

LE Magazine August 2001

## ABSTRACTS

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## DHEA

Dehydroepiandrosterone decreases mortality rate and improves cellular immune function during polymicrobial sepsis.

**OBJECTIVE:** Sepsis is associated with a marked depression of cellular immune function. The steroid hormone dehydroepiandrosterone (DHEA) is proposed to have immunoenhancing activities. We, therefore, investigated the effect of DHEA on the mortality rate and cellular immune functions in an experimental model of sepsis. **DESIGN:** Randomized animal study. **SETTING:** Level I trauma center, university research laboratory. **SUBJECTS:** Male NMRI mice. **INTERVENTIONS:** Mice were subjected to laparotomy (sham) or cecal ligation and puncture (CLP). Mice were treated with (sham/DHEA; CLP/DHEA) or without (sham; CLP) the steroid hormone DHEA (30 mg/kg sc). Animals were killed 48 hrs after the onset of sepsis. **MEASUREMENTS AND MAIN RESULTS:** The survival rate of septic mice was determined 24 and 48 hrs after onset of sepsis. Forty-eight hours after the septic challenge, a white blood cell count was performed and serum tumor necrosis factor (TNF)-alpha and interleukin (IL)-1beta concentrations were monitored using ELISA. Furthermore, the delayed type of hypersensitivity (DTH) reaction was evaluated on the basis of ear pinna swelling after dinitrofluorobenzene (DNFB) administration, and clinical variables (body weight, temperature, heart rate, fluid input/output, food intake) were monitored using metabolic cages. DHEA administration improved the survival rate (87% vs. 53% after 48 hrs;  $p < .001$ ). This was accompanied by a restoration of the depressed DTH reaction and a reduction in TNF-alpha serum concentrations (20.7 +/- 1.4 pg/mL vs. 32.4 +/- 6.6 pg/mL). **CONCLUSIONS:** These results demonstrate that DHEA administration leads to an increased survival following a septic challenge. The immunoenhancing effect of DHEA is accompanied by a reduction of TNF-alpha release and an improved activity of T-cellular immunity. DHEA administration may, therefore, be beneficial in systemic inflammation.

Crit Care Med 2001 Feb;29(2):380-4

Dehydroepiandrosterone (DHEA) reduces neuronal injury in a rat model of global cerebral ischemia.

**Introduction:** Many studies report an inverse correlation between levels of DHEA and neurological diseases. Exogenous DHEA protects hippocampal neurons against excitatory amino acid induced neurotoxicity. The purpose of this experiment is to evaluate the effect of DHEA in an animal model of transient but severe forebrain ischemia. **Methods:** At thirteen days prior to induction of ischemia, male Wistar rats were implanted with various doses of DHEA-placebo, 25 mg, 50 mg or 100 mg. Forebrain ischemia was induced for 10 minutes using a modified four-vessel occlusion technique, with hippocampal neuronal injury assessed at 7 days post-ischemically and expressed as a percentage of total cells. **Results:** Both normal and necrotic hippocampal CA(1) cells were counted. Percentages of hippocampal injury observed were 88+/-13% in animals treated with placebo, 84+/-8% in the 25 mg DHEA group, and 60+/-7% in the 50 mg DHEA group. Animals treated with 100 mg DHEA displayed a significant ( $P < 0.05$ ) reduction of hippocampal CA(1) cell injury at 60+/-7%. **Conclusion:** Treatment with a high dose, but not a low or moderate dose, of DHEA implantation reduces hippocampal CA(1) neuronal injury following severe but transient forebrain ischemia.

Brain Res 2001 Jan 12;888(2):263-266

Dehydroepiandrosterone in systemic lupus erythematosus.

DHEA has shown promise for the treatment of SLE in three controlled and several uncontrolled clinical trials, including one large multicenter study comprising nearly 200 patients. The main benefits of DHEA seem to be a decrease in corticosteroid requirements and improved overall symptomatology. Intriguing aspects of DHEA treatment in SLE that require further study are a possible bone protective effect and improvements in cognitive function. The most frequent side effect is mild acneiform dermatitis, and long-term concerns include lowered HDL cholesterol.

Rheum Dis Clin North Am 2000 May;26(2):349-62

Dehydroepiandrosterone prevents oxidative injury induced by transient ischemia/reperfusion in the brain of diabetic rats.

Both chronic hyperglycemia and ischemia/reperfusion (IR) cause an imbalance in the oxidative state of tissues. Normoglycemic

and streptozotocin (STZ)-diabetic rats were subjected to bilateral carotid artery occlusion for 30 min followed by reperfusion for 60 minutes. Rats had either been treated with dehydroepiandrosterone (DHEA) for 7, 14 or 21 days (2 or 4 mg/day per rat) or left untreated. Oxidative state, antioxidant balance, and membrane integrity were evaluated in isolated synaptosomes. IR increased the levels of reactive species and worsened the synaptic function, affecting membrane Na/K-ATPase activity and lactate dehydrogenase release in all rats. The oxidative imbalance was much severer when transient IR was induced in STZ-diabetic rats. DHEA treatment restored H<sub>2</sub>O<sub>2</sub>, hydroxyl radical, and reactive oxygen species to close to control levels in normoglycemic rats and significantly reduced the level of all reactive species in STZ-diabetic rats. Moreover, DHEA treatment counteracted the detrimental effect of IR on membrane integrity and function: the increase of lactate dehydrogenase release and the drop in Na/K-ATPase activity were significantly prevented in both normoglycemic and STZ-diabetic rats. The results confirm that DHEA, an adrenal steroid that is synthesized de novo by brain neurons and astrocytes, possesses a multitargeted antioxidant effect. They also show that DHEA treatment is effective in preventing both derangement of the oxidative state and neuronal damage induced by IR in experimental diabetes.

Diabetes 2000 Nov;49(11):1924-31

Dehydroepiandrosterone replacement in women with adrenal insufficiency.

**BACKGROUND:** The physiologic role of dehydroepiandrosterone in humans is still unclear. Adrenal insufficiency leads to a deficiency of dehydroepiandrosterone; we therefore, investigated the effects of dehydroepiandrosterone replacement, in patients with adrenal insufficiency. **METHODS:** In a double-blind study, 24 women with adrenal insufficiency received in random order 50 mg of dehydroepiandrosterone orally each morning for four months and placebo daily for four months, with a one-month washout period. We measured serum steroid hormones, insulin-like growth factor I, lipids, and sex hormone-binding globulin, and we evaluated well-being and sexuality with the use of validated psychological questionnaires and visual-analogue scales, respectively. The women were assessed before treatment, after one and four months of treatment with dehydroepiandrosterone, after one and four months of placebo, and one month after the end of the second treatment period. **RESULTS:** Treatment with dehydroepiandrosterone raised the initially low serum concentrations of dehydroepiandrosterone, dehydroepiandrosterone sulfate, androstenedione, and testosterone into the normal range; serum concentrations of sex hormone-binding globulin, total cholesterol, and high-density lipoprotein cholesterol decreased significantly. Dehydroepiandrosterone significantly improved overall well-being as well as scores for depression and anxiety. For the global severity index, the mean (+/-SD) change from base line was -0.18+/-0.29 after four months of dehydroepiandrosterone therapy, as compared with 0.03+/-0.29 after four months of placebo (P=0.02). As compared with placebo, dehydroepiandrosterone significantly increased the frequency of sexual thoughts (P=0.006), sexual interest (P=0.002), and satisfaction with both mental and physical aspects of sexuality (P=0.009 and P=0.02, respectively). **CONCLUSIONS:** Dehydroepiandrosterone improves well-being and sexuality in women with adrenal insufficiency.

N Engl J Med 1999 Sep 30;341(14):1013-20

Changes in cortisol/DHEA ratio in HIV-infected men are related to immunological and metabolic perturbations leading to malnutrition and lipodystrophy.

HIV-1 infection is associated with immune deficiency and metabolic perturbations leading to malnutrition and lipodystrophy. Because immune response and metabolic perturbations (protein and lipid metabolism) are partly regulated by glucocorticoids and DHEA, we determined serum cortisol and DHEA concentrations, and the cortisol/DHEA ratio in HIV-positive men, either untreated or receiving various antiretroviral treatments (ART), including highly active antiretroviral therapy (HAART). Cortisol levels were found increased in all patients, whatever the stage of the disease and independently of the ART treatment. In contrast, serum DHEA was elevated in the asymptomatic stage, and it was below normal values in AIDS patients, either untreated or mono-ART-treated. The DHEA level was low in HAART-treated patients with lipodystrophy (LD+) and highly increased in HAART-treated patients without lipodystrophy (LD-). Consequently, the cortisol/DHEA ratio was similar to controls in asymptomatic untreated or mono-ART-treated patients, but increased in AIDS patients. Interestingly, this ratio was increased in LD+ HAART-treated men, but normalized in LD-HAART-treated patients. Changes in the cortisol/DHEA ratio were negatively correlated with the in vivo CD4 T-cell counts, with the malnutrition markers, such as body-cell mass and fat mass, and with the increased circulating lipids (cholesterol, triglycerides, and apolipoprotein B) associated to the lipodystrophy syndrome. Our observations show that the cortisol/DHEA ratio is dramatically altered in HIV-infected men, particularly during the syndromes of malnutrition and lipodystrophy, and this ratio remains elevated whatever the antiretroviral treatment, including HAART. These findings have practical clinical implications, since manipulation of this ratio could prevent metabolic (protein and lipid) perturbations.

Ann N Y Acad Sci 2000;917:962-70

Inhibition of human immunodeficiency virus type-1 (HIV-1) replication by immunor (IM28), a new analog of dehydroepiandrosterone.

The inhibition of HIV-1 replication in vitro by Immunor 28 (IM28), an analog of dehydroepiandrosterone (DHEA), was monitored using the HIV-1 laboratory wild-type strain IIIB. Evaluation of the 50% inhibitory dose (IC<sub>50</sub>) revealed a decrease in HIV-1 replication giving an IC<sub>50</sub> value around 22 microM. The toxicity of the drug has been determined also, in MT2 cells and PBMCs. 60 microM of

IM28 provoked a 50% decrease in cell viability while DHEA caused the same decrease at 75 microM in MT2 cells. These values are 125 microM for IM28 in PBMCs and 135 microM for DHEA. Thus, DHEA is less toxic than IM28, but IM28 has a higher antiviral activity.

Nucleosides Nucleotides Nucleic Acids 2000 Oct-Dec;19(10-12):2019-24

Serum levels of interleukin-6 and dehydroepiandrosterone sulphate in response to either fasting or a ketogenic diet in rheumatoid arthritis patients.

**OBJECTIVE:** To investigate the effects of either a 7-day fast or a 7-day ketogenic diet upon serum interleukin-6 (IL-6) and dehydroepiandrosterone sulphate (DHEAS) in RA patients. **METHODS:** We measured serum concentrations of DHEAS and IL-6 in 23 RA patients with active disease, 10 of whom followed a 7-day sub-total fast and 13 of whom consumed a ketogenic diet (isoenergetic, carbohydrate < 40 g/day) for 7 days. Clinical and laboratory variables were measured at baseline, on day 7 and after re-feeding on day 21. Correlation analyses were used to assess the associations between serum IL-6, DHEAS and disease activity variables at each timepoint. **RESULTS:** Fasting, but not the ketogenic diet, decreased serum IL-6 concentrations by 37% ( $p < 0.03$ ) and improved disease activity at day 7. Both fasting and the ketogenic diet increased serum DHEAS levels by 34% as compared with baseline (both  $p < 0.006$ ). Levels of IL-6, but not DHEAS, correlated with several disease activity variables. **CONCLUSION:** Both fasting and a ketogenic diet significantly increased serum DHEAS concentrations in RA patients. Only fasting significantly decreased serum IL-6 levels and improved disease activity. As the increases in serum DHEAS were similar in response to both fasting and a ketogenic diet, it is unlikely that the fall in serum IL-6 or clinical improvements after fasting were directly related to increases in serum DHEAS. The fasting-induced fall in serum IL-6 may underlie the fall in CRP and ESR observed in RA patients in response to a 7-day fast.

Clin Exp Rheumatol 2000 May-Jun;18(3):357-62

Dehydroepiandrosterone, pregnenolone and sex steroids down-regulate reactive astroglia in the male rat brain after a penetrating brain injury.

Astrocytes are a target for steroid hormones and for steroids produced by the nervous system (neurosteroids). The effect of gonadal hormones and several neurosteroids in the formation of gliotic tissue has been assessed in adult male rats after a penetrating wound of the cerebral cortex and the hippocampal formation. The hormones testosterone, 17beta-estradiol and progesterone and the neurosteroids dehydroepiandrosterone, pregnenolone and pregnenolone sulfate resulted in a significant decrease in the accumulation of astrocytes in the proximity of the wound and in a decreased bromodeoxyuridine incorporation in reactive astrocytes. Of all steroids tested, dehydroepiandrosterone was the most potent inhibitor of gliotic tissue formation. These findings suggest that neurosteroids and sex steroids may affect brain repair by down-regulating gliotic tissue.

Int J Dev Neurosci 1999 Apr;17(2):145-51

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## ABSTRACTS

Severity of depression in abstinent alcoholics is associated with monoamine metabolites and dehydroepiandrosterone-sulfate concentrations.

Depressed mood increases the relapse risk of abstinent alcoholics; its neurobiological correlates may include reduced serotonin and norepinephrine turnover rates and increased cortisol concentrations during detoxification stress. Neurosteroids such as dehydroepiandrosterone and its sulfate (DHEA and DHEA-S) may antagonize cortisol action and may have mood-elevating effects on their own. We measured severity of depression with Beck's Depression Inventory (BDI) and Hamilton's Depression Rating Scale (HDRS), plasma concentrations of cortisol, DHEA and DHEA-S, and CSF concentrations of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA), the norepinephrine metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) and the dopamine metabolite homovanillic acid (HVA) in 21 abstinent alcoholics after 4 weeks of abstinence and in 11 age-matched healthy control subjects. Only CSF MHPG concentrations were reduced in alcoholics compared to control subjects (41.4 +/- 6.6 vs. 53.3 +/- 8.6 pmol/ml). Self-rated depression was significantly correlated with CSF MHPG (Spearman's  $R = +0.57$ ,  $P < 0.01$ ), CSF 5-HIAA ( $R = +0.51$ ,  $P < 0.05$ ) and plasma cortisol concentrations ( $R = +0.50$ ,  $P < 0.05$ ). Negative correlations were found between DHEA-S concentrations and both self-rated depression ( $R = -0.45$ ,  $P < 0.05$ ) and observer-rated depression ( $R = -0.55$ ,  $P < 0.05$ ). The ratio of DHEA-S to cortisol serum concentrations was also negatively correlated with depression (BDI:  $R = -0.55$ ,  $P < 0.01$ ; HDRS:  $R = -0.63$ ,  $P < 0.005$ ). Anxiety (Spielberger's State Anxiety Scale) was only associated with CSF MHPG concentrations ( $R = +0.58$ ,  $P < 0.01$ ). Our findings point to the importance of noradrenergic dysfunction in the pathogenesis of depression among abstinent alcoholics and indicate that their mood states may also be modulated by a low DHEA-S to cortisol ratio, hypothetically indicative of low stress protection capacities.

Psychiatry Res 1999 Dec 20;89(2):97-106

Improvement in mood and fatigue after dehydroepiandrosterone replacement in Addison's disease in a randomized, double blind trial.

Dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS) are adrenal precursors of steroid biosynthesis and centrally acting neurosteroids. Glucocorticoid and mineralocorticoid deficiencies in Addison's disease require life-long hormone replacement, but the associated failure of DHEA synthesis is not corrected. We conducted a randomized, double blind study in which 39 patients with Addison's disease received either 50 mg oral DHEA daily for 12 weeks, followed by a 4-week washout period, then 12 weeks of placebo, or vice versa. After DHEA treatment, levels of DHEAS and Delta(4)-androstenedione rose from subnormal to within the adult physiological range. Total testosterone increased from subnormal to low normal with a fall in serum sex hormone-binding globulin in females, but with no change in either parameter in males. In both sexes, psychological assessment showed significant enhancement of self-esteem with a tendency for improved overall well-being. Mood and fatigue also improved significantly, with benefit being evident in the evenings. No effects on cognitive or sexual function, body composition, lipids, or bone mineral density were observed. Our results indicate that DHEA replacement corrects this steroid deficiency effectively and improves some aspects of psychological function. Beneficial effects in males, independent of circulating testosterone levels, suggest that it may act directly on the central nervous system rather than by augmenting peripheral androgen biosynthesis. These positive effects, in the absence of significant adverse events, suggest a role for DHEA replacement therapy in the treatment of Addison's disease.

J Clin Endocrinol Metab 2000 Dec;85(12):4650-6

Dehydroepiandrosterone selectively inhibits production of tumor necrosis factor alpha and interleukin-6 in astrocytes.

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).

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

Altered salivary dehydroepiandrosterone levels in major depression in adults.

**BACKGROUND:** The authors sought to examine whether levels of dehydroepiandrosterone are abnormal in depression.

**METHODS:** Three groups of subjects aged 20 to 64 were studied: 44 major depressives, 35 subjects with partially or completely remitted depression, matched as far as possible for age and drug treatment, and 41 normal control subjects.

Dehydroepiandrosterone and cortisol in saliva were determined from specimens taken at 8:00 AM and 8:00 PM on 4 days.

**RESULTS:** The mean age of the three groups did not differ. Dehydroepiandrosterone was lowered at 8:00 AM and 8:00 PM compared with control subjects. Values for the remitted group were intermediate. Dehydroepiandrosterone levels at 8:00 AM correlated negatively with severity of depression and were not related to drug treatment or smoking, but decreased with age (as expected). Cortisol was elevated in depression in the evening. The molar cortisol/dehydroepiandrosterone ratio also differentiated those with depression from the control group. **CONCLUSIONS:** Lowered dehydroepiandrosterone levels are an additional state abnormality in adult depression. Adrenal steroid changes are thus not limited to cortisol. Because dehydroepiandrosterone may antagonize some effects of cortisol and may have mood improving properties, these findings may have significant implications for the pathophysiology of depression.

Biol Psychiatry 2000 Nov 15;48(10):989-95

Dehydroepiandrosterone and melatonin prevent Bacillus anthracis lethal toxin-induced TNF production in macrophages.

The lethal toxin of Bacillus anthracis, which is composed of two separate proteinaceous exotoxins, namely protective antigen and lethal factor, is central to the pathogenesis of anthrax. Low levels of this toxin are known to induce release of cytokines such as tumor necrosis factor alpha (TNF-alpha). In the present study we investigated the effect of dehydroepiandrosterone (DHEA), melatonin (MLT), or DHEA + MLT on production of lethal toxin-induced TNF-alpha in mouse peritoneal macrophages. We found that treatment with DHEA significantly inhibited the TNF-alpha production caused by anthrax lethal toxin. Exposure of MLT to anthrax lethal toxin-treated macrophages also decreased the release of TNF-alpha to the extracellular medium as compared to the control. However, combined use of DHEA and MLT also inhibited TNF-alpha release, but not more than single therapies. These results suggest that DHEA and MLT may have a therapeutic role in reducing the increased cytokine production induced by anthrax lethal toxin.

Cell Biol Toxicol 2000;16(3):165-74

## AGEs

An advanced glycation endproduct cross-link breaker can reverse age-related increases in myocardial stiffness.

Decreased elasticity of the cardiovascular system is one of the hallmarks of the normal aging process of mammals. A potential explanation for this decreased elasticity is that glucose can react nonenzymatically with long-lived proteins, such as collagen and lens crystallin, and link them together, producing advanced glycation endproducts (AGEs). Previous studies have shown that aminoguanidine, an AGE inhibitor, can prevent glucose cross-linking of proteins and the loss of elasticity associated with aging and diabetes. Recently, an AGE cross-link breaker (ALT-711) has been described, which we have evaluated in aged dogs. After 1 month of administration of ALT-711, a significant reduction (approximately 40%) in age-related left ventricular stiffness was observed [(57.1 +/- 6.8 mmHg x m(2)/ml pretreatment and 33.1 +/- 4.6 mmHg x m(2)/ml posttreatment (1 mmHg = 133 Pa)]. This decrease was accompanied by improvement in cardiac function.

Proc Natl Acad Sci U S A 2000 Mar 14;97(6):2809-13

New hypothesis on etiopathogenesis of Alzheimer syndrome. Advanced glycation end products (AGEs).

Despite intense efforts, it has not yet been possible to clarify the etiopathogenesis of Alzheimer's dementia. There are, however, hypotheses which focus on certain aspects of this type of dementia, characterized by particular neuropathological alterations and clinical correlates. Recently, evidence has accumulated that advanced glycation endproducts (AGEs) could play an important role in the etiology of the Alzheimer's syndrome. AGEs are generated by an irreversible reaction through the non-enzymatic, long-term glycosylation of proteins. They are strongly resistant to proteolytic processes and induce protein crosslinking. They could thus inhibit the physiological functions of many proteins. Moreover, it is suggested that they contribute to the transformation of the soluble form of beta-amyloid into its insoluble version. AGEs are also demonstrable in neurofibrillary tangles (NFTs). A further mechanism by which AGEs might be pathogenic is via their induction of oxidative stress. AGEs probably exert their pathological effects not only directly because of their chemical properties, but also by indirect receptor-mediated mechanisms. Further investigation of AGE-mediated mechanisms should reveal their role in the etiopathogenesis of the Alzheimer's syndrome and, finally, lead to the development of new pharmacological strategies aimed at inhibiting protein cross-linking.

Nervenarzt 1996 Nov;67(11):924-9

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Advanced glycation end products induce glomerular sclerosis and albuminuria in normal rats.

High levels of tissue advanced glycation end products (AGEs) that result from the spontaneous modification of proteins by glucose occur in diabetes and aging. To address the potential pathogenic role of AGEs in the glomerulosclerosis of diabetes or nephrosclerosis of aging, doses of AGE-modified rat albumin (25 mg per kg per day, i.v.) sufficient to elevate circulating AGE levels to the range of diabetic serum were administered daily to healthy rats alone or in combination with the AGE inhibitor aminoguanidine. After 5 months, the AGE content of renal tissues in AGE-treated rats rose to 50% above controls ( $P < 0.025$ ), whereas serum contained 2.8-fold greater AGE levels ( $P < 0.025$ ). Light and electron microscopy of kidneys from AGE-treated rats revealed a more than 50% increase in glomerular volume compared to controls ( $P < 0.001$ ), significant periodic acid/Schiff reagent-positive deposits, basement membrane widening, and mesangial extracellular matrix increase and indicated significant glomerulosclerosis compared to untreated ( $P < 0.002$ ) or albumin-treated controls ( $P < 0.002$ ). These changes were associated with significant loss of protein ( $P < 0.005$ ) and albumin ( $P < 0.002$ ) in the urine of AGE-treated rats compared to controls. Cotreatment with aminoguanidine markedly limited both the structural and functional defects. These *in vivo* data demonstrate that AGEs influence glomerular structure and function in a manner leading to glomerulosclerosis. The effects are AGE-specific, as they are ameliorated by a pharmacological AGE inhibitor, aminoguanidine.

Proc Natl Acad Sci U S A 1994 Nov 22;91(24):11704-8

An agent cleaving glucose-derived protein crosslinks *in vitro* and *in vivo*.

Glucose and other reducing sugars react with proteins by a nonenzymatic, post-translational modification process called nonenzymatic glycosylation or glycation. The sugar-derived carbonyl group adds to a free amine, forming a reversible adduct which over time rearranges to produce a class of products termed advanced-glycation end-products (AGEs). These remain irreversibly bound to macromolecules and can covalently crosslink proximate amino groups. The formation of AGEs on long-lived connective tissue and matrix components accounts largely for the increase in collagen crosslinking that accompanies normal ageing and which occurs at an accelerated rate in diabetes. AGEs can activate cellular receptors and initiate a variety of pathophysiological responses. They modify an appreciable fraction of circulating low-density lipoproteins preventing uptake of these particles by their high-affinity tissue receptors. Advanced glycation has also been implicated in the pathology of Alzheimer's disease. Because AGEs may form by a pathway involving reactive alpha-dicarbonyl intermediates, we investigated a potential pharmacological strategy for selectively cleaving the resultant glucose-derived protein crosslinks. We now describe a prototypic AGE crosslink "breaker", N-phenacylthiazolium bromide (PTB), which reacts with and cleaves covalent, AGE-derived protein crosslinks. The ability of PTB to break AGE crosslinks *in vivo* points to the importance of an alpha-dicarbonyl intermediate in the advanced glycation pathway and offers a potential therapeutic approach for the removal of established AGE crosslinks.

Nature 1996 Jul 18;382(6588):275-8

Pharmaceutical intervention of advanced glycation endproducts.

Recent studies have revealed that reducing sugars, such as glucose, react with proteins through non-enzymatic glycosylation to form irreversible, covalently cross-linked proteins known as advanced glycation endproducts (AGEs). Furthermore, it has been demonstrated that this naturally occurring process, accelerated in diabetics due to hyperglycaemia, impairs biological functions leading to cardiovascular disorders, as well as diabetic and age-related complications. Pharmaceutical intervention to prevent or reverse these complications have focused on inhibiting the formation of AGEs by compounds such as dimethyl-3-phenacylthiazolium chloride or breaking the glucose derived cross-links by selective cleavage. Intervention targeted at AGE crosslinks *in vivo* offers a way to interfere with age-related changes of tissues.

Novartis Found Symp 2001;235:202-12; discussion 212-6, 217-20

A cross-link breaker has sustained effects on arterial and ventricular properties in older rhesus monkeys.

Nonenzymatic glycosylation and cross-linking of proteins by glucose contributes to an age-associated increase in vascular and myocardial stiffness. Some recently synthesized thiazolium compounds selectively break these protein cross-links, reducing collagen stiffness. We investigated the effects of 3-phenacyl-4,5-dimethylthiazolium chloride (ALT-711) on arterial and left ventricular (LV) properties and their coupling in old, healthy, nondiabetic *Macaca mulatta* primates (age 21 +/- 3.6 years). Serial

measurements of arterial stiffness indices [i.e., aortic pulse wave velocity (PWV) and augmentation (AGI) of carotid arterial pressure waveform] as well as echocardiographic determinations of LV structure and function were made before and for 39 weeks after 11 intramuscular injections of ALT-711 at 1.0 mg/kg body weight every other day. Heart rate, brachial blood pressure, and body weight were unchanged by the drug. PWV and AGI decreased to a nadir at 6 weeks [PWV to 74.2 +/- 4.4% of baseline (B), P = 0.007; AGI to 41 +/- 7.3% of B, P = 0.046], and thereafter gradually returned to baseline. Concomitant increases in LV end diastolic diameter to 116.7 +/- 2.7% of B, P = 0.02; stroke volume index (SV(index)) to 173.1 +/- 40.1% of B, P = 0.01; and systolic fractional shortening to 180 +/- 29.7% of B, P = 0.01 occurred after drug treatment. The LV end systolic pressure/SV (index), an estimate of total LV vascular load, decreased to 60 +/- 12.1% of B (P = 0.02). The LV end systolic diameter/SV(index), an estimate of arterio-ventricular coupling, was improved (decreased to 54.3 +/- 11% of B, P < 0.002). Thus, in healthy older primates without diabetes, ALT-711 improved both arterial and ventricular function and optimized ventriculo-vascular coupling. This previously unidentified cross-link breaker may be an effective pharmacological therapy to improve impaired cardiovascular function that occurs in the context of heart failure associated with aging, diabetes, or hypertension, conditions in which arterial and ventricular stiffness are increased.

Proc Natl Acad Sci U S A 2001 Jan 30;98(3):1171-5

## B vitamins

### Hyperhomocysteinaemia and atherothrombosis.

Homocysteine (Hcy) is a sulfhydryl amino acid derived from the metabolic conversion of methionine that is dependent on vitamins (folic acid, B12 and B6) as cofactors or cosubstrates. In 1969, McCully first reported the presence of severe atherosclerotic lesions in patients with severe hyperhomocysteinaemia and hypothesized the existence of a pathogenic link between hyperhomocysteinaemia and atherogenesis. Several case-control and cross-sectional studies confirmed the initial hypothesis of McCully, showing that also moderate hyperhomocysteinaemia is associated with a heightened risk of occlusive arterial disease. Less consistent results have been reported by prospective cohort studies of subjects who were healthy at the time of their enrollment, whereas prospective cohort studies of patients with overt coronary artery disease or other risk conditions consistently confirmed the association between moderate hyperhomocysteinaemia and the risk of cardiovascular morbidity and mortality. More recently, an association between moderate hyperhomocysteinaemia and heightened risk of venous thromboembolism has been documented, suggesting that hyperhomocysteinaemia might be involved not only in atherogenesis, but also in thrombogenesis. The mechanisms by which hyperhomocysteinaemia might contribute to atherogenesis and thrombogenesis are incompletely understood. The mainstay of treatment of hyperhomocysteinaemia is folic acid, alone or in combination with vitamins B12 and B6. Although it is quite clear that vitamins effectively reduce the plasma levels of total Hcy, we do not yet know whether they will decrease the risk of vascular disease. The results of ongoing randomized, placebo-controlled, double-blinded trials on the effects of vitamins on thrombotic risk will help in defining whether the relationship between hyperhomocysteinaemia and thrombosis is causal, and will potentially have a dramatic impact on the prevention of thromboembolic events.

Ann Med 2000 Dec;32 Suppl 1:46-52

Distribution of and factors associated with serum homocysteine levels in children: child and adolescent trial for cardiovascular health.

**CONTEXT:** Although evidence suggests that homocysteine is a risk factor for cardiovascular disease in adults, little information exists on homocysteine levels in children. **OBJECTIVES:** To describe the distribution of serum homocysteine concentrations among children and to examine the association between homocysteine levels and several characteristics, including serum levels of folic acid and vitamins B12 and B6. **DESIGN:** Cross-sectional analysis. **SETTING:** School-based cohort from California, Louisiana, Minnesota, and Texas. **PARTICIPANTS:** A total of 3524 US schoolchildren, aged 13 and 14 years, from the Child and Adolescent Trial for Cardiovascular Health (completed in 1994). Measurement was conducted in 1997. **MAIN OUTCOME MEASURE:** Nonfasting serum total homocysteine concentration. **RESULTS:** The distribution of homocysteine values ranged from 0.1 to 25.7 micromol/L (median, 4.9 micromol/L). Geometric mean homocysteine concentration was significantly higher in boys (5.22 micromol/L) than girls (4.84 micromol/L); blacks (5.51 micromol/L) than whites (4.96 micromol/L) or Hispanics (4.93 micromol/L); nonusers of multivitamins (5.09 micromol/L) than users (4.82 micromol/L); and smokers (5.19 micromol/L) than nonsmokers (5.00 micromol/L). Serum homocysteine was significantly inversely correlated with serum levels of folic acid ( $r = -0.36$ ;  $P = .001$ ), vitamin B12 ( $r = -0.21$ ;  $P = .001$ ), and vitamin B6 ( $r = -0.18$ ;  $P = .001$ ). Serum homocysteine was not significantly associated with serum lipid levels or family history of cardiovascular disease and was only weakly related to body mass index and systolic blood pressure. After multivariate adjustment, homocysteine remained independently associated with sex, race, serum folic acid and vitamin B12 levels, and systolic blood pressure. **CONCLUSIONS:** The distribution of homocysteine levels in children is substantially lower than that observed for adults; however, a small percentage of children are still potentially at elevated risk for future cardiovascular disease. Serum folic acid may be an important determinant of homocysteine levels in children.

JAMA 1999 Apr 7;281(13):1189-96

Effects of folic acid and combinations of folic acid and vitamin B12 on plasma homocysteine concentrations in healthy, young women.

**BACKGROUND:** Elevated plasma homocysteine concentrations are considered to be a risk factor for vascular disease and fetal malformations such as neural tube defects. Recent studies have shown that plasma homocysteine can be lowered by folic acid in amounts corresponding to 1 to 2 times the recommended dietary allowance. Preliminary evidence indicates that vitamin B12 may be beneficial when included in supplements or in a food-fortification regimen together with folic acid. **OBJECTIVE:** We aimed to compare the homocysteine-lowering potential of a folic acid supplement with that of 2 supplements containing different doses of vitamin B12 in addition to folic acid. **DESIGN:** Female volunteers of childbearing age ( $n = 150$ ) received a placebo for 4 wk followed by a 4 wk treatment with either 400 microg folic acid, 400 microg folic acid + 6 microg vitamin B12, or 400 microg folic acid + 400 microg vitamin B12. **RESULTS:** Significant reductions ( $P < 0.001$ ) in plasma homocysteine were observed in all groups receiving vitamin treatment. The effect observed with the combination of folic acid + 400 microg vitamin B12 (total homocysteine, -18%) was significantly larger than that with a supplement containing folic acid alone (total homocysteine, -11%) ( $P < 0.05$ ). Folic acid in combination with a low vitamin B12 dose (6 microg) affected homocysteine as well (-15%). **CONCLUSIONS:** These results suggest that the addition of vitamin B12 to folic acid supplements or enriched foods maximizes the reduction of homocysteine and may

thus increase the benefits of the proposed measures in the prevention of vascular disease and neural tube defects.

Am J Clin Nutr 1998 Nov;68(5):1104-10

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## ABSTRACTS

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Improved vascular endothelial function after oral B vitamins: An effect mediated through reduced concentrations of free plasma homocysteine.

**BACKGROUND:** Hyperhomocysteinemia is an independent risk factor for coronary heart disease (CHD). Dietary supplementation with B vitamins lowers plasma homocysteine by up to 30%. However, little is known about the potential beneficial effects of homocysteine lowering on vascular function in patients with CHD. **METHODS AND RESULTS:** We investigated 89 men with CHD (aged 56 [range 39 to 67] years). Brachial artery flow-mediated dilatation (endothelium dependent) and nitroglycerin-induced dilatation (endothelium independent) were measured before and 8 weeks after treatment with either (1) folic acid (5 mg) and vitamin B12 (1 mg) daily (n=59) or (2) placebo (n=30). Total, protein-bound, and free plasma homocysteine, serum folate, and vitamin B12 were measured at baseline and at 8 weeks. Flow-mediated dilatation improved after treatment with B vitamins (2.5+/-3.2% to 4.0+/-3.7%, P=0.002) but not placebo (2.3+/-2.6% to 1.9+/-2.6%, P=0.5). Vitamin therapy lowered plasma concentrations of total homocysteine (from 13.0+/-3.4 to 9.3+/-1.9 micromol/L, P<0.001), protein-bound homocysteine (from 8.7+/-2.8 to 6.2+/-1.4 micromol/L, P<0.001), and free homocysteine (from 4.3+/-1.2 to 3.0+/-0.6 micromol/L, P<0.001) and raised concentrations of serum folate (from 10.3+/-4.3 to 31.2+/-10.8 ng/mL, P<0.001) and vitamin B12 (from 314+/-102 to 661+/-297 pg/mL, P<0.001). In regression analysis, improved flow-mediated dilatation correlated closely with the reduction in free plasma homocysteine (r=-0.26, P=0.001), independent of changes in protein-bound homocysteine, folate, and vitamin B12. Nitroglycerin-induced dilatation was unchanged after both B vitamins and placebo. **CONCLUSIONS:** Folic acid and vitamin B12 supplementation improves vascular endothelial function in patients with CHD, and this effect is likely to be mediated through reduced concentrations of free plasma homocysteine concentrations. Our data support the view that lowering homocysteine, through B vitamin supplementation, may reduce cardiovascular risk.

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Serum folate and cardiovascular disease mortality among US men and women.

**BACKGROUND:** Folate has been linked to cardiovascular disease (CVD) through its role in homocysteine metabolism. **OBJECTIVE:** To assess the relationship between serum folate and CVD mortality. **DESIGN:** In this prospective study, serum folate concentrations were measured on a subset of adults during the Second National Health and Nutrition Examination Survey (1976-1980) and vital status ascertained after 12 to 16 years. **SETTING AND PATIENTS:** A national probability sample consisting of 689 adults who were 30 to 75 years of age and did not have a history of CVD at baseline. **MAIN OUTCOME MEASURE:** Vital status was determined by searching national databases that contained information about US decedents. **RESULTS:** The associations between serum folate and CVD and all-cause mortality differed by diabetes status (P =.04 and P =.03, respectively). Participants without diabetes in the lowest compared with the highest serum folate tertile had more than twice the risk of CVD mortality after adjustment for age and sex (relative risk [RR], 2.64; 95% confidence interval [CI], 1.15-6.09). This increased risk for participants in the lowest tertile was attenuated after adjustment for CVD risk factors (RR, 2.28; 95% CI, 0.96-5.40). Serum folate tertiles were not significantly associated with total mortality, although the age- and sex-adjusted risk was increased for participants in the lowest compared with highest tertile (RR, 1.74; 95% CI, 0.96-3.15). Risk estimates for participants with diabetes were unstable because of the small sample size (n = 52). **CONCLUSION:** These data suggest that low serum folate concentrations are associated with an increased risk of CVD mortality among adults who do not have diabetes.

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Low serum folate but normal homocysteine levels in patients with atherosclerotic vascular disease and matched healthy controls.

Mild hyperhomocysteinemia has been considered a cardiovascular risk factor. However, recent prospective studies have not demonstrated that hyperhomocysteinemia or the underlying genetic defect on methylenetetrahydrofolate reductase is associated with a higher risk of coronary or peripheral artery disease. We compared serum homocysteine, folate, and vitamin B12 levels of patients with coronary and peripheral vascular disease with those of age- and sex-matched healthy individuals. Subjects taking multivitamins, with diabetes mellitus, or serum creatinine levels over 1.5 mg/dL were excluded from the study. Homocysteine was measured by fluorimetric high-performance liquid chromatography. Serum folate and vitamin B12 levels were measured by an ion-capture method. We studied 32 patients with peripheral vascular disease (10 female), aged 69.6 +/- 11 y, 24 age- and sex-matched control subjects, 52 patients with coronary artery disease (7 female), aged 59.5 +/- 10.4 y, and 42 age- and sex-matched control subjects. Serum homocysteine levels were 11.7 +/- 7.4 and 9.3 +/- 4.5 micromol/L in vascular patients and in the control counterparts, respectively (not significant). The levels for coronary patients and the control counterparts were 9.0 +/- 3.9 and 8.6

+/- 3.6 micromol/L, respectively (not significant). Folate levels were 4.48 +/- 2.42 and 7.14 +/- 4.04 ng/mL in vascular patients and control subjects, respectively ( $P < 0.02$ ); the levels in coronary patients and control counterparts were 5.15 +/- 1.9 and 6.59 +/- 2.49 ng/mL, respectively ( $P < 0.01$ ). No differences in vitamin B12 or tocopherol levels were observed between patients and control subjects. There were no differences in homocysteine levels, but lower serum folate levels were observed when comparing patients with atherosclerotic vascular disease and healthy control subjects.

Nutrition 2000 Jun;16(6):434-8

Dietary strategies for lowering homocysteine concentrations.

**BACKGROUND:** Elevated plasma total homocysteine (tHcy) concentrations are associated with increased risk of vascular disease, and there is a strong inverse association between dietary and blood folate and blood tHcy concentrations. Increased folate consumption may lower the risk of tHcy-mediated cardiovascular disease. **OBJECTIVES:** The objective was to determine the most appropriate means of increasing dietary folate to reduce plasma tHcy. **DESIGN:** Sixty-five free-living subjects aged 36 to 71 years with tHcy concentrations  $\geq 9$  micromol/L participated in a randomized, controlled trial to compare 3 approaches for increasing dietary folate to approximately 600 microg/d: folic acid supplementation, consumption of folic acid-fortified breakfast cereals, and increased consumption of folate-rich foods. **RESULTS:** An intake of 437 microg folic acid/d from supplements resulted in a 27-nmol/L increase in serum folate and a 21% reduction in tHcy, relative to the change in a control group. In subjects who consumed folic acid-fortified breakfast cereal, folate intake increased by an average of 298 microg, serum folate increased by 21 nmol/L, and tHcy concentrations decreased by 24%. Increased intakes of folate-rich foods resulted in a 418-microg increase in dietary folate, a 7-nmol/L increase in serum folate, and a 9% reduction in tHcy concentrations. The decrease in tHcy was negatively correlated ( $r = -0.66$ ) with the increase in serum folate. **CONCLUSIONS:** Daily consumption of folic acid-fortified breakfast cereals and the use of folic acid supplements appear to be the most effective means of reducing tHcy concentrations. The reduction in tHcy was significantly negatively correlated with the increase in serum folate, which may be a useful marker for measuring dietary change.

Am J Clin Nutr 2000 Jun;71(6):1448-54

Association of folate intake and serum homocysteine in elderly persons according to vitamin supplementation and alcohol use.

**BACKGROUND:** The serum total homocysteine concentration (tHcy), an indicator of folate status and a possible risk factor for vascular disease, is elevated with impaired renal function and poor vitamin B12 status, which are common in the elderly. **OBJECTIVE:** Our objective was to determine the association between tHcy, folate intake, alcohol consumption, and other lifestyle factors in elderly persons. **DESIGN:** This cross-sectional study used linear regression to model changes in tHcy. Subjects were 278 men and women aged 66 to 94 years studied in 1993. **RESULTS:** Total folate intake was negatively associated with tHcy in models adjusted for age, sex, serum creatinine, and serum albumin. We found an interaction between food folate intake and supplement use. Food folate intake had an inverse dose-response relation with tHcy that was limited to nonusers of supplements. Predicted tHcy was 1.5 micromol/L lower in users of supplements containing folate and vitamin B12 than in nonusers and was independent of food folate intake. We found a positive dose-response relation of coffee and tea intake with tHcy, a positive association for alcohol intake of  $\geq 60$  drinks/mo compared with low intake, and an interaction of alcohol use with folate intake and supplement use. Compared with alcohol users, nonusers had higher predicted tHcy and a lower inverse dose-response relation of food folate intake with tHcy. **CONCLUSIONS:** The inverse association between folate intake and tHcy was strongest among nonusers of supplements and among alcohol drinkers. Identifying modifiable factors related to tHcy, a possible risk factor for vascular disease, is especially important in elderly persons.

Am J Clin Nutr 2001 Mar;73(3):628-37

Determinants of plasma total homocysteine concentration in the Framingham Offspring cohort.

**BACKGROUND:** Established determinants of fasting total homocysteine (tHcy) concentration include folate and vitamin B12 status, serum creatinine concentration, and renal function. **OBJECTIVE:** Our objective was to examine the relation between known and suspected determinants of fasting plasma tHcy in a population-based cohort. **DESIGN:** We examined the relations between fasting plasma tHcy concentrations and nutritional and other health factors in 1960 men and women, aged 28 to 82 years, from the fifth examination cycle of the Framingham Offspring Study between 1991 and 1994, before the implementation of folic acid fortification. **RESULTS:** Geometric mean tHcy was 11% higher in men than in women and 23% higher in persons aged  $\geq 65$  y than in persons aged  $< 45$  y ( $P < 0.001$ ). tHcy was associated with plasma folate, vitamin B12, and pyridoxal phosphate ( $P$  for trend  $< 0.001$ ). Dietary folate, vitamin B-6, and riboflavin were associated with tHcy among non-supplement users ( $P$  for trend  $< 0.01$ ). The tHcy concentrations of persons who used vitamin B supplements were 18% lower than those of persons who did not ( $P < 0.001$ ). tHcy was positively associated with alcohol intake ( $P$  for trend = 0.004), caffeine intake ( $P$  for trend  $< 0.001$ ), serum creatinine ( $P$  for trend  $< 0.001$ ), number of cigarettes smoked ( $P$  for trend  $< 0.001$ ), and antihypertensive medication use ( $P < 0.001$ ). **CONCLUSIONS:** Our study confirmed, in a population-based setting, the importance of the known determinants of fasting tHcy and suggested that other dietary and lifestyle factors, including vitamin B6, riboflavin, alcohol and caffeine intakes as well as

smoking and hypertension, influence circulating tHcy concentrations.

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Vitamin intervention for stroke prevention (VISP) trial: rationale and design.

Elevated plasma levels of homocyst(e)ine [H(e)] are surprisingly common and strongly associated with endothelial dysfunction and a marked increase in vascular risk. Treatment with a combination of folic acid, pyridoxine (vitamin B6) and cobalamin (vitamin B12) reduces plasma H(e) levels in most cases, restores endothelial function, and regresses carotid plaque, but there is no evidence that such treatment will reduce clinical events. The Vitamin Intervention for Stroke Prevention (VISP) study is a double-masked, randomized, multicenter clinical trial designed to determine if, in addition to best medical/surgical management, high-dose folic acid, vitamin B6, and vitamin B12 supplements will reduce recurrent stroke compared to lower doses of these vitamins. Patients at least 35 years old with a nondisabling ischemic stroke within 120 days, and screening plasma H(e) > the 25th percentile of benchmark population data are eligible. Secondary endpoints are myocardial infarction or fatal coronary heart disease. This paper describes the design and rationale of the study.

Neuroepidemiology 2001 Feb;20(1):16-25

Multivitamin/mineral supplementation improves plasma B-vitamin status and homocysteine concentration in healthy older adults consuming a folate-fortified diet.

Elevated homocysteine has been identified as an independent risk factor for cardiovascular and cerebrovascular disease. Although multivitamin use has been associated with low plasma homocysteine concentrations in several observational studies, no clinical trials have been conducted using multivitamin/mineral supplements to lower homocysteine. We determined whether a multivitamin/mineral supplement formulated at about 100% Daily Value will further lower homocysteine concentration and improve B-vitamin status in healthy older adults already consuming a diet fortified with folic acid. In this randomized, double-blind, placebo-controlled trial, 80 free-living men and women aged 50 to 87 years with total plasma homocysteine concentrations of  $\geq 8$  micromol/L received either a multivitamin/mineral supplement or placebo for 56 d while consuming their usual diet. After the 8-wk treatment, subjects taking the supplement had significantly higher B-vitamin status and lower homocysteine concentration than controls ( $P < 0.01$ ). Plasma folate, pyridoxal phosphate (PLP) and vitamin B12 concentrations were increased 41.6, 36.5 and 13.8%, respectively, in the supplemented group, whereas no changes were observed in the placebo group. The mean homocysteine concentration decreased 9.6% in the supplemented group ( $P < 0.001$ ) and was unaffected in the placebo group. There were no significant changes in dietary intake during the intervention. Multivitamin/mineral supplementation can improve B-vitamin status and reduce plasma homocysteine concentration in older adults already consuming a folate-fortified diet.

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