALC, such as a direct effect on A-beta interaction with charged anionic phospholipids and/or stabilizing action on membranes, are also possible.

**Vitamin B(12) and folate in relation to the development of Alzheimer's disease.**

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Neurology 2001 May 8;56(9):1188-94

**OBJECTIVE:** To explore the associations of low serum levels of vitamin B(12) and folate with AD occurrence.

**METHODS:** A population-based longitudinal study in Sweden, the Kungsholmen

**PROJECT:** A random sample of 370 nondemented persons, aged 75 years and older and not treated with B(12) and folate, was followed for 3 years to detect incident AD cases. Two cut-off points were used to define low levels of vitamin B(12) (< or =150 and < or =250 pmol/L) and folate (< or =10 and < or =12 nmol/L), and all analyses were performed using both definitions. AD and other types of dementia were diagnosed by specialists according to DSM-III-R criteria.

**RESULTS:** When using B(12) < or =150 pmol/L and folate < or =10 nmol/L to define low levels, compared with people with normal levels of both vitamins, subjects with low levels of B(12) or folate had twice higher risks of developing AD (relative risk [RR] = 2.1, 95% CI = 1.2 to 3.5). These associations were even stronger in subjects with good baseline cognition (RR = 3.1, 95% CI = 1.1 to 8.4). Similar relative risks of AD were found in subjects with low levels of B(12) or folate and among those with both vitamins at low levels. A comparable pattern was detected when low vitamin levels were defined as B(12) < or =250 pmol/L and folate < or =12 nmol/L.

**CONCLUSIONS:** This study suggests that vitamin B(12) and folate may be involved in the development of AD. A clear association was detected only when both vitamins were taken into account, especially among the cognitively intact subjects. No interaction was found between the two vitamins. Monitoring serum B(12) and folate concentration in the elderly may be relevant for prevention of AD.

### **Tryptophan degradation and immune activation in Alzheimer's disease.**

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J Neural Transm 2000;107(3):343-53

Alzheimer's disease (AD) is likely associated with systemic immune activation. During immune response, interferon-gamma stimulates indoleamine 2,3-dioxygenase (IDO) converting tryptophan to N-formylkynurenine followed by kynurenine in an ensuing step. Thus, IDO activity is estimated by the kynurenine per tryptophan quotient (Kyn/Trp). In 21 patients suffering from AD, in 20 controls of similar age, and in 49 blood donors we measured serum tryptophan and kynurenine concentrations by HPLC. Lower tryptophan concentrations were found in elderly control subjects compared to blood donors (62.1 vs. 73.0 microM,  $p < 0.005$ ). Tryptophan concentrations tended to be still lower in AD patients (54.4 microM,  $p = 0.07$ ) compared to elderly controls. Enhanced tryptophan degradation in patients was reflected by significantly increased Kyn/Trp (46.1 vs. 34.1 in elderly controls,  $p < 0.05$ ). Correlations were found in patients between Kyn/Trp and concentrations of soluble immune markers in serum, i.e., neopterin, interleukin-2 receptor and tumor necrosis factor receptor (all  $p < 0.001$ ). Increased Kyn/Trp was associated with reduced cognitive performance. Tryptophan degradation due to immune activation may exert impact on the pathogenesis of AD.

## **The efficacy of ginkgo for elderly people with dementia and age-associated memory impairment: new results of a randomized clinical trial**

van Dongen MC, van Rossum E, Kessels AG, Sielhorst HJ, Knipschild PG  
Department of Epidemiology, Maastricht University, The Netherlands. [Record supplied by publisher]

J Am Geriatr Soc 2000 Oct;48(10):1183-94

**OBJECTIVES:** To evaluate the efficacy, the dose-dependence, and the durability of the effect of the ginkgo biloba special extract EGb 761 (ginkgo) in older people with dementia or age-associated memory impairment.

**DESIGN:** A 24-week, randomized, double-blind, placebo-controlled, parallel-group, multicenter trial.

**SETTING:** Homes for the elderly in the southern part of the Netherlands.

**PARTICIPANTS:** Older persons with dementia (either Alzheimer's dementia or vascular dementia; mild to moderate degree) or age-associated memory impairment (AAMI). 214 Participants were recruited from 39 homes for the elderly.

**INTERVENTION:** The participants were allocated randomly to treatment with EGb 761 (2 tablets per day, total dosage either 240 (high dose) or 160 (usual dose) mg/day) or placebo (0 mg/d). The total intervention period was 24 weeks. After 12 weeks of treatment, the initial ginkgo users were randomized once again to either continued ginkgo treatment or placebo treatment. Initial placebo use was prolonged after 12 weeks.

**MEASUREMENTS:** Outcomes were assessed after 12 and 24 weeks of intervention. Outcome measures included neuropsychological testing (trail-making speed (NAI-ZVT-G), digit memory span (NAI-ZN-G), and verbal learning (NAI-WL)), clinical assessment (presence and severity of geriatric symptoms (SCAG), depressive mood (GDS), self-perceived health and memory status (report marks)), and behavioral assessment (self-reported level of instrumental daily life activities).

**RESULTS:** An intention-to-treat analysis showed no effect on each of the outcome measures for participants who were assigned to ginkgo (n = 79) compared with placebo (n = 44) for the entire 24-week period. After 12 weeks of treatment, the combined high dose and usual dose ginkgo groups (n = 166) performed slightly better with regard to self-reported activities of daily life but slightly worse with regard to self-perceived health status compared with the placebo group (n = 48). No beneficial effects of a higher dose or a prolonged duration of ginkgo treatment were found. We could not detect any subgroup that benefited from ginkgo. Ginkgo use was also not associated with the occurrence of (serious) adverse events.

CONCLUSIONS: The results of our trial suggest that ginkgo is not effective as a treatment for older people with mild to moderate dementia or age-associated memory impairment. Our results contrast sharply with those of previous ginkgo trials.

**Cholinesterase inhibitors and Ginkgo extracts--are they comparable in the treatment of dementia? Comparison of published placebo-controlled efficacy studies of at least six months' duration.**

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Phytomedicine 2000 Jan;6(6):393-401

The efficacy of four cholinesterase inhibitors (tacrine, donepezil, rivastigmine, metrifonate) and Ginkgo special extract EGb 761 in Alzheimer's disease were compared. The differences in the effects of the active substance and placebo on cognition were measured on the ADAS-Cog scale, taking into account the different degrees of dementia in the various studies and the dropout rate due to adverse drug reactions. Efficacy, expressed as the delay in symptom progression or the difference in response rate between active substance and placebo, showed no major differences between the four cholinesterase inhibitors and the Ginkgo special extract. Only tacrine exhibited a high dropout rate due to adverse drug reactions. In view of this, the subject of new prescriptions should be critically reviewed. Second-generation cholinesterase inhibitors (donepezil, rivastigmine, metrifonate) and Ginkgo special extract EGb 761 should be considered equally effective in the treatment of mild to moderate Alzheimer's dementia.

**Protective effects of idebenone and alpha-tocopherol on beta-amyloid-(1-42)-induced learning and memory deficits in rats: implication of oxidative stress in beta-amyloid-induced neurotoxicity in vivo.**

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Eur J Neurosci 1999 Jan;11(1):83-90

Amyloid beta-peptide (A beta), the major constituent of the senile plaques in the brains of patients with Alzheimer's disease, is cytotoxic to neurons and has a central role in the pathogenesis of the disease. Previous studies have suggested that oxidative stress is involved in the mechanisms of A beta-induced neurotoxicity in vitro. In the present study, we examined whether oxidative stress contributes to learning and memory deficits caused by continuous intracerebroventricular infusion of A beta-(1-42). In the A beta-(1-42)-infused rats, spontaneous alternation behaviour in a Y-maze and spatial memory in a water maze task were significantly impaired, as compared with A beta-(40-1)-infused control rats. The retention of passive avoidance learning was also significantly impaired by treatment with A beta-(1-42). Potent antioxidants idebenone and alpha-tocopherol prevented the behavioural deficits in Y-maze and

water maze, but not passive avoidance, tasks in A beta-(1-42)-infused rats when they were repeatedly administered by mouth once a day from 3 days before the start of A beta infusion to the end of behavioural experiments. Lipid peroxide levels in the hippocampus and cerebral cortex of A beta-(1-42)-infused rats did not differ from those in control animals, and neither idebenone nor alpha-tocopherol affected the lipid peroxide levels. These results suggest that treatment with antioxidants such as idebenone and alpha-tocopherol prevents learning and memory deficits caused by A beta.

**Free radicals and lipid peroxidation do not mediate beta-amyloid-induced neuronal cell death.**

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Brain Res 1999 Nov 20;847(2):203-10

"beta Amyloid (Abeta)-induced free radical-mediated neurotoxicity" is a leading hypothesis as a cause of Alzheimer's disease (AD). Abeta increased free radical production and lipid peroxidation in PC12 nerve cells, leading to increased 4-hydroxy-2-nonenal (HNE) production and modification of specific mitochondrial target proteins, apoptosis and cell death. Pretreatment of the cells with isolated ginkgolides, the anti-oxidant component of Ginkgo biloba leaves, or vitamin E, prevented the Abeta-induced increase of reactive oxygen species (ROS). Ginkgolides, but not vitamin E, inhibited the Abeta-induced HNE modification of mitochondrial proteins. However, treatment with these anti-oxidants did not rescue the cells from Abeta-induced apoptosis and cell death. These results indicate that free radicals and lipid peroxidation may not mediate Abeta-induced neurotoxicity.

**The Ginkgo biloba extract EGb 761 rescues the PC12 neuronal cells from beta-amyloid-induced cell death by inhibiting the formation of beta-amyloid-derived diffusible neurotoxic ligands.**

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Brain Res 2001 Jan 19;889(1-2):181-90

beta Amyloid (Abeta) treatment induced free radical production and increased glucose uptake, apoptosis and cell death in PC12 nerve cells. Addition of the standardized extract of Ginkgo biloba leaves, EGb 761 together with the Abeta protein prevented, in a dose-dependent manner, the Abeta-induced free radical production, increased glucose uptake, apoptosis and cell death. However, pretreatment of the cells with EGb 761 did not rescue the cells from the Abeta-induced toxicity although it prevented the Abeta-induced reactive oxygen species generation. Moreover, the terpene and flavonoid-free EGb 761 extract, HE 208,

although inhibited the Abeta-induced increased glucose uptake, it failed to protect the cells from apoptosis and cytotoxicity induced by Abeta. In conclusion, these results indicate that the terpenoid and flavonoid constituents of EGb 761, acting probably in combination with components present in HE 208, are responsible for rescuing the neuronal cells from Abeta-induced apoptosis and cell death; their mechanism of action being distinct of their antioxidant properties. Because pre- and post-treatment with EGb 761 did not protect the cells from Abeta-induced neurotoxicity, we examined whether EGb 761 interacts directly with Abeta. Indeed, in vitro reconstitution studies demonstrated that EGb 761 inhibits, in a dose-dependent manner, the formation of beta-amyloid-derived diffusible neurotoxic soluble ligands (ADDLs), suggested to be involved in the pathogenesis of Alzheimer's disease.

**Alzheimer's amyloid beta-peptide associated free radicals increase rat embryonic neuronal polyamine uptake and ornithine decarboxylase activity: protective effect of vitamin E.**

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Neurosci Lett 1999 Mar 19;263(1):17-20

Recent evidence indicates that alterations in brain polyamine metabolism may be critical for nerve cell survival after a free radical initiated neurodegenerative process. It has been shown previously that A beta(1-42) and A beta(25-35) are toxic to neurons through a free radical dependent oxidative mechanism. Treatment of rat embryonic hippocampal neuronal cultures with A beta-peptides increased ornithine decarboxylase (ODC) activity and spermidine uptake, suggesting that oxidative stress upregulates the polyamine mechanism for the repair of free radical damage. Pretreatment of the cells with vitamin E prior to A beta exposure decreased ODC activity and spermidine uptake to control level. This study is the first to demonstrate that A beta treated cells show an increased polyamine metabolism in response to free radical mediated oxidative stress and that the free radical scavenger vitamin E prevents these attenuations. These results are discussed with reference to Alzheimer's disease.

**Essential fatty acids preparation (SR-3) improves Alzheimer's patients quality of life.**

Yehuda S, Rabinovtz S, Carasso RL, Mostofsky DI. Department of Psychology Bar-Ilan University, Ramat Gan, Israel.

Int J Neurosci 1996 Nov;87(3-4):141-9

In a number of previous reports we showed the salutary effects on rats of SR-3, a compound comprising a 1:4 ratio of n-3 and n-6 fatty acids. Improvements were noted in learning tasks, thermoregulation, recovery from neurotoxins, and seizure protection. Because we were impressed that these effects are related to changes in

membrane fluidity and neuronal functioning and because Alzheimer's Disease is also associated with lipid defects, we undertook a short term (4 week) double blind study with 100 Alzheimer patients (60 received SR-3 and 40 in a placebo control). The results indicated improvements in mood, cooperation, appetite, sleep, ability to navigate in the home, and short term memory. Overall improvement was reported for 49 patients, and in no case did a guardian report adverse effects to the compound. While not uniform or permanent, and while no mode of action for SR-3 can be precisely identified at this time, the promising results in quality of life for the patient and caregiver warrant further clinical trials and continued basic research into the neuropsychological substrate of the disease and its response to SR-3.

### **Essential fatty acids and the brain: possible health implications.**

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Int J Dev Neurosci 2000 Jul-Aug;18(4-5):383-99

Linoleic and alpha-linolenic acid are essential for normal cellular function, and act as precursors for the synthesis of longer chained polyunsaturated fatty acids (PUFAs) such as arachidonic (AA), eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), which have been shown to partake in numerous cellular functions affecting membrane fluidity, membrane enzyme activities and eicosanoid synthesis. The brain is particularly rich in PUFAs such as DHA, and changes in tissue membrane composition of these PUFAs reflect that of the dietary source. The decline in structural and functional integrity of this tissue appears to correlate with loss in membrane DHA concentrations. Arachidonic acid, also predominant in this tissue, is a major precursor for the synthesis of eicosanoids, that serve as intracellular or extracellular signals. With aging comes a likely increase in reactive oxygen species and hence a concomitant decline in membrane PUFA concentrations, and with it, cognitive impairment. Neurodegenerative disorders such as Parkinson's and Alzheimer's disease also appear to exhibit membrane loss of PUFAs. Thus it may be that an optimal diet with a balance of n-6 and n-3 fatty acids may help to delay their onset or reduce the insult to brain functions which these diseases elicit.

## 4. Anemia Thrombocytopenia Leukopenia

Preventative and curative options include:

Methylcobalamin, folic acid, iron, zinc, selenium, copper, fish oil, vitamin K, DHEA, nettle leaf extract, melatonin, multinutrient supplements, Vitamin A.

### **Anemia caused by vitamin B12 deficiency in subjects aged over 75 years: new hypotheses. A study of 20 cases. [Article in French]**

Andres E, Perrin AE, Kraemer JP, Goichot B, Demengeat C, Ruellan A, Grunenberger F, Constantinesco A, Schlienger JL. Service de medecine interne et nutrition, hopital de HautePierre, Strasbourg, France.

Rev Med Interne 2000 Nov;21(11):946-54

**PURPOSE:** New hypotheses have recently been developed on vitamin B12 deficiency and the frequently observed occurrence in the elderly subject of food cobalamin malabsorption, i.e., the non-dissociation of B12 and its carrier protein (ND B12), and the possibility of rectifying this imbalance by oral crystalline B12 supplementation. The aim of this study was therefore to confirm these hypotheses in a series of patients aged over 75 years with anemia due to B12 deficiency.

**METHODS:** A retrospective study was carried out over a 5-year period on patients aged over 75 years presenting with megaloblastic anemia (hemoglobin [Hb] < 12 g/dL) and vitamin B12/cobalamin deficiency (B12 < 160 pg/mL).

**RESULTS:** Twenty cases were analyzed. The average age of the patient population was 82.5 +/- 6 years, and the F/M sex ratio was 1:2. Mean Hb levels were 7.9 +/- 2.4 g/dL, mean serum B12 levels were 83 +/- 24 pg/mL, and mean homocysteinemic levels were 35 +/- 27 mumol/L. The diagnosis was as follows: food cobalamin malabsorption/ND B12 (n = 10), Biermer's disease/pernicious anemia (n = 5), malabsorption due to pancreatic insufficiency (n = 1), and low dietary B12 levels (n = 1). Disorders associated with ND B12 were: atrophic gastritis and Helicobacter pylori infection (n = 6), antacid or biguanide intake (n = 3), alcohol abuse (n = 2), or idiopathic syndrome (n = 2). In the patients who were followed up (n = 10), i.m. (n = 5) or oral (n = 5) administration of crystalline B12 resulted in the correction of hematological abnormalities.

**CONCLUSION:** In the elderly subject, food cobalamin/ND B12 malabsorption appears to be the main cause of B12 deficiency, and is frequently associated with atrophic gastritis. In these cases, administration of oral crystalline B12 may be an efficient means of treating this disorder.

### **Vitamin B12 deficiency in the elderly.**

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Annu. Rev. Nutr. 1999. 19:357-377

**KEY WORDS:** atrophic gastritis, hypochlorhydria, malabsorption of protein-bound vitamin B12, food fortification Vitamin B12 deficiency is estimated to affect 10%-15% of people over the age of 60, and the laboratory diagnosis is usually based on low serum vitamin B12 levels or elevated serum methylmalonic acid and homocysteine levels. Although elderly people with low vitamin B12 status frequently lack the classical signs and symptoms of vitamin B12 deficiency, e.g. megaloblastic anemia, precise evaluation and treatment in this population is important. Absorption of crystalline vitamin B12 does not decline with advancing age. However, compared with the younger population, absorption of protein-bound vitamin B12 is decreased in the elderly, owing to a high prevalence of atrophic gastritis in this age group. Atrophic gastritis results in a low acid-pepsin secretion by the gastric mucosa, which in turn results in a reduced release of free vitamin B12 from food proteins. Furthermore, hypochlorhydria in atrophic gastritis results in bacterial overgrowth of the stomach and small intestine, and these bacteria may bind vitamin B12 for their own use. The ability to absorb crystalline vitamin B12 remains intact in older people with atrophic gastritis. The 1998 recommended daily allowance for vitamin B12 is 2.4 µg, but elderly people should try to obtain their vitamin B12 from either supplements or fortified foods (e.g. fortified ready-to-eat breakfast cereals) to ensure adequate absorption from the gastrointestinal tract. Because the American food supply is now being fortified with folic acid, concern is increasing about neurologic exacerbation in individuals with marginal vitamin B12 status and high-dose folate intake.

### **The role of copper, molybdenum, selenium, and zinc in nutrition and health.**

Chan S, Gerson B, Subramaniam S. Quest Diagnostics Incorporated Nichols Institute, San Juan Capistrano, California, USA.

Clin Lab Med 1998 Dec;18(4):673-85

Copper, zinc, selenium, and molybdenum are involved in many biochemical processes supporting life. The most important of these processes are cellular respiration, cellular utilization of oxygen, DNA and RNA reproduction, maintenance of cell membrane integrity, and sequestration of free radicals. Copper, zinc, and selenium are involved in destruction of free radicals through cascading enzyme systems. Superoxide radicals are reduced to hydrogen peroxide by superoxide dismutases in the presence of copper and zinc cofactors. Hydrogen peroxide is then reduced to water by the selenium-glutathione peroxidase couple. Efficient removal of these superoxide free radicals maintains the integrity of membranes, reduces the risk of cancer, and slows the aging process. On the other hand, excess intake of these trace elements leads to disease and toxicity; therefore, a fine balance is essential for health. Trace element-deficient patients usually present with common symptoms such as malaise, loss of appetite, anemia,

infection, skin lesions, and low-grade neuropathy, thus complicating the diagnosis. Symptoms for intoxication by trace elements are general, for example, flu-like and CNS symptoms, fever, coughing, nausea, vomiting, diarrhea, anemia, and neuropathy. A combination of observation, medical and dietary history, and analyses for multiple trace elements is needed to pinpoint the trace element(s) involved. Serum, plasma, and erythrocytes may be used for the evaluation of copper and zinc status, whereas only serum or plasma is recommended for selenium. Whole blood is preferred for molybdenum. When trace element levels are inconsistent with medical evaluations, a test for activity of the suspected enzyme(s) would support the differential diagnosis. Furthermore, it is important to differentiate whether trace element deficiency or toxicity is the primary cause of the disorder, or is secondary to other underlying diseases. Only successful treatment of the primary disorder will lead to complete recovery. In the event of sample contamination during collection or analysis, the physician may be misled by falsely elevated results. Royal blue top evacuated tubes containing negligibly low concentrations of the trace element or acid-washed plastic sterilized syringes should be used for blood, serum, or plasma collection. Powdered gloves must be avoided. When possible, mineral supplements are not to be administered to the patient for a minimum of 3 days prior to sample collection. Serum and plasma specimens are to be transported in acid-washed polypropylene and polyethylene tubes. Analysis is performed in a controlled environment to minimize or eliminate contamination. During analysis, all laboratory wares should be acid-washed for decontamination. A detailed description of these precautions may be found in reviews by Aitio and Jarvisalo and by Chan and Gerson. Copper and zinc analysis on serum and plasma are commonly performed by flame atomic absorption spectrometry, inductively coupled plasma-atomic emission spectrometry, and inductively coupled plasma-mass spectrometry. Serum and plasma selenium levels are determined by graphite furnace atomic absorption with Zeeman background correction and neutron activation analysis. Molybdenum levels are best determined by neutron activation and highly sensitive inductively coupled plasma-mass spectrometry. The reader is referred to reviews by Tsalev and Jarvis.

**The omega-3 fatty acid docosahexaenoate reduces cytokine-induced expression of proatherogenic and proinflammatory proteins in human endothelial cells.**

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Arterioscler Thromb 1994 Nov;14(11):1829-36

The mechanisms by which dietary fatty acids can modulate atherogenesis and inflammation are poorly understood. Induction in endothelial cells of adhesion molecules for circulating leukocytes and of inflammatory mediators by cytokines probably contributes to the early phases of atherogenesis and inflammation. We report here that incorporation into cellular lipids of docosahexaenoic acid (DHA), a specific fatty acid of the omega 3 family, decreases cytokine-induced expression of endothelial leukocyte adhesion molecules, secretion of inflammatory

mediators, and leukocyte adhesion to cultured endothelial cells. DHA, but not eicosapentaenoic acid, decreased in a dose- and time-dependent fashion the expression of vascular cell adhesion molecule 1 (VCAM-1) induced by interleukin (IL)-1, tumor necrosis factor (TNF), IL-4, or bacterial lipopolysaccharide, with half-maximum inhibition at & 10  $\mu\text{mol/L}$ . This reduction required prolonged (24- to 96-hour) exposure of endothelial cells to DHA and correlated with the degree of DHA incorporation into cellular lipids. DHA also limited cytokine-stimulated endothelial cell expression of E-selectin and intercellular adhesion molecule 1 and the secretion of IL-6 and IL-8 into the medium but not the surface expression of constitutive surface molecules. Cyclooxygenase inhibition did not block the effect of DHA on VCAM-1. In parallel with reduced surface VCAM-1 protein expression, DHA reduced VCAM-1 mRNA induction by IL-1 or TNF. DHA treatment also reduced the adhesion of human monocytes and of monocytic U937 cells to cytokine-stimulated endothelial cells. These properties of DHA may contribute to antiatherogenic and anti-inflammatory effects of omega 3 fatty acids.

### **Anti-inflammatory effect of warfarin and vitamin K1.**

Eichbaum FW, Slemer O, Zyngier SB.

Naunyn Schmiedebergs Arch Pharmacol 1979 Jun 18;307(2):185-90

1. Sodium warfarin, given by oral or by parenteral route, displays a pronounced anti-inflammatory effect in the formaldehyde and carrageenan induced rat paw edema. This effect becomes patent not only when the warfarin application precedes the local injection of the irritant substance (prophylactic effect), but also when it is given to animals with already developed inflammatory reactions (therapeutic effect). 2. The active doses of Na warfarin lie between 0.5 and 5.0 mg/kg. Smaller as well as higher doses show a reduced anti-inflammatory effect. 3. A marked anti-inflammatory effect can be noted already 90 min after drug injection at a still normal prothrombin level. 4. Vitamin K1 (phylloquinone), given by oral or parenteral route, in doses from 1.6 mg/kg upwards, shows a marked anti-inflammatory effect both in the prophylactic and the therapeutic rat paw test. Vitamin K3 is devoid of any anti-inflammatory activity. 5. The anti-inflammatory effect of both sodium warfarin and of vitamin K1 in rats, is not interfered with by previous adrenalectomy.

### **Congenital dyschromia with erythrocyte, platelet and tryptophan metabolism abnormalities.**

Foldes C, Wallach D, Launay JM, Chirio R. Department of Dermatology, Hopital Saint-Louis, Paris, France.

J Am Acad Dermatol 1988 Oct;19(4):642-55

The case of a female child with a unique generalized congenital dyschromia is reported. She had hypopigmented skin, with hypomelanosis and hypomelanocytosis, and many pigmented macules, which consisted of epidermal

and dermal hypermelanosis without hypermelanocytosis. Biochemical investigations revealed normal catecholamine metabolism but abnormal tryptophan metabolism, including a decrease in blood serotonin and melatonin. A slight platelet storage pool disease was demonstrated, and a recurrent megaloblastic folate-related anemia occurred. The possible relationship between the pigmentary disease and the biochemical abnormalities is discussed. We suggest that this case represents a previously undescribed association of dyschromia, erythrocyte, platelet, and tryptophan metabolism abnormalities.

### **Melatonin prevents oxidative stress resulting from iron and erythropoietin administration.**

Herrera J, Nava M, Romero F, Rodriguez-Iturbe B. Renal Service and Laboratory, Hospital Universitario de Maracaibo and Instituto de Investigaciones Biomedicas, Fundacite-Zulia, Maracaibo, Venezuela.

Am J Kidney Dis 2001 Apr;37(4):750-7

Intravenous iron (Fe) and recombinant human erythropoietin (rHuEPO) are routine treatments in the management of anemia in patients with chronic renal failure. We investigated the oxidative stress acutely induced by these therapies and whether pretreatment with oral melatonin (MEL) would have a beneficial effect. Nine patients (four women) were studied within 1 month of entering a chronic hemodialysis program in the interdialytic period. Plasma malondialdehyde (MDA), red blood cell glutathione (GSH), and catalase (CAT) activity were measured in blood samples obtained before (baseline) and 1, 3, and 24 hours after the administration of Fe (100 mg of Fe saccharate intravenously over 1 hour) or rHuEPO (4,000 U intravenously). One hour before these treatments, patients were administered a single oral dose of MEL (0.3 mg/kg) or placebo. Each patient was studied on four occasions, corresponding to studies performed using either placebo or MEL in association with intravenous Fe and rHuEPO administration. Baseline data showed increased oxidative stress in patients with end-stage renal failure. Increments in oxidative stress induced by Fe were more pronounced at the end of the administration: MDA, baseline, 0.74 +/- 0.09 nmol/mL; 1 hour, 1.50 +/- 0.28 nmol/mL (< 0.001); GSH, baseline, 2.51 +/- 0.34 nmol/mg of hemoglobin (Hb); 1 hour, 1.66 +/- 0.01 nmol/mg Hb (< 0.001); and CAT activity, baseline, 27.0 +/- 5.7 kappa/mg Hb; 1 hour, 23.3 +/- 4.2 kappa/mg Hb (< 0.001). rHuEPO-induced increments in oxidative stress were more pronounced (< 0.001) at 3 hours (MDA, 1.24 +/- 0.34 nmol/mL; GSH, 1.52 +/- 0.23 nmol/mg Hb; CAT activity, 18.0 +/- 3.1 kappa/mg Hb). MEL administration prevented the changes induced by Fe and rHuEPO and had no adverse side effects. These studies show that intravenous Fe and rHuEPO in doses commonly used to treat anemia in chronic hemodialysis patients acutely generate significant oxidative stress. Oral MEL prevents such oxidative stress and may be of clinical use.

### **Effect of all-trans and 9-cis retinoic acid on growth and metastasis of xenotransplanted canine osteosarcoma cells in athymic mice.**

Hong SH, Kadosawa T, Mochizuki M, Matsunaga S, Nishimura R, Sasaki N. Pediatrics Oncology Branch, Division of Clinical Sciences, National Cancer Institute, Bethesda, MD 20892, USA.

Am J Vet Res 2000 Oct;61(10):1241-4

**OBJECTIVE:** To determine effects of all-trans and 9-cis retinoic acid (RA) on tumor growth and metastatic ability of canine osteosarcoma cells transplanted into athymic (nude) mice. **ANIMALS:** Forty-five 5-week-old female BALB/c nude mice.

**PROCEDURE:**  $1 \times 10^7$  POS osteosarcoma cells were transplanted subcutaneously into the intrascapular region of mice. All-trans RA (3 or 30 microg/kg of body weight in 0.1 ml of sesame oil), 9-cis RA (3 or 30 mg/kg in 0.1 ml of sesame oil), or sesame oil (0.1 ml; control treatment) were administered intragastrically 5 d/wk for 4 weeks beginning 3 days after transplantation (n = 4 mice/group) or after formation of a palpable tumor (5 mice/group). Tumor weight was estimated weekly by measuring tumor length and width, and retinoid toxic effects were evaluated daily. Two weeks after the final treatment, mice were euthanatized, and number of mice with pulmonary metastases was determined.

**RESULTS:** Adverse treatment effects were not detected. Tumor weight was less in mice treated with either dose of 9-cis RA than in control mice, although this difference was not significant. Treatment with 30 mg of 9-cis RA/kg initiated after tumor formation significantly reduced the incidence of pulmonary metastasis, compared with the control group.

**CONCLUSIONS AND CLINICAL RELEVANCE:** 9-cis RA decreased the incidence of pulmonary metastasis in nude mice transplanted with canine osteosarcoma cells and may be a potential adjunct therapy for treatment of osteosarcoma in dogs.

### **Dehydroepiandrosterone selectively inhibits production of tumor necrosis factor alpha and interleukin-6 [correction of interlukin-6] in astrocytes.**

Kipper-Galperin M, Galilly R, Danenberg HD, Brenner T. Laboratory of Neuroimmunology, Hadassah University Hospital, Jerusalem, Israel.

Int J Dev Neurosci 1999 Dec;17(8):765-75

Dehydroepiandrosterone (DHEA) is a native neurosteroid with immunomodulating activity. DHEA effectively protects animals from several viral, bacterial and parasitic infections and it was suggested that its age-associated decline is related with immunosenescence. In the present study we examined the ability of DHEA to inhibit the production of inflammatory mediators by mycoplasma-stimulated glial cells and to change the course of acute central nervous system (CNS) inflammatory disease in vivo. Addition of DHEA (10 microg/ml) markedly inhibited tumor necrosis factor alpha (TNFalpha) and interleukin-6 (IL-6) production (98 and 95%, respectively), whereas nitric oxide

(NO) and prostaglandin E2 (PGE2) production was not affected. However, daily administration of 0.5 mg DHEA to mice or 5 mg to rats did not change the clinical outcome of experimental autoimmune encephalomyelitis (EAE).

### **Meta-analysis of efficacy and tolerability data on iron protein succinylate in patients with iron deficiency anemia of different severity.**

Kopcke W, Sauerland MC. Institut für Medizinische Informatik und Biomathematik, Westfälische Wilhelms-Universität Münster, Germany.

Arzneimittelforschung 1995 Nov;45(11):1211-6

Iron protein succinylate (ITF 282, CAS 93615-44-2) is an iron derivative for the oral treatment of iron deficiency anemia. Its efficacy and tolerability have been proved in about 1800 patients, enrolled in 3 multicenter clinical trials. The first aim of this meta-analysis is to verify the increase of hemoglobin (Hb) in these patients (891 treated with ITF282, 644 treated with iron sulphate and 236 treated with iron-polystyrene sulphonate). The 3 studies show homogeneous Hb increases. ITF 282 appeared to provide, from time 0 to the 30th day of treatment, a similar or lesser increase in Hb in comparison to the reference drugs, while from the 30th day of treatment to the 60th day its efficacy was always greater than that of the reference medications. The data have been further analyzed by subdividing the patients in three classes, according to the severity of the anemia: basal Hb < or = 11 g/dl, < g/dl. During the 60-day treatment, both ITF 282 and the reference drugs induced the most significant increase in Hb in the patients affected by the most severe anemia. The meta-analytic evaluation of the 3 trials results has been extended to tolerability data. Most side effects were related to the gastrointestinal tract. Their incidence resulted significantly lower for ITF 282 than that for the reference drugs (9.4% vs. 20.4%, < 0.01). The comparative sub-analysis of the side effect distribution into the patients populations shows that ITF 282 is definitely better tolerated in pregnant women (relative risk 0.321, < 0.01). The time course of Hb increases and the tolerability data suggest a different mechanism by which ITF 282 and the reference drugs are effective. Since the main difference between ITF 282 and the reference drugs is the form in which the iron is presented to the gastrointestinal mucosa, it may be supposed that the reference drugs, providing free divalent iron ions for absorption, could induce some kind of irritative condition of the gastrointestinal mucosa, which results in a reduced long-term absorption capacity, as well as in a higher incidence of gastroenteric adverse events. ITF 282, providing protein-bound iron, would not permit the process supposed with divalent iron, thus resulting in prolonged absorption capacity (that is higher hemoglobin recovery) and higher gastrointestinal tolerability.

### **Is there a role for melatonin in supportive care?**

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Support Care Cancer 2002 Mar;10(2):110-6

Melatonin (MLT) is the main hormone released from the pineal gland and has proved to have physiological antitumor activity. MLT has been shown to exert anticancer activity through several biological mechanisms: antiproliferative action, stimulation of anticancer immunity, modulation of oncogene expression, and anti-inflammatory, anti-oxidant and anti-angiogenic effects. Several experimental studies have shown that MLT may inhibit cancer cell growth, and preliminary clinical studies seem to confirm its anticancer property in humans. In addition, MLT may have other biological effects, which could be useful in the palliative therapy of cancer, namely anticachectic, anti-asthenic and thrombopoietic activities. On this basis, the present clinical investigation was performed in an attempt at better definition of the therapeutic properties of MLT in human neoplasms. In a first clinical study, we evaluated the effects of MLT in a group of 1,440 patients with untreatable advanced solid tumors, who received supportive care alone or supportive care plus MLT. In a second study, we evaluated the influence of MLT on the efficacy and toxicity of chemotherapy in a group of 200 metastatic patients with chemotherapy-resistant tumor histotype, who were randomized to receive chemotherapy alone or chemotherapy plus MLT. In both studies, MLT was given orally at 20 mg/day during the dark period of the day. The frequency of cachexia, asthenia, thrombocytopenia and lymphocytopenia was significantly lower in patients treated with MLT than in those who received supportive care alone. Moreover, the percentage of patients with disease stabilization and the percentage 1-year survival were both significantly higher in patients concomitantly treated with MLT than in those treated with supportive care alone. The objective tumor response rate was significantly higher in patients treated with chemotherapy plus MLT than in those treated with chemotherapy alone. Moreover, MLT induced a significant decline in the frequency of chemotherapy-induced asthenia, thrombocytopenia, stomatitis, cardiotoxicity and neurotoxicity. These clinical results demonstrate that the pineal hormone MLT may be successfully administered in medical oncology in the supportive care of untreatable advanced cancer patients and for the prevention of chemotherapy-induced toxicity.

### **Immunotherapy with subcutaneous low-dose interleukin-2 plus melatonin as salvage therapy of heavily chemotherapy-pretreated ovarian cancer.**

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Oncol. Rep. 1996; 3(5): 947-9.

Preliminary results showed that IL-2 immunotherapy may be effective in the treatment of recurring advanced ovarian cancer. The pineal neurohormone melatonin (MLT) has been proven to amplify IL-2 efficacy by counteracting macrophage-mediated immunosuppression. On this basis, a pilot phase II study of low-dose IL-2 plus MLT was performed in advanced ovarian cancer patients progressing after at least 3 previous polychemotherapeutic lines. The study included 12 evaluable patients. IL-2 was injected subcutaneously at 3 million IU/day for 6 days/week for 4 weeks, by repeating the cycle after a 21-day rest

period in nonprogressing patients, MLT was given orally at 40 mg/day. No complete response was seen. A partial response was achieved in 2/12 (16%) patients. A stable disease was obtained in 5 other patients, whereas the remaining 5 patients progressed. The treatment was well tolerated. This preliminary study suggests that immunotherapy with low-dose IL-2 plus MLT may represent a well tolerated and promising therapy of advanced ovarian cancer progressing on standard medical treatments.

**Efficacy of the concomitant administration of the pineal hormone melatonin in cancer immunotherapy with low-dose IL-2 in patients with advanced solid tumors who had progressed on IL-2 alone.**

Lissoni P, Barni S, Cazzaniga M, Ardizzioia A, Rovelli F, Brivio F, Tancini G. Division of Radiation Oncology, San Gerardo Hospital, Monza, Italy.

Oncology 1994 Jul-Aug;51(4):344-7

Our preliminary studies in humans have shown that the pineal neurohormone melatonin (MLT) may enhance the antitumor activity of IL-2, by confirming the existence of a neuroendocrine control on cytokine effects. On this basis, a study was started to evaluate the influence of a concomitant administration of MLT and low-dose IL-2 in cancer patients, who had progressed during a previous immunotherapy with IL-2 alone. The study included 14 patients with advanced solid tumors (lung 6; kidney 4; stomach 2; liver 1; melanoma 1). IL-2 was given at a daily dose of 3 million IU s.c. for 6 days/week for 4 weeks. MLT was given orally at a daily dose of 40 mg every day, starting 7 days prior to IL-2. Objective tumor regression, consisting of a partial remission (PR), was achieved in 3/14 (21%) patients (lung 1; kidney 1; liver 1). Six other patients had a stable disease (SD), while the remaining 5 cases progressed. PR and SD were associated either with a significantly longer survival at 1 year, or with a significantly higher increase in lymphocyte and eosinophil mean number with respect to the patients with disease progression. This preliminary study suggests that advanced solid neoplasms resistant to IL-2 may become responsive to IL-2 therapy by a concomitant administration of the pineal hormone MLT, which could act by enhancing IL-2 antitumor immune effect and/or by increasing the susceptibility of cancer cells to the cytotoxicity mediated by IL-2-induced cytotoxic lymphocytes.

**A randomized study of chemotherapy with cisplatin plus etoposide versus chemoendocrine therapy with cisplatin, etoposide and the pineal hormone melatonin as a first-line treatment of advanced non-small cell lung cancer patients in a poor clinical state.**

Lissoni P; Paolorossi F; Ardizzioia A; Barni S; Chilelli M; Mancuso M; Tancini G; Conti A; Maestroni GJ Divisione di Radioterapia Oncologica, Ospedale S, Gerardo, Monza, Milan, Italy.

J Pineal Res (Denmark) Aug 1997, 23 (1) p15-9

Recent studies suggest that the pineal hormone melatonin may reduce chemotherapy -induced immune and bone marrow damage. In addition, melatonin may exert potential oncostatic effects either by stimulating host anticancer immune defenses or by inhibiting tumor growth factor production. On this basis, we have performed a randomized study of chemotherapy alone vs. chemotherapy plus melatonin in advanced non-small cell lung cancer patients (NSCLC) with poor clinical status. The study included 70 consecutive advanced NSCLC patients who were randomized to receive chemotherapy alone with cisplatin (20 mg/m<sup>2</sup>/day i.v. for 3 days) and etoposide (100 mg/m<sup>2</sup>/day i.v. for 3 days) or chemotherapy plus melatonin (20 mg/day orally in the evening). Cycles were repeated at 21-day intervals. Clinical response and toxicity were evaluated according to World Health Organization criteria. A complete response (CR) was achieved in 1/34 patients concomitantly treated with melatonin and in none of the patients receiving chemotherapy alone. Partial response (PR) occurred in 10/34 and in 6/36 patients treated with or without melatonin, respectively. Thus, the tumor response rate was higher in patients receiving melatonin (11/34 vs. 6/35), without, however, statistically significant differences. The percent of 1-year survival was significantly higher in patients treated with melatonin plus chemotherapy than in those who received chemotherapy alone (15/34 vs. 7/36, < 0.05). Finally, chemotherapy was well tolerated in patients receiving melatonin, and in particular the frequency of myelosuppression, neuropathy, and cachexia was significantly lower in the melatonin group. This study shows that the concomitant administration of melatonin may improve the efficacy of chemotherapy, mainly in terms of survival time, and reduce chemotherapeutic toxicity in advanced NSCLC, at least in patients in poor clinical condition.

### **Treatment of cancer chemotherapy-induced toxicity with the pineal hormone melatonin.**

Lissoni P, Tancini G, Barni S, Paolorossi F, Ardizzoia A, Conti A, Maestroni G. Division of Radiation Oncology, S. Gerardo Hospital, Monza (Milan), Italy.

Support Care Cancer 1997 Mar;5(2):126-9

Experimental data have suggested that the pineal hormone melatonin (MLT) may counteract chemotherapy-induced myelosuppression and immunosuppression. In addition, MLT has been shown to inhibit the production of free radicals, which play a part in mediating the toxicity of chemotherapy. A study was therefore performed in an attempt to evaluate the influence of MLT on chemotherapy toxicity. The study involved 80 patients with metastatic solid tumors who were in poor clinical condition (lung cancer: 35; breast cancer: 31; gastrointestinal tract tumors: 14). Lung cancer patients were treated with cisplatin and etoposide, breast cancer patients with mitoxantrone, and gastrointestinal tract tumor patients with 5-fluorouracil plus folates. Patients were randomised to receive chemotherapy alone or chemotherapy plus MLT (20 mg/day p.o. in the evening). Thrombocytopenia was significantly less frequent in patients concomitantly treated with MLT. Malaise and asthenia were also significantly less frequent in patients receiving MLT. Finally, stomatitis and neuropathy were less frequent in the MLT group, albeit without statistically significant differences. Alopecia and

vomiting were not influenced by MLT. This pilot study seems to suggest that the concomitant administration of the pineal hormone MLT during chemotherapy may prevent some chemotherapy-induced side-effects, particularly myelosuppression and neuropathy. Evaluation of the impact of MLT on chemotherapy efficacy will be the aim of future clinical investigations.

### **Ambulatory management of common forms of anemia.**

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Am Fam Physician 1999 Mar 15;59(6):1598-604

Anemia is a prevalent condition with a variety of underlying causes. Once the etiology has been established, many forms of anemia can be easily managed by the family physician. Iron deficiency, the most common form of anemia, may be treated orally or, rarely, parenterally. Vitamin B12 deficiency has traditionally been treated with intramuscular injections, although oral and intranasal preparations are also available. The treatment of folate deficiency is straightforward, relying on oral supplements. Folic acid supplementation is also recommended for women of child-bearing age to reduce their risk of neural tube defects. Current research focuses on folate's role in reducing the risk of premature cardiovascular disease.

### **Melatonin as biological response modifier in cancer patients.**

Neri B; de Leonardis V; Gemelli MT; di Loro F; Mottola A; Ponchiatti R; Raugeri A; Cini G Oncological Day Hospital, Department of Internal Medicine, University of Florence, Italy.

Anticancer Res (Greece) Mar-Apr 1998, 18 (2B) p1329-32

The neuroendocrine system modulates the immune response through neuropeptides and neurohormones, findings which point to the existence of a neuro-endocrine-immune system regulatory axis. At the same time, there is growing evidence that the pineal gland has anti-neoplastic properties, which include the action of its principal hormone, melatonin (MLT), on the immune system through the release of cytokines by activated T-cells and monocytes. The present study was carried out on 31 patients (19 males and 12 females, age range 46-73 years) with advanced solid tumors (7 gastric, 9 enteric, 8 renal, 5 bladder, 2 prostate) who either failed to respond to chemotherapy and radiotherapy or showed insignificant responses and were therefore shifted to MLT therapy (10 mg/die orally for 3 months). We obtained blood samples just before the start of MLT administration and after 30 days of therapy. Plasma was collected in EDTA tubes on ice, immediately centrifuged at 4 degrees C and stored frozen at -80 degrees C; samples were measured by immunoradiometric assays (Medgenix-Fleurus, Belgium) for tumor necrosis factor alpha (TNF), interleukin-1, 2 and 6 (IL-1, IL-2, IL-6) and interferon gamma (IFN). We used Student's paired t-test to compare each patient's cytokine circulating levels before and after MLT administration and found a significant differences ( $< 0.05$ ). After 3 months of

therapy, none of our patients displayed adverse reactions to MLT or had to discontinue treatment. Nineteen patients (61%) showed disease progression. The other 12 (39%), however, achieved disease stabilization with no further growth of either the primary tumor or of secondaries; moreover, they experienced an improvement in their general well-being, in terms of Tchekmedyan's criteria, associated with a significant decrease of IL-6 circulating levels. These findings are consistent with the hypothesis that MLT modulates immune function in cancer patients by activating the cytokine system which exerts growth-inhibitory properties over a wide range of tumor cell types. Furthermore, by stimulating the cytotoxic activity of macrophages and monocytes, MLT plays a critical role in host defence against the progression of neoplasia.

### **Some biological actions of alkylglycerols from shark liver oil.**

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J Altern Complement Med 1998 Spring;4(1):87-99

Shark liver oil has been used for over 40 years as both a therapeutic and preventive agent. The active ingredients in shark liver oil have been found to be a group of ether-linked glycerols known as alkylglycerols. Initial clinical use was for treating leukemias, and later to prevent radiation sickness from cancer x-ray therapy. Studies over the last 30 years have shown that alkylglycerols are multifunctional. The level of natural alkylglycerols rises within tumor cells, apparently in an effort to control cell growth. Recent studies indicate that the activation of protein kinase C, an essential step in cell proliferation, can be inhibited by alkylglycerols. This action suggests a competitive inhibition of 1,2-diacylglycerol by alkylglycerols. Further studies on the immunostimulatory action of alkylglycerols suggest a primary action on the macrophage. The process of macrophage activation has been demonstrated with both synthetic and natural alkylglycerols. While the exact mechanism has not been found, both an autocrine and paracrine system have been suggested. Shark liver is a major natural source of alkylglycerols, which have no known side effects in dosages of 100 mg three times a day. The information presented in this article suggests that alkylglycerols may be used both as an adjunct therapy in the treatment of neoplastic disorders and as an immune booster in infectious diseases.

### **Retinoids in pancreatic cancer.**

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Ann Oncol 1999;10 Suppl 4:197-200

Prognosis of advanced, unresectable pancreatic adenocarcinoma remains dismal and has not significantly improved over the past 20 years. In a broad panel of preclinical experimental settings we have therefore evaluated the effects of retinoids on human pancreatic carcinoma cells in vitro and in vivo. We found that

retinoid treatment results in inhibition of growth, induction of cellular differentiation and decreased adhesion to certain components of the extracellular matrix, all features compatible with a "less malignant" phenotype. Furthermore, retinoids act synergistically antiproliferative when combined with interferon-alpha. Using transient and stable genetic transfer studies we were able to identify two retinoid receptor subtypes responsible for mediating the growth inhibitory effects as well as retinoid sensitivity. In addition we observed a crucial functional interplay between the retinoid signalling pathway and the expression of a distinct protein kinase C isoenzyme, which determines the direction of the growth regulatory effects of retinoids. Based on these encouraging preclinical results we initiated a phase II clinical trial in which patients with advanced pancreatic carcinoma were treated with retinoic acid in combination with interferon-alpha. This therapeutic regimen was well tolerated and resulted in prolonged stable disease in approximately two thirds of the patients. In summary, these studies suggest that retinoids might be beneficial in the treatment of advanced pancreatic carcinoma patients based on their pleiotropic effects on tumor cell biology.

**Effectiveness of oral vitamin B12 therapy for pernicious anemia and vitamin B12 deficiency anemia.** [Article in Japanese]

Takasaki Y, Moriuchi Y, Tsushima H, Ikeda E, Koura S, Taguchi J, Fukushima T, Tomonaga M, Ikeda S. Department of Hematology, Sasebo City General Hospital, Sasebo, Japan.

Rinsho Ketsueki 2002 Mar;43(3):165-9

We investigated the efficacy of oral vitamin B12 (B12) therapy in patients with B12-deficiency anemia. Between June 1994 and June 2000, 17 patients, who were diagnosed as having B12-deficiency anemia and gave their informed consent, were enrolled in this study. Of these patients, 7 were further treated with a maintenance dose of methylcobalamin (1,500 micrograms daily for 7 days every 1-3 months). Correction of hematological and neurological abnormalities was prompt. The hemoglobin level and serum concentration of B12 were normalized within two months after starting the treatment. Recovery from neurological disturbance was observed within one month. To maintain a normal serum concentration of B12, a 7-day regime of administration was needed every month in 3 patients, every 2 months in 3 patients, and every 3 months in 1 patient. These results demonstrate the effectiveness of oral cobalamin therapy, and also that oral intermittent therapy is useful for maintaining a normal serum B12 concentration. Oral cobalamin therapy might be as effective as conventional injection therapy, and useful for long-term treatment.

**Dysregulation of melatonin metabolism in chronic renal insufficiency: role of erythropoietin-deficiency anemia.**

Vaziri ND, Oveisi F, Reyes GA, Zhou XJ. Department of Medicine, University of California, Irvine, USA.

Kidney Int 1996 Aug;50(2):653-6

Chronic renal failure (CRF) is associated with a variety of neurological and endocrine disorders. In this study, we examined the effect of CRF and the associated anemia on circadian variation of pineal hormone, melatonin. Animals were studied six weeks after 5/6 nephrectomy (CRF group, N = 26) or sham operation (control group, N = 28). A group of erythropoietin-treated CRF animals (CRF/EPO, N = 6) was included to discern the possible role of EPO-deficiency anemia. Compared with the normal control group, the CRF group showed a marked attenuation of the nocturnal surge in serum melatonin concentration. In addition, pineal gland melatonin content measured after a 12-hour dark cycle (< or = 2 lux) was significantly depressed in the CRF group when compared to that obtained in the control group. However, the CRF animals exhibited appropriate suppression of serum concentration and pineal tissue melatonin content in response to bright light (< or = 2500 lux). Administration of EPO led to correction of the CRF anemia and a marked improvement of the defective nocturnal rhythm of serum melatonin. Based on our results, experimental CRF is associated with a marked attenuation of the normal nocturnal surge of serum melatonin concentration. Regular EPO administration results in the correction of anemia and substantial reversal of this abnormality suggesting the partial role of EPO deficiency. The possible role of melatonin dysregulation in the pathophysiology of CRF and the potential value of melatonin supplementation in this condition is uncertain and awaits future investigations.

#### **Folic acid deficiency can cause severe anemia and pancytopenia.**

Brinch L, Tjonnfjord G, Ly B. Hematologisk seksjon, Medisinsk avdeling A, Rikshospitalet, Oslo.

Tidsskr Nor Laegeforen 1990 May 30;110(14):1830-1

Pancytopenia is occasionally a consequence of folate deficiency. The most important differential diagnostic considerations are haematologic malignancies, aplastic anaemia and vitamin B12 deficiency. We discuss the problem as exemplified by three patients. Bone marrow examination and determination of blood concentrations of vitamin B12 and folate will give the correct diagnosis.

#### **n-3 Polyunsaturated fatty acids and cytokine production in health and disease.**

Calder PC. Division of Human Nutrition, School of Biological Sciences, University of Southampton, UK.

Ann Nutr Metab 1997;41(4):203-34

Arachidonic-acid-derived eicosanoids modulate the production of pro-inflammatory and immunoregulatory cytokines. Overproduction of these cytokines is associated with both septic shock and chronic inflammatory diseases. The n-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid, which are found in fish oils, suppress the production of arachidonic-acid-derived eicosanoids and EPA is a substrate for the synthesis of

an alternative family of eicosanoids. Thus, dietary fats which are rich in n-3 PUFAs have the potential to alter cytokine production. Animal studies have provided a great deal of evidence that feeding plant or fish oils rich in n-3 PUFAs does alter the ex vivo production of tumour necrosis factor (TNF), interleukin 1 (IL-1), IL-6 and IL-2, but many contradictory observations have been made; it is most likely that the discrepancies in the literature result from differences in the cell types and experimental protocols used. Human studies provide more consistent data: several studies have shown that supplementation of the diet of healthy volunteers results in reduced ex vivo production of IL-1, IL-6, TNF and IL-2 by peripheral blood mononuclear cells. Similar findings have been made in patients with rheumatoid arthritis and multiple sclerosis. Animal studies indicate that dietary fish oil reduces the response to endotoxin and to pro-inflammatory cytokines, resulting in increased survival; such diets have been beneficial in some models of bacterial challenge, chronic inflammation and auto-immunity. These beneficial effects of dietary n-3 PUFAs may be of use as a therapy for acute and chronic inflammation and for disorders which involve an inappropriately activated immune response.

### **Polyunsaturated fatty acids and rheumatoid arthritis.**

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Curr Opin Clin Nutr Metab Care 2001 Mar;4(2):115-21

Rheumatoid arthritis is characterized by infiltration of T lymphocytes, macrophages and plasma cells into the synovium, and the initiation of a chronic inflammatory state that involves overproduction of proinflammatory cytokines and a dysregulated T-helper-1-type response. Eicosanoids synthesized from arachidonic acid and cytokines cause progressive destruction of cartilage and bone. The n-6 polyunsaturated fatty acid gamma-linolenic acid is the precursor of di-homo-gamma-linolenic acid. The latter and the n-3 polyunsaturated fatty acid eicosapentaenoic acid, which is found in fish oil, are able to decrease the production of arachidonic acid-derived eicosanoids and to decrease the production of proinflammatory cytokines and reactive oxygen species, and the reactivity of lymphocytes. A number of double-blind, placebo-controlled trials of gamma-linolenic acid and fish oil in rheumatoid arthritis have shown significant improvements in a variety of clinical outcomes. These fatty acids should be included as part of the normal therapeutic approach to rheumatoid arthritis. However, it is unclear what the optimal dosage of the fatty acids is, or whether there would be extra benefit from using them in combination.

### **Dietary polyunsaturated fatty acids and inflammatory mediator production.**

James MJ, Gibson RA, Cleland LG. Rheumatology Unit, Royal Adelaide Hospital, Adelaide, Australia, and the Department of Pediatrics and Child Health, Flinders Medical Center, Bedford Park, Australia.

Am J Clin Nutr 2000 Jan;71(1 Suppl):343S-8S

Many antiinflammatory pharmaceutical products inhibit the production of certain eicosanoids and cytokines and it is here that possibilities exist for therapies that incorporate n-3 and n-9 dietary fatty acids. The proinflammatory eicosanoids prostaglandin E(2) (PGE(2)) and leukotriene B(4) (LTB(4)) are derived from the n-6 fatty acid arachidonic acid (AA), which is maintained at high cellular concentrations by the high n-6 and low n-3 polyunsaturated fatty acid content of the modern Western diet. Flaxseed oil contains the 18-carbon n-3 fatty acid alpha-linolenic acid, which can be converted after ingestion to the 20-carbon n-3 fatty acid eicosapentaenoic acid (EPA). Fish oils contain both 20- and 22-carbon n-3 fatty acids, EPA and docosahexaenoic acid. EPA can act as a competitive inhibitor of AA conversion to PGE(2) and LTB(4), and decreased synthesis of one or both of these eicosanoids has been observed after inclusion of flaxseed oil or fish oil in the diet. Analogous to the effect of n-3 fatty acids, inclusion of the 20-carbon n-9 fatty acid eicosatrienoic acid in the diet also results in decreased synthesis of LTB(4). Regarding the proinflammatory cytokines, tumor necrosis factor alpha and interleukin 1beta, studies of healthy volunteers and rheumatoid arthritis patients have shown <math>\approx 90\%</math> inhibition of cytokine production after dietary supplementation with fish oil. Use of flaxseed oil in domestic food preparation also reduced production of these cytokines. Novel antiinflammatory therapies can be developed that take advantage of positive interactions between the dietary fats and existing or newly developed pharmaceutical products.

**Docosahexaenoic acid ingestion inhibits natural killer cell activity and production of inflammatory mediators in young healthy men.**

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Lipids 1999 Apr;34(4):317-24

The purpose of this study was to examine the effects of feeding docosahexaenoic acid (DHA) as triacylglycerol on the fatty acid composition, eicosanoid production, and select activities of human peripheral blood mononuclear cells (PBMNC). A 120-d study with 11 healthy men was conducted at the Metabolic Research Unit of Western Human Nutrition Research Center. Four subjects (control group) were fed the stabilization diet throughout the study; the remaining seven subjects were fed the basal diet for the first 30 d, followed by 6 g DHA/d for the next 90 d. DHA replaced an equivalent amount of linoleic acid; the two diets were comparable in their total fat and all other nutrients. Both diets were supplemented with 20 mg D alpha-tocopherol acetate per day. PBMNC fatty acid composition and eicosanoid production were examined on day 30 and 113; immune cell functions were tested on day 22, 30, 78, 85, 106, and 113. DHA feeding increased its concentration from 2.3 to 7.4 wt% in the PBMNC total lipids, and decreased arachidonic acid concentration from 19.8 to 10.7 wt%. It also lowered prostaglandin E2 (PGE2) and leukotriene B4 (LTB4) production, in response to lipopolysaccharide, by 60-75%. Natural killer cell activity and in vitro secretion of interleukin-1beta and tumor necrosis factor alpha were significantly reduced by

DHA feeding. These parameters remained unchanged in the subjects fed the control diet. B-cell functions as reported here and T-cell functions that we reported previously were not altered by DHA feeding. Our results show that inhibitory effects of DHA on immune cell functions varied with the cell type, and that the inhibitory effects are not mediated through increased production of PGE2 and LTB4.

**Docosahexaenoic and eicosapentaenoic acids inhibit human lymphoproliferative responses in vitro but not the expression of T cell surface activation markers.**

Khalfoun B, Thibault G, Lacord M, Gruel Y, Bardos P, Lebranchu Y. Groupe "Interactions Hote-Greffon", Laboratoire d'Immunologie, Faculte de Medecine, Tours, France.

Scand J Immunol 1996 Mar;43(3):248-56

The effects of polyunsaturated fatty acids (PUFAs: docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids) on induced lymphocyte proliferation and expression of CD25alpha chain of interleukin-2 receptor, CD71 and HLA-DR were investigated. PUFAs had no effect on phytohaemagglutinin (PHA)-induced lymphocyte agglutination, but they strongly inhibited the lymphoproliferative response to PHA. This inhibitory effect is PUFA dose-dependent and seems to be more potent with DHA than EPA. Pre-incubation experiments showed that lymphocytes cultured with PUFAs for 6 h then washed and exposed to PHA, still inhibited lymphocyte proliferation. The authors also showed that this inhibitory activity was time dependent but became nonsignificant when PUFAs were added after 48 h lymphocyte culture. The addition of excess exogenous human recombinant rIL-2 partly restored PHA-lymphocyte proliferation inhibited by EPA but not by DHA. On the other hand, the authors showed that PUFAs did not inhibit IL-2 stimulated lymphocyte proliferation. The addition of PUFAs to cell culture medium had no inhibitory action on the PHA-induced lymphocyte expression of CD25, CD71 and HLA-DR. Furthermore, this effect appeared independent of eicosanoid synthesis or peroxide formation. Indeed, the inclusion of aspirin and vitamin E in the culture medium did not prevent the inhibitory effects of PUFAs on lymphocyte proliferation. Regardless of the mechanism of action, the inhibitory effect of PUFAs on activated lymphocytes may explain why some clinical trials of fish oil supplemented diets containing high amounts of DHA and EPA have been successful in improving the health status of patients suffering from inflammatory and autoimmune disorders.

**Correlation between traditional Chinese medicine classification of 53 patients with aplastic anemia and varieties of hemopoietic progenitor cells in vitro culture.** [Article in Chinese]

Luo XS. Dept. of Hematology, Affiliated Hospital of Zhejiang College of TCM, Hangzhou.

Zhongguo Zhong Xi Yi Jie He Za Zhi 1992 Mar;12(3):139-41, 131

Aplastic anemia can be classified distinctively three types as progenitor depletive; immunosuppressive and androgenic sensitive. Using bone marrow culture in vitro which had been accomplished in our laboratory, 53 patients with aplastic anemia were also classified according to TCM term in three groups as Yin deficiency, Yang deficiency and both Yin and Yang deficiency, and the correlation was observed between TCM classification and lab character of these patients. The results showed that the number of CFU-GM, CFU-E and BFU-E in Yang deficiency group was significantly higher than that in the other two groups (P less than 0.01 and P less than 0.05). It also showed that the sensitivity of progenitor cells to androgenic hormones of Yang deficiency group was preferential to all (P less than 0.005 and P less than 0.05). The percentage of immunosuppressive type of aplastic anemia in Yin deficiency group was much higher than those in the other two groups (P less than 0.005). These observations suggested that TCM classification for aplastic anemia in this paper has objective material foundation.

### **Biochemical effects of a diet containing foods enriched with n-3 fatty acids.**

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Rheumatology Unit, Royal Adelaide Hospital, Adelaide, Australia.

Am J Clin Nutr 2000 Jul;72(1):42-8

**BACKGROUND:** Results of many studies indicate that consumption of n-3 fatty acids can benefit persons with cardiovascular disease and rheumatoid arthritis. However, encapsulated fish oil is unlikely to be suited to lifetime daily use and recommendations to increase fish intake have not been effective.

**OBJECTIVE:** The objective was to examine the effectiveness of a diet that incorporates foods rich in n-3 fatty acids in elevating tissue concentrations of eicosapentaenoic acid and in suppressing the production of inflammatory mediators.

**DESIGN:** Healthy male volunteers were provided with foods that were enriched in alpha-linolenic acid (cooking oil, margarine, salad dressing, and mayonnaise) and eicosapentaenoic and docosahexaenoic acids (sausages and savory dip) and with foods naturally rich in n-3 fatty acids, such as flaxseed meal and fish. Subjects incorporated these products into their food at home for 4 wk. Fatty acid intakes, cellular and plasma fatty acid concentrations, and monocyte-derived eicosanoid and cytokine production were measured.

**RESULTS:** Analyses of dietary records indicated that intake of eicosapentaenoic acid plus docosahexaenoic acid averaged 1.8 g/d and intake of alpha-linolenic acid averaged 9.0 g/d. These intakes led to an average 3-fold increase in eicosapentaenoic acid in plasma, platelet, and mononuclear cell phospholipids. Thromboxane B(2), prostaglandin E(2), and interleukin 1beta synthesis decreased by 36%, 26%, and 20% (P < 0.05), respectively.

**CONCLUSIONS:** Foods that are strategically or naturally enriched in n-3 fatty acids can be used to achieve desired biochemical effects without the ingestion of

supplements or a change in dietary habits. A wide range of n-3-enriched foods could be developed to support large-scale programs on the basis of the therapeutic and disease-preventive effects of n-3 fatty acids.

**Clinical roles of vitamins in hematopoietic disorders.** [Article in Japanese]

Matsuda M, Kanamaru A. Third Department of Internal Medicine, Kinki University School of Medicine.

Nippon Rinsho 1999 Oct;57(10):2349-55

Vitamins are essential organisms which promote various metabolisms and physiological systems. Several vitamins play important roles in hematopoietic system. Vitamin B12, C and folic acid are associated with DNA synthesis of erythroid nucleus, the deficiency of which causes the megaloblastic anemia. Some megaloblastic anemia and sideroblastic anemia might response to vitamin B1 and B6, respectively. Vitamin K participates in some coagulation factors in coagulation-fibrinolysis system. It has been reported that vitamins A, D and K potentially differentiate leukemic cells and then induce the apoptosis, suggesting that they would be new therapeutic agents in acute leukemia.

**Effect of intravenous infusion of omega-3 and omega-6 lipid emulsions on equine monocyte fatty acid composition and inflammatory mediator production in vitro.**

McCann ME, Moore JN, Carrick JB, Barton MH. Department of Physiology, College of Veterinary Medicine, University of Georgia, Athens 30602, USA.

Shock 2000 Aug;14(2):222-8

The effect of intravenous administration of lipid emulsions enriched with omega-3 (n3) and omega-6 (n6) fatty acids on equine monocyte phospholipid fatty acid composition and the synthesis of inflammatory mediators in vitro was evaluated. In a randomized crossover design, horses were infused intravenously with 20% lipid emulsions containing n3 or n6 fatty acids. Monocytes were isolated from the horses before and 0 h, 8 h, 24 h, and 7 days after lipid infusion. Monocyte fatty acid analysis demonstrated incorporation of the parenteral n3 and n6 fatty acids in monocyte phospholipids immediately after infusion, with changes in the fatty acid composition persisting for up to 7 days after infusion. In vitro production of the inflammatory mediators thromboxane B2/thromboxane B3 (TXB<sub>2</sub>/TXB<sub>3</sub>) and tumor necrosis factor-alpha (TNFalpha) by peripheral blood monocytes was diminished by n3 lipid infusion and was unchanged or increased by n6 lipid infusion. The results of this study demonstrate that short-term infusions of n3 and n6 fatty acid-enriched lipid emulsions alter the fatty acid composition of equine monocyte phospholipids and modify the inflammatory response of these cells in vitro. These results also support further investigation into the use of parenteral n3 fatty acids as part of the supportive therapy of patients with multiple organ dysfunction (MODS) or systemic inflammatory response syndrome (SIRS).

### **Effect of zinc supplementation on serum testosterone level in adult male sickle cell anemia subjects.**

Prasad AS, Abbasi AA, Rabbani P, DuMouchelle E.

Am J Hematol 1981;10(2):119-27

Previously, we have documented primary testicular failure in adult male subjects with sickle cell anemia. We have also reported the occurrence of zinc deficiency and suggested that androgen deficiency may be related to zinc deficiency in such patients. In this study, we present data with respect to the effect of oral zinc supplementation on serum testosterone levels in adult male patients with sickle cell anemia. An increase in serum testosterone, neutrophil zinc, and neutrophil alkaline phosphatase activity was observed in the zinc-supplemented group in comparison with the group on placebo. Additionally, body weight increased and serum lactic dehydrogenase activity decreased in response to zinc supplementation. We conclude that androgen deficiency in adult male subjects with sickle cell anemia is correctable with zinc supplementation and that the determination of neutrophil zinc and alkaline phosphatase activity in the neutrophils may be utilized as good indicators of body zinc status in such subjects.

### **Interleukin 6 production by lipopolysaccharide-stimulated human fibroblasts is potently inhibited by naphthoquinone (vitamin K) compounds.**

Reddi K, Henderson B, Meghji S, Wilson M, Poole S, Hopper C, Harris M, Hodges SJ. Department of Oral and Maxillofacial Surgery, Eastman Dental Institute for Oral Healthcare Sciences, London.

Cytokine 1995 Apr;7(3):287-90

Naphthoquinone vitamins (vitamin K) are widely recognized for their role in the gamma-carboxylation of specific glutamyl residues in coagulation, anti-coagulation and extra-hepatic proteins. Recently, however, there have been reports that these compounds can exert actions other than those normally associated with protein gamma-carboxylation. These observations suggest that naphthoquinones may have effects on the production of inflammatory mediators including cytokines. Fibroblasts are now recognized as a rich source of cytokines and we have examined the effect of various naphthoquinones on the production of interleukin 6 (IL-6) by lipopolysaccharide-stimulated human gingival fibroblasts. Compounds examined in this study include: phylloquinone (K1), menaquinone-4 (K2), menadione (K3), 2,3-dimethoxy-1,4-naphthoquinone (DMK) and a synthetic product of vitamin K catabolism, 2-methyl, 3-(2-methyl)-hexanoic acid-1,4-naphthoquinone (KCAT). All of these compounds are capable of inhibiting IL-6 production with a rank order of potency: KCAT > K3 > DMK > K2 > K1. The most potent compound, KCAT, inhibited IL-6 production with an IC<sub>50</sub> of 3 x 10<sup>-7</sup>M. The mechanism of action of these naphthoquinones on fibroblast IL-6 production is unknown. Given that K3 and KCAT are inactive in the gamma-carboxylation reaction, we suggest that this activity is not essential for

the inhibition of IL-6 production and that activity may be related to the redox capacity of these naphthoquinones.

### **Serum erythropoietin and erythroid activity in vitamin B12 deficiency.**

Remacha AF, Bellido M, Garcia-Die F, Marco N, Ubeda J, Gimferrer E.  
Hematology Department, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain.

Haematologica 1997 Jan-Feb;82(1):67-8

We studied erythropoiesis in 31 patients with vitamin B12 deficiency by measuring serum erythropoietin (s-Epo), serum transferrin receptor (s-TfR, taken as an index of total erythroid activity), reticulocyte count, and the reticulocyte maturation index (RMI). s-Epo and s-TfR were measured with commercial immunoassays, whereas reticulocyte count and RMI were determined by flow cytometry. s-Epo (123 +/- 196 U/L) and s-TfR (4.1 +/- 2 mg/L) levels were increased in patients with vitamin B12 deficiency. The absolute reticulocyte counts were decreased (29 +/- 18 x 10<sup>9</sup>/L) with a relative increase in the most immature fractions (RMI: 29.6 +/- 18%). A significant negative relationship was found between s-Epo and Hb level (r = -0.65, p < 0.0001). On the average, however, s-Epo was inappropriately low for the degree of anemia, since the observed/predicted (O/P) s-Epo ratio was 0.80 +/- 0.28 in vitamin B12 deficiency vs 1.00 +/- 0.16 in a group of patients with iron deficiency anemia. It is concluded that at least a portion of patients with vitamin B12 deficiency have serum erythropoietin levels that are inappropriately low for the degree of anemia.

### **Plant extracts from stinging nettle (*Urtica dioica*), an antirheumatic remedy, inhibit the proinflammatory transcription.**

Riehemann K, Behnke B, Schulze-Osthoff K. Department of Internal Medicine I, Medical Clinics, University of Tübingen, Germany.

FEBS Lett 1999 Jan 8;442(1):89-94

Activation of transcription factor NF-kappaB is elevated in several chronic inflammatory diseases and is responsible for the enhanced expression of many proinflammatory gene products. Extracts from leaves of stinging nettle (*Urtica dioica*) are used as antiinflammatory remedies in rheumatoid arthritis. Standardized preparations of these extracts (IDS23) suppress cytokine production, but their mode of action remains unclear. Here we demonstrate that treatment of different cells with IDS23 potently inhibits NF-kappaB activation. An inhibitory effect was observed in response to several stimuli, suggesting that IDS23 suppressed a common NF-kappaB pathway. Inhibition of NF-kappaB activation by IDS23 was not mediated by a direct modification of DNA binding, but rather by preventing degradation of its inhibitory subunit IkappaB-alpha. Our results suggest that part of the antiinflammatory effect of *Urtica* extract may be ascribed to its inhibitory effect on NF-kappaB activation.

**Alteration of tumor necrosis factor-alpha production by macrophages from mice fed diets high in eicosapentaenoic and docosahexaenoic fatty acids.**

Somers SD, Erickson KL. Division of Immunology, James N. Gamble Institute of Medical Research, Cincinnati, Ohio 45319.

Cell Immunol 1994 Feb;153(2):287-97

Dietary exposure to n-3 fats found in marine fish oils are known to reduce certain inflammatory conditions. Although depressed prostaglandin E2 (PGE2) production is thought to be a major mechanism of the beneficial effects, the direct effects of n-3 fatty acids on inflammatory macrophage function are not well understood. In this study, production of the inflammatory monokine, tumor necrosis factor-alpha (TNF alpha), by isolated murine macrophages was assessed following a 3-week feeding with diets containing either 10% menhaden fish oil as a source of n-3 fatty acids or, as a control and source of n-6 fatty acids, 10% safflower oil. Cultures of peritoneal macrophages from mice fed diets with n-3 fatty acids had more TNF alpha activity 24 hr after in vitro stimulation with bacterial lipopolysaccharide than did macrophages from mice fed the n-6-containing diet. The onset and maximal synthesis of bioactive TNF alpha and down-regulation of messenger RNA for TNF alpha appeared to be similar for the two diets, suggesting that macrophages from mice fed a diet high in n-6 but not n-3 fatty acids were capable of removing active TNF alpha from culture media. Experiments in which PGE2 was added exogenously indicated that the removal of TNF alpha from culture supernatant by macrophages was induced by lower concentrations of PGE2 than that associated with termination of production, and that n-3 fatty acid diets caused a selective loss in the clearance mechanism. These results demonstrate a specific alteration of PGE2-mediated regulation of macrophage-produced TNF alpha by n-3 fatty acids.

**Dietary supplementation with very long-chain n-3 fatty acids in man decreases expression of the interleukin-2 receptor (CD25) on mitogen-stimulated lymphocytes from patients with inflammatory skin diseases.**

Soyland E, Lea T, Sandstad B, Drevon A. Section for Dietary Research, University of Oslo, Norway.

Eur J Clin Invest 1994 Apr;24(4):236-42

T-cell activation and cytokine production play an important role in several chronic inflammatory diseases. Because n-3 fatty acids exert beneficial effects on the clinical state of some of these diseases, we examined the effect of dietary supplementation of n-3 fatty acids on T-cell proliferation, expression of CD25 (interleukin-2 receptor alpha-chain), secretion of interleukin-2, interleukin-6 and tumour necrosis factor from T-cells from patients with psoriasis and atopic dermatitis. During 4 months, 21 patients supplied 6 g of highly concentrated ethyl esters of EPA and DHA in gelatin capsules daily to their diet. In the control group 20 patients supplied 6 g per day of corn oil in gelatin capsules to their diet. Eicosapentaenoic acid (20:5, n-3) of serum phospholipids increased from 14 (min

4-max 42) to 81 (min 59-max 144) mg l-1 (P < 0.01) in patients with atopic dermatitis receiving n-3 fatty acids, and from 25 (min 7-max 66) to 74 (min 46-max 142) mg l-1 (P < 0.01) in patients with psoriasis, whereas docosahexaenoic acid (22:6, n-3) increased from 65 (min 46-max 120) to 92 (min 54-max 121) mg l-1 (P < 0.05) and from 81 (min 38-max 122) to 92 (min 63-max 169) mg l-1 (NS) in atopic and psoriatic patients, respectively. The changes in the serum phospholipid fatty acid profile in the groups receiving n-3 fatty acids, correlate to the dietary intake of corresponding fatty acids. (ABSTRACT TRUNCATED AT 250 WORDS)

**Association of humoral markers of inflammation and dehydroepiandrosterone sulfate or cortisol serum levels in patients with chronic inflammatory bowel disease.**

Straub RH, Vogl D, Gross V, Lang B, Scholmerich J, Andus T. Department of Internal Medicine I, University Medical Center, Regensburg, Germany.

Am J Gastroenterol 1998 Nov;93(11):2197-202

**OBJECTIVES:** Dehydroepiandrosterone sulfate (DHEAS) and cortisol are multifunctional adrenal hormones with immunomodulating properties. DHEAS levels were found to be very low in chronic inflammatory diseases. This study aimed to shed more light on the interrelation between DHEAS and cortisol (and humoral markers of inflammation) in chronic inflammatory bowel disease.

**METHODS:** DHEAS and cortisol serum levels were measured by ELISA in the serum of 66 normal subjects, 115 patients with Crohn's disease (CD) and 64 patients with ulcerative colitis (UC). Humoral markers of inflammation and disease activity scores were assessed by standard techniques.

**RESULTS:** DHEAS was lower in patients with CD (p < 0.005) and UC (p < 0.005) than in controls, which was, in part, dependent on previous corticosteroid treatment (p < 0.01). In CD patients, z-normalized DHEAS was inversely correlated with blood sedimentation rate (p = 0.017). Z-normalized DHEAS was negatively correlated with interleukin-6 (IL-6) in the form of a trend (p = 0.068), and z-normalized DHEAS was significantly positively correlated with hemoglobin (p = 0.001) but not with the Crohn's disease activity index. Cortisol, however, was positively correlated with blood sedimentation rate (p = 0.034) and C-reactive protein (p = 0.006). In contrast, in UC patients no such correlation of z-normalized DHEAS or cortisol and parameters of humoral inflammatory activity or Rachmilewitz index exist.

**CONCLUSIONS:** DHEAS as a marker of inflammation was low in CD and UC. In CD patients, low DHEAS and high cortisol serum levels were associated with higher humoral inflammatory activity. With respect to humoral inflammatory activity in CD patients, DHEAS and cortisol seem to be inversely regulated, which may have an impact on several immune functions, such as IL-6 secretion.

## **Replacement therapy with DHEA plus corticosteroids in patients with chronic inflammatory diseases-substitutes of adrenal and sex hormones.**

Straub RH, Scholmerich J, Zietz B. Laboratory of Neuroendocrinoimmunology, Department of Internal Medicine I, University Hospital, Franz-Josef-Strauss-Allee 11, D-93042 Regensburg, Germany. Rainer.Straub@klinik.uni-regensburg.de

Z Rheumatol 2000;59 Suppl 2:II/108-18

A dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis was found in animal models of chronic inflammatory diseases, and the defect was located in more central portions of the HPA axis. This defect of neuroendocrine regulatory mechanisms contributes to the onset of the model disease. Since these first observations in animal models were made, evidence has accumulated that the possible defect in the HPA axis in humans is more distal to the hypothalamus or pituitary gland: In chronic inflammatory diseases, such as rheumatoid arthritis, an alteration of the HPA stress response results in inappropriately low cortisol secretion in relation to adrenocorticotropic hormone (ACTH) secretion. Furthermore, it has recently been shown that the serum levels of another adrenal hormone, dehydroepiandrosterone (DHEA), were significantly lower after ACTH stimulation in patients with rheumatoid arthritis without prior corticosteroids than in healthy controls. These studies clearly indicate that chronic inflammation alters, particularly, the adrenal response. However, at this point, the reason for the specific alteration of adrenal function in relation to pituitary function remains to be determined. Since one of the down-regulated adrenal hormones, DHEA, is an inhibitor of cytokines due to an inhibition of nuclear factor-kappa B (NF-kappa B) activation, low levels of this hormone may be deleterious in chronic inflammatory diseases. We have recently demonstrated that DHEA is a potent inhibitor of IL-6, which confirmed an earlier study in mice. Since IL-6 is an important factor for B lymphocyte differentiation, the missing down-regulation of this cytokine, and others such as TNF, may be a significant risk factor in rheumatic diseases. Since in these patients, administration of prednisolone or the chronic inflammatory process itself alters adrenal function, endogenous adrenal hormones in relation to proinflammatory cytokines change. Furthermore, these mechanisms may also lead to shifts in steroidogenesis which have been demonstrated in chronic inflammatory diseases. It was repeatedly demonstrated that the serum level of the sulphated form of DHEA (DHEAS) was significantly lower in patients with chronic inflammatory diseases. Since DHEAS is the pool for peripheral sex steroids, such as testosterone and 17 beta-estradiol, lack of this hormone leads to a significant sex hormone deficiency in the periphery. This overview will demonstrate mechanisms why DHEAS is reduced in chronic inflammatory diseases. The importance of DHEAS deficiency will be demonstrated with respect to osteoporosis. As a consequence, we suggest a combined therapy with corticosteroids plus DHEA in chronic inflammatory diseases.

**Serum dehydroepiandrosterone (DHEA) and DHEA sulfate are negatively correlated with serum interleukin-6 (IL-6), and DHEA inhibits IL-6 secretion**

**from mononuclear cells in man in vitro: possible link between endocrinosenescence and immunosenescence.**

Straub RH, Konecna L, Hrach S, Rothe G, Kreutz M, Scholmerich J, Falk W, Lang B. Department of Internal Medicine I, University Medical Center, Regensburg, Germany. rainer.straub@klinik.uni-regensburg.de

J Clin Endocrinol Metab 1998 Jun;83(6):2012-7

Interleukin-6 (IL-6) is one of the pathogenetic elements in inflammatory and age-related diseases such as rheumatoid arthritis, osteoporosis, atherosclerosis, and late-onset B cell neoplasia. In these diseases or during aging, the decrease in production of sex hormones such as dehydroepiandrosterone (DHEA) is thought to play an important role in IL-6-mediated pathogenetic effects in mice. In humans, we investigated the correlation of serum levels of DHEA, DHEA sulfate (DHEAS), or androstenedione (ASD) and IL-6, tumor necrosis factor-alpha, or IL-2 with age in 120 female and male healthy subjects (15-75 yr of age). Serum DHEA, DHEAS, and ASD levels significantly decreased with age (all  $P < 0.001$ ), whereas serum IL-6 levels significantly increased with age ( $P < 0.001$ ). DHEA/DHEAS and IL-6 (but not tumor necrosis factor-alpha or IL-2) were inversely correlated (all patients:  $r = -0.242/-0.312$ ;  $P = 0.010/0.001$ ). In female and male subjects, DHEA and ASD concentration dependently inhibited IL-6 production from peripheral blood mononuclear cells ( $P = 0.001$ ). The concentration-response curve for DHEA was U shaped (maximal effective concentration,  $1-5 \times 10^{-8}$  mol/L), which may be the optimal range for immunomodulation. In summary, the data indicate a functional link between DHEA or ASD and IL-6. It is concluded that the increase in IL-6 production during the process of aging might be due to diminished DHEA and ASD secretion. Immunosenescence may be directly related to endocrinosenescence, which, in turn, may be a significant cofactor for the manifestation of inflammatory and age-related diseases.

**Cytokine secretion in whole blood of healthy subjects following oral administration of *Urtica dioica* L. plant extract.** [Article in German]

Teucher T, Obertreis B, Rutkowski T, Schmitz H. Strathmann AG & Co., Hamburg.

Arzneimittelforschung 1996 Sep;46(9):906-10

Twenty healthy volunteers ingested for 21 days 2 capsules b.i.d. of an IDS 23/1 containing nettle leaf extract (Rheuma-Hek). Before and after 7 and 21 days the basal and the lipopolysaccharide (LPS) stimulated tumor necrosis factor-alpha (TNF-alpha), interleukin-1 beta (IL-1 beta) and interleukin-6 (IL-6) concentrations were measured ex vivo. In vitro the effects of IDS 23/1 on the release of these cytokines were determined. Additionally basal interleukin-4 (IL-4) and interleukin-10 (IL-10) levels were recorded. Orally taken the test drug has ex vivo no effect on basal levels of TNF-alpha, IL-1 beta, IL-4, IL-6 or IL-10 which were always below detection limits. After 7 and 21 days ingestion ex vivo

a decrease of LPS stimulated TNF-alpha release of 14.6 and 24.0%, respectively, was observed. IL-1 beta was reduced for 19.2 and 39.3%. In vitro IDS 23/1 added to whole blood resulted in an exceeded inhibition of LPS stimulated TNF-alpha and IL-1 beta secretion which correlated with the duration of the drug ingestion. Using the highest tested IDS 23/1 concentration the inhibition reached 50.5 (day 0) to 79.5% (day 21) for TNF-alpha and 90.0 (day 0) to 99.2% (day 21) for IL-1 beta, respectively. IDS 23/1 induced a pronounced release of IL-6 in absence of LPS only in vitro. The detected IL-6 concentrations were comparable to those after LPS stimulation, additive effects could not be observed. The absence of detectable IL-6 concentrations in whole blood ex vivo after oral ingestion of the tested drug as well as the differences in the inhibition patterns for TNF-alpha and IL-1 beta ex vivo and ex vivo in vitro suggest that the extract contains different pharmacological effective compounds with varying bioavailabilities.

### **Dietary docosahexaenoic acid suppresses inflammation and immunoresponses in contact hypersensitivity reaction in mice.**

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Lipids 2000 Jan;35(1):61-9

This study was designed to examine the immunomodulatory effects of dietary docosahexaenoic acid (DHA) in the absence of eicosapentaenoic acid (EPA). We investigated the effects of feeding dietary DHA ethyl ester (DHA-Et) (97% pure) at levels of 4.8 wt% of the total diet and of feeding EPA ethyl ester (EPA-Et) (99% pure) at 4.8 wt% on the inflammatory response in the challenge phase of the contact hypersensitivity reaction (CHR) in the ears of mice sensitized with 2,4-dinitro-1-fluorobenzene (DNFB). The effect of DHA-Et on T lymphocytes at the CHR site was examined using anti-CD4 antibodies. Furthermore, we examined the cytokines formed at the CHR site on the mRNA level. It was found that 24 h after the challenge, DHA-Et but not EPA-Et reduced the ear swelling. Infiltration of inflammatory cells, in particular, CD4-positive T lymphocytes, into the ears in the challenge phase of CHR was observed. DHA-Et reduced the infiltration of CD4-positive T lymphocytes into the ears. DHA-Et also decreased the expression of interferon-gamma, interleukin (IL)-6, IL-1beta, and IL-2 mRNA in ears. These observations suggest that DHA, but not EPA, may exert an antiinflammatory and immunosuppressive effect. The immunosuppressive effectiveness of fish oil may be attributed mainly to DHA.

### **Suppression of tumor growth and metastasis by dietary fish oil combined with vitamins E and C and cisplatin.**

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Cancer Chemother Pharmacol 2001;47(1):34-40

**PURPOSE:** The anticancer activity of omega-3 polyunsaturated fatty acids (omega-3 PUFA) has been shown in a large number of studies. This study was undertaken to analyze the combined effect of omega-3 PUFA and antioxidative vitamins on the level of spontaneous metastatic dissemination. The supportive effect of this dietary combination on chemotherapy with cisplatin (CP) was determined in parallel.

**METHODS:** C57BL/6J mice bearing the Lewis lung carcinoma 3LL were fed ad libitum one of three isocaloric diets containing 5% soybean oil supplemented with 40 mg/kg alpha-tocopherol acetate (SO diet), or 4% fish oil plus 1% corn oil, and basal amounts of vitamin E (FO diet) or FO diet supplemented with vitamins E and C (FO+E+C diet). These diets were tested in combination with the conventional cytotoxic agent CP in a series of regimens. Tumor growth, feed consumption, body weight, lung metastasis and lung histology were followed.

**RESULTS:** Both the FO dietary groups showed significantly lower tumor development than the SO group in all examined parameters, indicating that omega-3 PUFA have anticancer activity. However, the FO diet, in comparison with the FO+E+C diet induced a significantly slower rate of tumor growth, and lower metastatic load, as reflected in lung weight. The decrease in the anticancer activity of FO by the addition of vitamins E and C suggests that in situ oxidation of omega-3 PUFA underlies their anticancer action. It is thus proposed that oxidized omega-3 PUFA accumulates in the membranes and the cytosol of tumor cells, reducing their vitality and eventually leading to their death. No signs of anorexia or cachexia were observed in either FO group, in contrast to the SO group. CP treatment with the SO diet had no apparent therapeutic effect, while with the FO diets it reduced the metastatic load. The best regimen of this combined treatment was FO diet followed by CP treatment with FO diet supplemented with vitamins E and C after resection of the primary growth. This regimen could be translated to a combined therapy for human cancer.

**CONCLUSIONS:** Diets enriched with omega-3 PUFA may have beneficial anticancer effects in particular when containing only basal amounts of antioxidants such as vitamin E or C. Furthermore, the addition of drugs which promote oxidation of omega-3 PUFA, such as ferrous salts (e.g. as prescribed for the treatment of anemia), may further increase these effects. However, the supportive effect of omega-3 PUFA in chemotherapy (e.g. with CP) increases when vitamins E and C are also included.

**Folates in human nutrition. Different clinical situations in which folate deficiencies exist.** [Article in Spanish]

Zarazaga A, Garcia de Lorenzo A, Montanes P, Culebras JM. Servicio de Cirugia General, Hospital Universitario, La Paz, Madrid.

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The alimentary surveys carried out on various sectors of the population in industrialized countries have shown the existence of chronic clinically silent

deficiency in micronutrients. In some cases, as in folates, their lability against conservation techniques, the change in alimentary habits, the abuse of alcohol and the great quantity of frequently used drugs which interfere in their absorption, diminish their content in the diet and their bio-availability. The appearance of macrocytic anemia is a late deficiency sign, and therefore in situations of an increase need and in patients included in the risk groups, a supplemental intake must be given in order to avoid irreversible lesions if it is not possible to monitor the folate levels. There are risk groups in which various etiological factors come into play, acting at a different metabolic level on the folates and making more difficult their dietetic or pharmacological compensation even if supply is considerably increased. We studied these factors independently and in each specific situation (old people, patients with liver disease, alcoholics, pregnant women and nursing mothers, neonates, children, malabsorption syndromes, gastrectomy, AIDS, anaesthesia and patients being treated with antifolic medication), evaluating their mechanisms of action and their potentiation in determined specific situations